Pontifícia Universidade Católica do Rio Grande do Sul
Faculdade de Biociências
Programa de Pós-Graduação em Biologia Celular e Molecular

KELLY JULIANA SEIBT BENDER

Avaliação do potencial neuroprotetor de fármacos antipsicóticos em alterações bioquímicas, moleculares e comportamentais induzidas por antagonista de receptor NMDA (MK-801) em peixe zebra (*Danio rerio*)

Porto Alegre
2011

KELLY JULIANA SEIBT BENDER

Avaliação do potencial neuroprotetor de fármacos antipsicóticos em alterações bioquímicas, moleculares e comportamentais induzidas por antagonista de receptor NMDA (MK-801) em peixe zebra (*Danio rerio*)

Tese apresentada como requisito para obtenção do grau de Doutor pelo Programa de Pós-Graduação em Biologia Celular e Molecular da Faculdade de Biociências da Pontifícia Universidade Católica do Rio Grande do Sul.

Orientadora: Profª Drª Carla Denise Bonan

Porto Alegre
2011

KELLY JULIANA SEIBT BENDER

Avaliação do potencial neuroprotetor de fármacos antipsicóticos em alterações bioquímicas, moleculares e comportamentais induzidas por antagonista de receptor NMDA (MK-801) em peixe zebra

Tese apresentada como requisito para obtenção do grau de Doutor pelo Programa de Pós-Graduação em Biologia Celular e Molecular da Faculdade de Biociências da Pontifícia Universidade Católica do Rio Grande do Sul.

Aprovada em: ____de_____de_____.

BANCA EXAMINADORA:

_____
Prof. Dra. Fernanda Bueno Morrone - PUCRS

_____
Profa. Dra Iraci Lucena da Silva Torres - UFRGS

_____
Prof. Dra. Maria Rosa Chitolina Schetinger - UFSM

Porto Alegre
2011

Aos meus pais, Clovis e Ilse

Ao meu esposo Luis Felipe

Que em muitos momentos acreditaram mais em

mim do que eu mesma, dedico este trabalho.

AGRADECIMENTOS

À minha orientadora Carla Bonan, por ter me proporcionado momentos de crescimento e de conhecimento. Agradeço pelas palavras de incentivo e afeto, que me incentivaram nos momentos mais difíceis. Só cheguei até aqui porque você sempre acreditou em mim e isso me fez seguir em frente. A você o meu carinho, admiração e eterna gratidão.

À minha família, em especial aos meus pais, que se esforçaram para me dar a melhor educação possível. Por sempre estarem ao meu lado em minhas decisões, e por me ensinarem que o estudo é o alicerce de quem somos. Muito obrigada, amo vocês.

Ao meu esposo, Luis Felipe, que nunca deixou de acreditar em mim e me incentivar. Obrigada pela tolerância com que suportou as minhas longas ausências durante todo o curso. Obrigada pelo amor e pela atenção. Eu te amo muito.

À minha amiga Renata da Luz Oliveira, por me ajudar na pesquisa, pela paciência, amizade e companheirismo, e principalmente pelo ombro amigo e palavras de incentivo. Você foi fundamental para a conclusão de cada etapa desse trabalho. Muito obrigada.

Aos colegas do Laboratório de Neuroquímica e Psicofarmacologia da PUCRS, com os quais compartilhei um pouco da minha vida: alegrias, dúvidas e incertezas, além de inesquecíveis momentos de carinho e amizade. Com vocês vivi dias inesquecíveis.

Aos professores, Rosane, Monica e Mauricio, pela convivência, dicas, amizade e incentivo. Muito obrigada.

À PUCRS pela bolsa de estudo concedida.

"Quero, um dia, dizer às pessoas que nada foi em vão, que o amor existe

que vale a pena se doar às amizades e às pessoas

que a vida é bela sim

e que eu sempre dei o melhor de mim...

E que valeu a pena!!!"

Mário Quintana

RESUMO

A esquizofrenia é uma doença mental grave caracterizada por sintomas positivos, negativos e déficits cognitivos que ainda é pouco compreendida. A redução da neurotransmissão glutamatérgica por antagonistas dos receptores NMDA mimetiza os sintomas da esquizofrenia. Muitos modelos animais têm mostrado sua importância para o estudo dessa doença, e o peixe-zebra tem sido proposto como um modelo promissor para estudar os efeitos *in vivo* de várias drogas e descobrir novos alvos farmacológicos. Neste estudo caracterizamos a síndrome comportamental produzida pela exposição ao antagonista do receptor NMDA, MK-801, no peixe zebra, e investigamos a capacidade dos fármacos antipsicóticos em reverter estes sintomas. MK-801 (20 μM) aumentou o comportamento locomotor que foi medido pelo número de linhas cruzadas, a distância percorrida e a velocidade média no aquário teste, após 15, 30 e 60 min de exposição. Os antipsicóticos sulpirida, olanzapina e haloperidol reverteram as alterações locomotoras induzidas pelo MK-801 em todos os parâmetros testados, e em doses que administrado isoladamente não tiveram efeito sobre a atividade locomotora. Modelos de interação social e déficits cognitivos em animais pode ser de grande utilidade para o desenvolvimento de novos tratamentos para os sintomas negativos e cognitivos da esquizofrenia. Os resultados mostraram que o MK-801 (5 μM) administrado antes do treino impediu a formação da memória, enquanto ambos os antipsicóticos atípicos sulpirida (250 μM) e olanzapina (50 μM) melhoraram a amnésia. A mesma alteração foi observada na tarefa de interação social, onde os antipsicóticos atípicos reverteram o déficit de interação social induzida pelo MK-801, enquanto o antipsicótico tipico testado, o haloperidol (9 μM), não foi capaz de reverter esse déficit comportamental. Algumas evidências sugerem que mudanças no sistema purinérgico, mais especificamente na atividade adenosinérgica, poderiam estar envolvidos na fisiopatologia da esquizofrenia. Nesse estudo, mostramos que o tratamento com haloperidol (9 μM) foi capaz de diminuir a hidrólise de ATP (35%), enquanto que não houve mudanças significativas na hidrólise de ADP e AMP em membranas cerebrais. A atividade da ADA em frações de membrana também foi inibida significativamente (38%) após o tratamento com haloperidol, quando comparado ao grupo controle. Além disso, a exposição ao haloperidol também promoveu uma diminuição na expressão gênica das NTPDases (*entpd2_mq* e *entpd3) e* adenosina desaminase *(adal)*. Considerando que a enzima Na^+, K^+-ATPase é essencial para a função cerebral normal, avaliamos os efeitos do MK-801 e farmacos antipsicóticos na atividade desta enzima. Nossos resultados mostraram que o tratamento com MK-801 diminuiu significativamente a atividade da Na^+, K^+-ATPase, e todos os antipsicóticos testados impediram tais efeitos. Sabe-se que o estresse oxidativo pode estar associado com a fisiopatologia da esquizofrenia e que a Na^+,K^+-ATPase é particularmente suscetível ao ataque de radicais livres. Mostramos que o tratamento com MK-801 não alterou as espécies reativas de oxigênio/nitrogênio pelo ensaio de oxidação 2'7'-diclorofluorosceína (DCF), mas aumentou os níveis de substâncias reativas ao ácido tiobarbitúrico (TBARS). Os antipsicóticos haloperidol, sulpirida e olanzapina preveniram o aumento nos níveis de TBARS induzidos pelo MK-801. Portanto, demonstramos que o peixe zebra pode apresentar algumas características comportamentais e bioquímicas observadas na esquizofrenia, sendo considerado um promissor modelo animal capaz de contribuir na obtenção de informações sobre potenciais tratamentos e características da doença.

Palavras-chave: peixe-zebra, antipsicóticos, MK-801, esquizofrenia, estresse oxidativo.

ABSTRACT

Schizophrenia is a severe mental illness characterized by positive and negative symptoms and cognitive deficits. This pathology is still poorly understood. Reduction of glutamatergic neurotransmission by NMDA receptor antagonists mimics disease symptoms. Many animal models have shown their importance in the study of this disease and the zebrafish has been proposed as a promissor model to study the *in vivo* effects of several drugs and to discover new pharmacological targets. In this study we characterized the behavioral syndrome produced by the NMDA receptor antagonist, MK-801, exposure in zebrafish and investigated the ability of antipsychotic drugs to reverse the schizophrenia-like symptoms. MK-801 (20 μM) increased the locomotor behavior as measured by the number of line crossings, distance traveled, and the mean speed in the tank test after 15, 30, and 60 min of exposure. The antipsychotics sulpiride, olanzapine, and haloperidol counteracted MK-801-induced hyperactivity on all parameters analyzed and at doses that, given alone, had no effect on spontaneous locomotor activity. Modeling social interaction and cognitive impairment in animals can be of great benefit in the effort to develop novel treatments for negative and cognitive symptoms of schizophrenia. Results showed that MK-801 (5 μM) given pre-training hindered memory formation while both atypical antipsychotics sulpiride (250 μM) and olanzapine (50 μM) improved MK-801-induced amnesia. The same change was observed in the social interaction task, where atypical antipsychotics reversed the MK-801-induced social interaction deficit whereas the typical antipsychotic haloperidol (9 μM) was ineffective to reverse those behavioral deficits. Some evidence suggests that changes in the purinergic system, more specifically in adenosinergic activity, could be involved in the physiopathology of schizophrenia. In this study, we demonstrated that haloperidol treatment (9 μM) was able to decrease ATP hydrolysis (35%), whereas there were no significant changes in ADP and AMP hydrolysis in brain membranes. Adenosine deaminase activity in membrane fractions was significantly inhibited (38%) after haloperidol treatment when compared to the control group. Furthermore, haloperidol exposure also led to a decrease in NTPDase gene expression (*entpd2_mq* and *entpd3*), and adenosine deaminase (*adal*). Considering that the enzyme Na^+,K^+-ATPase is essencial to brain normal function, we evaluated the effect of MK-801 and antipsychotic drugs on activity this enzyme. Our results showed that MK-801 treatment significantly decreased Na^+,K^+-ATPase activity, and all antipsychotics tested prevented such effects. Moreover, it is known that oxidative stress may be associated with the physiopathology of schizophrenia and the Na^+,K^+-ATPase is particularly susceptible to free radical attack. We showed that MK-801 treatment did not alter reactive oxygen/nitrogen species by 2′7′-dichlorofluorscein (DCF) oxidation assay, but increased the levels of thiobarbituric acid reactive substances (TBARS), when compared to controls. The antipsychotics sulpiride, olanzapine, and haloperidol prevented the increase of TBARS caused by MK-801. Therefore, we demonstrated that zebrafish might present some behavioral and biochemical features observed in schizophrenia, being considered a promising animal model able to contribute for providing information on potential treatments and disease characteristics.

Keywords: zebrafish, antipsychotics, MK-801, schizophrenia, oxidative stress.

LISTA DE ABREVIATURAS

ADA – adenosina desaminase

ADP – adenosina 5´- difosfato

AMP – adenosina 5´- monofosfato

AMPc – adenosina 5´- monofosfato cíclico

ATP – adenosina 5´- trifosfato

Ca^{+2} – cálcio

CAT - catalase

DCF - 2´7´diclorofluoresceína

DCFH-DA - 2´7´diclorofluoresceína diacetato

DSM IV – manual diagnóstico e estatístico de transtornos mentais

E-NPP – ectonucleotídeo pirofosfatase/fosfodiesterase

EPS – sintomas extrapiramidais

ERO – espécies reativas de oxigênio

GABA – ácido γ-amino butírico

GPI – glicosilfosfatidilinositol

GSH – glutationa reduzida

K^{+} - potássio

MDA – malondialdeído

Mg^{+2} – magnésio

MK-801 – maleato de dizocilpina

mRNA – RNA mansageiro

Na^{+} – sódio

NMDA – N-metil-D-aspartato

NTPDase – nucleosídeo trifosfato difosfoidrolase

SNC – sistema nervoso central

SOD – superóxido dismutase

NMDA – N-metil-D-aspartato

TBARS – substâncias reativas ao ácido tiobarbitúrico

5-HT – 5-hidroxitriptamina (serotonina)

LISTA DE FIGURAS

SUMÁRIO

1. INTRODUÇÃO

1.1. Peixe zebra (*Danio rerio*)

O peixe zebra, também conhecido como paulistinha ou *zebrafish*, é um pequeno teleósteo (3-5cm) de água doce da família *Cyprinidae.* Esse peixe é caracterizado por seu tamanho pequeno e um padrão de coloração distinto, baseado em listras horizontais, claras e escuras e alternadas (Spence et al., 2008) (Figure 1). Nos últimos anos, o número de pesquisas desenvolvidas utilizando este modelo animal tem aumentado consideravelmente. O peixe zebra possui alta homologia genética (70-80%) com roedores e humanos (Barbazuk et al., 2000; Miklósi & Andrew, 2006) e tem sido utilizado como uma importante ferramenta para a realização de estudos toxicológicos, pesquisa transgênica, evolução do genoma vertebrado, teratologia (Yang et al., 2009), mutagênese, neurociências (Becker & Becker, 2008) e também tem sido alvo de estudos farmacológicos (Bencan et al., 2009; Egan et al., 2009) e comportamentais (Mathur & Guo, 2010; Sison & Gerlai, 2011).

Figura 1. Peixe zebra (*Danio rerio*)

O peixe zebra possui uma série de características favoráveis que o tornam particularmente acessível à manipulação experimental. As fêmeas se reproduzem o ano todo e os animais podem ser mantidos e facilmente controlados em grande número por um custo baixo. A desova das fêmeas geralmente ocorre a cada 2-3 dias e uma única desova pode conter centenas de ovos (Spence et al., 2008). Seus ovos são opticamente transparentes e a fecundação é externa, o que facilita os estudos de embriogênese nessa espécie, já que todas as fases de desenvolvimento podem ser vistas e monitoradas por meio do uso de um microscópio (Beis & Stainier, 2006). Seu desenvolvimento é rápido, sendo que no prazo de 36 horas os embriões apresentam precursores de todos os principais órgãos. Apenas cinco dias após a fertilização, as larvas de peixe zebra já apresentam capacidade de buscar alimento e evitar o

perigo, demonstrando um comportamento anti-predatório. Além disso, o peixe zebra apresenta a capacidade de absorver compostos adicionados à água, dispensando assim a necessidade de tratamentos por meio de de protocolos invasivos (Goldsmith, 2004; Berghmans et al., 2007). Além das inúmeras vantagens do peixe zebra citadas acima, seu cérebro é anatomicamente e funcionalmente semelhante ao de mamíferos (Guo, 2009) e muitos neurotransmissores já foram documentados nesta espécie (Kaslin & Panula, 2001; Rico et al., 2003; Edwards & Boehmler et al., 2004; Rosemberg et al., 2007).

O interesse pelo peixe zebra pode ser observado pelo vasto número de laboratórios que utilizam este peixe como modelo experimental em suas pesquisas (Kabashi et al., 2011) e pelo crescimento exponencial do número de estudos publicados que envolvem esta espécie (Lieschke & Currie, 2007). Devido às suas peculiaridades reprodutivas e às suas características morfológicas e fisiológicas, esta espécie desperta o interesse pela oportunidade de acelerar o processo da descoberta de novas drogas (Stern & Zon, 2003; Kabashi et al., 2011), além de estar sendo considerado um modelo promissor para estudos envolvendo diversas doenças (Best & Alderton, 2008; Wong et al., 2010).

1.1.1. Estudos comportamentais utilizando o peixe zebra

A utilização do peixe zebra para pesquisa em neurociências tem crescido muito na última década, principalmente na biologia do comportamento (Blaser & Peñalosa, 2011, Padilla et al., 2011). O estudo do comportamento desta espécie é um campo relativamente novo, e muitas vezes os testes utilizados são adaptações de protocolos já estabelecidos em roedores, como teste de campo aberto, preferência por claro-escuro, labirinto em cruz e testes de exposição ao predador (Levin et al., 2007; Saverino & Gerlai, 2008; Sison & Gerlai, 2010; Champagne et al., 2010; Grossman et al., 2010; Maximino et al., 2010; Stewart et al., 2010). As pesquisas que abordam comportamento no peixe zebra tratam de diferentes tarefas, tais como: preferência por claro/escuro (Serra et al., 1999; Blank et al., 2009), agressividade (Gerlai et al., 2000), atividade locomotora/comportamento exploratório (Gerlai *et al*., 2000; Swain et al., 2004; Levin et al., 2007), memória (Levin & Chen, 2004; Blank et al., 2009), vício (Gerlai et al., 2000; Ninkovic & Bally-Cuif., 2006), preferência social (Gerlai et al., 2000; Gerlai 2003), medo/ansiedade (Gerlai et al., 2000; Levin et al, 2007; Blaser et al., 2009), entre outros parâmetros (Gerlai et al. 2000; Blank et al., 2009; Egan et al., 2009; Kurta & Palestis, 2010; Stewart et al., 2010).

Estudos envolvendo aprendizado e memória neste modelo permitem a identificação de genes e seus produtos envolvidos com os processos cognitivos de maneira fácil, barata e eficiente e de baixo custo (Guo, 2004; Al-Imari & Gerlai, 2008). Diversos estudos sobre o processo cognitivo têm sido desenvolvidos nessa espécie (Blank et al., 2009). Darland e Dowling (2001) desenvolveram uma tarefa de preferência de lugar induzido por cocaína para estudar o comportamento do peixe zebra. Williams et al. (2002) e Bilotta et al. (2005) estudaram tarefas relacionadas à recompensa por alimento e verificaram que o peixe zebra aprende de forma rápida, demonstrando altos níveis de acertos logo após o início do treino. A esquiva inibitória é uma tarefa cujos parâmetros bioquímicos e farmacológicos estão bem estudados em mamíferos, em especial em roedores (Barros et al., 2002; Izquierdo et al., 2006). Esta tarefa permite avaliar a memória de trabalho (WM), de curta duração (STM) e de longa duração (LTM) (Izquierdo & Medina, 1995). Em 2009, Blank et al. desenvolveram a tarefa de esquiva inibitória para o peixe zebra. Por ser a tarefa mais amplamente utilizada em estudos com roedores e, portanto, aquela usada para extrapolação de resultados para humanos, este estudo padronizou um novo paradigma de esquiva inibitória em peixe zebra a fim de permitir a avaliação dos mecanismos celulares e moleculares da formação de memória em vertebrados (Blanck et al., 2009).

Piato et al. (2010) desenvolveram um modelo de estresse crônico em peixe zebra e, após submeterem os animais a 7 ou 14 dias de estresse, avaliaram parâmetros comportamentais e marcadores de estresse. Os animais estressados permaneceram mais tempo na parte inferior do aquário, mostrando claro efeito ansiogênico do estresse (Piato et al., 2010). Gerlai (2003) observou alterações na atividade motora induzida pelo tratamento com álcool a 0,25 e 0,50%. Neste mesmo trabalho, Gerlai testou a preferência por grupo, mostrando que o álcool diminuiu a coesão social entre o grupo, fazendo com que os peixes passassem mais tempo perto do aquário vazio do que do aquário estímulo, onde estavam 15 peixes para induzir a interação social (Gerlai, 2003).

Como descrito anteriormente, o zebrafish representa um modelo vertebrado com grande potencial para estudos comportamentais. A padronização de novos testes com o peixe zebra fornece meios promissores para descobertas de novas drogas e até mesmo para o melhor entendimento de diversas doenças, como por exemplo, a esquizofrenia.

1.2. Esquizofrenia

Esquizofrenia é o termo usado para descrever uma doença mental que acomete em torno de 1% da população mundial (Ross et al., 2006). A manifestação inicial da doença ocorre em jovens, entre 15 e 25 anos, e em geral causa profundos danos na qualidade de vida profissional, intelectual e especialmente social dos pacientes (Szymanski et al., 1995; Dawe et al., 2009). A incidência é discretamente maior entre os homens (Buchanan & Carpenter, 2005), sendo que nas mulheres os sintomas se manifestam em média cinco anos mais tarde (Hafner et al., 1998). O risco de desenvolvimento da doença é maior quando há histórico familiar, especialmente se há parentesco de primeiro grau ou mais de um membro da família afetado (Dawe et al., 2009).

A doença foi descrita pela primeira vez por Kraepelin, em seu livro texto de 1893, como *dementia praecox* (demência precoce) por causar um sério comprometimento cognitivo em pacientes relativamente jovens (Meltzer et al., 1999). Em 1911, Eugen Bleuler, um psiquiatra suíço, trocou o termo por esquizofrenia que descreveu como um grupo de psicoses caracterizadas por alterações específicas de pensamento, sentimentos e relação com o mundo (Ban, 2004). Atualmente, o DSM-IV classifica a esquizofrenia, para fim de diagnóstico, como uma perturbação com duração mínima de 6 meses com a presença de delírios, alucinações, discurso desorganizado, comportamento amplamente alterado ou catatônico, bem como a falta de afeto e comprometimento cognitivo (DSM-IV-TR, 2002; Bowie & Harvey, 2006).

Os sintomas da esquizofrenia podem ser classicamente divididos em positivos e negativos (Lewis & Lieberman, 2000). Dentre os sintomas positivos, estão incluídos alucinações (visual e auditiva), delírios, desorganização severa do pensamento e do discurso; já os sintomas negativos referem-se à diminuição ou perda das funções normais, tais como, perda de energia, da motivação, diminuição do afeto, isolamento social, entre outros (Ross et al., 2006; van Os & Kapur, 2009). Além desses sintomas, a atividade cognitiva do esquizofrênico é anormal, apresentando incoerências e desconexões de raciocínio, com grande repercussão na linguagem (Pinkham et al., 2003; Morgan et al., 2004).

A fisiopatologia completa do transtorno é desconhecida, porém, diversos fatores sugerem que a esquizofrenia seja um transtorno poligênico onde atuam vulnerabilidades ambientais e do neurodesenvolvimento (Farber et al., 1995; Lewis & Lieberman, 2000). Diversos estudos demonstraram a existência de desequilíbrios em vários sistemas de neurotransmissores nesta patologia, permitindo assim a elaboração de algumas teorias para tentar explicar melhor essa doença (Krystal et al., 2002).

A hipótese dopaminérgica foi desenvolvida a partir da observação de que a anfetamina, um agonista dopaminérgico, poderia induzir sintomas psicóticos em pessoas saudáveis e exacerbar alucinações, delírios e distúrbios do pensamento em pacientes esquizofrênicos (Abi-Dargham & Laruelle, 2005). Além disto, drogas com a capacidade de bloquear os receptores pós-sinápticos dopaminérgicos reduzem os sintomas psicóticos em pacientes com esquizofrenia (Abi-Dargham & Laruelle, 2005; Buchanan & Carpenter, 2005), sendo que a potência das medicações antipsicóticas tem relação direta com sua capacidade de bloquear receptores dopaminérgicos do tipo D2 (Seeman & Tallerico, 1998).

O sistema glutamatérgico também parece ter papel importante na fisiopatologia da doença, sendo um dos principais neurotransmissores estudados na esquizofrenia (Boison et al., 2011). Dentre as evidências científicas que levaram a pensar na participação do glutamato na fisiopatologia da esquizofrenia, destacam-se os níveis diminuídos de glutamato no líquor de portadores de esquizofrenia (Kim et al., 1980). Além disso, estudos mostram que antagonistas dos receptores glutamatérgicos NMDA (como cetamina, fenciclidina e MK-801) são capazes de produzir tanto sintomas positivos como negativos e cognitivos da doença (Krystal et al., 2002; Powel & Miyakawa, 2006).

Outra hipótese da esquizofrenia seria a serotoninérgica, onde a primeira evidência foi o resultado da observação de que a dietilamida do ácido lisérgico (LSD), uma droga com estrutura similar à da serotonina e com grande afinidade pelos receptores $5HT_{2A}$, possui propriedades alucinógenas, induzindo efeitos similares a alguns sintomas da esquizofrenia (Domino et al., 2004). Assim como LSD, psilocina e psilocibina, as quais são substâncias alucinógenas presentes em cogumelos, também induzem alucinações e também agem no sistema serotoninérgico reforçando esta hipótese (Whitaker et al., 1981). Outra evidência indireta que reforça a idéia do envolvimento dos receptores serotoninérgicos na esquizofrenia surgiu da associação entre o perfil de afinidade e observações clínicas dos antipsicóticos atípicos. Comparando-se com os antipsicóticos típicos que essencialmente ligam-se em receptores D_2, os antipsicóticos atípicos em sua maioria apresentam maior afinidade por receptores $5HT_{2A}$, o que pode estar associado ao seu menor potencial de causar sintomas extrapiramidais e seu maior efeito nos sintomas negativos (Meltzer, 2003).

Entretanto, é pouco provável que apenas um neurotransmissor ou receptor seja responsável pelas variadas alterações observadas na esquizofrenia. Além disso, a co-modulação existente entre os diversos sistemas de neurotransmissores torna ainda menos plausível a proposta de que um único sistema possa ser o responsável pela doença. É o caso da adenosina, um neuromodulador do sistema purinérgico, que regula a atividade de diversos

neurotransmissores, incluindo dopamina e glutamato (Boison et al., 2011). Com base em evidências experimentais e observações clínicas foi proposta a hipótese adenosinérgica da esquizofrenia, que constitui um elo entre as outras hipóteses, principalmente a dopaminérgica e a glutamatérgica (Lara & Souza, 2000).

A hipótese adenosinérgica da esquizofrenia foi primeiramente proposta por Lara e Souza (2000). Eles sugeriram que uma disfunção no sistema purinérgico resultaria em uma atividade reduzida da adenosina, o que poderia ser a explicação para o desequilíbrio entre a neurotransmissão dopaminérgica e glutamatérgica, que são marcos característicos da esquizofrenia (Lara & Souza, 2000; Lara et al., 2006). A hipótese foi amplamente baseada em dados preliminares, sugerindo uma atividade benéfica terapêutica de um derivado da purina, o alopurinol, em pacientes com esquizofrenia e uma eficácia moderada contra o comportamento agressivo (Lara et al., 2000).

1.2.1 Modelos animais de esquizofrenia

Devido à dificuldade de simular os sintomas positivos em animais experimentais, como alucinações, paranóias e delírios, os modelos animais para os sintomas positivos da esquizofrenia em sua maioria avaliam o comportamento estereotipado e a agitação, que são mais facilmente mensuráveis e que mimetizam o estado de comportamento observado nos pacientes (Geyer & Ellenbroek, 2003). Os antagonistas de receptores NMDA (fenciclidina, cetamina e MK-801) de glutamato são utilizados para a indução de modelos de sintomas positivos, já que produzem um quadro de agitação e comportamento estereotipado. Além disso, essas drogas induzem delírios e alucinações psicóticas em humanos (Coyle et al., 2003).

A anedonia e o déficit de interação social são os sintomas negativos mais estudados em modelos animais. Considera-se que as medidas de interação social em roedores são diretamente análogas às medidas em humanos (Powell & Miakawa, 2006). Por este motivo, há um crescente interesse no aperfeiçoamento e utilização desse teste. Outro teste bastante aplicado é a medida da locomoção, que é utilizada como um modelo de psicose em testes com camundongos e ratos (Ninan & Kulkarni, 1999; Geyer & Ellenbroek, 2003). A administração sistêmica de MK-801 em camundongos é também indicada como um bom modelo farmacológico para esquizofrenia (Ninan & Kulkarni, 1999; O'Neill & Shaw, 1999), uma vez que em humanos esse antagonista é capaz de reproduzir tanto os efeitos positivos como os negativos da doença (Goff & Coyle, 2001). Diversos estudos realizados com ratos tratados

com fármacos antipsicóticos têm mostrado que antipsicóticos típicos e atípicos podem reverter os sintomas positivos, negativos e o déficit cognitivo gerados pelos antagonistas NMDA (Geyer et al., 2001; Amitai et al., 2007). Assim, estudos mostram que a administração de antipsicóticos pode reverter déficits causados pelos antagonistas glutamatérgicos, sendo um modelo promissor para a busca de novos fármacos com esta aplicação terapêutica (Heresco-Levy, 2003; Sun et al., 2009).

1.3. Fármacos Antipsicóticos

Desde a sua introdução, em 1952, os antipsicóticos são a base do tratamento da esquizofrenia. Atualmente, são classificados como típicos (primeira geração) ou atípicos (segunda geração). Os antipsicóticos típicos, os primeiros introduzidos na terapêutica, são antagonistas de alta afinidade de receptores D_2 (Abi-Dargham & Lauruelle, 2005). Apesar da melhora relativa dos sintomas, especialmente os positivos, o bloqueio de receptores dopaminérgicos também induz vários efeitos adversos, como acatisia, discinesia tardia, distonia (aguda e crônica), sintomas extrapiramidais (EPS), como rigidez, bradicinesia e tremores (Miyamoto et al., 2005). Além disso, os antipsicóticos típicos em geral exercem pouco efeito sobre os sintomas negativos e cognitivos (Gardner et al., 2005). Os representantes mais importantes desse grupo são o haloperidol e a clorpromazina.

Na década de 1970, foi descoberta a clozapina, o primeiro antipsicótico de segunda geração ou atípico. Entretanto, no final da década de 1970, essa medicação foi retirada de mercado em vários países devido ao risco de agranulocitose. Somente em 1988 este fármaco foi reintroduzido em função da sua eficácia diferenciada em quadros refratários a outros antipsicóticos (Kane et al., 1988). Os antipsicóticos atípicos foram lançados no mercado com a proposta de superioridade em relação a eficácia, especialmente na melhora dos sintomas negativos e a ausência de EPS, possibilitando melhor adesão ao tratamento a longo prazo e conseqüente redução de taxas de internações hospitalares (Kane et al., 1988; Breier et al., 2005). Os fármacos atípicos diferem farmacologicamente dos típicos pela sua baixa afinidade pelos receptores dopaminérgicos D_2 e pela alta afinidade por receptores de outras vias neuronais, como os serotoninérgicos, histamínicos, adrenérgicos e colinérgicos (Miyamoto et al., 2005). A denominação atípica vem justamente do fato de que esses fármacos apresentam um comportamento atípico em relação ao padrão dos agentes clássicos, não induzindo EPS, também conhecida como síndrome neuroléptica em humanos, ou induzindo catalepsia em modelos experimentais (Kapur & Mamo, 2003). Além da vantagem da diminuição de EPS,

foi sugerido inicialmente que esses novos compostos fossem eficazes quanto aos sintomas negativos. Com isso, a introdução destes novos fármacos no tratamento de esquizofrenia foi acompanhada de grande expectativa. Entretanto, muitos pacientes também não respondem a esses medicamentos, e o efeito sobre sintomas negativos e cognitivos não se mostrou muito eficaz (Murphy et al., 2006). Entre os representantes dessa classe temos a clozapina, risperidona, olanzapina, sulpirida e quetiapina (Kane et al., 2002; Simpson et al., 2005).

1.4. Sistema purinérgico

Os nucleosídeos e nucleotídeos exercem um importante papel de moléculas sinalizadoras extracelulares em vários tecidos, por meio de dos receptores purinérgicos (Burnstock, 2008). A adenosina 5'-trifosfato, ou ATP, é um nucleotídeo trifosfatado existente em todas as células e está envolvido na regulação de vários processos fisiológicos e patológicos no meio extracelular (Bours et al., 2006). O ATP é armazenado em vesículas pré-sinápticas e após a despolarização neuronal é liberado atuando em receptores específicos na membrana pós-sináptica, sendo considerado um neurotransmissor (Burnstock, 1972). O ATP pode ser co-liberado juntamente com vários outros neurotransmissores, tais como a acetilcolina, o glutamato, a noradrenalina, a serotonina e o ácido γ-amino butírico (GABA) (Di Iorio et al., 1998; Burnstock, 2004).

O ATP exerce suas funções por meio de da ativação de receptores purinérgicos do tipo P2. Este grupo de purinoreceptores é subdivido em duas famílias distintas: P2X e P2Y (Abbracchio et al., 2009). A família P2X consiste em receptores ionotrópicos que apresentam permeabilidade rápida e seletiva para cátions (Na^+, K^+ e Ca^{2+}) e está dividida em sete membros (P2X1-7), que estão distribuídos em neurônios, células gliais e musculatura lisa (North, 2002; Burnstock, 2004). A família P2Y consiste em receptores metabotrópicos e foram funcionalmente descritos oito membros ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ e $P2Y_{14}$), que apresentam uma ampla distribuição nos tecidos e sistemas, tais como: vascular, nervoso e cardíaco (Erb et al., 2006; Burnstock, 2007) (Figura 2).

Em situações patofisiológicas, a liberação de ATP e a expressão de receptores purinérgicos pelas células estão consideravelmente aumentadas (Guido et al., 2008). Como este nucleotídeo não é capaz de atravessar as membranas biológicas por difusão ou transporte ativo, o controle de sua concentração extracelular é realizado pela ação das ectonucleotidases que catalisam sua degradação à adenosina (Robson et al., 2006; Schetinger et al., 2007).

A adenosina é um nucleosídeo de purina descrito classicamente como um neuromodulador (Fredholm, 2003). A concentração extracelular de adenosina é um fator determinante dos efeitos neuromoduladores desta molécula (Antonioli et al., 2008). A adenosina exerce seus efeitos por meio de da ativação de receptores purinérgicos do tipo P1 (Fredholm et al., 2001). Este nucleosídeo atua sobre uma família de quatro subtipos de receptores: A_1, A_{2A}, A_{2B} e A_3, os quais são acoplados a proteína G e exibem sete domínios transmembrana formados por aminoácidos hidrofóbicos (Abbracchio et al., 2009; Muller & Jacobson, 2011).

A adenosina tem papel relevante na neuromodulação regulando a liberação de vários neurotransmissores, agindo tanto pré quanto pós-sinapticamente (Cunha, 2001; Dunwiddie & Masino, 2001; Ribeiro et al., 2003). A regulação da liberação de neurotransmissores excitatórios por esta molécula tem se tornado importante em diversos processos patológicos, pois a adenosina pode limitar o dano causado pela excitotoxicidade destes neurotransmissores, exercendo assim uma ação protetora no sistema nervoso central (SNC) (Zimmermann et al., 1998; Dunwiddie & Masino, 2001).

Devido a este papel neuromodulador, a adenosina está envolvida na regulação de importantes mecanismos no SNC (Cunha, 2001; Boison et al., 2010), sendo reconhecida como um importante modulador da neurotransmissão excitatória e agente neuroprotetor em diferentes patologias relacionadas ao SNC, tais como na isquemia, hipóxia (Fredholm, 1997; Ribeiro et al., 2003), epilepsia (Boison, 2005; Vianna et al., 2005), doença de Parkinson (Fredduzzi et al., 2002) e na esquizofrenia (Lara et al., 2001; Boison et al,. 2011; Gomes et al., 2011).

Além de ser formada a partir da hidrólise do ATP, por meio de da ação das ectonucleotidases, a adenosina pode ser produzida no meio intracelular e transportada para o meio extracelular por meio de de transportadores específicos bidirecionais, que mantêm os níveis intracelulares e extracelulares de adenosina em equilíbrio (Latini & Pedata, 2001). A adenosina extracelular também pode ser formada a partir da degradação do monofosfato cíclico de adenosina (AMPc) (Latini & Pedata, 2001), e sua concentração extracelular é um fator importante em seu efeito neuromodulador (Antonioli et al., 2008).

No peixe zebra, já foram realizadas a clonagem e a caracterização molecular dos receptores P2X (Egan et al., 2000; Diaz-Hernandes et al., 2002). Kucenas et al. (2003) mostraram que a subunidade P2X possui nove membros, sendo destes seis ortólogos a genes dos receptores P2X de mamíferos, dois parálogos e um gene ainda precisa ser devidamente classificado (Kucenas et al., 2003). Os subtipos dos receptores P2X do peixe zebra contêm

resíduos altamente conservados, os quais são encontrados nas subunidades de mamíferos. Até o momento, na família de receptores P2Y foram identificados oito proteínas funcionais (Ralevic & Bursntock, 1998; Lazarowski et al., 2003; Illes & Ribeiro, 2004), e apenas foram identificados receptores P2Y1 em trombócitos de peixe zebra (Gregory & Jagadeeswaran, 2002). Recentemente, um estudo identificou os receptores de adenosina A_{2A} no peixe zebra (Boehmler et al., 2009).

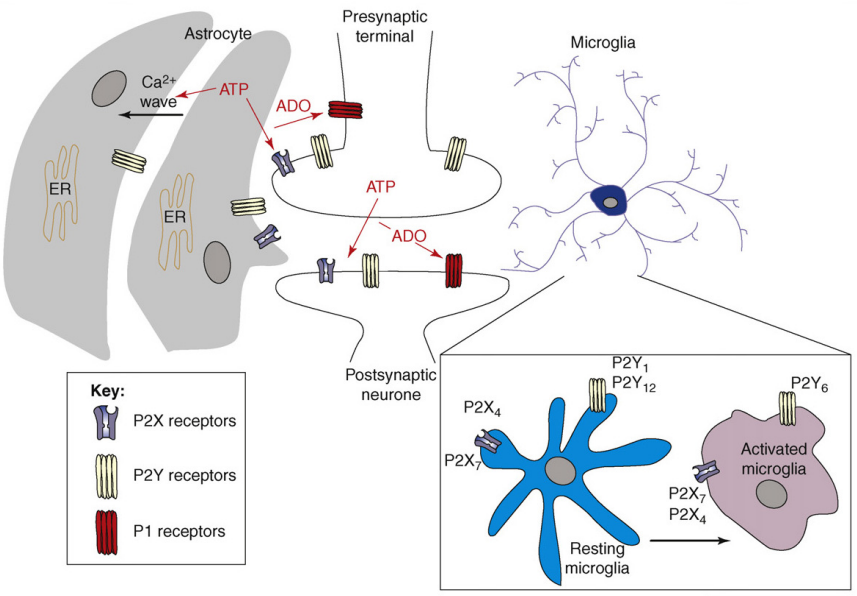

Figura 2. Representação esquemática da sinalização purinérgica no SNC. O ATP liberado liga-se a receptores P2 localizados em membranas pós-sinápticas ou pré-sinápticas e em astrócitos. A degradação do ATP origina a adenosina, que atua em receptores P1. O ATP também pode ser liberado a partir de astrócitos, onde inicia e propaga o fluxo de Ca^{+2} na sinalização glia-neurônio, que também pode ser controlada pela adenosina. Figura obtida de Abbracchio et al. (2009).

1.4.1 Ectonucleotidases

Os nucleotídeos extracelulares são degradados por uma cascata de hidrólise constituída por uma variedade de enzimas que estão localizadas na superfície celular, chamadas de ectonucleotidases. Dentre elas, destacam-se a família das nucleosídeo trifosfato difosfoidrolases (NTPDases), a ecto-5'-nucleotidase e a ecto-nucleotídeo pirofosfatase/fosfodiesterase (E-NPP), as quais são enzimas capazes de alterar os níveis de ATP, ADP, AMP e adenosina (Zimmermann, 2001; Robson et al., 2006).

As NTPDases realizam a hidrólise de nucleotídeos trifosfatados e difosfatados (Zimmermann, 2001). Essa família de enzimas é composta por oito membros (NTPDases 1-8) sendo que quatro das NTPDases (NTPDase1, 2, 3, 8) estão localizadas na superfície das células, com um sítio catalítico extracelular. As NTPDases5 e 6 apresentam localização intracelular e as NTPDases4 e 7 estão localizadas no meio intracelular com seus sítios ativos direcionados para o lúmen das organelas citoplasmáticas. As NTPDases compartilham 5 domínios altamente conservados denominados regiões conservadas de apirase (Zimmermann, 2001; Robson et al., 2006). Em termos de hidrólise de nucleotídeos, a NTPDase1 hidrolisa ATP e ADP igualmente (Heine et al., 1999). A enzima NTPDase2 hidrolisa 30 vezes mais ATP do que o ADP. A NTPDase3 e a NTPDase8 preferem o ATP em relação ao ADP numa razão de hidrólise de aproximadamente 3:1 e 2:1, respectivamente (Chadwick et al., 1998; Bigonnesse et al., 2004). A NTPDase4α tem uma alta preferência por UTP e TTP, enquanto que a NTPDase4β apresenta alta preferência por CTP e UDP. A função dessas NTPDases ainda não é clara (Zimmermann, 2001). A NTPDase5 tem uma preferência na hidrólise de nucleotídeos na seguinte ordem: UDP>GDP = IDP>>ADP = CDP, enquanto que a NTPDase6 tem a seguinte preferência: GDP>IDP>>UDP = CDP>>ADP. Evidências indicam que a NTPDase5 e a NTPDase6 participam das reações de glicosilação envolvidas nos processos de dobramento de glicoproteínas (Zimmermann, 2001). A NTPDase7 prefere nucleotídeos trifosfatados como substratos (Zimmermann, 2001). As NTPDases hidrolisam tanto ATP como ADP, formando AMP na presença de íons Ca^{+2} e Mg^{+2} (Robson et al., 2006).

A 5´-nucleotidase constitui uma família de enzimas com distribuição tecidual ampla e com capacidade de produzir nucleosídeos a partir de nucleotídeos monofosfatados AMP, GMP ou UMP. Diferentes distribuições sub-celulares são encontradas para os membros da família das 5´- nucleotidases, existindo formas solúveis e formas ancoradas à membrana (Bianchi & Spychala, 2003; Hunsucker et al., 2005). A participação da ecto-5´-nucleotidase na via das ectonucleotidases exerce um papel modulador sobre a produção de adenosina extracelular, sendo a enzima marca-passo desta cascata enzimática (Zimmermann, 2000; Cunha, 2001). Assim, de acordo com a sua localização tecidual, ela desempenha importantes funções como, por exemplo, no controle da agregação plaquetária, na regulação do tônus vascular e também na neuromodulação e neuroproteção do sistema nervoso (Zimmermann et al., 1998; Dunwiddie & Masino, 2001). Um estudo mostrou que o tratamento crônico com clozapina, mas não haloperidol, aumenta a atividade da ecto-5'-nucleotidase em estriado de ratos (Lara et al., 2001).

No peixe zebra, estudos demonstraram a presença de NTPDases e uma ecto-5'-nucleotidase em membranas cerebrais. Estas duas enzimas foram caracterizadas como cátion-dependentes, apresentando atividade máxima à temperatura de 37 °C, pH ótimo entre 7,2 e 8,0, K_M na faixa de micromolar e uma ampla especificidade por outros nucleotídeos (Rico et al., 2003; Senger et al., 2004). Rico et al. (2006) identificaram três isoformas da NTPDase2, que foram nomeadas como NTPDase2mv, NTPDase2mq e NTPDase2mg em peixe zebra (Rico et al., 2006). Appelbaum et al. (2007) clonaram e caracterizaram o padrão de expressão da NTPDase3 em peixe zebra (Appelbaum et al., 2007). Rosemberg et al. (2010) verificaram a presença de diferentes membros da família das NTPDases (NTPDases 1, 2, 3, 4, 5, 6, 8) em cérebro, coração e fígado de peixe zebra. As NTPDase1 e NTPDase2 também foram encontradas em fotorreceptores, células horizontais e células ganglionares em retina de peixe zebra (Ricatti et al., 2009). Estas enzimas desempenham uma função essencial na neurotransmissão purinérgica, controlando a disponibilidade e os níveis de nucleotídeos e nucleosídeos extracelulares e, consequentemente, a ativação dos purinoreceptores P2 e P1 (Zimmermann, 2001).

1.4.2. Adenosina desaminase

A adenosina extracelular pode ser liberada pelas células ou ser proveniente da hidrólise do ATP. A sua concentração pode ser controlada via transportadores celulares, sua fosforilação à AMP pela adenosina quinase (AK) ou por meio de de sua desaminação à inosina pela adenosina desaminase (ADA, EC 3.5.4.4) (Latini & Pedata, 2001). A ADA é uma enzima envolvida no metabolismo das purinas por catalisar a conversão da adenosina e da deoxiadenosina a inosina e deoxiinosina, respectivamente (Franco et al., 1997) (Figura 3).

A ADA é encontrada como uma enzima citosólica e também pode ser expressa na superfície celular como uma ectoenzima. Dois membros clássicos da ADA estão descritos, sendo eles primeiramente denominados como ADA1 e ADA2. Estudos têm demonstrado que os membros apresentam características cinéticas distintas, o que faz com que ambos possam desempenhar uma função diferenciada nos organismos (Iwaki-Egawa et al., 2004; Zavialov & Engström, 2005).

Embora a existência de dois membros da ADA tenha sido previamente descrita, uma análise filogenética das sequências de diferentes organismos revelou uma nova família de proteínas relacionada com a ADA1 e ADA2, a qual foi denominada ADAL (Rosemberg et al., 2007). Todos estes membros, juntamente com a ADA presente em alguns fungos e bactérias e

a adenosina-5'-monofosfato desaminase, foram classificados como subfamílias pertencentes ao grupo das adenil-desaminases. Por apresentar sítios de aminoácidos importantes relacionados à desaminação de adenosina e motivos conservados entre as subfamílias da ADA, é sugerido que a ADAL também possa participar da clivagem de adenosina à inosina (Maier et al., 2005). Contudo, não existem estudos demonstrando a atividade ou expressão deste membro das adenil-desaminases em mecanismos de injúria e neuroproteção em nenhum modelo animal, até o presente momento.

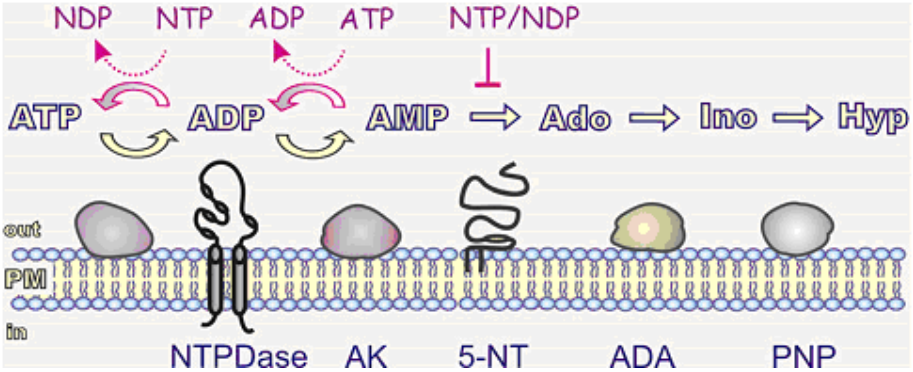

Figura 3. Representação esquemática da degradação extracelular de purinas. As enzimas que atuam na conversão de ATP à adenosina incluem as NTPDases, 5' nucleotidase e ADA. A PNP (purina nucleosídeo fosforilase) hidrolisa a adenosina, produzindo hipoxantina. A AK (adenosina quinase) fosforila a adenosina em AMP. Figura adaptada de Yegutkin (2008).

Rosemberg et al. (2007) realizaram o mapeamento do padrão de expressão de genes relacionados a adenosina desaminase em vários tecidos de peixe zebra (Rosemberg et al., 2007), bem como a caracterização cinética da atividade da ADA em cérebro de peixe zebra (Rosemberg et al., 2008). Brustein et al. (2007) mostraram que a atividade da ADA em pacientes esquizofrênicos que realizam tratamento com drogas antipsicóticas, em especial a clozapina, encontra-se alterada (Brustein et al., 2007).

1.5. Na^+,K^+-ATPase (EC 3.6.3.9)

A Na^+,K^+-ATPase ou bomba de Na^+ é uma proteína transmembrana presente em grande quantidade no tecido cerebral. Esta proteína hidrolisa ATP, gerando energia para o transporte iônico pela membrana plasmática, ou seja, exporta íons Na^+ da célula e importa íons K^+, mantendo o gradiente iônico necessário para a excitabilidade neuronal e a regulação do volume celular (Erecinska et al., 2004; Illarionova et al., 2010).

Um grande número de funções celulares está acoplado à manutenção das concentrações intracelulares e extracelulares de Na^+ e K^+, tais como controle do volume celular, a excitabilidade neuronal, a atividade de enzimas citosólicas, a contração muscular, além de auxiliar no movimento de outros íons e compostos por meio de da membrana como, por exemplo, a glicose, os aminoácidos e os neurotransmissores (Kaplan, 2002; Jorgensen et al., 2003).

Considerando a importância da Na^+,K^+-ATPase para o funcionamento normal do SNC, a inibição da sua atividade tem sido associada a diversas condições fisiológicas e patológicas, como a isquemia cerebral (Wyse et al., 2000), a doença de Alzheimer (Lees, 1993; Hattori et al., 1998) e os mecanismos de memória em ratos (Wyse et al., 2004). Estudos com animais mostram que substâncias psicoativas como a anfetamina (Zugno et al., 2009), fluoxetina (Zanatta et al., 2001), selegilina (Carageorgius et al., 2003), haloperidol, carbamazepina e lítio (Wood et al., 1989) estimulam a atividade da Na^+,K^+-ATPase. Além disso, dados na literatura mostram que essa enzima é inibida por radicais livres, produtos de lipoperoxidação e alterações na fluidez da membrana (Dobrota et al., 1999; Kurella et al., 1999; Chakraborty et al., 2003).

Rajarao et al. (2002) demonstraram que isoformas da Na^+,K^+-ATPase são diferencialmente expressas em sistema nervoso central e órgãos sensoriais durante a embriogênese de zebrafish (Rajarao et al., 2002). Nesse mesmo estudo os autores identificaram e clonaram oito subunidades α e cinco subunidade β de Na^+,K^+-ATPases em peixe zebra (Rajarao et al., 2002).

1.6. Radicais Livres e Estresse Oxidativo

O radical livre é definido como qualquer átomo, grupo de átomos ou molécula que possua um ou mais elétrons desemparelhados girando em seus orbitais externos. Isso o torna muito instável e lhe confere a propriedade de ser uma espécie química altamente agressiva e de vida curta (Andreazza, 2004). Uma vez formados, os RLs buscam estabilizar-se, cedendo ou captando um elétron de espécies vizinhas (Schanaider, 2000). Cria-se uma reação em cadeia que termina por alterar a conformação, a estrutura ou as funções de proteínas, fosfolipídios de membrana, ácidos nucléicos e outros componentes celulares (Riegel, 2002).

Várias são as fontes geradoras de RLs. As ERO - termo utilizado para designar radicais (ânion superóxido e radical hidroxila) e alguns não radicais derivados do oxigênio

(peróxido de hidrogênio e oxigênio singleto) - são formadas principalmente durante a respiração celular, pela redução incompleta (2-5%) do oxigênio molecular (Salvador & Henriques, 2004; Halliwell & Gutteridge, 2007). Fisiologicamente, essas espécies participam de funções importantes, como a fagocitose, a sinalização celular, a regulação de proteínas e a plasticidade sináptica (Serrano & Klann, 2004; Halliwell & Gutteridge, 2007). Entretanto, quando em excesso, as ERO podem oxidar diversas biomoléculas, como os lipídios, as proteínas e o DNA (Halliwell & Gutteridge, 2007). Com relação aos efeitos prejudiciais das reações oxidantes ao organismo, os RLs podem promover lipoperoxidação, podem causar a oxidação de proteínas de baixa densidade (LDL), podem reagir com proteínas, levando à sua inativação e podem também reagir com DNA e RNA, levando a mutações somáticas e a distúrbios de transcrição (Delanty & Dichter, 1998).

A fim de evitar os efeitos danosos das espécies reativas, o nosso organismo dispõe de mecanismos eficientes para a detoxificação desses agentes oxidantes, conhecidos como defesas antioxidantes. Essas podem ser divididas em enzimáticas e não enzimáticas. As principais enzimas antioxidantes são superóxido dismutase (SOD), a catalase (CAT) e a glutationa peroxidase (GSH-Px). As defesas antioxidantes não enzimáticas incluem principalmente a GSH, o ácido ascórbico (vitamina C), o α-tocoferol (vitamina E), e os polifenóis, a melatonina, a bilirrubina, o urato, o ácido lipóico e os estrógenos (Salvador & Henriques, 2004; Halliwell & Gutteridge, 2007).

Em condições fisiológicas, há um balanço entre os mecanismos oxidativos e as defesas antioxidantes; porém, em certas condições patológicas pode haver um desequilíbrio entre esses dois sistemas, onde ocorre a produção de uma série de substâncias pró-oxidantes, acima da capacidade antioxidante do organismo, favorecendo a ocorrência do estresse oxidativo (Dadheech et al., 2008). Todos os tecidos são vulneráveis ao estresse oxidativo, porém o SNC é particularmente sensível por causa da alta taxa de consumo de oxigênio, dos elevados níveis de lipídios poliinsaturados capazes de sofrer peroxidação lipídica e da auto-oxidação de alguns neurotransmissores (Obata, 2002; Halliwell, 2006).

Evidências crescentes indicam que o estresse oxidativo desempenha um importante papel em várias condições clínicas, como doença de Parkinson, e doença de Alzheimer (Paraskevas et al., 2003) e a esquizofrenia (Owe-Larsson et al., 2011; Yao & Reddy, 2011). Alguns estudos relatam problemas com a defesa antioxidante e aumento da peroxidação lipídica em pacientes esquizofrênicos livres de tratamento (Arvindakshan et al., 2003; Reddy et al., 2003) e naqueles tratados cronicamente com neurolépticos típicos (Herken et al., 2001). Pacientes com esquizofrenia apresentam baixos níveis das enzimas antioxidantes e da

capacidade antioxidante total quando comparado a controles saudáveis, gerando estresse oxidativo e peroxidação lipídica. Essa condição piora com o aumento da idade, tabagismo e com o estilo de vida (Dadheeck et al., 2008; Mico et al., 2011).

2. OBJETIVOS

2.1. Objetivo geral

Considerando que: (1) o peixe zebra é um importante modelo experimental em estudos farmacológicos, bioquímicos, comportamentais e patológicos, e que (2) a esquizofrenia é uma doença complexa cujos mecanismos de ação são pouco esclarecidos; o presente estudo objetiva analisar alterações comportamentais, bioquímicas e moleculares em um modelo experimental induzido pelo MK-801 que mimetiza sintomas da esquizofrenia, e assim avaliar o efeito protetor de fármacos antipsicóticos nas alterações induzidas pelo MK-801 no peixe zebra.

2.2 Objetivos específicos

- Avaliar a ação do haloperidol, sulpirida, olanzapina e MK-801 sobre parâmetros comportamentais do peixe zebra, como atividade locomotora, interação social e memória;
- Verificar a possível influência dos fármacos antipsicóticos sobre as alterações comportamentais induzidas pelo MK-801;
- Verificar o efeito do tratamento *in vivo* com fármacos antipsicóticos e MK-801, sobre as atividades e expressão gênica das NTPDases e 5´-nucleotidase em SNC do peixe zebra;
- Verificar o efeito do tratamento *in vivo* com haloperidol, sulpirida, olanzapina e MK-801 sobre a atividade e expressão gênica da ADA em SNC do peixe zebra;
- Verificar o efeito do tratamento *in vivo* com haloperidol, sulpirida, olanzapina e MK-801 sobre a atividade da enzima Na^+K^+-ATPase em SNC do peixe zebra;
- Avaliar o efeito do haloperidol, sulpirida e olanzapina isoladamente e após tratamento com MK-801 sobre parâmetro de lipoperoxidação, por meio de da medida das espécies reativas ao acido tiobarbitúrico (TBARS), e níveis de formação de radicais livres, em cérebro de peixe zebra.

3. Artigos Científicos

CAPÍTULO I

ARTIGO CIENTÍFICO

Antipsychotic drugs prevent the motor hyperactivity induced by psychotomimetic MK-801 in zebrafish (Danio rerio)

Kelly J. Seibt, Renata L. Oliveira, Fernanda F. Zimmermann, Katiúcia M. Capiotti, Maurício R. Bogo, Gabriele Ghisleni, Carla D. Bonan.

Artigo publicado no periódico Behavioural Brain Research
214 (2010): 417–422

Author's personal copy

Behavioural Brain Research 214 (2010) 417–422

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Antipsychotic drugs prevent the motor hyperactivity induced by psychotomimetic MK-801 in zebrafish (*Danio rerio*)

Kelly Juliana Seibt[a], Renata da Luz Oliveira[a], Fernanda Francine Zimmermann[a], Katiúcia Marques Capiotti[a], Maurício Reis Bogo[b,c], Gabriele Ghisleni[a,*], Carla Denise Bonan[a,c,**]

[a] Neurochemistry and Psychopharmacology Laboratory, Department of Molecular and Cellular Biology, Faculty of Biosciences, Pontifical Catholic University of Rio Grande do Sul, Avenida Ipiranga, 6681, 90619-900 Porto Alegre, RS, Brazil
[b] Genomics and Molecular Biology, Faculty of Biosciences, Pontifical Catholic University of Rio Grande do Sul, Avenida Ipiranga, 6681, 90619-900 Porto Alegre, RS, Brazil
[c] National Institute for Translational Medicine (INCT-TM), 90035-003 Porto Alegre, RS, Brazil

ARTICLE INFO

Article history:
Received 13 March 2010
Received in revised form 8 June 2010
Accepted 13 June 2010
Available online 20 June 2010

Keywords:
MK-801
Antipsychotics
Zebrafish
Locomotor activity
Anxiety
Schizophrenia

ABSTRACT

Glutamate N-methyl-D-aspartate (NMDA) receptor antagonists, such as dizocilpine (MK-801), elicit schizophrenia-like symptoms in humans and a behavioral syndrome in rodents, characterized by hyperlocomotion and stereotyped actions, which is antagonized by antipsychotic drugs. Animal models of schizophrenia have been established and used for the development of new antipsychotic drugs. In this work we characterized the behavioral effects of MK-801 and investigated the effect of typical and atypical antipsychotic treatments on locomotor activity as well on the hyperlocomotion induced by MK-801 in zebrafish. MK-801 (20 μM) increased the locomotor behavior as measured by the number of line crossings, distance traveled, and the mean speed in the tank test after 15, 30, and 60 min of exposure. All tested antipsychotics counteracted MK-801-induced hyperactivity on all parameters analyzed and at doses that, given alone, had no effect on spontaneous locomotor activity. The results suggest a similar profile between typical and atypical antipsychotics in the reversal of locomotor disorders induced by MK-801. Moreover, an anxiolytic effect was verified at 30 and 60 min of MK-801 exposure, which was not reversed by antipsychotics tested in this work. In addition, olanzapine, which alone caused an anxiolytic response, when given with MK-801 potentiated the latter's effect on anxiety. In this work we demonstrated the value of the zebrafish, a simple to use animal model, in developing some behavioral features observed in schizophrenia, which may indicate a new approach for drug screening.
© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Locomotor behavior in vertebrates, such as walking or swimming, relies upon neural networks in the brain and spinal cord [47]. *Danio rerio*, the so-called zebrafish, exhibits several features that have made it an increasingly popular research system to examine the development and functioning of these networks [10,18]. Consequently, this animal has become consolidated as a model system in neurochemical, toxicological, and behavioral studies [21,61,63]. Compared to rodent models, zebrafish have many practical advantages, including a high fertility level (up to 200 eggs in one mating), small size, rapid generation time (days as opposed to weeks), and optical transparency during early embryogenesis [28,67]. There is a paucity of studies on complex behavior in zebrafish, even though it is recognized as having great potential as a model for understanding the genetic basis of human behavioral disorders [26,56], for the investigation of neuropharmacological mechanisms in mammals, and for applications in drug discovery [33,55].

Schizophrenia is a complex psychiatric disorder which is characterized by three main types of symptoms: positive (e.g. hallucinations, delusions), negative (e.g. social withdrawal, anhedonia), and cognitive deficits (e.g. impaired working memory and attention) [5]. Motor symptoms are frequent in schizophrenia and about 50% of psychotic patients display at least one motor symptom [57]. Increasing evidence supports the view that the development of motor systems in schizophrenic patients is impaired at very early life stages [41]. For example, motor development during infancy and early childhood was shown to be delayed or deviant in subjects later to suffer from schizophrenia [59]. In addition, several studies reported motor disturbances in both treated and untreated schizophrenia [19,46,72]. Changes in several neurotransmitter systems as well as neuroanatomical changes have been reported in the

* Corresponding author. Tel.: +55 51 3353 4158; fax: +55 51 3320 3568.
** Corresponding author at: Neurochemistry and Psychopharmacology Laboratory, Department of Molecular and Cellular Biology, Faculty of Biosciences, Pontifical Catholic University of Rio Grande do Sul, Avenida Ipiranga, 6681, 90619-900 Porto Alegre, RS, Brazil. Tel.: +55 51 3353 4158; fax: +55 51 3320 3568.
E-mail addresses: ghisleni.g@gmail.com (G. Ghisleni), cbonan@pucrs.br (C.D. Bonan).

0166-4328/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2010.06.014

Author's personal copy

brains of schizophrenic patients [73]. Nevertheless, the pathophysiology of schizophrenia remains unclear and this lack of information might be attributed in part to the difficulties in modeling this disorder [62].

At least two classes of psychotomimetics have been used as a model of schizophrenia in animals: dopamine agonists, such as amphetamine, which produce and exacerbate positive symptoms [36,65], and NMDA (N-methyl-D-aspartate) antagonists, such as ketamine, phencyclidine (PCP) or MK-801 (dizocilpine maleate) that can cause a profile of behavioral changes that are similar to the positive, negative, and cognitive symptoms of schizophrenia [23,49,60]. It is well known that systemic administration of the non-competitive NMDA receptor antagonist MK-801 causes an increase in rodent locomotion and, at higher doses, stereotypic behaviors including head weaving and uncoordinated, ataxic gaits [14,16].

Behavioral abnormalities induced in rodents by NMDA, such as hyperactivity, are prevented by antipsychotic drugs, more potently by the atypical than the typical type [12,23,29]. The principal mechanism of action of typical antipsychotics is the blockade of central DA receptors [58]. By contrast, atypical antipsychotics, while less potent than their typical counterparts in blocking central D2 receptors, have affinity for a wide range of other receptors including D1, D4, 5-HT2A, 5-HT6, α1, H1, and M1 [30]. Typical antipsychotic medications, although effective in treating psychotic symptoms, are limited by their propensity to cause higher rates of motor side effects [2], leading to non-adherence to treatment and increasing the risk of relapse [31]. Meanwhile, the main disadvantages of using atypical antipsychotic medications include higher costs and adverse effects on metabolism [39,69], although they present fewer extrapyramidal side effects and do alleviate negative symptoms while improving cognitive deficits [70].

In the present study we characterized the behavioral syndrome produced by MK-801 exposure in the zebrafish. Since various antipsychotics have been reported to modulate MK-801-induced hyperlocomotion, we also examined the effects of typical and atypical antipsychotics and their interaction with the MK-801-induced behavioral changes in zebrafish through the analysis of swimming activity and anxiety responses using the novel tank diving test.

2. Materials and methods

2.1. Animals

Adult wild type zebrafish strains (3–5 cm) of both sexes were obtained from a specialized commercial supplier (Redfish, RS, Brazil) and were of genetically heterogeneous (randomly bred) stock. The fish were acclimatized to the laboratory environment for at least 14 days and housed in a 50-l thermostated aquarium filled with continuously unchlorinated water at a targeted temperature of 28 ± 2 °C, with constant filtration and aeration (7.20 mg O_2/l) and a density of up to five animals per liter [71]. Animals were kept on a day:night cycle of 14:10 h and fed twice a day with flaked fish food that was supplemented with live brine shrimp.

Fish were manipulated healthy and free of any signs of disease, according to the "Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health (NIH publication No. 85–23, revised 1996). The Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul (PUCRS) approved the protocol under license number CEUA 09/00135.

2.2. Pharmacological treatments

A group of animals was individually exposed in a 300-ml beaker to 20 μM MK-801 hydrogen maleate (Sigma–Aldrich, Brazil), dissolved in tank water, for 15, 30 or 60 min before analysis in the tank diving behavioral test. Control animals were maintained individually in a 300-ml beaker with tank water for the same time as the MK-801 treatment.

The effects of typical and atypical antipsychotics were investigated in the same way as MK-801 in the apparatus test. Groups of animals were individually treated for 15 or 30 min in a 300-ml beaker with 9 μM haloperidol, 100 μM olanzapine or 250 μM sulpiride (all from Sigma–Aldrich, Brazil). Tank water was used as the vehicle for haloperidol and olanzapine and tank water with 5% DMSO was used as the vehicle to dissolve sulpiride.

To assess the effects of antipsychotics on MK-801-induced behavioral changes in the zebrafish, the following treatments were performed: (i) a control group was exposed to tank water in a beaker for 30 min; (ii) a MK-801 group was exposed to 20 μM MK-801 for 30 min; and (iii) a MK-801 plus antipsychotic group was pre-treated in a beaker with 20 μM MK-801 for the first 15 min and subsequently the same animals were transferred to another beaker containing 20 μM MK-801 plus 9 μM haloperidol or 100 μM olanzapine or 250 μM sulpiride, and remained there for an additional 15 min.

The MK-801 and haloperidol doses were chosen based on previous studies with zebrafish [68,25]. The doses of other antipsychotic agents used in this study were chosen based on drug potencies observed in human [45,34] and rat [27,54] studies.

2.3. Behavioral assessment

Behavioral testing of drug effects took place during the light phase between 10:00 a.m. and 5:00 p.m. Animals were individually placed in the experimental tank (30 cm × 15 cm × 10 cm, length × height × width) immediately after the pharmacological manipulation and were first habituated to the tank for 30 s, as previously described [22]. There was no drug exposure during behavioral experiments. The animals' locomotor activity was recorded on video for 5 min after the habituation period and simultaneously analyzed using the ANY-Maze recording software (Stoelting Co., Wood Dale, IL, USA). The tank was divided into equal sections with four vertical lines and one horizontal line, and the following behavior patterns were measured: number of line crossings (vertical and horizontal lines), distance traveled and mean speed. The time spent in each tank position (bottom vs. upper levels) was considered as the index of anxiety. This task exploits the natural tendency for zebrafish to spend most of the time at the bottom when introduced into a novel environment and then gradually to extend the swimming range, over a period of minutes, to include the upper portions of the test tank [37]. A longer time spent in the bottom and less time spent in the top part of the tank indicates heightened anxiety [37]. Visual observations throughout the experimental periods allow the documentation of erratic movements, defined as sharp changes in direction or velocity and repeated rapid darting behaviors [37]. In addition, these movements may be manifested by bouts of vertical swimming or sideways swimming, suggesting a problem with coordination [25].

2.4. Statistical analysis

Data are expressed as mean ± S.E.M. The behavioral effects of MK-801, antipsychotic drugs, and MK-801 plus antipsychotics were examined by one-way ANOVA, followed by Newman–Keuls *post hoc* test. A significant difference was attributed to p values less than 0.05.

3. Results

3.1. MK-801 induces changes in locomotor activity

Distinct parameters of zebrafish swimming activity were examined in the tank diving behavioral test. As indicated by the number of line crossings in the apparatus there was increased locomotor activity of animals treated with MK-801 in both the 15 (76.6%) and 30 min (100.6%) groups when compared with the control group (170.5 ± 20 line crossings) (Fig. 1A). MK-801 treatment (15, 30, and 60 min) increased the distance traveled (89.4%, 81.9%, and 85.1%, respectively) and the mean speed (89.8%, 99.8%, and 85.1%, respectively) in relation to control animals (11.4 ± 1.1 m; 0.04 ± 0.004 m/s, respectively) (Fig. 1B and C). Fig. 1D shows that when compared with the control group there was a significant, gradual increase in the time spent in the upper portion of the test tank in animals exposed to MK-801 for 30 (373.9%) or 60 min (464.7%), which may be interpreted as an indicator of anxiolytic behavior. As can be seen in Fig. 1E, there was no change in the behavioral parameters with MK-801 treatment when the data were subjected to minute-by-minute analysis. A representative trace of control and MK-801 treatment time-effects is shown in Fig. 1F. After assessing the effects of MK-801 on the zebrafish behavior we chose 30 min of MK-801 exposure as an adequate time to investigate the interaction with antipsychotics.

3.2. Effects of antipsychotic drug administration on the locomotor activity

Haloperidol, sulpiride, and olanzapine administered alone for 15 or 30 min before test sessions did not affect the number of line crossings, distance traveled and mean speed (Fig. 2A–C). However,

Author's personal copy

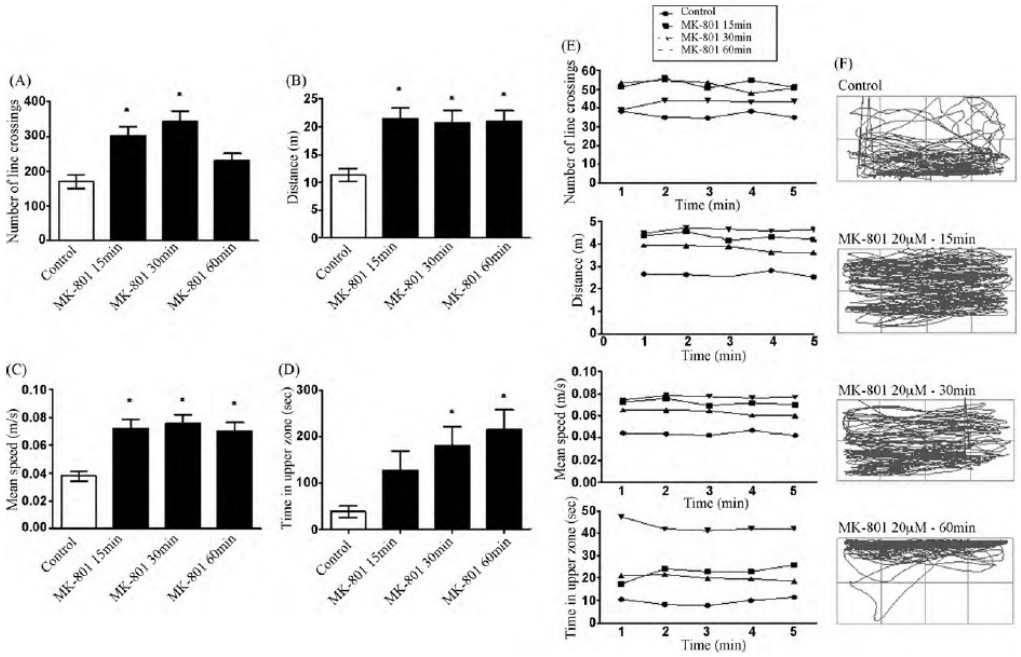

Fig. 1. Effect of exposure to 20 μM MK-801 for different times (15, 30 or 60 min) on the number of line crossings (A), distance traveled (B), mean speed (C), and time spent in the upper zone (D) determined during 5 min of videorecording in the tank diving behavioral test. (E) Minute-by-minute analysis; (F) representative traces. Data were expressed as mean ± S.E.M. of 10 animals for each group and were analyzed by one-way ANOVA followed by Newman–Keuls post hoc test. $^{*}p < 0.05$ denotes a significant difference from the control group.

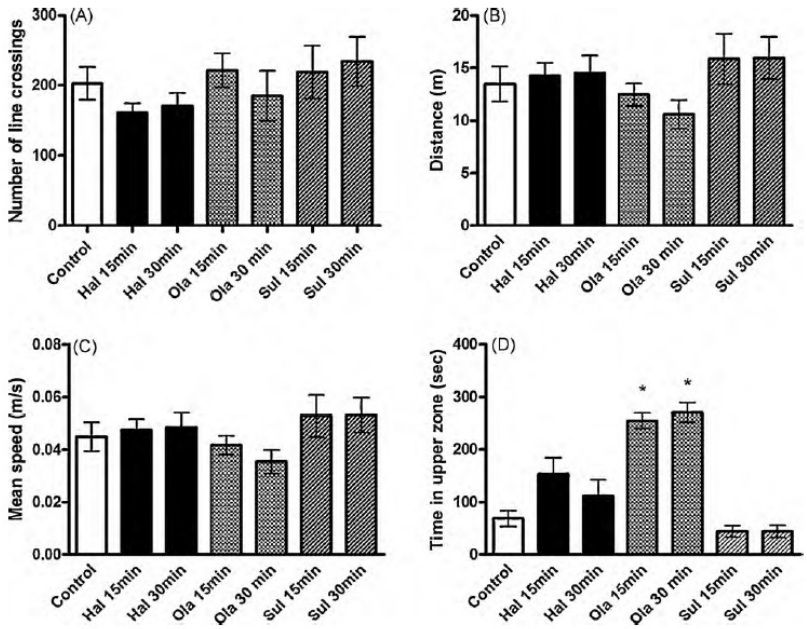

Fig. 2. Effect of 15 and 30 min treatment with haloperidol (Hal; 9 μM), olanzapine (Ola; 100 μM) and sulpiride (Sul; 250 μM) on the number of line crossings (A), distance traveled (B), mean speed (C), and time spent in the upper zone (D) determined during 5 min of videorecording in the tank diving behavioral test. Data were expressed as mean ± S.E.M. of 10 animals for each group and were analyzed by one-way ANOVA followed by Newman–Keuls post hoc test. $^{*}p < 0.05$ denotes a significant difference from the control group.

Author's personal copy

in relation to the anxiolytic profile, only olanzapine at 15 (269.7%) and 30 min (293.2%) induced a significant anxiolytic response (control group, 68.8 ± 15.02 s) (Fig. 2D).

3.3. Behavioral changes induced by MK-801 pretreatment are reversed by co-administration with different antipsychotic drugs

Based on pronounced MK-801-induced hyperlocomotor behavior, we examined the effects on behavioral activity of co-administration of MK-801 with typical or atypical antipsychotic drugs. When co-administered with MK-801 both classes of antipsychotic drugs were able to reverse the increase in the number of line crossings, distance traveled, and mean speed promoted by 30 min of MK-801 exposure (Fig. 3A–C, respectively).

However, all antipsychotic drugs tested in the co-treatment with MK-801 failed to reverse the anxiolytic effect of MK-801 determined by the time spent in the upper zone of the tank (Fig. 3D). Moreover, it is important to note that olanzapine, when co-administered with MK-801, actually potentiated the anxiolytic effect of the NMDA antagonist (Fig. 3D).

4. Discussion

The use of preclinical tests and animal models is essential in understanding and developing the pharmacological profile of novel antipsychotics. It is now recognized that zebrafish possess a great deal of similarity to mammals and are an extremely useful model for screening compounds at several stages of the drug discovery process [7]. Therefore, the present study was designed to determine if MK-801, previously described as a psychotomimetic drug of schizophrenia, causes behavioral changes in zebrafish.

Schizophrenia is a psychotic disorder marked by severely impaired thinking, emotions, and behaviors that affects about 1% of the general population worldwide. Although the etiology remains unknown, studies with animal models have begun to reveal possible mechanisms. It is difficult to produce animal models of hallucinations and delusions [24]; however, impairments in cognition, memory, emotion and social interaction, impaired sensorimotor gating, and hyperactivity in response to different drugs may be investigated [42,50,64]. Kilts [35] have described drug-induced hyperactivity as corresponding to psychomotor agitation, which is also a characteristic of schizophrenia, and studies have shown that MK-801 and PCP increase locomotor activity and induce stereotypic effects, which can be reversed with both typical and atypical antipsychotics [15,20,50].

Since the effects of NMDA antagonists on locomotor activity are well known and the latter is altered in schizophrenia, this behavioral measure was used in the present study. Our data show that locomotor activity in zebrafish was increased after MK-801 administration, in agreement with previous results obtained in mammals [13,11]. The assessment of locomotor activity may be a valuable method to identify the effect of antipsychotics on behavioral changes induced by psychotomimetics, and it has previously been demonstrated that antipsychotic agents, including haloperidol, clozapine, and olanzapine antagonize MK-801-induced hyperlocomotion. However, haloperidol exerted this antagonistic effect only at a dose that also decreased spontaneous activity, whereas clozapine and olanzapine reduced MK-801-induced hyperactivity at doses that had no effect on spontaneous activity [51,53]. Moreover, although haloperidol tended to decrease the locomotor activity, the three antipsychotics tested in this study caused no significant changes in this parameter when tested alone. A possible reason for this effect could be the unusual body position exhibited in swimming during the behavioral test after exposure to a typical antipsychotic. While control animals maintain a position parallel to the water surface during swimming, we observed that animals treated with haloperidol were unable to maintain such a position, indicating a lack of postural balance, as described in previous studies [25]. In addition, we found that haloperidol-treated zebrafish exhibited erratic swimming patterns, manifested by bouts of vertical swimming or sideways swimming, suggesting a problem with coordination.

The mechanisms by which antagonists of serotonin 5-HT2A receptors, such as sulpiride and olanzapine, inhibit MK-801-induced hyperlocomotion have not been fully elucidated. Some neurochemical data suggest that NMDA antagonists increase serotonin release, which in turn activates serotonin 5-HT2A receptors on glutamatergic neurons in the cortex to release glutamate [1,3]. The increased glutamate release may act on post-synaptic AMPA/kainate receptors causing changes in behavioral responsiveness and inducing the neuropathological changes observed with NMDA antagonist exposure [48,52]. Su et al. [66] showed that the atypical antipsychotic risperidone inhibits NMDA antagonist-induced glutamate release in the medial prefrontal cortex by blocking serotonin 5-HT2A receptors on glutamatergic terminals, leading to attenuation of the activity of cortico-subcortical glutamatergic neurons. This attenuation, in turn, decreases MK-801-induced hyperlocomotion [66]. Therefore, it is possible that sulpiride and olanzapine inhibit the MK-801-induced hyperlocomotion through a similar mechanism.

Clearly, other mechanisms may also be involved in the inhibitory effect of antipsychotic drugs on MK-801-induced hyperlocomotion. As we know, atypical antipsychotics block not only serotonin 5-HT2A receptors but also dopamine D2, α1-adrenoreceptors, and histamine H1 receptors [38], and therefore these agents may attenuate MK-801-induced hyperlocomotion via such blockade. Many reports have shown that atypical antipsychotics are more potent than typical antipsychotic drugs in inhibiting MK-801-, phencyclidine- or ketamine-induced locomotor activity [12,29,53]. Our findings demonstrate that haloperidol, sulpiride, and olanzapine acutely inhibited MK-801-induced hyperlocomotion, differing only in their influence upon the anxiolytic effect of MK-801.

Additionally, our results showed an MK-801-induced anxiolytic profile in zebrafish after 30 and 60 min of exposure. This is in agreement with previous studies conducted in mammals, in which rats treated with MK-801 and submitted to the elevated plus-maze test presented an increase in time spent in the open arms, indicative of an anxiolytic-like effect [6,17,32]. A further notable finding in our study was the anxiolytic effect presented after 15 and 30 min of exposure to olanzapine, although this drug did not have any effect on locomotor activity. Atypical antipsychotic drugs (risperidone, olanzapine, and quetiapine) have been increasingly used to treat anxiety-related disorders in addition to their use in the treatment of psychosis. Indirect evidence suggests that the effects of clozapine on allopregnanolone, a progesterone metabolite, may be responsible for its anxiolytic effect. It has been found that clozapine and olanzapine increase allopregnanolone in rat cerebral cortex and hippocampus in a dose-dependent manner [43,44], and since allopregnanolone acts as a positive modulator of the $GABA_A$ receptor [40] and shows a strong anxiolytic effect in the elevated plus-maze task and the Geller–Seifter conflict test [4,8,9], it is possible to suggest that clozapine or olanzapine-induced elevations in allopregnanolone contribute to their anxiolytic-like effect. Finally, our results show that haloperidol and sulpiride did not cause significant changes in the behavioral parameters observed.

In conclusion, both typical and atypical antipsychotics attenuated MK-801-induced hyperlocomotion in zebrafish at doses that did not reduce spontaneous locomotor activity. Testing the efficacy of drugs in alleviating MK-801-induced behavioral changes is one of the experimental approaches for screening a new therapeutically

Author's personal copy

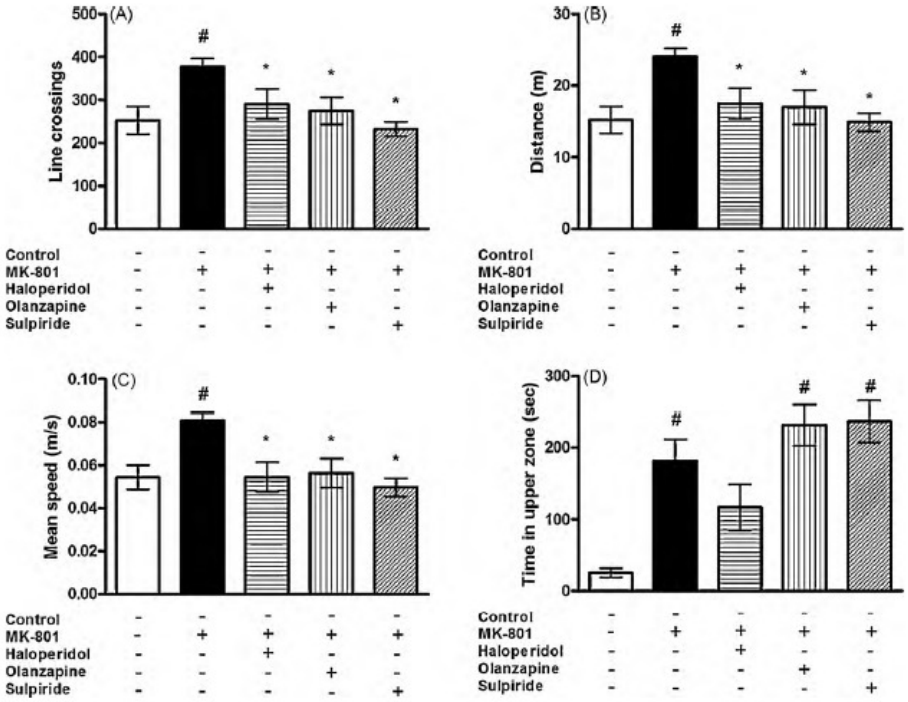

Fig. 3. Effect of 15 min pretreatment with 20 μM MK-801 and later 15 min co-treatment with 20 μM MK-801 and haloperidol (Hal; 9 μM), olanzapine (Ola; 100 μM), or sulpiride (Sul; 250 μM) on the number of line crossings (A), distance traveled (B), mean speed (C), and time spent in the upper zone (D) determined during 5 min of videorecording in the tank diving behavioral test. Data were expressed as mean ± S.E.M. of 10 animals for each group and were analyzed by one-way ANOVA followed by Newman–Keuls post hoc test. $^{\#}p < 0.05$ denotes a significant difference from the control group; $^{*}p < 0.05$ denotes a significant difference from the MK-801 treatment.

relevant compound, and merits further investigation in this animal model.

Acknowledgments

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) and by the FINEP research grant "Rede Instituto Brasileiro de Neurociência (IBN-Net)" # 01.06.0842-00.

References

[1] Adams BW, Moghaddam B. Effect of clozapine, haloperidol, or M100907 on phencyclidine-activated glutamate efflux in the prefrontal cortex. Biol Psychiatry 2001;50:750–7.
[2] Adams CE, Coutinho ES, Davis J, Duggan L, Leucht S, Li C, et al. Cochrane schizophrenia group. Schizophr Bull 2008;34:259–65.
[3] Aghajanian GK, Marek GJ. Serotonin, via 5-HT2A receptors, increases EPSCs in layer V pyramidal cells of prefrontal cortex by an asynchronous mode of glutamate release. Brain Res 1999;825:161–71.
[4] Akwa Y, Purdy RH, Koob GF, Britton KT. The amygdala mediates the anxiolytic-like effect of the neurosteroid allopregnanolone in rat. Behav Brain Res 1999;106:119–25.
[5] Austin J. Schizophrenia: an update and review. J Genet Couns 2005;14:329–40.
[6] Bertoglio LJ, Carobrez AP. Anxiolytic-like effects of NMDA/glycine-B receptor ligands are abolished during the elevated plus-maze trial 2 in rats. Psychopharmacology (Berl) 2003;170:335–42.
[7] Best JD, Alderton WK. Zebrafish: an in vivo model for the study of neurological diseases. Neuropsychiatr Dis Treat 2008;4:567–76.
[8] Bitran D, Klibansky DA, Martin GA. The neurosteroid pregnanolone prevents the anxiogenic-like effect of inescapable shock in the rat. Psychopharmacology (Berl) 2000;151:31–7.
[9] Brot MD, Akwa Y, Purdy RH, Koob GF, Britton KT. The anxiolytic-like effects of the neurosteroid allopregnanolone: interactions with GABA(A) receptors. Eur J Pharmacol 1997;325:1–7.
[10] Brustein E, Saint-Amant L, Buss RR, Chong M, McDearmid JR, Drapeau P. Steps during the development of the zebrafish locomotor network. J Physiol (Paris) 2003;97:77–86.
[11] Bubenikova-Valesova V, Svoboda J, Horacek J, Vales K. The effect of a full agonist/antagonist of the D1 receptor on locomotor activity, sensorimotor gating and cognitive function in dizocilpine-treated rats. Int J Neuropsychopharmacol 2009;1–11.
[12] Carlsson M, Carlsson A. Interactions between glutamatergic and monoaminergic systems within the basal ganglia—implications for schizophrenia and Parkinson's disease. Trends Neurosci 1990;13:272–6.
[13] Chartoff EH, Heusner CL, Palmiter RD. Dopamine is not required for the hyperlocomotor response to NMDA receptor antagonists. Neuropsychopharmacology 2005;30(1):324–33.
[14] Clineschmidt BV, Martin GE, Bunting PR, Papp NL. Central sympathomimetic activity of(+)-5-methyl-10,11-dihydro-5*H*-dibenzo [d]cycloheptene-5,10-immine(MK-801), a substance with potent anticonvulsant, central sympathomimetic, and apparent anxiolytic properties. Drug Dev Res 1982;2:135–45.
[15] Corbett R, Camacho F, Woods AT, Kerman LL, Fishkin RJ, Brooks K, et al. Antipsychotic agents antagonize non-competitive N-methyl-D-aspartate antagonist-induced behaviors. Psychopharmacology (Berl) 1995;120:67–74.
[16] Deutsch SI, Rosse RB, Mastropaolo J. Behavioral approaches to the functional assessment of NMDA-mediated neural transmission in intact mice. Clin Neuropharmacol 1997;20:375–84.
[17] Dunn RW, Corbett R, Fielding S. Effects of 5-HT1A receptor agonists and NMDA receptor antagonists in the social interaction test and the elevated plus maze. Eur J Pharmacol 1989;169:1–10.
[18] Fetcho JR, Bhatt DH. Genes and photons: new avenues into the neuronal basis of behavior. Curr Opin Neurobiol 2004;14:707–14.
[19] Flyckt L, Sydow O, Bjerkenstedt L, Edman G, Rydin E, Wiesel FA. Neurological signs and psychomotor performance in patients with schizophrenia, their relatives and healthy controls. Psychiatry Res 1999;86:113–29.
[20] Gattaz WF, Schummer B, Behrens S. Effects of zotepine, haloperidol and clozapine on MK-801-induced stereotypy and locomotion in rats. J Neural Transm Gen Sect 1994;96:227–32.

Author's personal copy

[21] Gerlai R, Fernandes Y, Pereira T. Zebrafish (*Danio rerio*) responds to the animated image of a predator: towards the development of an automated aversive task. Behav Brain Res 2009;201:318–24.
[22] Gerlai R, Lahav S, Guo S, Rosenthal A. Drinks like a fish: zebra fish (*Danio rerio*) as a behavior genetic model to study alcohol effects. Pharmacol Biochem Behav 2000;67:773–82.
[23] Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology (Berl) 2001;156:117–54.
[24] Geyer MA, Moghaddam B. Animal models relevant to schizophrenia disorders. In: Davis KL, Charney D, Coyle JT, Nemeroff C, editors. Neuropsychopharmacology: the fifth generation of progress. Washington, DC: Lippincott Williams & Wilkins; 2002. p. 689–701.
[25] Giacomini NJ, Rose B, Kobayashi K, Guo S. Antipsychotics produce locomotor impairment in larval zebrafish. Neurotoxicol Teratol 2006;28:245–50.
[26] Guo S. Linking genes to brain, behavior and neurological diseases: what can we learn from zebrafish? Genes Brain Behav 2004;3:63–74.
[27] Ichikawa J, Kuroki T, Dai J, Meltzer HY. Effect of antipsychotic drugs on extracellular serotonin levels in rat medial prefrontal cortex and nucleus accumbens. Eur J Pharmacol 1998;351:163–71.
[28] Irons TD, Macphail RC, Hunter DL, Padilla S. Acute neuroactive drug exposures alter locomotor activity in larval zebrafish. Neurotoxicol Teratol 2010;32:84–90.
[29] Jentsch JD, Roth RH. The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 1999;20:201–25.
[30] Jones DNC, Gartlon JE, Minassian A, Perry W, Geyer M. In: McArthur RA, Borsini F, editors. Developing new drugs for schizophrenia: from animals to the clinic. Animal and translational models for CNS drug discovery volume 1: psychiatric disorders. New York: Elsevier; 2008. p. 199–261.
[31] Kane JM. Schizophrenia. N Engl J Med 1996;334:34–41.
[32] Karcz-Kubicha M, Jessa M, Nazar M, Plaznik A, Hartmann S, Parsons CG, et al. Anxiolytic activity of glycine-B antagonists and partial agonists—no relation to intrinsic activity in the patch clamp. Neuropharmacology 1997;36:1355–67.
[33] Kari G, Rodeck U, Dicker AP. Zebrafish: an emerging model system for human disease and drug discovery. Clin Pharmacol Ther 2007;82:70–80.
[34] Kiang M, Daskalakis ZJ, Christensen BK, Remington G, Kapur S. Actigraphic measurement of the effects of single-dose haloperidol and olanzapine on spontaneous motor activity in normal subjects. J Psychiatry Neurosci 2003;28:293–9.
[35] Kilts CD. The changing roles and targets for animal models of schizophrenia. Biol Psychiatry 2001;50:845–55.
[36] Laruelle M, Abi-Dargham A. Dopamine as the wind of the psychotic fire: new evidence from brain imaging studies. J Psychopharmacol 1999;13:358–71.
[37] Levin ED, Bencan Z, Cerutti DT. Anxiolytic effects of nicotine in zebrafish. Physiol Behav 2007;90:54–8.
[38] Leysen JE, Janssen PM, Megens AA, Schotte A. Risperidone: a novel antipsychotic with balanced serotonin-dopamine antagonism, receptor occupancy profile, and pharmacologic activity. J Clin Psychiatry 1994;55:5–12.
[39] Lindenmayer JP, Nathan AM, Smith RC. Hyperglycemia associated with the use of atypical antipsychotics. J Clin Psychiatry 2001;62:30–8.
[40] Majewska MD. Steroid regulation of the GABAA receptor: ligand binding, chloride transport and behaviour. Ciba Found Symp 1990;153:83–97.
[41] Manschreck TC, Maker BA, Candela SF. Earlier age of first diagnosis in schizophrenia is related to impaired motor control. Schizophr Bull 2004;30:351–60.
[42] Marquis JP, Audet MC, Doré FY, Goulet S. Delayed alternation performance following subchronic phencyclidine administration in rats depends on task parameters. Prog Neuropsychopharmacol 2007;31:1108–12.
[43] Marx CE, VanDoren MJ, Duncan GE, Lieberman JA, Morrow AL. Olanzapine and clozapine increase the GABAergic neuroactive steroid allopregnanolone in rodents. Neuropsychopharmacology 2003;28:1–13.
[44] Marx CE, Shampine LJ, Duncan GE, VanDoren MJ, Grobin AC, Massing MW, et al. Clozapine markedly elevates pregnenolone in rat hippocampus, cerebral cortex, and serum: candidate mechanism for superior efficacy? Pharmacol Biochem Behav 2006;84:598–608.
[45] McClelland GR, Cooper SM, Pilgrim AJ. A comparison of the central nervous system effects of haloperidol, chlorpromazine and sulpiride in normal volunteers. Br J Clin Pharmacol 1990;30:795–803.
[46] McCreadie RG, Srinivasan TN, Padmavati R, Thara R. Extrapyramidal symptoms in unmedicated schizophrenia. J Psychiatr Res 2005;39:261–6.
[47] McKeown KA, Downes GB, Hutson LD. Modular laboratory exercises to analyze the development of zebrafish motor behavior. Zebrafish 2009;6:179–85.
[48] Moghaddam B, Adams B, Verma A, Daly D. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. J Neurosci 1997;17:2921–7.
[49] Murray J. Phencyclidine (PCP): a dangerous drug, but useful in schizophrenia research. J Psychol 2002;136:319–27.
[50] Newcomer JW, Farber NB, Jevtovic-Todorovic V, Selke G, Melson AK, Hershey T, et al. Ketamine-induced NMDA receptor hypofunction as a model of memory impairment and psychosis. Neuropsychopharmacology 1999;20:106–18.
[51] Ninan I, Kulkarni SK. Preferential inhibition of dizocilpine-induced hyperlocomotion by olanzapine. Eur J Pharmacol 1999;368:1–7.
[52] Olney JW, Newcomer JW, Farber NB. NMDA receptor hypofunction model of schizophrenia. J Psychiatr Res 1999;33:523–33.
[53] O'Neill MF, Shaw G. Comparison of dopamine receptor antagonists on hyperlocomotion induced by cocaine, amphetamine, MK-801 and the dopamine D1 agonist C-APB in mice. Psychopharmacology (Berl) 1999;145:237–50.
[54] Parikh V, Terry AV, Khan MM, Mahadik SP. Modulation of nerve growth factor and choline acetyltransferase expression in rat hippocampus after chronic exposure to haloperidol, risperidone, and olanzapine. Psychopharmacology 2004;172:365–74.
[55] Parng C, Seng WL, Semino C, McGrath P. Zebrafish: a preclinical model for drug screening. Assay Drug Dev Technol 2002;1:41–8.
[56] Patton EE, Zon LI. The art and design of genetic screens: zebrafish. Nat Rev Genet 2001;2:956–66.
[57] Peralta V, Cuesta MJ. Motor features in psychotic disorders. II. Development of diagnostic criteria for catatonia. Schizophr Res 2001;47:117–26.
[58] Porsolt RD, Moser PC, Castagne V. Behavioral indices in antipsychotic drug discovery. J Pharmacol Exp Ther 2010;333:632–8.
[59] Ridler K, Veijola JM, Tanskanen P, Miettunen J, Chitnis X, Suckling J, et al. Fronto-cerebellar systems are associated with infant motor and adult executive functions in healthy adults but not in schizophrenia. Proc Natl Acad Sci USA 2006;103:15651–6.
[60] Rujescu D, Bender A, Keck M, Hartmann AM, Ohl F, Raeder H, et al. A pharmacological model for psychosis based on N-methyl-d-aspartate receptor hypofunction: molecular, cellular, functional and behavioral abnormalities. Biol Psychiatry 2006;59:721–9.
[61] Seibt KJ, Oliveira RL, Rico EP, Dias RD, Bogo MR, Bonan CD. Typical and atypical antipsychotics alter acetylcholinesterase activity and *ache* expression in zebrafish (*Danio rerio*) brain. Comp Biochem Physiol C 2009;150:10–5.
[62] Seillier A, Giuffrida A. Evaluation of NMDA receptor models of schizophrenia: divergences in the behavioral effects of sub-chronic PCP and MK-801. Behav Brain Res 2009;204:410–5.
[63] Senger MR, Rosemberg DB, Rico EP, Arizi MB, Dias RD, Bogo MR, et al. In vitro effect of zinc and cadmium on acetylcholinesterase and ectonucleotidase activities in zebrafish (*Danio rerio*) brain. Toxicol In Vitro 2006;20:954–8.
[64] Simpson SM, Menard JL, Reynolds JN, Beninger RJ. Post-weaning social isolation increases activity in a novel environment but decreases defensive burying and subchronic MK-801 enhances the activity but not the burying effect in rats. Pharmacol Biochem Behav 2010;95:72–9.
[65] Snyder SH. Amphetamine psychosis: a 'model' schizophrenia mediated by catecholamines. Am J Psychiatry 1973;130:61–7.
[66] Su YA, Si TM, Zhou DF, Guo CM, Wang XD, Yang Y, et al. Risperidone attenuates MK-801-induced hyperlocomotion in mice via the blockade of serotonin 5-HT2A/2C receptors. Eur J Pharmacol 2007;564:123–30.
[67] Sumanasa S, Lin S. Zebrafish as a model system for drug target screening and validation. Drug Discov Today Targets 2004;3:89–96.
[68] Swain HA, Sigstad C, Scalzo FM. Effects of dizocilpine (MK-801) on circling behavior, swimming activity, and place preference in zebrafish (*Danio rerio*). Neurotoxicol Teratol 2004;26:725–9.
[69] Taylor DM, McAskill R. Atypical antipsychotics and weight gain—a systemic review. Acta Psychiatr Scand 2000;101:416–32.
[70] Voruganti L, Cortese L, Ouyewumi L, Cernovsky Z, Zirul S, Awad A. Comparative evaluation of conventional and new antipsychotic drugs with reference to their subjective tolerability, side effect profile and impact on quality of life. Schizophr Res 2000;43:135–45.
[71] Westerfield M. The zebrafish book. 5th ed. Eugene: University of Oregon Press; 2007.
[72] Whitty P, Clarke M, Browne S, McTigue O, Kamali M, Feeney L, et al. Prospective evaluation of neurological soft signs in first-episode schizophrenia in relation to psychopathology: state versus trait phenomena. Psychol Med 2003;33:1479–84.
[73] Wong AH, Van Tol HH. Schizophrenia: from phenomenology to neurobiology. Neurosci Biobehav Rev 2003;27:269–306.

CAPÍTULO II

ARTIGO CIENTÍFICO

Antipsychotic drugs reverse MK-801-induced cognitive and social interaction deficits in zebrafish (Danio rerio)

Kelly J. Seibt; Ângelo L. Piato, Renata L. Oliveira, Katiúcia M Capiotti, Monica R. Vianna, Carla D. Bonan.

Artigo publicado no periódico Behavioural Brain Research 224 (2011): 135-139

Behavioural Brain Research 224 (2011) 135–139

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Antipsychotic drugs reverse MK-801-induced cognitive and social interaction deficits in zebrafish (*Danio rerio*)

Kelly Juliana Seibt[a,c], Angelo Luis Piato[a,d], Renata da Luz Oliveira[a,c], Katiucia Marques Capiotti[a,c], Monica Ryff Vianna[b,c], Carla Denise Bonan[a,c,*]

[a] *Laboratório de Neuroquímica e Psicofarmacologia, Departamento de Biologia Celular e Molecular, Programa de Pós-Graduação em Biologia Celular e Molecular. Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga, 6681, 90619-900, Porto Alegre, RS, Brazil*

[b] *Laboratório e Biologia e Desenvolvimento do Sistema Nervoso, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga 6681, 90619-900, PortoAlegre, RS, Brazil*

[c] *Instituto Nacional de Ciência e Tecnologia Translacional em Medicina (INCT-TM), 90035-003, Porto Alegre, RS, Brazil*

[d] *Programa de Pós-Graduação em Medicina e Ciências da Saúde, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga, 6690, 90610-000, Porto Alegre, RS, Brazil*

ARTICLE INFO

Article history:
Received 15 March 2011
Received in revised form 26 May 2011
Accepted 30 May 2011

Keywords:
MK-801
Antipsychotic
Memory
Zebrafish
Schizophrenia

ABSTRACT

Schizophrenia is a severe mental illness characterized by positive and negative symptoms and cognitive deficits. Reduction of glutamatergic neurotransmission by NMDA receptor antagonists mimics symptoms of schizophrenia. Modeling social interaction and cognitive impairment in animals can be of great benefit in the effort to develop novel treatments for negative and cognitive symptoms of schizophrenia. Studies have demonstrated that these behavioral changes are, in some cases, sensitive to remediation by antipsychotic drugs. The zebrafish has been proposed as a candidate to study the *in vivo* effects of several drugs and to discover new pharmacological targets. In the current study we investigated the ability of antipsychotic drugs to reverse schizophrenia-like symptoms produced by the NMDA receptor antagonist MK-801. Results showed that MK-801 (5 μM) given pre-training hindered memory formation while both atypical antipsychotics sulpiride (250 μM) and olanzapine (50 μM) improved MK-801-induced amnesia. The same change was observed in the social interaction task, where atypical antipsychotics reversed the MK-801-induced social interaction deficit whereas the typical antipsychotic haloperidol (9 μM) was ineffective to reverse those behavioral deficits. Therefore, MK-801-treated zebrafish showed some behavioral features observed in schizophrenia, such as cognitive and social interaction deficits, which were reverted by current available atypical drugs.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Schizophrenia is a devastating psychiatric disorder characterized by positive and negative symptoms and cognitive deficits [55]. Positive symptoms refer to newly acquired behaviors, including delusions, hallucinations, and thought disorders whereas the negative symptoms refer to impairments or losses in normal behavior and include deficits in social interaction, emotional expression, and motivation. Cognitive deficits usually manifest as impaired attention/information processing, problem-solving, processing speed, verbal and visual learning, memory, and working memory [51,54].

Alterations in several neurotransmitter systems and neuroanatomical characteristics have been reported in schizophrenic patients [65]. Different theories have attempted to clarify the aetiology of schizophrenia but the exact causes of this complex and multifactorial mental disorder remain unknown. This lack of information might be attributed in part to the difficulties in modeling the disorder. One of the best characterized animal models of schizophrenia is based on NMDA hypofunction [22]. This model is based on observations that NMDA antagonists, such as phencyclidine and MK-801, can mimic the complexity of positive, negative, and cognitive symptoms of the disease [46,60].

MK-801 is a non-competitive antagonist of NMDA subtype of glutamate receptors and acts by means of open-channel blockade [61]. When given to animals, NMDA antagonists cause changes that resemble some features of schizophrenia, including hyperlocomotion, stereotypical behavior, memory impairment [6,9,35,49], and social withdrawal [16,57]. These behavioral changes are, in some cases, blocked by antipsychotic drugs [8,58].

Antipsychotic drugs are widely used for the treatment of psychotic symptoms in patients with several brain disorders, including schizophrenia. Typical (first-generation) antipsychotics alleviate psychotic symptoms, but lead to severe motor side effects due to

* Corresponding author at: Laboratório de Neuroquímica e Psicofarmacologia, Departamento de Biologia Celular e Molecular, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul. Avenida Ipiranga, 6681, 90619-900 Porto Alegre, RS, Brazil. Tel.: +55 51 3353 4158; fax: +55 51 3320 3568.
E-mail address: cbonan@pucrs.br (C.D. Bonan).

0166-4328/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2011.05.034

the blockade of dopamine D_2 receptors [38]. Over the past decade, atypical (second-generation) antipsychotics have been increasingly used in the treatment of schizophrenia in preference to 'conventional' typical drugs [15]. Atypical antipsychotics, although less potent in blocking central D_2 receptors, have affinity for a wide range of other receptors including dopaminergic D_1 and D_4, serotonergic 5-HT_{2A} and 5-HT_6, adrenergic α_1, histaminergic H_1, and muscarinergic M_1 [37].

The teleost *Danio rerio*, popularly known as zebrafish, have many inherent advantages as a model organism, such as low cost, easy handling and maintenance as compared to other vertebrate models and 70–80% genetic homology to humans [7,19,30]. Therefore, zebrafish may be an ideal vertebrate model system for numerous human diseases, where genetic and biological mechanisms of the diseases may be studied [18,23,34]. Zebrafish embryos are permeable to drugs and can easily be manipulated using well-established genetic and molecular approaches [39]. Transparency of zebrafish embryos and early larvae allows direct visualization of tissue morphogenesis as it occurs in a live organism [7]. In addition, zebrafish behavior can be easily observed and quantified in a controlled environment [45]. Behavior-based chemical screens in zebrafish may improve our understanding of neurobiology and drug action and accelerate the pace of psychiatric drug discovery [41,56].

It is well known that systemic administration of the non-competitive NMDA receptor antagonist MK-801 causes an increase in rodent locomotion [14], and this effect was fully replicated in a previous study from our laboratory using the zebrafish as animal model [58]. The present study was designed to determine if MK-801 also causes memory and social interaction impairments in zebrafish. Since various antipsychotics have been reported to modulate MK-801-induced changes, we also examined the ability of typical and atypical antipsychotics to reverse the deleterious effects of MK-801. Therefore, our work evaluated the potential of zebrafish as a reliable animal model to study NMDA antagonist-induced cognitive deficits and negative symptoms and a possible new approach for drug screening.

2. Material and methods

2.1. Animals and maintenance

Adult male zebrafish (<8 months old) were obtained from a local commercial supplier (Redfish, RS, Brazil). All fish were kept in 50 l housing tanks with AquaSafe® (Tetra) conditioned water continuously aerated (7.20 mgO_2/l) at 25 ± 2 °C, under a 14–10 h light/dark photoperiod in at a density of up to five animals per liter. Animals were acclimated for at least 2 weeks before the experiments and were fed three times a day with commercial flake food (TetraMin Tropical Flake®). Groups consisted of 11–12 animals and different animals were used for each experiment. All protocols were approved by the Institutional Animal Care Committee (09/00135, CEUA–PUCRS) and followed Brazilian legislation, the guidelines of the Brazilian Collegium of Animal Experimentation (COBEA), and the Canadian Council for Animal Care (CCAC) Guide on the care and use of fish in research, teaching, and testing.

2.2. Chemicals

Haloperidol, sulpiride, olanzapine, MK-801 hydrogen maleate and dimethylsulfoxide (DMSO) were used. The antypsychotics used were from clinical grade/suppliers, while MK-801 and DMSO were purchased from Sigma–Aldrich (St. Louis, USA). Tank water was used as the vehicle for haloperidol and olanzapine and tank water with 5% DMSO was used as the vehicle to sulpiride.

2.3. Pharmacological treatment

Immediately before the behavioral tests, fish were treated either individually (for inhibitory avoidance protocol) or in groups of five (for social interaction protocol) in 500-ml beakers for 30 min, which are divided in two consecutive 15 min-periods, as follows: (a) In the first 15 min-exposure period, animals were exposed to tank water (CTRL) or 5 μM MK-801. (b) In the second 15 min-exposure, animals were treated with tank water or with one of the antipsychotic drugs (9 μM haloperidol, 50 μM olanzapine or 250 μM sulpiride). Therefore, the following experimental treatments were tested: (i) CTRL plus CTRL, (ii) CTRL plus HAL, (iii) CTRL plus OLA, (iv) CTRL plus SUL, (v) MK-801 plus CTRL, (vi) MK-801 plus HAL, (vii) MK-801 plus OLA (viii) MK-801 plus SUL. An additional control group receiving 5% DMSO was simultaneously tested in both inhibitory avoidance and social interaction tests, showing identical responses to those of the water-treated control group (data not shown).

The MK-801 and haloperidol doses were chosen based on previous studies with zebrafish [25,58,63]. The doses of other antipsychotic agents used in this study were chosen based on drug potencies observed in humans [40,47], rats [33,53], and zebrafish [58,59]. Previous studies have shown that haloperidol, sulpiride, and olanzapine *per se* did not induce changes in the locomotion in zebrafish [58].

2.4. Behavioral analysis

2.4.1. Inhibitory avoidance

Long-term memory was evaluated using an inhibitory avoidance (IA) protocol described in detail by Blank et al. [11]. Briefly, a glass tank (18 cm × 9 cm × 7 cm length × height × width) divided in two equally sized compartments, designated here as dark and white, by a sliding guillotine-type partition (9 cm × 7 cm) was used. The tank water level was 3 cm and the partition raised 1 cm above the tank floor to allow zebrafish to swim freely from one side of the tank to the other. Two electrodes extending through the wall height and placed on each end side of the dark walls attached to an 8 V stimulator administered a final 3 ± 0.2 V AC shock when manually activated. On training session, animals were placed in the white side of the tank while the partition between compartments was closed. After 1 min of adaptation with the new environment the partition was raised, allowing fish to cross to the dark side of the tank. When animals entered the dark side with their entire body the sliding partition was closed and a pulsed electric shock administered for 5 s. Fish were then removed from the apparatus and placed in the dedicated temporary tank. Animals were tested 24 h after training. The test session repeated the training protocol except that no shock was administered and animals immediately removed from the dark compartment. The latency to completely enter the dark compartment was measured on both sessions and the test latencies used as an index of retention.

2.4.2. Social interaction

The zebrafish is a schooling fish that may exhibit preference for its conspecifics under certain circumstances. The rationale behind using a group of five fish as subjects is that this social setting biases behavior toward schooling. Fish were placed in groups of five in a small experimental tank (30 cm × 15 cm × 10 cm length × height × width). On one side of the experimental tank an empty fish tank was placed, and on the other side a tank of identical size held 15 zebrafish, hereon designed "stimulus fish". The experimental fish were allowed to acclimate to the experimental tank for a 30 s period, after which their behavior was video recorded. In order to quantify their preference between the "stimulus fish" side of their tank in detriment of the empty tank, the experimental fish tank was divided in two equal sections and the amount of time the five experimental fish spent on the side of the tank closer to the conspecific school was measured using an event recorder program [24].

2.5. Statistical analysis

Results are expressed as mean ± S.E.M. In the social interaction test comparisons between groups were made by two-way ANOVA followed by Tukey's *post hoc* test. Inhibitory avoidance data are expressed as medians ± interquartile ranges and data were analyzed by Kruskal–Wallis non-parametric analysis of variance followed by Mann–Whitney *U*-test (two-tailed) for comparisons among treatment groups. Significance was set at $p < 0.05$. All data were evaluated with SPSS 18.0 for Windows.

3. Results

Fig. 1 shows the effects of haloperidol (HAL), sulpiride (SUL), and olanzapine (OLA) on MK-801-induced amnesia in the inhibitory avoidance task. Significant and consistent differences ($p < 0.05$, Wilcoxon) between training and test sessions were observed in all groups except for MK-801 and MK-801 plus HAL ($p > 0.05$). These results show that only the atypical antipsychotics (sulpiride and olanzapine) were able to reverse the memory impairment induced by MK-801.

Fig. 2 shows the effects of HAL, SUL, and OLA on MK-801-induced social interaction impairment in zebrafish. As expected, MK-801 significantly decreased ($p < 0.001$) the time of social interaction when compared to controls, whereas HAL, SUL, and OLA *per se* were devoid of effects. However, when zebrafish were treated with HAL, SUL, and OLA after MK-801 pre-treatment, a two-way ANOVA revealed a main effect of pre-treatment

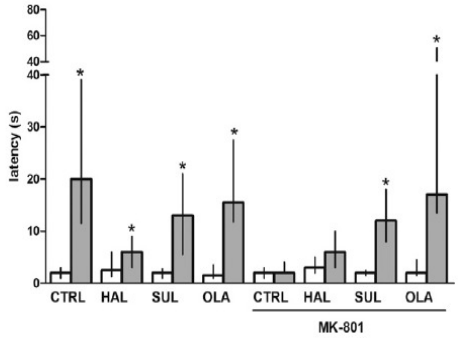

Fig. 1. Effects of haloperidol (HAL), sulpiride (SUL) and olanzapine (OLA) on MK-801-induced amnesia in the inhibitory avoidance task. Significant differences (*$p < 0.05$, Wilcoxon test) between training and test sessions were observed in all groups, except for MK-801 and MK + HAL ($p > 0.05$). Data are expressed as medians ± interquartile ranges and were analyzed by Kruskal–Wallis non-parametric analysis of variance followed by Mann–Whitney *U*-test (two-tailed) for comparisons among treatment groups.

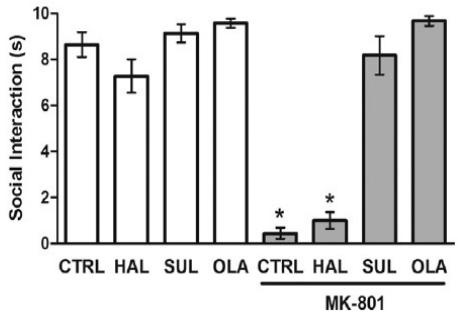

Fig. 2. Effects of haloperidol (HAL), sulpiride (SUL) and olanzapine (OLA) on MK-801-induced social interaction deficits in zebrafish. Data are expressed as mean ± S.E.M. *$p < 0.01$ × control group.

($F_{(1,67)} = 87.4$, $p < 0.001$), treatment ($F_{(3,67)} = 46.2$, $p < 0.001$), and of pre-treatment × treatment interaction ($F_{(3,65)} = 25.6$, $p < 0.001$). *Post hoc* analyses indicated that treatment with SUL and OLA, but not with HAL, reversed the effects of MK-801 in the social interaction test. Therefore, atypical antipsychotic drugs reversed MK-801-induced cognitive and social interaction deficits in zebrafish. No changes in latency for inhibitory avoidance and social interaction were induced by DMSO (data not shown).

4. Discussion

Schizophrenic patients suffer from enduring and persistent symptoms, as well as deficiencies in cognitive abilities and social interaction. Given the negative impact of cognitive and social dysfunction on long-term function and quality of life, the lack of effective treatment is clearly a key unmet clinical need [27]. There is evidence that, at least some of the pathology and symptomatology (particularly cognitive and negative symptoms) of schizophrenia results from a dysfunction of the glutamatergic system, which may be modeled in animals through the use of NMDA receptor antagonists.

There is a growing interest in zebrafish as a model organism in behavioral pharmacology [29,64]. Zebrafish, a vertebrate model organism amenable to high throughput screening, is an attractive system to model and study the mechanisms underlying human diseases. In a previous work [58], our group characterized the behavioral effects of MK-801 and investigated the effect of typical and atypical antipsychotic treatments on locomotor activity as well as on the hyperlocomotion induced by MK-801 in this animal model. In the present study, using social interaction and memory deficits as key schizophrenia-like symptoms, we demonstrated that acute MK-801 treatment induced a deficit in cognition and social interaction in zebrafish, which was completely restored by acute administration of atypical antipsychotics, but not by a typical antipsychotic.

It is currently accepted that the glutamatergic neurotransmitter system plays an important role in the aetiopathogenesis of schizophrenia, as supported by findings on various aspects of neural substrates ranging from molecular interactions to the neuronal networks in the human brain [26,42]. Moreover, administration of non-competitive antagonists of NMDA glutamate receptors (phencyclidine, ketamine, and MK-801) has been reported to induce behavioral abnormalities related to symptoms of schizophrenia, such as impairment of information processing and attention, as well as hyperlocomotion in response to a novel environment, which are all ameliorated by antipsychotic use [4,14,58].

Modeling cognitive impairment in animals can be of great benefit in the effort to develop novel treatments for psychotic, negative, and cognitive symptoms of schizophrenia. Previous studies have shown that acute MK-801 impairs working memory in the delayed alternation task in rodents [9,67] whereas no information is available on the behavioral effects of MK-801 chronic treatment and/or withdrawal. Acute phencyclidine treatment, other NMDA receptor antagonist, impairs performance at rats in the Morris maze, and this effect can be reversed by clozapine and other atypical antipsychotics, but not by haloperidol [17]. In accordance with these studies, we found that acute treatment with MK-801 caused memory impairment in the inhibitory avoidance task, and MK-801-induced cognitive impairment was ameliorated by atypical antipsychotics, such as sulpiride and olanzapine, but not by typical, such as haloperidol.

According to Rung et al. [57], social deficits can be considered a core negative symptom in schizophrenia. Social withdrawal in rats, in response to non-competitive NMDA-receptor antagonists, is an accepted model for negative symptoms of schizophrenia [10,20,57]. Haloperidol, a typical antipsychotic, does not reverse acute NMDA antagonist-induced deficits in social investigation [12], and conflicting data exist for clozapine, which is either inactive [12] or effecting in reversing social investigation deficits [10]. Acute treatment with the atypical drugs ziprasidone and aripiprazole has been reported to reverse subchronic phencyclidine-induced deficits in social investigation, whereas similar treatment with haloperidol or clozapine has no effect [60]. In this study, sulpiride and olanzapine prevented the MK-801-induced social withdrawal in zebrafish whereas haloperidol was ineffective. The fact that social interaction is diminished by NMDA glutamate antagonists in zebrafish and improved by some atypical antipsychotics suggests that NMDA antagonist-reduced social interaction might represent an useful model for evaluating novel antipsychotics in this species.

Neurochemical data suggest that NMDA antagonists increase serotonin release, which in turn activates serotonin 5-HT_{2A} receptors on glutamatergic neurons in the cerebral cortex to release glutamate [2,3]. The increased glutamate release may act on post-synaptic AMPA/kainate receptors causing changes in behavioral responsiveness and inducing the neuropathological changes observed with NMDA antagonist exposure [48,52]. Despite the fact that atypical antipsychotics do not directly target glutamatergic receptors, the ability to modulate the glutamatergic system has been proposed as the basis of olanzapine and sulpiride atypical profile [32,43]. Atypical antipsychotics show marked polypharmacology, with affinities for a wide range of other receptors.

Evins et al. [21] have shown that clozapine, an atypical antipsychotic, alters serum glutamate concentrations [21], facilitates NMDA-mediated neurotransmission [5], and antagonizes NMDA antagonist-mediated behaviors. Thus, this drug may also facilitate NMDAR activity indirectly through inhibition of the glycine transporter, up-regulating glycine binding to its positive modulatory site on the NMDAR [36]. However, it remains unclear whether the increased NMDA receptor binding can be attributed to increased NMDAR expression, an alteration of receptor conformation or affinity for MK-801 [28]. Behaviorally, chronic administration of clozapine restores NMDAR function after phencyclidine treatment [50] and reverses the prepulse inhibition (PPI) deficits induced by ketamine and MK-801 [1,13,44]. Su et al. [62] showed that the atypical antipsychotic risperidone inhibits NMDA antagonist-induced glutamate release in the medial prefrontal cortex by blocking serotonin 5-HT_{2A} receptors on glutamatergic terminals, leading to attenuation of the activity of cortico-subcortical glutamatergic neurons [62]. Therefore, it is possible that atypical drugs such as olanzapine and sulpiride inhibit MK-801-induced social interaction and cognitive impairments through a similar mechanism.

Monoaminergic, cholinergic, GABAergic and peptidergic neurons can be identified early on zebrafish development, developing in similar spatial and temporal patterns than those observed in mammals [31]. Additionally, zebrafish maintains the typical brain structural organization observed in vertebrates, enabling extrapolation of findings from zebrafish to mammals, despite specific embryological aspects and relative position of homologous structures [for a detailed review see Ref. [66]]. The general subdivision of vesicles during brain development and their resulting structures are evolutionary conserved, and zebrafish shows telencephalic regions that are homologous to the hippocampus and amygdala, critical for memory and emotion processing, in addition to multiple diencephalic nuclei, reinforcing zebrafish advantages in screens for drugs with potential neural effects [31,66]. Despite some proteomic and neuroanatomical differences between zebrafish and mammals, the effects of drugs that act on the dopaminergic and serotonergic systems (receptor agonists or antagonists, transporter inhibitors, etc.) on zebrafish behavior are similar to those observed in rodents [19,25,58]. However, there are no studies evaluating affinity, efficacy and transduction mechanisms for duplicated zebrafish receptors in relation to different drugs, such antipsychotics.

The behavioral syndrome produced by NMDA antagonists has been widely used as an animal model to study the mechanism of action of conventional and atypical antipsychotics. Our interest to establish this behavioral syndrome in zebrafish was supported by numerous practical advantages of this species in behavioral experimentation combined to its substantial genetic similarity to mammals. In this work we demonstrated that MK-801-treated zebrafish mimics some behavioral features observed in schizophrenia, such as cognitive and social interaction deficits, which responded positively to available atypical treatments, supporting the use of this animal model for drug screening.

Acknowledgements

This work was supported by DECIT/SCTIEMS through Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS, Proc. 10/0036-5 – PRONEX/Conv. 700545/2008), and by the FINEP research grant "Rede Instituto Brasileiro de Neurociência (IBN-Net)" 01.06.0842-00. K.J.S. was a recipient of a fellowship from Programa PROBOLSAS/PUCRS. R.L.O. was a recipient of a fellowship from CAPES. A.L.P and K.M.C were recipients of CNPq fellowships. M R.V. and C.D.B. were also recipient of Research scholarship from CNPq.

References

[1] Abdul-Monim Z, Reynolds GP, Neill JC. The effect of atypical and classical antipsychotics on sub-chronic PCP-induced cognitive deficits in a reversal-learning paradigm. Behav Brain Res 2006;169:263–73.
[2] Adams BW, Moghadam B. Effect of clozapine, haloperidol, or M100907 on phencyclidine-activated glutamate efflux in the prefrontal cortex. Biol Psychiatry 2001;50:750–7.
[3] Aghajanian GK, Marek GJ. Serotonin, via 5-HT2A receptors, increases EPSCs in layer v pyramidal cells of prefrontal cortex by an asynchronous mode of glutamate release. Brain Res 1999;825:161–71.
[4] Amitai N, Semenova S, Markou A. Cognitive–disruptive effects of the psychotomimetic phencyclidine and attenuation by atypical antipsychotic medications in rats. Psychopharmacology 2007;193:521–37.
[5] Arvanov VL, Liang X, Schwartz J, Grossman S, Wang RY. Clozapine and haloperidol modulate N-methyl-D-aspartate- and non-N-methyl-D-aspartate receptor mediated neurotransmission in rat prefrontal cortical neurons in vitro. J Pharmacol Exp Ther 1997;283:226–34.
[6] Bakshi VP, Geyer MA. Multiple limbic regions mediate the disruption of prepulse inhibition produced in rats by the noncompetitive NMDA antagonist dizocilpine. J Neurosci 1998;18:8394–401.
[7] Barbazuk WB, Korf I, Kadavi C, Heyen J, Tate S, Wun E, et al. The syntenic relationship of the zebrafish and human genomes. Genome Res 2000;10:1351–8.
[8] Bardgett ME. Behavioral models of atypical antipsychotic drug action. In: Csernansky JG, Laurello J, editors. Atypical antipsychotics: from bench to bedside. New York: Marcel Dekker; 2004. p. 61–93.
[9] Bardgett ME, Points M, Ramsey-Faulkner C, Topmiller J, Roflow J, McDaniel T, et al. The effects of clonidine on discrete-trial delayed spatial alternation in two rat models of memory loss. Neuropsychopharmacology 2008;33:1980–91.
[10] Becker A, Grecksch G. Ketamine-induced changes in rat behaviour: a possible animal model of schizophrenia. Test of predictive validity. Prog Neuro-Psychopharmacol Biol Psychiatry 2004;28(8):1267–77.
[11] Blank M, Guerim LD, Cordeiro RF, Vianna MR. A one-trial inhibitory avoidance task to zebrafish: rapid acquisition of an NMDA-dependent long-term memory. Neurobiol Learn Mem 2009;92(4):529–34.
[12] Boulay D, Depoortère R, Louis C, Perrault G, Griebel G, Soubrié P. SSR 181507, a putative atypical antipsychotic with dopamine D2 antagonist and 5-HT1A agonist activities: improvement of social interaction deficits induced by phencyclidine in rats. Neuropharmacology 2004;46(8):1121–9.
[13] Bubenikova V, Votava M, Horacek J, Palenicek T, Dockery C. The effect of zotepine, risperidone, clozapine and olanzapine on MK-801-disrupted sensorimotor gating. Pharmacol Biochem Behav 2005;80:591–6.
[14] Bubenikova-Valesova V, Horacek J, Vrajova M, Höschl C. Models of schizophrenia in humans and animals based on inhibition of NMDA receptors. Neurosci Biobehav Rev 2008;32:1014–23.
[15] Crossley NA, Constante M, McGuire P, Power P. Efficacy of atypical v. typical antipsychotics in the treatment of early psychosis: meta-analysis. Br J Psychiatry 2010;196(6):434–9.
[16] de Moura Linck V, Herrmann AP, Goerck GC, Iwu MM, Okunji CO, Leal MB, et al. The putative antipsychotic alstonine reverses social interaction withdrawal in mice. Prog Neuro-Psychopharmacol Biol Psychiatry 2008;32:1449–52.
[17] Didriksen M, Skarsfeldt T, Arnt J. Reversal of PCP-induced learning and memory deficits in the Morris' water maze by sertindole and other antipsychotics. Psychopharmacology (Berl) 2007;193:225–33.
[18] Dodd A, Curtis PM, Williams LC, Love DA. Zebrafish: bridging the gap between development and disease. Hum Mol Genet 2000;9(16):2443–9.
[19] Egan RJ, Bergner CL, Hart PC, Cachat JM, Canavello PR, Elegante MF, et al. Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. Behav Brain Res 2009;205(1):38–44.
[20] Ellenbroek BA, Cools AR. Animal models for the negative symptoms of schizophrenia. Behav Pharmacol 2000;11(3–4):223–33.
[21] Evins AE, Amico ET, Shih V, Goff DC. Clozapine treatment increases serum glutamate and aspartate compared to conventional neuroleptics. J Neural Transm 1997;104:761–6.
[22] Farber NB. The NMDA receptor hypofunction model of psychosis. Ann N Y Acad Sci 2003;1003:119–30.
[23] Gerlai R, Lahav M, Guo S, Rosental A. Drinks like a fish: zebrafish (*Danio rerio*) as a behavior genetic model to study alcohol effects. Pharmacol Biochem Behav 2000;67(4):773–82.
[24] Gerlai R. Zebra fish: an uncharted behavior genetic model. Behav Genet 2003;33(5):461–8.
[25] Giacomini NJ, Rose B, Kobayashi K, Guo S. Antipsychotics produce locomotor impairment in larval zebrafish. Neurotoxicol Teratol 2006;28:245–50.
[26] Goff DC, Coyle JT. The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. Am J Psychiatry 2001;158:1367–77.
[27] Goldberg TE, Gold JM. Neurocognitive deficits in schizophrenia. London, UK: Blackwell; 1995.
[28] Gray L, van den Buuse M, Scarr E, Dean B, Hannan AJ. Clozapine reverses schizophrenia-related behaviours in the metabotropic glutamate receptor 5 knockout mouse: association with N-methyl-D-aspartic acid receptor up-regulation. Int J Neuropsychopharmacol 2009;12(1):45–60.
[29] Grossman L, Stewart A, Gaikwad S, Utterback E, Wu N, Dileo J, et al. Effects of piracetam on behavior and learning in adult zebrafish. Brain Res Bull 2011;85(1–2):58–63.

[30] Guo S. Linking genes to brain, behavior and neurological diseases: what can we learn from zebrafish? Genes Brain Behav 2004;3:63–74.
[31] Guo S. Using zebrafish to assess the impact of drugs on neural development and function. Expert Opin Drug Discov 2009;4(7):715–26.
[32] Heresco-Levy U. Glutamatergic neurotransmission modulation and the mechanisms of antipsychotic atypicality. Prog Neuropsychopharmacol Biol Psychiatry 2003;27:1113–23.
[33] Ichikawa J, Kuroki T, Dai J, Meltzer HY. Effect of antipsychotic drugs on extracellular serotonin levels in rat medial prefrontal cortex and nucleus accumbens. Eur J Pharmacol 1998;351:163–71.
[34] Ivetac I, Becanovic J, Krishnapillai V. Zebrafish: genetic tools and genomics. Asia-Pac J Mol Biol Biotechnol 2000;8:1–11.
[35] Jacobs PS, Taylor BM, Bardgett ME. Maturation of locomotor and Fos responses to NMDA antagonists, PCP and MK-801. Dev Brain Res 2000;122:91–5.
[36] Javitt DC. Glutamate as a therapeutic target in psychiatric disorders. Mol Psychiatry 2004;9:984–97.
[37] Jones DNC, Gartlon JE, Minassian A, Perry W, Geyer M. Developing new drugs for schizophrenia: from animals to the clinic. Animal and translational models for CNS drug discovery. In: McArthur RA, Borsini F, editors. Psychiatric disorders, vol. 1. New York: Elsevier; 2008. p. 199–261.
[38] Kapur S, Zipursky R, Jones C, et al. Relationship between dopamine D2 occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. Am J Psychiatry 2000;157:514–20.
[39] Kari G, Rodeck U, Dicker AP. Zebrafish: an emerging model system for human disease and drug discovery. Clin Pharmacol Ther 2007;82:70–80.
[40] Kiang M, Daskalakis ZJ, Christensen BK, Remington G, Kapur S. Actigraphic measurement of the effects of single-dose haloperidol and olanzapine on spontaneous motor activity in normal subjects. J Psychiatry Neurosci 2003;28:293–9.
[41] Kokel D, Peterson RT. Chemobehavioural phenomics and behaviour-based psychiatric drug discovery in the zebrafish. Brief Funct Genomic Proteomic 2008;7(6):483–90.
[42] Kristiansen LV, Huerta I, Beneyto M, Meador-Woodruff JH. NMDA receptors and schizophrenia. Curr Opin Pharmacol 2007;7:48–55.
[43] Large CH. Do NMDA receptor antagonist models of schizophrenia predict the clinical efficacy of antipsychotic drugs? J Psychopharmacol 2007;21:283–301.
[44] Levin ED, Petro A, Caldwell DP. Nicotine and clozapine actions on pre-pulse inhibition deficits caused by N-methyl-D-aspartate (NMDA) glutamatergic receptor blockade. Prog Neuropsychopharmacol Biol Psychiatry 2005;29(4):581–6.
[45] Levin ED, Bencan Z, Cerutti DT. Anxiolytic effects of nicotine in zebrafish. Physiol Behav 2007;90(1):54–8.
[46] Li JT, Su YA, Guo CM, Feng Y, Yang Y, Huang RH, et al. Persisting cognitive deficits induced by low-dose, subchronic treatment with MK-801 in adolescent rats. Eur J Pharmacol 2011;652(1–3):65–72.
[47] McClelland GR, Cooper SM, Pilgrim AJ. A comparison of the central nervous system effects of haloperidol, chlorpromazine and sulpiride in normal volunteers. Br J Clin Pharmacol 1990;30:795–803.
[48] Moghaddam B, Adams B, Verma A, Daly D. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathways from NMDA receptor-blocked to dopaminergic and cognitive disruptions associated with the prefrontal cortex. J Neurosci 1997;7:921–7.
[49] Nilsson M, Waters S, Waters N, Carlsson A, Carlsson ML. A behavioural pattern analysis of hypoglutamatergic mice – effects of four different antipsychotic agents. J Neural Transm 2001;108:1181–96.
[50] Ninan I, Jardemark KE, Wang RY. Olanzapine and clozapine but not haloperidol reverse subchronic phencyclidine-induced functional hyperactivity of N-methyl-D-aspartate receptors in pyramidal cells of the rat medial prefrontal cortex. Neuropharmacology 2003;44:462–72.
[51] Nuechterlein KH, Barch DM, Gold JM, Goldberg TE, Green MF, Heaton RK. Identification of separable cognitive factors in schizophrenia. Schizophr Res 2004;72:29–39.
[52] Olney JW, Newcomer JW, Farber NB. NMDA receptor hypofunction model of schizophrenia. J Psychiat Res 1999;33:523–33.
[53] Parikh V, Terry AV, Khan MM, Mahadik SP. Modulation of nerve growth factor and choline acetyltransferase expression in rat hippocampus after chronic exposure to haloperidol, risperidone, and olanzapine. Influence of long-lasting administration of neuroleptics on cortical NMDA. Psychopharmacology (Berl) 2004;172:365–74.
[54] Pearlson GD. Neurobiology of schizophrenia. Ann Neurol 2000;48:556–66.
[55] Porsolt RD, Moser PC, Castagne V. Behavioral indices in antipsychotic drug discovery. J Pharmacol Exp Ther 2010;333(3):632–8.
[56] Rihel J, Prober DA, Arvanites A, Lam K, Zimmerman S, Jang S, et al. Zebrafish behavioral profiling links drugs to biological targets and rest/wake regulation. Science 2010;327(5963):348–51.
[57] Rung JP, Carlsson A, Ryden MK, Carlsson ML. (+)-MK-801 induced social withdrawal in rats, a model for negative symptoms of schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 2005;29:827–32.
[58] Seibt KJ, Oliveira Rda L, Zimmermann FF, Capiotti KM, Bogo MR, Ghisleni G, et al. Antipsychotic drugs prevent the motor hyperactivity induced by psychotomimetic MK-801 in zebrafish (*Danio rerio*). Behav Brain Res 2010;214(2):417–22.
[59] Sison M, Gerlai R. Behavioral performance altering effects of MK-801 in zebrafish (*Danio rerio*). Behav Brain Res 2011;220(2):331–7.
[60] Snighda S, Neill JC. Efficacy of antipsychotics to reverse phencyclidine-induced social interaction deficits in female rats-a preliminary investigation. Behav Brain Res 2008;187:489–94.
[61] Stuchlík A, Petrásek T, Vales K. Effect of alpha(1)-adrenergic antagonist prazosin on behavioral alterations induced by MK-801 in a spatial memory task in Long-Evans rats. Physiol Res 2009;58:733–40.
[62] Su YA, Si TM, Zhou DF, Guo CM, Wang XD, Yang Y, et al. Risperidone attenuates MK-801-induced hyperlocomotion in mice via the blockade of serotonin 5-HT 2A/2C receptors. Eur J Pharmacol 2007;564:123–30.
[63] Swain HA, Sigstad C, Scalzo FM. Effects of dizocilpine (MK-801) on circling behavior, swimming activity, and place preference in zebrafish (*Danio rerio*). Neurotoxicol Teratol 2004;26:725–9.
[64] Tsang M, Zebrafish:. A tool for chemical screens. Birth Defects Res C Embryo Today 2010;90(3):185–92 [Review].
[65] Wong AH, Van Tol HH. Schizophrenia: from phenomenology to neurobiology. Neurosci Biobehav Rev 2003;27:269–306.
[66] Wullimann MF, Mueller T. Teleostean and mammalian forebrains contrasted: evidence from genes to behavior. J Comp Neurol 2004;475:143–62.
[67] Zajaczkowski W, Czyrak A, Wedzony K. A competitive antagonist of NMDA receptors CGP 40116 attenuates experimental symptoms of schizophrenia evoked by MK-801. Pol J Pharmacol 2003;55:703–11.

CAPÍTULO III

ARTIGO CIENTÍFICO

Investigation on the effects of antipsychotics on ectonucleotidase and adenosine deaminase in zebrafish brain

Kelly J. Seibt; Renata L. Oliveira; Mario R. Senger; Mauricio R. Bogo;
Carla D. Bonan.

Manuscrito a ser submetido ao periódico Neurochemical Research

Investigation on the effects of antipsychotics on ectonucleotidase and adenosine deaminase in zebrafish brain

Kelly Juliana Seibt[a,e], Renata da Luz Oliveira[a,e], Mario Roberto Senger [b,c], Mauricio Reis Bogo [d,e], Carla Denise Bonan[a,e,*]

[a] Laboratório de Neuroquímica e Psicofarmacologia, Departamento de Biologia Celular e Molecular, Programa de Pós-Graduação em Biologia Celular e Molecular, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul. Avenida Ipiranga, 6681, 90619-900 Porto Alegre, RS, Brazil.

[b] Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Programa de Pós-Graduação em Ciências Biológicas: Bioquímica, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos 2600-Anexo, 90035-003 Porto Alegre, RS, Brazil.

[c] Laboratório de Bioquímica de Proteínas e Peptídeos, Instituto Oswaldo Cruz - FIOCRUZ, Av. Brasil, 4365, Pavilhão Leonidas Deanne, Sala 309, 21045-900, Rio de Janeiro, RJ, Brazil.

[d] Laboratório de Biologia Genômica e Molecular, Departamento de Biologia Celular e Molecular, Programa de Pós-Graduação em Biologia Celular e Molecular, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga, 6681, 90619-900 Porto Alegre, RS, Brazil

[e] Instituto Nacional de Ciência e Tecnologia Translacional em Medicina (INCT-TM), 90035-003, Porto Alegre, RS, Brazil.

Addresses for correspondence: Bonan, C.D. (cbonan@pucrs.br) Avenida Ipiranga, 6681, 90619-900 Porto Alegre, RS, Brazil Tel.: +55 51 3353 4158; Fax: +55 51 3320 3568.

Abstract

Antipsychotic agents are widely used for the treatment of psychotic symptoms in patients with several brain disorders, such as schizophrenia. Atypical and typical antipsychotics differ in parts considerably regarding their clinical and side-effects profile. Haloperidol is a representative typical antipsychotic drug and has potent dopamine receptor antagonistic; however, various atypical antipsychotics have been developed, were an important advance in the treatment of schizophrenia and other psychotic disorders. Purine nucleotides and nucleosides, such as ATP and adenosine, represent a ubiquitous class of extracellular signaling molecules crucial for normal functioning of the nervous system. Indirect findings suggest that changes in the purinergic system, more specifically in adenosinergic activity, could be involved in the physiopathology of schizophrenia. Here we investigated the effects of typical and atypical antipsychotics on ectonucleotidase and adenosine deaminase (ADA) activities, followed by an analysis of gene expression patterns in zebrafish brain. Haloperidol treatment (9 μM) was able to decrease ATP hydrolysis (35%), whereas there were no significant changes in ADP and AMP hydrolysis in brain membranes. Adenosine deamination in membrane fractions was significantly inhibited (38%) after haloperidol treatment when compared to the control group; however, no changes were observed in ADA soluble fractions after exposure to all antipsychotics tested. Haloperidol exposure also led to a decrease in *entpd2_mq*, *entpd3*, and *adal* mRNA transcripts. These findings demonstrate that the haloperidol is an inhibitor of ectonucleotidases and ADA in zebrafish brain, suggesting that purinergic signaling may also be a target of pharmacological effects promoted by this drug.

Keywords: Ectonucleotidases; zebrafish; antipsychotic; adenosine deaminase.

1. Introduction

Schizophrenia is a neurodevelopmental disorder which afflicts about 1% of the human population worldwide, and is caused by both genetic and environmental factors (McGrath et al., 2004; van Os et al., 2009). Atypical and typical antipsychotics differ in parts considerably regarding their clinical and side-effects profile (Heiser et al., 2007). Haloperidol is a representative typical antipsychotic drug and has potent dopamine receptor antagonistic activity (Ishida et al., 2009); however, various atypical antipsychotics have been developed, were an important advance in the treatment of schizophrenia and other psychotic disorders (Meltzer et al. 2002). Their main advantages include better tolerability, especially regarding extrapyramidal symptoms, efficacy in a wider range of symptoms (Volavka et al. 2002) and increase in quality of life (Karow and Naber 2002). Drug therapy for schizophrenia aims to reduce symptoms in the acute phase and maintain long-term symptomatic remission during periods of stabilization (Pani, 2009).

Despite intensive research, the etiology of schizophrenia remains puzzling. The role of extracellular purines and purinoreceptors in the pathophysiology of several neurological disorders is the focus of a rapidly expanding area of research. ATP is a fast excitatory neurotransmitter co-released with other neurotransmitters in the central nervous system (CNS) (Burnstock, 2009). The inactivation of ATP-mediated signaling is exerted by ectonucleotidases, which include the nucleoside triphosphate diphosphohydrolase (NTPDase) family and an ecto-5'-nucleotidase (Massé et al., 2006; Zimmermann, 2001). Indirect findings propose that alterations involving the purinergic system could be implicated in the schizophrenia, since adenosine, the final product of ectonucleotidase cascade, plays a modulatory role in dopaminergic and glutamatergic systems (Lara and Souza, 2000; Lara et al., 2006). Extracellular adenosine concentrations can be regulated by neural cell uptake through bi-directional nucleoside transporters followed by phosphorylation to AMP by

adenosine kinase, or deamination to inosine by ADA (Franco et al., 1997; Fredholm et al., 2005; Rosemberg et al., 2007). This process is mostly intracellular, but studies showed that ADA is also associated with cell membranes (Franco et al., 1997). This ecto-ADA is colocalized with adenosine A_1 and A_{2B} receptors, being essential for controlling P1 signaling (Herrera et al., 2001; Saura et al., 1998). Additionally, it has been shown that adenosine A_{2A} receptors reduce the affinity of dopaminergic D_2 receptors for dopamine, a probable mechanism underlying the antipsychotic-like profile of adenosine agonists (Cunha et al., 2008; Wardas, 2008).

Zebrafish is a promising vertebrate model for studying numerous human diseases and drug-related mechanisms (Gerlai et al., 2000; Morris, 2009). Ionotropic P2X receptors have already been characterized in this species (Kucenas et al., 2003) as well as the expression of adenosine A_2 receptors in developing zebrafish embryos (Boehmler et al., 2009). Moreover, studies from our laboratory demonstrated the presence of ectonucleotidase and ADA activities in zebrafish brain (Rico et al., 2003; Rosemberg et al., 2008; Senger et al., 2004). Previous study showed that antipsychotics inhibited *in vitro* NTPDase activities in zebrafish brain (Seibt et al., 2009), suggesting that these enzymes might be sensitive to these drugs. Thus, the purpose of this study was to investigate the acute effects of typical and atypical antipsychotics on ectonucleotidase and ADA activities in zebrafish brain and to evaluate their gene expression pattern analysis.

2. Materials and methods

2.1. Animals

Wild-type adult (<8 months old) zebrafish (*Danio rerio*) of both sexes were obtained from a specialist supplier (Redfish Agroloja, RS, Brazil). Animals were kept at a density of up to five animals per liter in 50L housing tanks containing tap water previously treated with

Tetra's AquaSafe® (to neutralize chlorine, chloramines, and heavy metals present in the water that could be harmful to fish) and continuously aerated (7.20 mg O_2/L) at 25 ± 2 ºC under a 14/10 h light/dark photoperiod. Animals were acclimated for at least 2 weeks before the experiments. They were fed three times a day with TetraMin Tropical Flakes fish food®. The procedures were previously approved by the Animal Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul (PUCRS) under license number CEUA 09/00135.

2.2. Chemicals

Sulpiride, haloperidol, olanzapine, nucleotides (ATP, ADP and AMP), adenosine, Trizma base, EDTA, EGTA, sodium citrate, Coomassie Blue G, bovine serum albumin, malachite green, ammonium molybdate, polyvinyl alcohol, nucleotides, and calcium chloride were purchased from Sigma (St. Louis, MO, USA). Magnesium chloride, phenol, and sodium nitroprusside were purchased from Merck (Darmstadt, Germany). TRIzol, SuperScript™ III First-Strand Synthesis SuperMix, Taq Platinum, GelRed and Low DNA Mass Ladder were purchased from Invitrogen (Carlsbad, CA, USA). All other reagents used were of analytical grade.

2.3. Drug treatments

For the treatment, fish were transferred to 1L aquariums and exposed to water containing sulpiride (250 µM), olanzapine (100 µM), or haloperidol (9 µM) for 2 h. The haloperidol dose and time of treatment *in vivo* were chosen based on previous studies in zebrafish (Giacomini et al., 2006). The doses of other antipsychotic agents used in this study were chosen according to our previous study, which have shown that haloperidol, sulpiride, and olanzapine *per se* did not induce changes in the locomotion in zebrafish (Seibt et al., 2010).

2.4. Preparation of soluble and membrane fractions

Brain samples were obtained as described previously (Rico et al., 2003; Rosemberg et al., 2007). Zebrafish were cryoanesthetized and immediately euthanized by decapitation, and whole brains dissected. For each sample, five zebrafish brains were pooled and then homogenized in a glass-Teflon homogenizer according to the protocol for each enzyme assay. For NTPDase and ecto-5'-nucleotidase assays, zebrafish brains were homogenized in 60 vol. (v/w) of chilled Tris–citrate buffer (50 mM Tris–citrate, 2 mM EDTA, 2 mM EGTA, pH 7.4). For ADA experiments, brains were homogenized in 20 vol. (v/w) of chilled phosphate-buffered saline (PBS), containing 2 mM EDTA and 2 mM EGTA, pH 7.4. The brain membranes were prepared as described previously by Barnes et al. (1993). In brief, the homogenates were centrifuged at 800g for 10 min and the supernatant fraction was subsequently centrifuged for 25 min at 40 000g. The resultant supernatant and the pellet obtained corresponded to the soluble and membrane fractions, respectively. For soluble ADA activity experiments, the supernatant was collected and kept on ice for enzyme assays. The pellets of membrane preparations were frozen in liquid nitrogen, thawed, resuspended in the respective buffers and centrifuged for 20 min at 40 000 g. This freeze–thaw–wash procedure was used to ensure lysis of the brain vesicle membranes. The final pellets were resuspended and used for enzyme assays. All samples were maintained at 2-4 °C throughout preparation.

2.5. Adenosine deaminase assays

Ecto- and cytosolic-ADA activities were determined as described previously (Rosemberg et al., 2008). The brain fractions (5–10 μg protein) were added to the reaction mixture containing 50 mM sodium phosphate buffer (pH 7.0) and 50 mM sodium acetate buffer (pH 5.0) for soluble and membrane fractions, respectively, in a final volume of 200 μL.

The samples were preincubated for 10 min at 37 °C and the reaction was initiated by the addition of substrate (adenosine) to a final concentration of 1.5 mM. The reaction was stopped after 75 min (soluble fraction) and 120 min (membrane fraction) by the addition of 500 µL phenol-nitroprusside reagent (50.4 mg of phenol and 0.4 mg of sodium nitroprusside/mL). Adenosine deaminase activity was determined spectrophotometrically by measuring the ammonia produced over a fixed time using a Berthelot reaction as previously reported (Weisman et al., 1988). In order to correct for non-enzymatic hydrolysis of the substrates controls were employed with the addition of the enzyme preparation after mixing with phenol-nitroprusside reagent. The reaction mixtures were immediately added to 500 µL of alkaline-hypochlorite reagent (sodium hypochlorite to 0.125% available chlorine, in 0.6 M NaOH) and vortexed. Samples were incubated at 37 °C for 15 min and the colorimetric assay was carried out at 635 nm. Incubation times and protein concentrations were chosen in order to ensure the linearity of the reactions. Specific activity was expressed as nmol of NH_3. min^{-1}. mg $protein^{-1}$. The ADA activity was expressed as nmol $NH_3.min^{-1}.mg^{-1}$of protein. All enzyme assays were carried out on at least four separate occasions, with each one performed in triplicate.

2.6. Ectonucleotidase assays

NTPDase and 5'-nucleotidase assays were performed as described previously (Rico et al., 2003; Senger et al., 2004). Zebrafish brain membranes (3 µg protein for NTPDase and 5 µg protein for 5'-nucleotidase) were added to the reaction mixture containing 50 mM Tris–HCl (pH 8.0) and 5 mM $CaCl_2$ (for the NTPDase activity) or 50 mM Tris–HCl (pH 7.2) and 5 mM $MgCl_2$ (for the 5'-nucleotidase activity) in a total volume of 200 µL. The samples were preincubated for 10 min at 37 °C before starting the reaction by the addition of substrate (ATP, ADP or AMP) to a final concentration of 1 mM. The reaction was stopped after 30 min

with 200 μL trichloroacetic acid at a final concentration of 5%. The samples were chilled on ice for 10 min and 1 mL of a colorimetric reagent composed of 2.3% polyvinyl alcohol, 5.7% ammonium molybdate, and 0.08% malachite green was added in order to determine the inorganic phosphate (Pi) released (Chan et al., 1986). The Pi release was quantified spectrophotometrically at 630 nm and the specific activity was expressed as nmol Pi min^{-1} mg $protein^{-1}$. In order to correct for non-enzymatic hydrolysis of the substrates, controls were used with the addition of the enzyme preparation after the addition of trichloroacetic acid. All enzyme assays were carried out on at least four separate occasions, with each one performed in triplicate.

2.7. Protein determination

Protein was measured using Coomassie Blue as the color reagent (Bradford, 1976) and bovine serum albumin was used as standard.

2.8. Molecular analysis

For analysis by reverse transcription-polymerase chain reaction (RT-PCR), zebrafish *entpd1*, three different forms of *entpd2* (*entpd2_mg*, *entpd2_mq*, *entpd2_mv*) (Rico et al., 2006), *entpd3* (Appelbaum et al., 2007), *ada1*, *ada2-1*, *ada2-2* and *adal* (Rosemberg et al., 2007) and *β-actin* (Chen et al., 2004) primers were used as described previously. The optimal annealing temperatures were also tested (Table 1). TRIzol® reagent (Invitrogen) was employed to isolate total zebrafish brain RNA in accordance with the manufacturer's instructions. RNA was quantified spectrophotometrically and samples were adjusted to 160 ng/μL. cDNA species were synthesized with the SuperScriptTM First-Strand (Synthesis System for RT-PCR) kit (Invitrogen) following the supplier's instructions. PCR reactions were performed as described previously (Rico et al., 2006; Rosemberg et al., 2007). A

negative control was included for each set of PCR reactions. PCR products were analyzed on a 1% agarose gel containing GelRed® and visualized with ultraviolet light. The β-actin gene was amplified for normalization and the Invitrogen 1 kb Plus DNA ladder was used as a molecular marker in order to confirm the fragment size. The band intensities were measured by optical densitometry and the enzyme/β-actin mRNA ratios were established for each treatment using the software ImageJ 1.37 for Windows after running all PCR products in a single gel.

2.9. Statistical analysis

Results are expressed as means ± S.D. Data were analyzed by one-way analysis of variance (one-way ANOVA) followed by Tukey multiple range post-hoc test, considering $P \leq 0.05$ as significant. SPSS 16.0 was used for statistical analysis.

3. Results

Nucleotidase activities in zebrafish brain membranes were determined after acute typical or atypical antipsychotic treatments. The animals were exposed to haloperidol (9 μM), sulpiride (250 μM), and olanzapine (100 μM) for 2 hours. Olanzapine and sulpiride had no significant effect on ATP hydrolysis in zebrafish brain. However, haloperidol treatment did inhibit ATP hydrolysis (35%, $P<0.05$) when compared to the control group (Fig. 1A). There were no significant changes in ADP hydrolysis (Fig. 1A) and AMP hydrolysis (Fig. 1B) after exposure to all the antipsychotics tested.

The effect of antipsychotic drug treatments on ADA activity was examined in both soluble and membrane fractions from zebrafish brain (Fig. 2). The results showed that olanzapine and sulpiride did not alter ADA activity in either fraction. However, haloperidol

significantly decreased ecto-ADA activity (38%, $P<0.05$) when compared to control. In contrast, the soluble ADA activity was not altered by the drugs at the concentrations tested.

In order to determine if the decrease in NTPDase and ADA activities could be a consequence of transcriptional control, RT-PCR analysis was performed when alterations in NTPDase and ADA activities were observed after haloperidol treatment (Table 1). For this reason, the ecto-5´-nucleotidase activity was not analyzed. The expression patterns after acute haloperidol treatment are presented (Fig. 3A and 3B). Haloperidol exposure led to a decrease in *entpd2_mq* (45%, $P<0.05$), *entpd3* (24%, $P<0.05$) and *adal* (33%, $P<0.05$) mRNA transcript levels, whereas *entpd2_mv*, *entpd2_mg*, *entpd1*, *ada1*, *ada2-1* and *ada2-2* transcripts were not affected.

4. Discussion

Extracellular adenosine levels and the degree of receptor activation depend on the rate of formation, diffusion, and degradation of adenine nucleotides (ATP, ADP, and AMP) and the nucleoside adenosine (Brundege and Dunwiddie, 1997). In the present study, there was a significant inhibition of ATP hydrolysis and adenosine deamination in zebrafish brain after acute treatment with haloperidol. Considering the existence of antagonistic intramembrane interaction between adenosine A_{2A} and D_2 receptors and the role of ectonucleotidases and ADA as the enzyme members of the pathway responsible for the production and degradation of extracellular adenosine, it is important to clarify the role of these enzymes in schizophrenia and their interactions with therapeutic agents used in the management of this disorder. Therefore, this study investigated the effect of haloperidol, sulpiride, and olanzapine on ectonucleotidase and ADA activities, followed by the analysis of their gene expression patterns in zebrafish brain.

Adenosinergic activity may play a role in schizophrenia, especially because adenosine modulates several neurotransmitter systems (Burnstock, 2008; Lara et al., 2006). Previous studies have shown that activation of the adenosine A_1 receptor inhibits the release of several neurotransmitters, such as serotonin, glutamate, acetylcholine, and dopamine, and decreases neuronal activity by post-synaptic hyperpolarization (Dunwiddie and Mansino, 2001). The proposed adenosine dysfunction in schizophrenia, leading to a synaptic adenosinergic deficit, could be due to receptor alterations or altered metabolism, i.e. decreased production/release or increased degradation/uptake of adenosine (Lara et al., 2006). There has been a growing interest in purinergic neurotransmission and neuromodulation in different regions of the brain and spinal cord (Burnstock, 2007; North and Verkhratsky, 2006), and the involvement of ATP receptors in schizophrenia has been discussed in relation to reports that antipsychotic drugs, such as haloperidol, chlorpromazine, and fluspirilene are able to inhibit ATP-evoked responses mediated by P2X receptors (Inoue et al., 1996). It was suggested that ATP might facilitate dopaminergic neurotransmission and that various antipsychotic drugs suppress dopaminergic hyperactivity through inhibition of P2X receptor-mediated effects.

Regarding the adenosine involvement in schizophrenia there have been reports of adenosine–dopamine interactions (Cunha et al., 2008; Wardas, 2008). For example, it is noteworthy that activation of adenosine A_{2A} receptors reduces the affinity of dopaminergic D_2 receptors for dopamine, and this is the probable mechanism underlying the antipsychotic-like profile of adenosine agonists (Ferré, 1997), the hyperdopaminergic effect of caffeine (Ferré, 1997; Ferré, 2008) and the exacerbation of psychotic symptoms by caffeine in schizophrenic patients (Lucas et al., 1990). The demonstration of an increase in basal D_2 receptor occupancy by dopamine in schizophrenic patients (Abi-Dargham et al., 2000; Seeman et al., 2006) is compatible with a decreased adenosinergic tone, which via A_{2A}-D_2 receptor interaction increases the affinity of D_2 receptors for dopamine (Férre, 1997; Svenningsson et al., 1999).

Moreover, striatal dopamine release is known to be under tonic inhibition by adenosine acting on presynaptic A_1 receptors (Borycz et al., 2007; Golembiowska and Zylewska, 1998), which is in line with the increased release of dopamine in schizophrenia (Laruelle et al., 2000). It was also observed that the ability of clozapine to induce *c-fos* expression is blocked by A_{2A} receptor antagonists (Pinna et al., 1999) and this antipsychotic also affected the ectonucleotidase pathway responsible for the formation of ATP-derived adenosine, which acts on A_{2A} receptors (Lara et al., 2001). Therefore, these observations are consistent with the importance of the modulation of ectonucleotidase and ADA activities by haloperidol, since the control of adenosine levels is involved in the manipulation of activation of the A_{2A} receptor, which, in turn, might help to restore adequate dopaminergic signaling.

ADA activity is a target of investigation into the biochemical regulation of behavior. Ozyur et al. (2006) reported that ADA activity was significantly increased in the prefrontal cortex of rats in an MK-801-induced experimental psychosis model. Interestingly, chronic treatment with the adenosine receptor antagonist caffeine, which induces adaptive changes to a low endogenous adenosine signal, significantly reduced the hyperlocomotor and amnesic effects of MK-801 in mice (Dall'Igna et al., 2003; de Oliveira et al., 2005). ADA activity is involved in the regulation of adenosine levels in the extracellular milieu and also interacts with A_1 receptors (Franco et al., 1997). When we analyzed the effect of sulpiride, haloperidol, and olanzapine on ADA activity, only haloperidol produced a significant inhibition of this enzyme activity. Furthermore, taking into consideration that the control of the adenosinergic signaling can also be exerted by adenosine uptake via bi-directional transporters and by adenosine kinase in mammals (Boison, 2006, Latini & Pedala, 2001), further studies are important to demonstrate the impact of these mechanisms in the modulation of adenosine levels in zebrafish.

Drug interaction with biological membranes influences the bilayer structure, consequently modulating processes that range from membrane-bound enzyme activity and receptor binding to membrane permeability and transport (Carfagna and Muhoberac, 1993). Various studies have demonstrated that antipsychotic drugs have high affinity for biological membranes due to their amphipathic and amphiphilic properties, and this implies that antipsychotic drugs can interact with membrane lipid organization. It is known that antipsychotic intercalation in the membrane can alter the membrane lipid dynamics, possibly leading to modification of the receptor response (Tessier et al., 2008). Accordingly, it is possible to suggest that changes in membrane structure induced by antipsychotics might be responsible for the inhibitory effect observed on NTPDase and ADA activities in zebrafish brain membranes.

Another possibility is that the inhibitory effect of haloperidol on NTPDases and ADA may occur via transcriptional mechanisms. It is known that treatment with various classes of antipsychotic drugs may result in a common, final pathway of changes in gene expression in the brain. Our findings demonstrated that animals submitted to haloperidol treatment presented significant changes in NTPDase and ADA gene expression patterns. Haloperidol exposure decreased *entpd2_mq* (45%, $P<0.05$) and *entpd3* (24%, $P<0.05$) mRNA transcript levels, whereas *entpd2_mv*, *entpd2_mg* and *entpd1* mRNA transcript levels apparently were not affected. Considering that ATP is the preferential substrate for NTPDase2 and NTPDase3 (Zimmmermann, 2001), it is possible to suggest that the decrease in the mRNA transcript levels for *entpd2_mq* and *entp3* is related to the decrease in ATP hydrolysis observed after haloperidol treatment. Previous work from our group has already revealed the expression pattern of ADA-related genes (*ada1*, *ada2a*, *ada2b*, *and adal*) in zebrafish brain (Rosemberg et al., 2007). In this study, haloperidol treatment significantly decreased mRNA transcript levels for *adal* (33%, $P<0.05$), but *ada1*, *ada2-1* and *ada2-2* did not change, suggesting that

the observed alteration in ADA activity might be related to changes in the gene expression level.

Extracellular nucleotides and nucleosides are important signaling molecules that require effective mechanisms for their signal regulation (Yegutkin, 2008). This regulation is exerted by a broad range of nucleotide-degrading and interconverting extracellular enzymes (Abbracchio et al., 2009; Zimmermann, 2006). Our findings show that a typical antipsychotic drug, such as haloperidol, might modulate the ectonucleotidase and ADA pathway, an important source of extracellular adenosine. These results indicate that adenosine degradation and production might be a pharmacological target for this class of drugs and this finding suggests the existence of another mechanism of action involving the purinergic system, which can influence their final effects.

Acknowledgments

This work was supported by DECIT/SCTIEMS through Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS, Proc. 10/0036-5, conv. 700545/2008), and by the FINEP research grant "Rede Instituto Brasileiro de Neurociência (IBN-Net)" 01.06.0842-00. K.J.S. was a recipient of a fellowship from Programa PROBOLSAS/PUCRS. R.L.O. was a recipient of a fellowship from PIBIC/CNPq/PUCRS. M.R.S was the recipient of a fellowship PAPDRJ – CAPES/FAPERJ. The authors also would like to thank Cladinara Sarturi for technical assistance.

References

Abbracchio MP, Burnstock G, Verkhratsky A, Zimmermann H (2009). Purinergic signaling in the nervous system: an overview. Trends. Neurosci. 32(1), 19–29.

Abi-Dargham A, Rodenhiser J, Printz D, Zea-Ponce Y, Gil R, Kegeles LS, Weiss R, Cooper TB, Mann JJ, Van Heertum RL, Gorman JM, Laruelle M (2000). Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. Proc. Natl. Acad. Sci. USA 97, 8104–8109.

Appelbaum L, Skariahm G, Mourrain P, Mignot E (2007). Comparative expression of P2X receptors and ecto-nucleoside triphosphate diphosphohydrolase 3 in hypocretin and sensory neurons in zebrafish. Brain. Res. 1174, 66–75.

Barnes JM, Murphy PA, Kirkham D, Henley JM (1993). Interaction of guanine nucleotides with [3H] kainate and 6-[3H] cyano-7-nitroquinoxaline-2,3-dione binding in goldfish brain. J. Neurochem. 61, 1685–1691.

Boehmler W, Petko J, Woll M, Frey C, Thisse B, Thisse C, Canfield VA, Levenson R (2009). Identification of zebrafish A2 adenosine receptors and expression in developing embryos. Gene. Expr. Patterns. 9, 144-51.

Boison D (2006). Adenosine kinase, epilepsy and stroke: mechanisms and 398 therapies. Trends Pharmacol. Sci. 27 (12) 652–658.

Borycz J, Pereira MF, Melani A, Rodrigues RJ, Köfalvi A, Panlilio L, Pedata F, Goldberg SR, Cunha RA, Ferré S (2007). Differential glutamate-dependent and glutamate-independent adenosine A1 receptor mediated modulation of dopamine release in different striatal compartments. J. Neurochem. 101, 355–363.

Bradford MM (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 72, 218–254.

Brundege JM, Dunwiddie TV (1997). Role of adenosine as a modulator of synaptic activity in the central nervous system. Adv. Pharmacol. 39, 353-391.

Burnstock G (2007). Physiology and pathophysiology of purinergic neurotransmission. Physiol. Rev. 87, 659–797.

Burnstock G (2008). Purinergic signalling and disorders of the central nervous system, Nat. Rev. Drug Discov. 7 (7), 575–590.

Burnstock G (2009). Purinergic signalling: past, present and future. Braz. J. Med. Biol. Res. 42, 3–8.

Carfagna MA, Muhoberac BB (1993). Interaction of tricyclic drug analogs with synaptic plasma membranes: structure–mechanism relationships in inhibition of neuronal Na+/K(+)-ATPase activity. Mol. Pharmacol. 44, 129–141.

Chan KM, Delfert D, Junger KD (1986). A direct colorimetric assay for Ca2+- stimulated ATPase activity. Anal. Biochem. 157, 375–380.

Chen WY, John JAC, Lin CH, Lin HF, Wu SC, Lin CH, Chang CY (2004). Expression of metallothionen gene during embryonic and early larval development in zebrafish. Aquat. Toxicol. 69, 215–227.

Cunha RA, Ferre S, Vaugeois JM, Chen JF (2008). Potential therapeutic interest of adenosine A2A receptors in psychiatric disorders. Curr. Pharm. Des. 14 (15), 1512–24.

Dall'Igna OP, Da Silva AL, Dietrich MO, Hoffmann A, de Oliveira RV, Souza DO, Lara DR (2003). Chronic treatment with caffeine blunts the hyperlocomotor but not cognitive effects of theN-methyl-d-aspartate receptor antagonist MK-801 in mice. Psychopharmacol. (Berl.) 166, 258–263.

de Oliveira RV, Dall'Igna OP, Tort AB, Schuh JF, Neto PF, Santos Gomes MW, Souza DO, Lara DR (2005). Effect of subchronic caffeine treatment on MK-801-induced changes in locomotion, cognition and ataxia in mice. Behav. Pharmacol. 16, 79–84.

Dunwiddie TV, Mansino SA (2001). The role and regulation of adenosine in central nervous system. Annu. Rev. Neurosci. 24, 31-55.

Franco R, Casado V, Ciruela F, Saura C, Mallol J, Canela EI, Lluis C (1997). Cell surface adenosine deaminase: much more than an ecto-enzyme. Prog. Neurobiol. 52, 283–294.

Ferré S (1997). Adenosine–dopamine interactions in the ventral striatum. Implications for the treatment of schizophrenia. Psychopharmacology 2, 107–20.

Ferré S (2008). An update on the mechanisms of the psychostimulant effects of caffeine. J. Neurochem. 105(4), 1067-79.

Fredholm BB, Chen JF, Cunha RA, Svenningsson P, Vaugeois JM (2005). Adenosine and brain function. Int. Rev. Neurobiol. 63, 191–270.

Gerlai R, Lahav S, Guo S, Rosenthal A (2000). Drinks like a fish: Zebra fish (Danio rerio) as a behavior genetic model to study alcohol effects. Pharmacol. Biochem. Behav. 67, 773–782.

Giacomini NJ, Rose B, Kobayashi K, Guo S (2006). Antipsychotics produce locomotor impairment in larval zebrafish. Neurotoxicol. Teratol. 28, 245–250.

Golembiowska K, Zylewska A (1998). Agonists of A1 and A2A adenosine receptors attenuate methamphetamine-induced overflow of dopamine in rat striatum. Brain. Res. 2, 202–209.

Heiser P, Enning F, Krieg JC, Vedder H (2007). Effects of haloperidol, clozapine and olanzapine on the survival of human neuronal and immune cells in vitro. J Psychopharmacol. 21(8):851-6.

Herrera C, Casadó V, Ciruela F, Schofield P, Mallol J, Lluis C, Franco R (2001). Adenosine A2B receptors behave as an alternative anchoring protein for cell surface adenosine deaminase in lymphocytes and cultured cells. Mol. Pharmacol. 59, 127–134.

Inoue K, Koizumi S, Ueno S (1996). Implication of ATP receptors in brain functions. Prog. Neurobiol. 50, 483–492.

Ishida T, Obara Y, Kamei C (2009). Effects of Some Antipsychotics and a Benzodiazepine Hypnotic on the Sleep-Wake Pattern in an Animal Model of Schizophrenia. J Pharmacol Sci 111, 44 – 52.

Karow A, Naber D (2002) Subjective well-being and quality of life under atypical antipsychotic treatment. Psychopharmacology 162:3–10.

Kucenas S, Li Z, Cox JA, Egan TM, Voigt MM (2003). Molecular characterization of the zebrafish P2X receptor subunit gene family. Neurosci. 121, 935-945.

Lara DR, Dall'Igna OP, Ghisolfi ES, Brunstein MG (2006). Involvement of adenosine in the neurobiology of schizophrenia and its therapeutic implications. Prog. Neuropsychopharmacol. Biol. Psychiatry 30, 617–629.

Lara DR, Souza DO (2000). Schizophrenia: a purinergic hypothesis. Med. Hypotheses 54, 157–166.

Lara DR, Vianna MR, de Paris F, Quevedo J, Oses JP, Battastini AM, Sarkis JJ, Souza DO (2001). Chronic treatment with clozapine, but not haloperidol, increase striatal ecto-5'-nucleotidase activity in rats. Neuropsychobiol. 44(2), 99-102.

Laruelle M (2000). The role of endogenous sensitization in the pathophysiology of schizophrenia: implications from recent brain imaging studies. Brain Res. Rev. 31 (2-3), 371–84.

Latini S., Pedata F. Adenosine in the central nervous system: release mechanisms and extracellular concentrations, J. Neurochem. 79 (3) (2001) 463–484.

Lucas PB, Pickar D, Kelsoe J, Rapaport M, Pato C, Hommer D (1990). Effects of the acute administration of caffeine in patients with schizophrenia. Biol. Psychiatry 1, 35–40.

Massé K, Eason R, Bhamra S, Dale N, Jones EA (2006). Comparative genomic and expression analysis of the conserved NTPDase gene family in Xenopus. Genomics 87(3), 366-381.

McGrath J, Saha S, Welham J, El Saadi O, MacCauley C, Chant D (2004). A systematic review of the incidence of schizophrenia: the distribution of rates and the influence of sex, urbanicity, migrant status and methodology. BMC Med. 28, 2:13.

Meltzer HY, Davidson M, Glassman AH, Vieweg WV (2002). Assessing cardiovascular risks versus clinical benefits of atypical antipsychotic drug treatment. J Clin Psychiatry 63:25–29.

Morris JA (2009). Zebrafish: a model system to examine the neurodevelopmental basis of schizophrenia. Prog. Brain Res. 179, 97-106.

North RA, Verkhratsky A (2006). Purinergic transmission in the central nervous system. Pflugers. Arch. 452, 479–485.

Ozyurt B, Sarsilmaz M, Akpolat N, Ozyurt H, Akyol O, Herken H, Kus I (2007). The protective effects of omega-3 fatty acids against MK-801-induced neurotoxicity in prefrontal cortex of rat. Neurochem. Int. 50(1), 196-202.

Pani L (2009). The need for individualised antipsychotic drug therapy in patients with schizophrenia. Eur. Rev. Med. Pharmacol. Sci. 13, 453-9.

Pinna A, Wardas J, Cozzolino A, Morelli M (1999). Involvement of adenosine A2A receptors in the induction of c-fos expression by clozapine and haloperidol. Neuropsychopharmacol. 20, 44–51.

Rico EP, Rosemberg DB, Senger MR, Arizi MB, Bernardi GF, Dias RD, Bogo MR, Bonan CD (2006). Methanol alters ecto-nucleotidases and acetylcholinesterase in zebrafish brain. Neurotoxicol. Teratol. 28, 489–496.

Rico EP, Senger MR, Fauth MG, Dias RD, Bogo MR, Bonan CD (2003). ATP and ADP hydrolysis in brain membranes of zebrafish (Danio rerio). Life Sci. 73, 2071–2082.

Rosemberg DB, Rico EP, Guidoti MR, Dias RD, Souza DO, Bonan CD, (2007). Adenosine deaminase-related genes: molecular identification, tissue expression pattern and truncated

alternative splice isoform in adult zebrafish (Danio rerio). Life Sci. 81 (21–22), 1526–1534.

Rosemberg DB, Rico EP, Senger MR, Dias RD, Bogo MR, Bonan CD (2008). Kinetic characterization of adenosine deaminase activity in zebrafish (Danio rerio) brain, Comp. Biochem. Physiol. B 151 (1), 96–101.

Saura CA, Mallol J, Canela EI, Lluis C, Franco R (1998). Adenosine deaminase and A1 adenosine receptors internalize together following agonist-induced receptor desensitization. J. Biol. Chem. 273, 17610–17617.

Seeman P, Schwarz J, Chen JF, Szechtman H, Perreault M, McKnight GS, Roder JC, Quirion R, Boksa P, Srivastava LK, Yanai K, Weinshenker D, Sumiyoshi T (2006). Psychosis pathways converge via D2high dopamine receptors. Synapse 60, 319–346.

Seibt KJ, Oliveira RL, Rico EP, Dias RD, Bogo MR, Bonan CD (2009). Antipsychotic drugs inhibit nucleotide hydrolysis in zebrafish (Danio rerio) brain membranes. Toxicol. In Vitro 23 (1), 78-82

Seibt KJ, Oliveira R da L, Zimmermann FF, Capiotti KM, Bogo MR, Ghisleni G, Bonan CD (2010). Antipsychotic drugs prevent the motor hyperactivity induced by psychotomimetic MK-801 in zebrafish (Danio rerio). Behav Brain Res. 214(2):417-22.

Senger MR, Rico EP, Dias RD, Bogo MR, Bonan CD (2004). Ecto-5'-nucleotidase activity in brain membranes of zebrafish (Danio rerio). Comp. Biochem. Physiol. 139 (2), 203–207.

Svenningsson P, Le Moine C, Fisone G, Fredholm BB (1999). Distribution, biochemistry and function of striatal adenosine A2A receptors. Prog. Neurobiol. 59, 355–396.

Tessier C, Nuss P, Staneva G, Wolf C (2008). Modification of membrane heterogeneity by antipsychotic drugs: an X-ray diffraction comparative study. J. Colloid Interface Sci. 320, 469–475.

van Os J, Kapur S (2009). Schizophrenia. Lancet. 22, 374 (9690): 635-45.

Volavka J, Czobor P, Sheitman B, Lindenmayer JP, Citrome L, McEvoy JP, Cooper TB, Chakos M, Lieberman JA (2002). Clozapine, olanzapine, risperidone, and haloperidol in the treatment of patients with chronic schizophrenia and schizoaffective disorder. Am J Psychiatry 159:255–262.

Wardas J (2008). Potential role of adenosine A2A receptors in the treatment of schizophrenia. Front.Biosci.13, 4071–96.

Weisman MI, Caiolfa VR, Parola AH (1988). Adenosine deaminase-complexing protein from bovine kidney. Isolation of two distinct subunits. J. Biol. Chem. 263 (11), 5266–5270.

Yegutkin GG (2008). Nucleotide- and nucleoside-converting ectoenzymes: important modulators of purinergic signalling cascade. Biochim. Biophys. Acta 1783, 673–694.

Zimmermann H (2001). Ecto-nucleotidases: some recent developments and a note on nomenclature. Drug Dev. Res. 52, 44–56.

Zimmermann H (2006). Ectonucleotidases in the nervous system. Novartis Found. Symp.276, 113–128.

Figure legends:

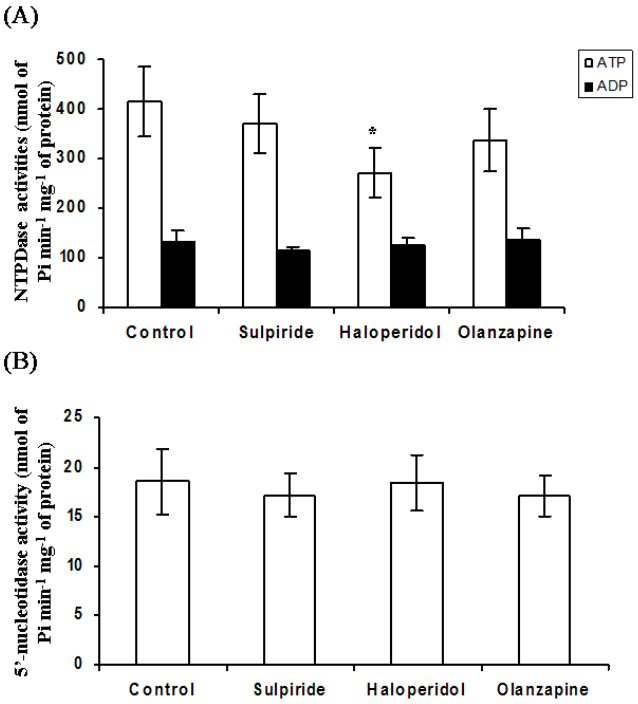

Figure 1: *In vivo* effect of sulpiride, haloperidol, and olanzapine on (A) NTPDase and (B) 5'-nucleotidase activities in zebrafish brain membranes. ATP, ADP, and AMP hydrolysis were determined. Bars represent the mean ± S.D. of at least four different experiments, each one performed in triplicate. The symbol (*) indicates a difference when compared to the control group. Data were analyzed by one-way ANOVA followed by Tukey test as post-hoc, considering $P<0.05$ as significant.

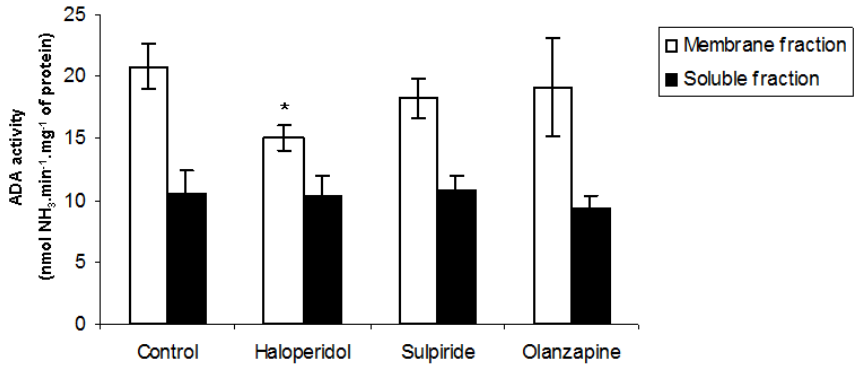

Figure 2: Effect of acute (2 h) antipsychotic drugs exposure on soluble and membrane-bound ADA activity from zebrafish brain. Data were expressed as mean ± S.D. of four independent experiments, each one performed in triplicate. The asterisk represents a significant difference from the control group (one-way ANOVA, followed by Tukey's test as post-hoc, $P<0.05$).

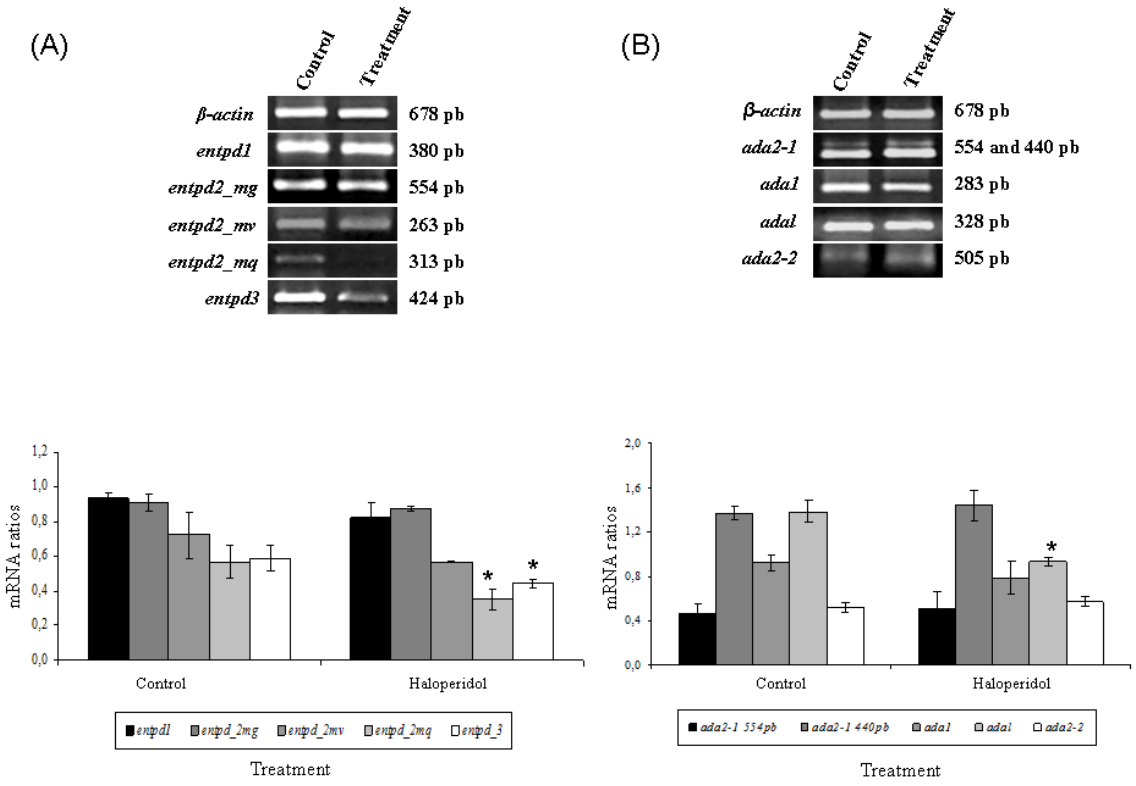

Figure 3: Effect of haloperidol exposure on NTPDase and ADA transcripts. The figure shows (A) *β-actin*, *entpd1*, *entpd2_mg*, *entpd2_mq*, *entpd2_mv*, *entpd3* and (B) *β-actin, ada1*, *adal*, *ada2-1* and *ada2-2* mRNA expression in adult zebrafish and the enzyme/ β-actin mRNA ratios obtained by optical densitometry. Three independent experiments were performed, with entirely consistent results. *Significantly different from control (P<0.05, ANOVA followed by Tukey post-hoc).

Table 1: Primer sequences and PCR amplification conditions

Enzymes	Sequences (5´- 3´)	Annealing temperature (ºC)	PCR product (bp)	GenBank accession number
entpd1	CCCATGGCACAGGCCGGTTG (forward) GCAGTCTCATGCCAGCCGTG (reverse)	54	380	AAH78240
*entpd2_mg**	GGAAGTGTTTGACTCGCCTTGCACG (forward) CAGGACACAAGCCCTTCCGGATC (reverse)	62	554	XP_697600
*entpd2_mq**	CCAGCGGATTTAGAGCACGCTG (forward) GAAGAACGGCGGCACGCCAC (reverse)	62	313	XP_687722
*entpd2_mv**	GCTCATTTAGAGGACGCTGCTCGTG (forward) GCAACGTTTTCGGCAGGCAGC (reverse)	62	263	AAH78419
entpd3	TACTTTCTTTGGACAGAGCAACCCTG (forward) AAGCATATAGCCCAGGGACCAGG (reverse)	62	424	ABR15509
ada1	CAGGTCCATTCTGTGCTGCATGCGTC (forward) AAGTGTGTGGTATCCGTGCCCAATGC (reverse)	58	283	AAH76532
*ada2-1***	AAGACAAGGGTTTTAACCTGCCCTAC (forward) CTCCTTTCTTTGACTTGGCAATGTGC (reverse)	63	554 and 440	AAL40922
ada2-2	CTGAAGATGAAGGAAATCACCCTTTCACC (forward) TGTCTTCATAAAGCTCTTTCAAACCCTGG (reverse)	54	505	XP_687719
adal	TCATTCAAGAGTTTGCGGCAGATGG (forward) TTGGCTTTCTGAAGTGCAGCGAGC (reverse)	61	328	NP_001028916
β-actin	GTCCCTGTACGCCTCTGGTCG (forward) GCCGGACTCATCGTACTCCTG (reverse)	54	678	AAC13314

* Corresponds to the first two amino acid residues of the protein sequence.
** The same primers amplified a truncated *ada*2-1 splice isoform (*ada*2-1/T).

CAPÍTULO IV

ARTIGO CIENTÍFICO

MK-801 alters Na^+, K^+-ATPase activity and oxidative status in zebrafish brain: reversal by antipsychotic drugs

Kelly Juliana Seibt, Renata da Luz Oliveira**,** Luiz Eduardo Savio, Denis Broock Rosemberg,

Emilene BS Scherer, Felipe Schmitz, Ângela T. Wyse,

Carla Denise Bonan.

Artigo aceito para publicação no periódico Journal of Neural Transmission, 2011

J Neural Transm
DOI 10.1007/s00702-011-0745-z

BASIC NEUROSCIENCES, GENETICS AND IMMUNOLOGY - ORIGINAL ARTICLE

MK-801 alters Na^+, K^+-ATPase activity and oxidative status in zebrafish brain: reversal by antipsychotic drugs

Kelly Juliana Seibt · Renata da Luz Oliveira · Denis Broock Rosemberg · Luiz Eduardo Baggio Savio · Emilene B. S. Scherer · Felipe Schmitz · Angela T. S. Wyse · Carla Denise Bonan

Received: 6 September 2011 / Accepted: 20 November 2011
© Springer-Verlag 2011

Abstract Schizophrenia is a debilitating mental disorder with a global prevalence of 1% and its etiology remains poorly understood. In the current study we investigated the influence of antipsychotic drugs on the effects of MK-801 administration, which is a drug that mimics biochemical changes observed in schizophrenia, on Na^+, K^+-ATPase activity and some parameters of oxidative stress in zebrafish brain. Our results showed that MK-801 treatment significantly decreased Na^+, K^+-ATPase activity, and all antipsychotics tested prevented such effects. Acute MK-801 treatment did not alter reactive oxygen/nitrogen species by 2′7′-dichlorofluorscein (H2DCF) oxidation assay, but increased the levels of thiobarbituric acid reactive substances (TBARS), when compared with controls. Some antipsychotics such as sulpiride, olanzapine, and haloperidol prevented the increase of TBARS caused by MK-801. These findings indicate oxidative damage might be a mechanism involved in the decrease of Na^+, K^+-ATPase activity induced by MK-801. The parameters evaluated in this study had not yet been tested in this animal model using the MK-801, suggesting that zebrafish is an animal model that can contribute for providing information on potential treatments and disease characteristics.

Keywords Schizophrenia · Antipsychotics · MK-801 · Na^+, K^+-ATPase · TBARS · DCF

K. J. Seibt · R. da Luz Oliveira · C. D. Bonan (✉)
Programa de Pós-Graduação em Biologia Celular e Molecular
Laboratório de Neuroquímica e Psicofarmacologia,
Departamento de Biologia Celular e Molecular, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga, 6681, Porto Alegre, RS 90619-900, Brazil
e-mail: cbonan@pucrs.br

K. J. Seibt · R. da Luz Oliveira · C. D. Bonan
Instituto Nacional Translacional em Medicina (INCT-TM),
Porto Alegre, RS 90035-003, Brazil

D. B. Rosemberg · L. E. B. Savio · E. B. S. Scherer · F. Schmitz · A. T. S. Wyse
Programa de Pós-Graduação em Bioquímica, Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde,
Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos 2600-Anexo, Porto Alegre, RS 90035-003, Brazil

D. B. Rosemberg
Instituto Nacional de Ciência e Tecnologia em Excitotoxicidade e Neuroproteção (INCT-EN), Porto Alegre,
RS 90035-003, Brazil

Introduction

Schizophrenia is a severe neuropsychiatric illness with a lifetime prevalence of approximately 1% whose etiology remains poorly understood (Boison et al. 2011; Yu et al. 2011). The disease is characterized by a broad range of mental and neuropsychological dysfunctions including (i) positive symptoms characterized by functional excesses such as delusions, hallucinations, and disorganized thinking; (ii) affective symptoms, such as depression or mania; (iii) negative symptoms, characterized by loss of normal functions, such as anhedonia, blunted affect, and social withdrawal, and (iv) cognitive symptoms, reflecting deterioration of memory, selective attention, and executive functions (Ross et al. 2006; van Os and Kapur 2009). One of the best characterized animal models of schizophrenia is based on NMDA hypofunction (Farber 2003). This model is based on observations that NMDA antagonists, such as phencyclidine and MK-801, can mimic the complexity of positive, negative, and cognitive symptoms of the disease (Li et al. 2011; Rujescu et al. 2006). MK-801 is a

Published online: 06 December 2011

non-competitive antagonist of NMDA subtype of glutamate receptors and acts by means of open-channel blockade (Stuchlík et al. 2009).

Although the pathophysiological mechanisms underlying this disorder remain unclear, many studies point towards an involvement of oxidative stress in the neuropathological processes of schizophrenia (Gama et al. 2008; Ng et al. 2008; Zhang et al. 2010). Oxidative stress, which results from an impaired redox balance, is supposed to be one of the major causes for schizophrenia (Mahadik and Scheffer 1996; Fenton et al. 2000). Shifting of the redox balance towards oxidative stress may occur due to excessive generation of free radicals, decreased antioxidant activities, or a combination of both (Singh et al. 2008). Recent findings indicate the role of changes of membrane phospholipids and fatty acids induced by oxidative stress in etiopathogenic mechanisms in schizophrenia (Fendri et al. 2006; Yao et al. 2001). Lipid peroxidation assessed by TBARS was shown to be increased in the plasma of drug-free and medicated schizophrenic patients (Akyol et al. 2002).

Na^+, K^+-ATPase (EC 3.6.1.37), also known as the sodium pump, is a major membrane protein responsible for generating the membrane potential. The active transport of Na^+ and K^+ ions in the central nervous system (CNS) is indispensable to regulate neuronal excitability and cellular volume (Kaplan 2002; Aperia 2007). This enzyme consumes about 40–60% of ATP produced in brain (Erecinska et al. 2004). Disturbance in Na^+, K^+-ATPase density and/or activity might induce significant damage on brain function. Studies show that its activity is altered in various brain disorders, such as ischemia (Wyse et al. 2000), neurodegenerative (Yu 2003; Vignini et al. 2007) and neuropsychiatric diseases (Goldstein et al. 2006). In addition, a decline of Na^+, K^+-ATPase activity has been attributed to induce lipid changes and associated oxidative damage (Chakraborty et al. 2003; Dencher et al. 2007).

Zebrafish represents an attractive vertebrate model in Developmental Biology, Genetics, Pharmacology, and Neuroscience (Gerlai et al. 2000; Grossman et al. 2010; Cachat et al. 2010). The zebrafish have many inherent advantages as a model organism, such as low cost, easy handling and maintenance as compared with other vertebrate models, and 70–80% genetic homology to humans (Barbazuk et al. 2000; Egan et al. 2009). Behavior-based chemical screens in zebrafish may improve our understanding of neurobiology and drug action and accelerate the pace of psychiatric drug discovery (Kokel and Peterson 2008; Rihel et al. 2010). Considering (i) oxidative stress is likely to be involved in the pathophysiology of schizophrenia, (ii) Na^+, K^+-ATPase activity is critical for normal brain function, being altered in many disorders, and (iii) zebrafish has been used as a model for studying several diseases, we investigate the effect of MK-801, a drug used for eliciting schizophrenia-like symptoms, on Na^+, K^+-ATPase activity and reactive oxygen/nitrogen species levels in zebrafish brain. The protective effect of antipsychotic drugs was also evaluated.

Materials and methods

Animals

Adult wild-type zebrafish strains (3–5 cm) of both sexes were obtained from a specialized commercial supplier (Redfish, RS, Brazil) and were of genetically heterogeneous (randomly bred) stock. The fish were acclimatized to the laboratory environment for at least 14 days and housed in a 50-L thermostated aquarium filled with continuously unchlorinated water at a targeted temperature of 28 ± 2°C, with constant filtration and aeration (7.20 mg O_2/L) and a density of up to five animals per liter (Westerfield 2007). Animals were kept on a day:night cycle of 14:10 h and fed twice a day with flaked fish food that was supplemented with live brine shrimp.

Fish were manipulated healthy and free of any signs of disease, according to the ''Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health (NIH publication No 85–23, revised 1996). The ethics committee of the Pontifical Catholic University of Rio Grande do Sul (PUCRS) approved the protocol under license number CEUA 09/00135.

Pharmacological treatments

A group of five animals were transferred and kept in 500-mL beakers for 30 min divided in two consecutive 15-min periods, as follows: (a) In the first 15-min exposure period, animals were exposed to tank water or 20 μM MK-801; (b) During the final 15-min-exposure, animals were treated with tank water or with one of the following antipsychotic drugs: 9 μM haloperidol (HAL), 100 μM olanzapine (OLA), or 250 μM sulpiride (SUL). Therefore, the following experimental groups were tested: (i) water plus water, (ii) water plus HAL, (iii) water plus OLA, (iv) water plus SUL, (v) MK-801 plus water, (vi) MK-801 plus HAL, (vii) MK-801 plus OLA (viii) MK-801 plus SUL. The MK-801 and haloperidol doses were chosen based on previous studies with zebrafish (Giacomini et al. 2006; Swain et al. 2004; Seibt et al. 2010). Moreover, our group showed that haloperidol, sulpiride, and olanzapine per se did not induce changes in the locomotion in zebrafish (Seibt et al. 2010).

Membrane preparation

Brain membranes were prepared according to the method previously described (Barnes et al. 1993). Briefly, whole zebrafish brains were homogenized in 60 volumes (v/w) of chilled Tris-citrate buffer (50 mM Tris, 2 mM EDTA, 2 mM EGTA, pH 7.4, adjusted with citric acid) in a glass-Teflon homogenizer. The homogenate was centrifuged at 1,000*g* for 10 min and the pellet was discarded. After removing the nuclear and cell debris, the supernatant was centrifuged for 25 min at 40,000*g*. The resultant pellet was frozen in liquid nitrogen, thawed, resuspended in Tris-citrate buffer, and centrifuged for 20 min at 40,000*g*. The final pellet was resuspended and used for the enzyme assays. The material was maintained at 2–4°C throughout preparation.

Protein determination

Protein was measured by the Coomassie Blue method (Bradford 1976), using bovine serum albumin as standard.

Na^+K^+ATPase activity

Na^+, K^+-ATPase activity was determined as previously reported (Tsakiris and Deliconstantinos 1984). The enzyme preparation (3–5 µg protein) was added to the reaction mixture containing 5 mM $MgCl_2$, 80 mM NaCl, 20 mM KCl, and 40 mM Tris–HCl buffer, pH 7.4, in a final volume of 200 µL. The reaction was started by the addition of ATP (vanadium-free disodium salt) to a final concentration of 3 mM and stopped after 5 min with addition of 200 µL trichloroacetic acid 10%. The control was assessed under the same conditions, except that 1 mM ouabain was added to the reaction medium. The Na^+, K^+-ATPase activity was calculated by determining the difference between these two assays. The released inorganic phosphate (Pi) was measured by the colorimetric Malachite Green method (Chan et al. 1986) and results were expressed as nmol Pi/min/mg protein.

Measurement of lipid peroxidation

Lipid peroxidation was measured through determination of thiobarbituric acid reactive substances (TBARS) according to the colorimetric assay previously described (Ohkawa et al. 1979). Briefly, samples and reagents were added in the following order: 100 µL of tissue supernatant; 25 µL of SDS 8.1%; 190 µL of 20% acetic acid in aqueous solution (v/v) pH 3.5; and 190 µL of 0.8% thiobarbituric acid. The mixture was vortexed and the reaction was carried out in a boiling water bath for 1 h. TBARS were determined by the absorbance at 535 nm and calculated as nmol of malondialdehyde (MDA) formed per milligram of protein.

Dichlorofluorescein (H2DCF) oxidation assay

Reactive oxygen/nitrogen species production was measured following the method based on 2′7′-dichlorofluorscein oxidation (Lebel et al. 1992). Briefly, homogenates from total zebrafish brain (60 µL) were incubated for 30 min at 37°C in the dark with 240 µL of 100 µM 2′7′-dichlorofluorscein diacetate solution in a 96-well plate. H2DCF-DA is cleaved by cellular esterases and H2DCF formed is eventually oxidized by ROS or RNS present in samples. The last reaction produces the fluorescent compound DCF, which was measured at 488 nm excitation and 525 nm emission, and the results were represented by nmol DCF/mg protein. A calibration curve was performed with purified DCF as standard.

Statistical analysis

Data were expressed as means ± SEM. The Na^+, K^+-ATPase activity and TBARS data were analyzed by two-way analysis of variance (ANOVA), followed by Bonferroni's multiple test range as post hoc. The DCFH data was analyzed by one-way analysis of variance (ANOVA), followed by Tukey's test. A value of $p < 0.05$ was considered significant.

Results

At first, we tested 20 µM MK-801 during 15 and 30 min of exposure in order to determine the time exposure to further experiments related to antipsychotic effects. Our results demonstrated that there was a decrease [52.5%; $F(2,9) = 34.56$; $p < 0.01$] of Na^+, K^+-ATPase activity in the group submitted to 15 min of exposure to MK-801 (195 ± 33 nmol Pi. min^{-1} mg^{-1} protein) in relation to the control group (410 ± 52 nmol Pi. min^{-1} mg^{-1} protein). However, there were no changes on Na^+, K^+-ATPase activity after 30-min MK-801 exposure (405 ± 13 nmol Pi. min^{-1} mg^{-1} protein) when compared with control group (410 ± 52 nmol Pi. min^{-1} mg^{-1} protein). Therefore, we have chosen 15 min-exposure to MK-801 for the subsequent experiments with antipsychotics.

We investigated the effect of acute administration of MK-801 and antipsychotic drugs on Na^+, K^+-ATPase activity. Figure 1 shows that MK-801/water significantly decreased Na^+, K^+-ATPase activity [$F(1,11) = 13.18$, $p < 0.002$]. Water/sulpiride and water/olanzapine alone did not alter this parameter. However, water/haloperidol treatment significantly increased Na^+, K^+-ATPase activity [$F(3,23) = 18{,}91$, $p < 0.001$]. In addition, the treatment with MK-801/olanzapine [$F(3,23) = 93.64$, $p < 0.0001$], MK-801/sulpiride [$F(3,23) = 25.64$, $p < 0.0001$], and

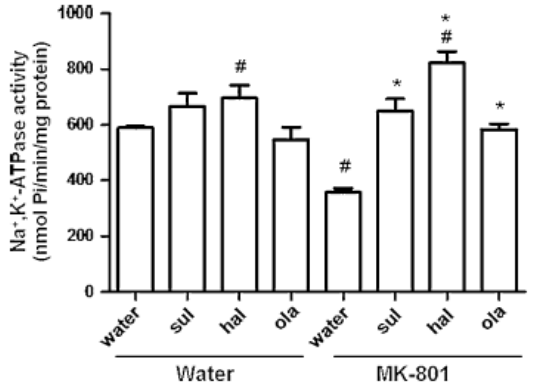

Fig. 1 Effect of MK-801 and antipsychotic drug treatment on Na^+, K^+-ATPase activity in zebrafish brain. Data are expressed as mean ± SEM for 5–7 animals in each group. #Significant difference compared to water/water. *Significant difference compared to MK-801/water

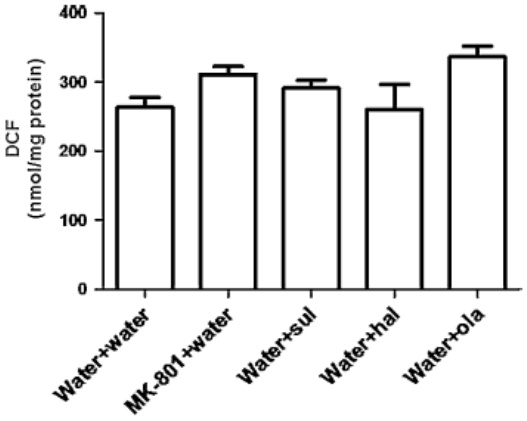

Fig. 2 Effect of acute administration of antipsychotic drugs and MK-801 on the reactive species levels in zebrafish brain. *Data* are expressed as mean ± SEM for 5–7 animals in each group. Results are expressed in nmol/mg protein

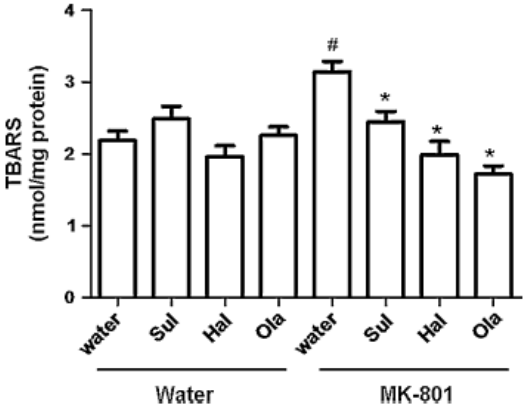

Fig. 3 In vivo effect of acute administration of antipsychotic drugs and MK-801 on TBARS in zebrafish brain. Data are expressed as mean ± SEM for 5–7 animals in each group. Results are expressed in nmol MDA/mg protein. #Significant difference compared with water/water. *Significant difference compared with MK-801/water

MK-801/haloperidol [$F(3,23) = 68.95$, $p < 0.0001$] reverted MK-801/water-induced effects on Na^+, K^+-ATPase activity. Only MK-801/olanzapine and MK-801/sulpiride maintained the enzyme activity at the same levels of control group. DMSO 5%, used as the vehicle to sulpiride, was tested alone and together with MK-801 and Na^+, K^+-ATPase activity was not altered (data not shown).

We also investigated the effect of MK-801/water on some parameters of oxidative stress, such as production of reactive oxygen/nitrogen species and TBARS in zebrafish brain. Figure 2 shows that MK-801/water and water/antipsychotic drugs did not change the levels of this marker in zebrafish brain [$F(4,27) = 2.750$; $P > 0.09$]. Figure 3 shows that acute MK-801/water administration significantly increased TBARS levels in zebrafish brain, when compared with controls (water-water treated) [$F(1,11) = 8.68$; $P < 0.01$]. Our data also showed that the treatment with MK-801/antipsychotic drugs, sulpiride [$F(3,23) = 10.31$; $P < 0.01$], haloperidol [$F(3,23) = 8.53$; $P < 0.01$], and olanzapine [$F(3,23) = 31.07$; $P < 0.001$], reverted this effect (Fig. 3).

Discussion

Schizophrenia is a brain disorder that has been intensively studied for over a century; however, its etiology and multifactorial pathophysiology remain a puzzle (Yao and Keshavan 2011). Previous studies have demonstrated that Na^+, K^+-ATPase activity may be altered in both neurodegenerative (Grisar 1984; Wyse et al. 2000) and psychiatric disorders (Kurup and Kurup 2002; Goldstein et al. 2006).

Na^+, K^+-ATPase is a membrane enzyme responsible for the generation of the membrane potential through the active transport of sodium and potassium ions in the CNS indispensable to maintain neuronal excitability, present at elevated concentrations in brain (Aperia 2007). Previous studies have shown a significant decrease in Na^+, K^+-ATPase activity in patients with schizophrenia (Rybakowski and Lehmann, 1994; Kurup and Kurup 2002). In the present study, we evaluated the effects of MK-801 and antipsychotic drugs on Na^+, K^+-ATPase activity in zebrafish brain. Our results showed that MK-801 decreased Na^+, K^+-ATPase activity and that antipsychotic

drugs (haloperidol, sulpiride and olanzapine) reverted this effect. Our findings have also shown that haloperidol administration increased Na^+, K^+-ATPase activity in zebrafish brain. These data are consistent with previous studies that showed haloperidol increased Na^+, K^+-ATPase activity in the rat brain (Wood et al. 1989). Other studies have demonstrated similar effects on other drugs, such as amphetamine (Zugno et al. 2009), fluoxetine (Zanatta et al. 2001), selegiline (Carageorgiou et al. 2003), carbamazepine, and lithium (Wood et al. 1989).

Oxidative stress occurs when there is an imbalance between reactive oxygen species (ROS) generation and antioxidant defenses in favor of the former. There are multiple pathological consequences of increased ROS production. Oxidative stress and the oxidative changes in different biomolecules may be involved in the pathology of schizophrenia (Berk et al. 2008). It has been verified that Na^+, K^+-ATPase is particularly susceptible to free radical attack since its inhibition has been correlated with changes in plasma membrane lipid composition (Dencher et al. 2007), and in other amino acid residues caused by free radicals or lipid peroxidation (Potts et al. 2006; Siems et al. 1996).

To verify whether reactive oxidative stress could play a role on MK-801-mediated effects, TBARS and H_2DCF assays were performed. Our results showed that acute administration of MK-801 and antipsychotic drugs did not alter the 2′,7′-Dichlorofluorescein oxidation. However, the results showed that MK-801 increased TBARS levels in brain from zebrafish treated with MK-801. We also observed that administration of antipsychotic drugs after MK-801 treatment significantly reversed the increase on TBARS levels. These data are consistent with previous studies showing that oxidative stress and alterations in antioxidant enzymes have long been described in the pathophysiology of schizophrenia (Reddy and Yao 1996; Grignon and Chianetta 2007). In addition, Dietrich-Muszalska et al has shown that the level of TBARS was significantly increased in plasma of patients with schizophrenia (Dietrich-Muszalska et al. 2005; Dietrich-Muszalska and Olas 2007), whereas the activities of antioxidant defense enzymes were diminished (Dietrich-Muszalska and Olas 2007). Other studies have shown that chronic treatment with antipsychotics increased free radical production and oxidative stress (Balijepalli et al. 2001). In addition, chronic use of antipsychotics is also reported to cause a decrease in the activity of antioxidant enzymes, superoxide dismutase, and catalase (Cadet et al. 1987).

In summary, we demonstrated that MK-801 administration decreased Na^+, K^+-ATPase activity in zebrafish brain and that distinct typical and atypical antipsychotics reverted this effect. The same effect is also evaluated when testing the TBARS parameter of oxidative stress. Together, these findings support the involvement of reactive oxygen/nitrogen species and/or lipid peroxidation in the MK-801-elicited effects on Na^+, K^+-ATPase. This data show that treatment with MK-801 in zebrafish might mimic some biochemical changes observed in schizophrenic patients, suggesting that the zebrafish is an animal model that can contribute for providing information about potential treatments and disease characteristics.

Acknowledgments This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS, Proc. 1018580). And by DECIT/SCTIE-MS through CNPq and FAPERGS, (Proc. 10/0036-5 – PRONEX/Conv. 700545/2008).

References

Akyol O, Herken H, Uz E, Fadillioglu E, Unal S, Sogutt S, Ozyurt H, Savaş HÁ (2002) The indices of endogenous oxidative and antioxidative processes in plasma from schizophrenic patients. The possible role of oxidant/antioxidant imbalance. Prog Neuropsychopharmacol Biol Psychiatry 26:995–1005

Aperia A (2007) New roles for an old enzyme: Na, K-ATPase emerges as an interesting drug target. J Intern Med 261:44–52

Balijepalli S, Kenchappa RS, Boyd MR, Ravindranath V (2001) Protein thiol oxidation by haloperidol results in inhibition of mitochondrial complex I in brain regions: comparison with atypical antipsychotics. Neurochem Int 38:425–435

Barbazuk WB, Korf I, Kadavi C, Heyen J, Tate S, Wun E, Bedell JA, McPherson JD, Johnson SL (2000) The syntenic relationship of the zebrafish and human genomes. Genome Res 10:1351–1358

Barnes JM, Murphy PA, Kirkham D, Henley JM (1993) Interaction of guanine nucleotides with [3H]kainate and 6-[3H]cyano-7-nitroquinoxaline-2,3-dione binding in goldfish brain. J Neurochem 61:1685–1691

Berk M, Copolov D, Dean O, Lu K, Jeavons S, Schapkaitz I, Anderson-Hunt M, Judd F, Katz P, Ording-Jespersen S, Little J, Conus P, Cuenod M, Do KQ, Bush AI (2008) *N*-acetyl cysteine as a glutathione precursor for schizophrenia–A double-blind, randomized, placebo- controlled trial. Biol Psychiatry 64:361–368

Boison D, Singer P, Shen HY, Feldon J, Yee BK (2011) Adenosine hypothesis of schizophrenia—Opportunities for pharmacotherapy. Neuropharmacology (Epub ahead of print)

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:218–254

Cachat J, Stewart A, Grossman L, Gaikwad S, Kadri F, Chung KM, Wu N, Wong K, Roy S, Suciu C, Goodspeed J, Elegante M, Bartels B, Elkhayat S, Tien D, Tan J, Denmark A, Gilder T, Kyzar E, Dileo J, Frank K, Chang K, Utterback E, Hart P, Kalueff AV (2010) Measuring behavioral and endocrine responses to novelty stress in adult zebrafish. Nat Protoc 5(11):1786–1799

Cadet JL, Lohr JB, Jeste DV (1987) Tardive dyskinesia and schizophrenic burnout: the possible involvement of cytotoxic free radicals. In: Henn FA, DeLisi LE (eds) Handbook of Schizophrenia: the neurochemistry and pharmacology of Schizophrenia. Amsterdam, Elsevier, pp 425–438

Carageorgiou H, Zarros A, Tsakiris S (2003) Selegiline long-term effects on brain acetylcholinesterase, Na^+, K^+-ATPase activities, antioxidant status and learning performance of aged rats. Pharmacol Res 48:245–251

Chakraborty H, Sen P, Sur A, Chatterjee U, Chakrabarti S (2003) Age-related oxidative inactivation of Na^+, K^+-ATPase in rat brain crude synaptosomes. Exp Gerontol 38:705–710

Chan KM, Delfer D, Junger KD (1986) A direct colorimetric assay for Ca2+-stimulated ATPase activity. Anal Biochem 157:375–380

Dencher NA, Frenzel M, Reifschneider NH, Sugawa M, Krause F (2007) Proteome alterations in rat mitochondria caused by aging, Ann. NY Acad Sci 1100:291–298

Dietrich-Muszalska A, Olas B (2007) Isoprostanes as indicators of oxidative stress in schizophrenia. World J Biol Psychiatry 4:1–6

Dietrich-Muszalska A, Olas B, Rabe-Jablonska J (2005) Oxidative stress in blood platelets from schizophrenic patients. Platelets 16:386–391

Egan RJ, Bergner CL, Hart PC, Cachat JM, Canavello PR, Elegante MF, Elkhayat SI, Bartels BK, Tien AK, Tien DH, Mohnot S, Beeson E, Glasgow E, Amri H, Zukowska Z, Kalueff AV (2009) Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. Behav Brain Res 205(1): 38–44

Erecinska M, Cherian S, Silver IA (2004) Energy metabolism in mammalian brain during development. Prog Neurobiol 73:397–445

Farber NB (2003) The NMDA receptor hypofunction model of psychosis. Ann NY Acad Sci 1003:119–130

Fendri C, Mechri A, Khiari G, Othman A, Kerkeni A, Gaha L (2006) Oxidative stress involvement in schizophrenia pathophysiology. Encephale 32:244–252

Fenton WS, Hibbeln J, Knable M (2000) Essential fatty acids, lipid membrane abnormalities, and the diagnosis and treatment of schizophrenia. Biol Psychiatry 47:8–21

Gama CS, Salvador M, Andreazza AC, Lobato MI, Berk M, Kapczinski F, Belmonte-de-Abreu PS (2008) Elevated serum thiobarbituric acid reactive substances in clinically symptomatic schizophrenic males. Neurosci Lett 433(3):270–273

Gerlai R, Lahav M, Guo S, Rosenthal A (2000) Drinks like a fish: zebra fish (*Danio rerio*) as a behavior genetic model to study alcohol effects. Pharmacol Biochem Behav 67(4):773–782

Giacomini NJ, Rose B, Kobayashi K, Guo S (2006) Antipsychotics produce locomotor impairment in larval zebrafish. Neurotoxicol Teratol 28:245–250

Goldstein I, Levy T, Galili D, Ovadia H, Yirmiya R, Rosen H, Lichtstein D (2006) Involvement of Na^+, K^+-ATPase and endogenous digitalis-like compounds in depressive disorders. Biol Psychiatry 60:491–499

Grignon S, Chianetta JM (2007) Assessment of malondialdehyde levels in schizophrenia: a meta-analysis and some methodological considerations. Prog Neuropsychopharmacol Biol Psychiatry 31(2):365–369

Grisar T (1984) Glial and neuronal Na^+-K^+ pump in epilepsy. Ann Neurol 16:S128–S134

Grossman L, Utterback E, Stewart A, Gaikwad S, Chung KM, Suciu C, Wong K, Elegante M, Elkhayat S, Tan J, Gilder T, Wu N, Dileo J, Cachat J, Kalueff AV (2010) Characterization of behavioral and endocrine effects of LSD on zebrafish. Behav Brain Res 214(2):277–284

Jianfeng Yu, Dake Qi, Mengjuan Xing, Ruixi Li, Kaida Jiang, Yuwen Peng, Donghong Cui (2011) MK-801 induces schizophrenic behaviors through downregulating Wnt signaling pathways in male mice. Brain Res 1385:281–292

Kaplan JH (2002) Biochemistry of Na, K-ATPase. Annu Ver Biochem 71:511–535

Kokel D, Peterson RT (2008) Chemobehavioural phenomics and behaviour-based psychiatric drug discovery in the zebrafish. Brief Funct Genomic Proteomic 7(6):483–490

Kurup AR, Kurup PA (2002) Membrane Na^+, K^+-ATPase mediated cascade in bipolar mood disorder, major depressive disorder, and schizophrenia-relationship to hemispheric dominance. Int J Neurosci 112:965–982

Lebel CP, Ischiropoulos H, Bondy SC (1992) Evaluation of the probe 2′,7′-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. Chem Res Toxicol 5:227–231

Li JT, Su YA, Guo CM, Feng Y, Yang Y, Huang RH et al (2011) Persisting cognitive deficits induced by low-dose, subchronic treatment with MK-801 in adolescent rats. Eur J Pharmacol 652(1–3):65–72

Mahadik SP, Scheffer RE (1996) Oxidative injury and potential use of antioxidants in schizophrenia. Prostaglandins Leukot Essent Fatty Acids 55:45–54

Ng F, Berk M, Dean O, Bush AI (2008) Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. Int J Neuropsychopharmacol 11(6):851–876

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358

Potts MB, Koh SE, Whetstone WD, Walker BA, Yoneyama T, Claus CP, Manvelyan HM, Noble-Haeusslein LJ (2006) Traumatic injury to the immature brain: inflammation, oxidative injury, and iron-mediated damage as potential therapeutic targets. NeuroRx 3:143–153

Reddy RD, Yao JK (1996) Free radical pathology in schizophrenia: a review. Prostaglandins Leukot Essent Fatty Acids 55(1–2):33–43

Rihel J, Prober DA, Arvanites A, Lam K, Zimmerman S, Jang S, Haggarty SJ, Kokel D, Rubin LL, Peterson RT, Schier AF (2010) Zebrafish behavioral profiling links drugs to biological targets and rest/wake regulation. Science 327(5963):348–351

Ross CA, Margolis RL, Reading SA, Pletnikov M, Coyle JT (2006) Neurobiology of schizophrenia. Neuron 52(1):139–153 Review

Rujescu D, Bender A, Keck M, Hartmann AM, Ohl F, Raeder H et al (2006) A pharmacological model for psychosis based on N-methyl-d-aspartate receptor hypofunction: molecular, cellular, functional and behavioral abnormalities. Biol Psychiatry 59:721–729

Rybakowski JK, Lehmann W (1994) Decreased activity of erythrocyte membrane ATPases in depression and schizophrenia. Biol Psychiatry 30:11–14

Seibt KJ, Oliveira Rda L, Zimmermann FF, Capiotti KM, Bogo MR, Ghisleni G, Bonan CD (2010) Antipsychotic drugs prevent the motor hyperactivity induced by psychotomimetic MK-801 in zebrafish (*Danio rerio*). Behav Brain Res 214(2):417–422

Siems WG, Hapner SJ, van Kuijk F (1996) 4-hydroxynonenal inhibits Na(+)–K(+)-ATPase, Free Radic. Biol Med 20:215–223

Singh OP, Chakraborty I, Dasgupta A, Datta S (2008) A comparative study of oxidative stress and interrelationship of important antioxidants in haloperidol and olanzapine treated patients suffering from schizophrenia. Indian J Psychiatry 50(3):171–176

Stuchlík A, Petrásek T, Vales K (2009) Effect of alpha(1)-adrenergic antagonist prazosin on behavioral alterations induced by MK-801 in a spatial memory task in Long-Evans rats. Physiol Res 58:733–740

Swain HA, Sigstad C, Scalzo FM (2004) Effects of dizocilpine (MK-801) on circling behavior, swimming activity, and place preference in zebrafish (*Danio rerio*). Neurotoxicol Teratol 26:725–729

Tsakiris S, Deliconstantinos G (1984) Influence of phosphatidylserine on (Na^+ + K^+)-stimulated ATPase and acetylcholinesterase activities of dog brain synaptosomal plasma membranes. Biochem J 220(1):301–307

van Os J, Kapur S (2009) Schizophrenia. Lancet 374(9690):635–645

Vignini A, Nanetti L, Moroni C, Tanase L, Bartolini M, Luzzi S, Provinciali L, Mazzanti L (2007) Modifications of platelet from Alzheimer disease patients: a possible relation between

membrane properties and NO metabolites. Neurobiol Aging 28:987–994

Westerfield M (2007) The zebrafish book, 5th edn. University of Oregon Press, Eugene

Wood AJ, Elphick M, Grahame-Smith DG (1989) Effect of lithium and of other drugs used in the treatment of manic illness on the cation-transporting properties of Na^+, K^+-ATPase in mouse brain synaptosomes. J Neurochem 52(4):1042–1049

Wyse ATS, Streck EL, Worm P, Wajner A, Ritter F, Netto CA (2000) Preconditioning prevents the inhibition of Na^+, K^+-ATPase activity after brain ischemia. Neurochem Res 25:969–973

Yao JK, Keshavan MS (2011) Antioxidants, redox signaling, and pathophysiology in schizophrenia: an integrative view. Antioxid Redox Signal 15(7):2011–2035

Yao JK, Reddy RD, van Kammen DP (2001) Oxidative damage and schizophrenia: an overview of the evidence and its therapeutic implications. CNS Drugs 15:287–310

Yu SP (2003) Na^+, K^+-ATPase: the new face of an old player in pathogenesis and apoptotic/hybrid cell death. Biochem Pharmacol 66:1601–1609

Zanatta LM, Nascimento FC, Barros SV, Silva GR, Zugno AI, Netto CA, Wyse AT (2001) In vivo and in vitro effect of imipramine and fluoxetine on Na^+, K^+-ATPase activity in synaptic plasma membranes from the cerebral cortex of rats. Braz J Med Biol Res 34:1265–1269

Zhang M, Zhao Z, He L, Wan C (2010) A meta-analysis of oxidative stress markers in schizophrenia. Sci China Life Sci 53(1): 112–124

Zugno AI, Valvassori SS, Scherer EB, Mattos C, Matté C, Ferreira CL, Rezin GT, Wyse AT, Quevedo J, Streck EL (2009) Na^+, K^+-ATPase activity in an animal model of mania. J Neural Transm 116(4):431–436

4. DISCUSSÃO

A esquizofrenia é uma doença neuropsiquiátrica grave que afeta cerca de 1% da população mundial (Maj & Sartorius, 2005). Apesar dos avanços importantes nas últimas décadas, existem muitos aspectos a serem compreendidos sobre a doença e suas intervenções farmacológicas, a fim de minimizar os sintomas dessa patologia. Desde a descoberta dos primeiros antipsicóticos na década de 50, no século passado, a terapia farmacológica da esquizofrenia não avançou de forma impactante. A indústria farmacêutica deste então vem apenas buscando desenvolver medicamentos baseados nos mecanismos de ação de antipsicóticos existentes, deixando de pesquisar ações diferenciadas e que realmente possam revolucionar o tratamento da esquizofrenia. Sendo assim, estudos sobre novos mecanismos de ação e estratégias de intervenção farmacológica são promissores. Em busca de um melhor entendimento sobre as alterações causadas pela esquizofrenia e os tratamentos farmacológicos usados na clínica, neste estudo analisamos as ações de três fármacos antipsicóticos (haloperidol, sulpirida e olanzapina) e do MK-801, uma substância que mimetiza alguns sintomas da esquizofrenia, sobre parâmetros bioquímicos e comportamentais em peixe zebra.

O uso de testes pré-clinicos e modelos animais são essencias para o conhecimento e desenvolvimento de perfis farmacológicos de novos fármacos. O peixe zebra tem ganhado destaque no meio científico e demonstra grande potencial para estudos envolvendo diferentes doenças e também para a triagem de novos medicamentos. Portanto, na primeira etapa deste trabalho avaliamos se o MK-801, é capaz de induzir mudanças comportamentais que mimetizam a esquizofrenia neste modelo animal. O desenvolvimento de modelos animais capazes de induzir alucinações e delírios é uma tarefa difícil de ser desenvolvida. No entanto, alterações na cognição, interação social e locomoção em resposta a diferentes substâncias podem ser facilmente investigadas. Kilts (2001) descreveu a hiperatividade induzida por fármacos como uma característica comum na esquizofrenia. A administração aguda de antagonista de receptores NMDA, tais como MK-801, induz em roedores comportamentos que podem ser associados a sintomas positivos (hiperlocomoção), cognitivos (déficits de memória) e negativos (déficit de interação social) (Powell & Miyakawa, 2006). Considera-se que a exposição aos antagonistas de receptores NMDA representa o modelo farmacológico mais completo e abrangente de esquizofrenia em animais (Manahan-Vaughan et al., 2008). Partindo disso, nosso objetivo no primeiro capítulo dessa tese foi produzir a hiperlocomoção induzida pelo MK-801 em peixe zebra que é uma alteração comportamental observada na esquizofrenia. Nossos dados mostram que a atividade locomotora no peixe zebra foi aumentada após a administração de MK-801 (20 μM), nos três períodos de tempo de

exposição testados (15, 30 e 60 minutos), dados esses que estão de acordo com estudos realizados anteriormente em outros modelos animais (Chartoff et al., 2005; Bubenikova-Valesova et al., 2009). Após a indução da hiperlocomoção no peixe zebra, utilizamos essa ferramenta para avaliar o comportamento de diferentes antipsicóticos frente a essa alteração, ou seja, sobre o aumento no número de linhas cruzadas, distância percorrida, velocidade média e ainda sobre o tempo gasto na parte superior do aquário. Estudos anteriores já haviam demonstrado que a hiperlocomoção induzida por MK-801 pode ser prevenida por drogas antipsicóticas, e que os antipsicóticos atípicos são mais potentes do que os antipsicóticos típicos na inibição da hiperlocomoção induzida por antagonistas NMDA (Jentsch & Roth, 1999; O'Neill & Shaw, 1999). Entretanto, nossos resultados demonstram que o haloperidol, sulpirida e olanzapina inibiram igualmente a hiperlocomoção induzida por MK-801, diferindo apenas em sua influência sobre o efeito ansiolítico promovido pelo MK-801, que foi observado após 30 e 60 min de exposição. O efeito ansiolítico do MK-801 foi observado anteriormente em ratos tratados com MK-801 e submetidos ao teste de labirinto (Karcz-Kubicha et al., 1997; Bertoglio & Carobrez, 2003). Outro resultado observado nesse estudo foi o efeito ansiolítico apresentado após 15 e 30 min de exposição à olanzapina, embora esta droga não tenha qualquer efeito sobre a atividade locomotora. Em busca de uma explicação para o efeito ansiolítico, observa-se que antipsicóticos atípicos (risperidona, olanzapina e quetiapina) têm sido cada vez mais utilizados para tratar distúrbios relacionados à ansiedade, além de seu uso no tratamento de psicose, justificando o efeito ansiolítico observado após tratamento com olanzapina no peixe zebra (Sun et al., 2010; Vulink et al., 2011). Assim, podemos concluir que ambos os antipsicóticos, típicos e atípicos, atenuaram a hiperlocomoção induzida pelo MK-801 no peixe zebra de forma semelhante. Testar a eficácia de medicamentos para minimizar alterações comportamentais induzidas MK-801 é uma relevante abordagem experimental e merece uma investigação ainda mais aprofundada neste modelo animal.

Portanto, no segundo capítulo deste estudo continuamos analisando as alterações comportamentais induzidas pelo MK-801 em peixe zebra. Nós avaliamos a ação do MK-801 em dois parâmetros comportamentais: cognição e interação social, uma vez que pacientes esquizofrênicos sofrem de sintomas duradouros e persistentes, bem como deficiências na cognição e interação social, que costumam afetar a sua vida social (Abdul-Monim et al., 2006; de Moura Linck et al., 2008). Dado o impacto negativo da disfunção cognitiva e social sobre a qualidade de vida dos pacientes esquizofrênicos, a falta de tratamento eficaz é claramente uma necessidade não atendida pela clínica (Goldberg & Gold, 1995). Para analisar os déficits

cognitivos no peixe zebra, utilizamos o protocolo desenvolvido por Blank et al. (2009). Para tanto, o treino consistiu de apenas uma sessão onde os animais foram colocados individualmente no aquário de esquiva inibitória. Ao cruzar para o lado escuro do aquário um choque elétrico foi administrado por 5s e estes animais retirados imediatamente do aquário. Na sessão de teste, realizada 24 h depois, seguiu-se o mesmo protocolo, porém nenhum choque foi utilizado. As latências de entrada no lado escuro para o treino e o teste foram consideradas como indicadoras de retenção da memória. O resultado do presente estudo demonstrou que o tratamento com MK-801 induziu um déficit na mémoria do peixe zebra, e estes déficits foram totalmente revertidos pela administração aguda de antipsicóticos atípicos, mas não por antipsicótico típico. Esses dados estão de acordo com dados apresentados em estudos anteriores onde demonstraram que a administração aguda de MK-801 prejudica a memória de trabalho em roedores (Zajaczkowsky et al., 2003; Bardgett et al., 2008), e também que o tratamento com fenciclidina, outro antagonista do receptor NMDA, prejudica o desempenho de ratos no labirinto de Morris, sendo que esse efeito pode ser revertido pela clozapina, sertindol, risperidona, mas não por haloperidol (Didriksen et al., 2007).

O déficit de interação social é um dos sintomas negativos mais marcantes da esquizofrenia (Rung et al., 2005; Becker & Grecksch, 2004), e os modelos animais que utilizam antagonistas glutamatérgicos tais como fenciclidina e MK-801, para modular tal sintoma, vem sendo cada vez mais explorados (de Moura Linck et al., 2008). Para analisar a interação social, utilizamos uma técnica desenvolvida por Gerlai (2003), onde os peixes são colocados em contato com um aquário vazio de um lado, e de outro lado um aquário "estímulo", ou seja, um aquário com 15 peixes para estimular a interação social. Como resultado desse teste, os peixes tratados com MK-801 apresentaram uma diminuição na interação social, e somente os fármacos atípicos testados conseguiram reverter esse efeito, ou seja, a sulpirida e olanzapina foram eficazes em reverter os déficits de interação social induzidos pelo MK-801, enquanto que o haloperidol foi incapaz de promover tal efeito. Outro estudo mostrou que o haloperidol não reverte os déficits de interação social induzidos por antagonista NMDA (Boulay et al., 2004). Uma possivel explicação para os resultados obtidos nesse estudo é o fato de que antagonistas NMDA aumentam a liberação de serotonina, que por sua vez aumenta os níveis de glutamato pela ativação dos receptores 5-HT_{2A} nos neurônios glutamatérgicos. Assim, o aumento da liberação de glutamato pode atuar nos receptores pós-sinápticos AMPA/Kainato causando alteraçãos nas respostas comportamentais e influenciando assim nas respostas induzidas pela exposição aos antagonistas NMDA

(Aghajanian & Marek, 1999; Adams et al., 2001). Esses dados podem ter relação com a diferença de efeitos observados entre antipsicóticos típicos e atípicos, uma vez que sabemos que antipsicóticos atípicos não atuam nos receptores glutamatérgicos, mas atuam sobre diversos outros neurotransmissores que podem interferir nesses efeitos (Large, 2007). Su et al. (2007) mostraram que a risperidona, um antipsicótico atípico, inibe a liberação de glutamato induzida por antagonista NMDA pelo bloqueio de receptores de serotonina 5-HT_{2A} nos terminais glutamatérgicos. Assim, é possivel que os antipsicóticos atípicos testados neste estudo inibiram os prejuízos na interação social e déficits cognitivos induzidos pelo MK-801 por meio de de mecanismos similares.

A administração aguda de MK-801, como citada anteriormente, representa o modelo farmacológico mais completo e abrangente de esquizofrenia (Manahan-Vaughan et al., 2008), e têm sido amplamente utilizada como modelo animal para estudos envolvendo diversas doenças e também para entender melhor o mecanismo de ação de diferentes fármacos. Nosso interesse em investigar essas alterações comportamentais em peixe zebra foi apoiada por numerosas vantagens práticas desta espécie na experimentação comportamental combinada à sua semelhança genética com os mamíferos. A figura abaixo (Figura 4) representa de forma simplificada os resultados obtidos nos dois primeiros capítulos dessa tese.

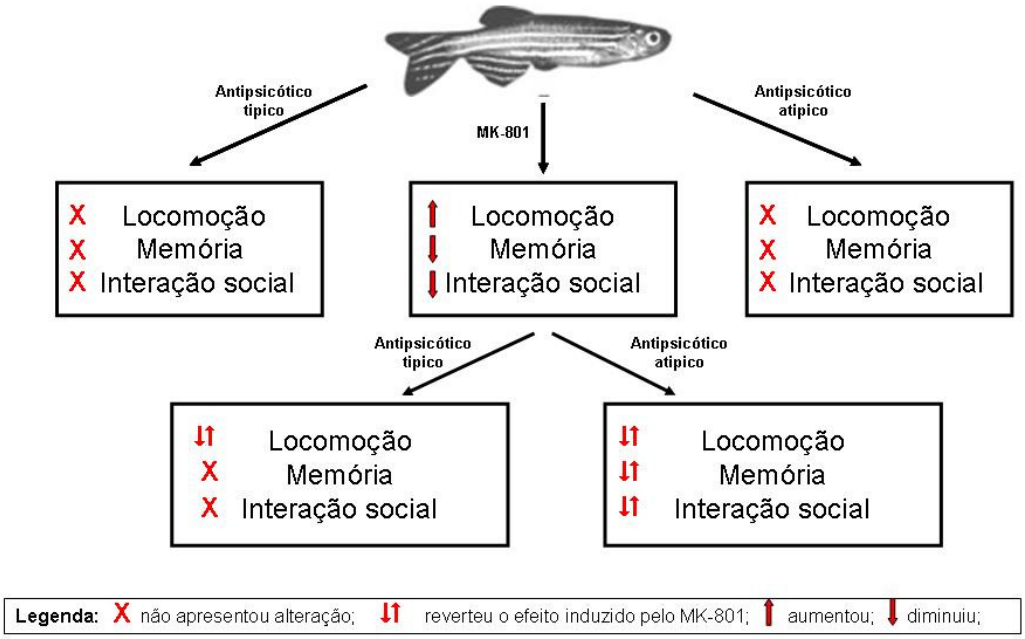

Figura 4 – Esquema representativo das alterações comportamentais induzidas por MK-801 e fármacos antipsicóticos no peixe zebra.

O modelo de disfunção purinérgica proposto por Lara e Souza (2000) sugere que uma hipofunção da sinalização promovida pela adenosina é compatível com muitos sintomas da esquizofrenia. Baseado na relação existente entre o sistema purinérgico e a esquizofrenia, no terceiro capítulo dessa tese vamos abordar o sistema purinérgico, que é uma importante via de sinalização no SNC, amplamente distribuído e envolvido em diversos mecanismos de controle neuronal (Zimmermann, 2008). No sistema purinérgico, o ATP atua como um neurotransmissor excitatório (principalmente ativando receptores P2X) e também tem envolvimento na proliferação, crescimento e diferenciação celular (atuando em receptores P2Y) (Burnstock, 2008). O ATP é sintetizado e armazenado em terminais pré-sinápticos e liberado na fenda sináptica sob estímulos nervosos (Abbracchio et al., 2009). O ATP liberado pode ser rapidamente degradado pelas ectonucleotidases, gerando o neuromodulador adenosina (Burnstock, 2008). Considerando que as ectonucleotidases controlam os níveis de ATP e do neuromodulador adenosina, e que estudos anteriores propuseram uma relação entre os níveis desse neuromodulador e a esquizofrenia, o estudo dessas enzimas torna-se

importante para um melhor entendimento desta patologia. Além disso, os níveis de adenosina podem ser controlados por meio de de sua desaminação à inosina pela adenosina deaminase (Latini & Pedata, 2001), uma enzima envolvida no metabolismo das purinas por catalisar a conversão da adenosina e da deoxiadenosina a inosina e deoxiinosina, respectivamente (Franco et al., 1997).

Existe uma carência de um melhor entendimento sobre os mecanismos envolvidos na esquizofrenia, tornando assim necessária a melhor caracterização de diferentes vias de sinalização nos processos relacionados a essa doença, pois identificando os mecanismos envolvidos, pode-se, por exemplo, buscar a triagem de novos fármacos. A farmacoterapia atual da esquizofrenia baseia-se nas hipóteses dopaminérgicas e glutamatérgicas, que enfatizam a contribuição de hiperfunção dopaminérgica e a hipofunção glutamatérgica na fisiopatologia da doença (Gordon, 2010; Heinz & Schlagenhauf, 2010). A adenosina no seu papel de regulador homeostático pode modular tanto a neurotransmissão dopaminérgica e glutamatérgica. Neste contexto, Lara & Souza (2000) propuseram uma hipótese purinérgica para a esquizofrenia. Eles sugeriram que uma disfunção no sistema purinérgico resultaria em redução da atividade adenosinérgica como uma possível explicação para o desequíbrio entre a neurotransmissão dopaminérgica e a glutamatérgica que são marcos característicos da esquizofrenia. Essa hipótese sugere que um desequilíbrio no neuromodulador adenosina pode criticamente determinar susceptibilidade à esquizofrenia (Lara & Souza, 2000; Lara et al, 2006). Sabendo dessa relação, no terceiro capítulo dessa tese resolvemos avaliar o efeito do MK-801 sobre as enzimas ectonucleotidases e ADA. Entretando, nenhum efeito na atividade dessas enzimas foi observado após exposição ao MK-801 no peixe zebra. Partindo desse resultado, resolvemos avaliar se os fármacos antipsicóticos eram capazes de alterar o comportamento dessas enzimas. Nossos resultados mostraram que o haloperidol, único fármaco antipsicótico típico estudado, alterou a atividade de ambas as enzimas, demonstrando sua influência sobre os níveis de ATP e adenosina. Entretanto, ambos os fármacos antipsicóticos atípicos testados neste estudo, olanzapina e sulpirida, não promoveram alteração nesta atividade enzimática. Estudos têm demonstrado que os fármacos antipsicóticos têm alta afinidade por membranas biológicas devido às suas propriedades anfipáticas e anfifílicas, e isso implica que o haloperidol pode interagir com a organização de lipídios de membrana, possivelmente levando à modificação na resposta do receptor (Tessier et al., 2008). Outra possibilidade é que o efeito inibitório do haloperidol sobre a atividade das NTPDases e ADA pode ocorrer por meio de de mecanismos de controle transcricional. Nossos achados demonstraram que os animais submetidos ao tratamento com haloperidol

apresentaram mudanças significativas nos padrões de expressão de genes das NTPDases e ADA. Exposição ao haloperidol diminuiu os níveis de transcritos de mRNA dos primers *entpd2_mq* (45%) e *entpd3* (24%), enquanto *entpd2_mv*, *entpd2_mg* e os níveis de *entpd1* aparentemente não foram afetados. Considerando que o ATP é o substrato preferencial para NTPDase2 e NTPDase3 (Zimmmermann, 2001), é possível sugerir que a diminuição nos níveis de transcritos de mRNA para *entpd2_mq* e *entpd3* está relacionada com a diminuição da hidrólise de ATP observadas após tratamento com haloperidol. Neste estudo, também observamos que o tratamento com haloperidol diminuiu significativamente os níveis de transcrição para o primer *adal* (33%), mas *ada1*, *ada2-1* e *ada2-2* não se alteraram, sugerindo que a mudança observada na atividade da ADA pode também estar relacionada a alterações na expressão gênica.Os nucleotídeos e nucleosídeos extracelulares são importantes moléculas sinalizadoras que necessitam de mecanismos eficazes para a sua regulação (Yegutkin, 2008). Nossos resultados mostram que um fármaco antipsicótico típico, o haloperidol, pode modular enzimas que são responsáveis pelos níveis de adenosina extracelular. Estes resultados indicam que a degradação e a produção de adenosina podem ser alvos farmacológicos para esta classe de fármacos, sugerindo assim uma possível relação entre o sistema purinérgico e a ação dos fármacos antipsicóticos.

Considerando a importância da Na^+,K^+-ATPase para o funcionamento normal do SNC, uma vez que a inibição da sua atividade tem sido associada a diversas condições fisiológicas e patológicas (Wyse et al., 2000; Wyse et al., 2004), no quarto capítulo desta tese analisamos o efeito do MK-801 e fármacos antipsicóticos sobre a atividade da Na^+, K^+-ATPase e medidas de estresse oxidativo. Estudos anteriores mostraram uma diminuição significativa na atividade da Na^+, K^+- ATPase em pacientes com esquizofrenia (Rybakowski & Lehmann, 1994; Kurup & Kurup, 2002). Nossos resultados estão de acordo com estudos anteriores, pois mostramos que o MK-801 diminuiu a atividade da Na^+, K^+-ATPase e que os fármacos antipsicóticos testados (haloperidol, sulpirida e olanzapina) reverteram este efeito. Nossos resultados também mostraram que a administração de haloperidol isoladamente produziu um aumento na atividade da Na^+, K^+- ATPase no cérebro de peixe zebra. Estes dados também estão de acordo com estudos anteriores que mostraram que o haloperidol aumentou a atividade desta enzima no cérebro de ratos (Wood et al., 1989). Efeitos similares têm sido observados com outras drogas, como anfetaminas (Zugno et al., 2009), fluoxetina (Zanatta et al., 2001), a selegilina (Carageorgiou et al. 2003), carbamazepina e lítio (Wood et al., 1989) .

Sabe-se que a enzima Na^+, K^+-ATPase é particularmente suscetível ao ataque de radicais livres e por isso a sua inibição tem sido correlacionada com alteração nos níveis de radicais livres (Dencher et al., 2007). Portanto, partindo dos resultados obtidos nesse estudo onde tivemos alterações nos níveis dessa enzima, resolvemos analisar as espécies reativas ao ácido tiobarbitúrico (TBARS) e os níveis de oxidação da DCF (parâmetros de estresse oxidativo) (Potts et al., 2006; Siems et al., 1996). Estresse oxidativo ocorre quando há um desequilíbrio entre as espécies reativas de oxigênio (ROS) e a geração de defesas antioxidantes em favor do primeiro. Nossos resultados mostraram que a administração aguda de MK-801 e drogas antipsicóticas não alteraram os níveis do DCF. Entretanto, os resultados desse estudo mostraram que MK-801 aumentou os níveis de TBARS no cérebro de peixe zebra e que a administração de drogas antipsicóticas, típicas e atípicas, conseguiu reverter o aumento nesses níveis. Estes dados são consistentes com estudos anteriores que mostraram que o estresse oxidativo e as alterações nas enzimas antioxidantes têm sido descritos na fisiopatologia da esquizofrenia (Reddy & Yao 1996; Grignon & Chianetta 2007).

A figura abaixo (figura 5) demonstra de forma simplificada os resultados obtidos nos dois últimos capítulos dessa tese.

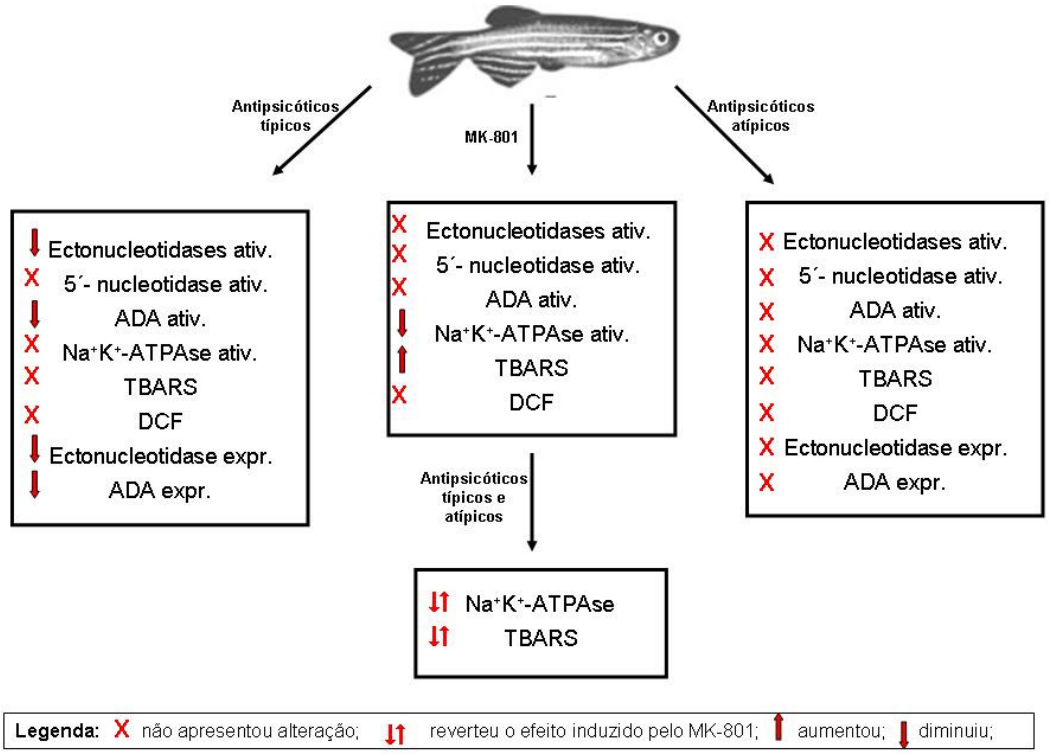

Figura 5 – Esquema representativo das alterações bioquímicas induzidas por MK-801 e fármacos antipsicóticos no peixe zebra.

Portanto, os dados apresentados nesta tese mostram que o tratamento com MK-801 no peixe zebra pode mimetizar alterações bioquímicas e comportamentais observadas em pacientes com esquizofrenia, os quais podem ser revertidos por fármacos antipsicóticos, sugerindo que o peixe zebra é um modelo animal que pode contribuir para a descoberta de potencias tratamentos e também ajudar a compreender melhor os mecanismos envolvidos nesta doença.

REFERÊNCIAS BIBLIOGRÁFICAS

Abbracchio MP, Burnstock G, Verkhratsky A, Zimmermann H. Purinergic signalling in the nervous system: an overview. Trends Neurosci 2009; 32:19-29.

Abdul-Monim Z, Reynolds GP, Neill JC. The effect of atypical and classical antipsychotics on sub-chronic PCP-induced cognitive deficits in a reversallearning paradigm. Behav Brain Res 2006; 169:263–273.

Abi-Dargham A, Laruelle M. Mechanisms of action of second generation antipsychotic drugs in schizophrenia: insights from brain imaging studies. Eur Psychiatry 2005; 20(1):15-27.

Adams BW, Moghadam B. Effect of clozapine, haloperidol, or M100907 on phencyclidine-activated glutamate efflux in the prefrontal cortex. Biol Psychiatry 2001; 50:750–757.

Aghajanian GK, Marek GJ. Serotonin, via 5-HT2A receptors, increases EPSCs in layer v pyramidal cells of prefrontal cortex by an asynchronous mode of glutamate release. Brain Res 1999; 825:161–171.

Al-Imari L, Gerlai R. Sight of conspecifics as reward in associative learning in zebrafish (Danio rerio). Behav Brain Res 2008; 189(1):216-219.

Amitai N, Semenova S, Markou A. Cognitive–disruptive effects of the psychotomimetic phencyclidine and attenuation by atypical antipsychotic medications in rats. Psychopharmacology 2007; 193: 521–537.

Andreazza, A. C., Soares, D. G., Kehl, L. F., Lima-Borella, M. L., Salvador, M. Transtornos Neuropsiquiátricos E Estresse Oxidativo. In: Izquierdo, I., Quevedo, J., Kapczinski, F. Bases Biológicas dos Transtornos Psiquiátricos. Porto Alegre: Artmed p.489-496, 2004.

Antonioli L, Fornai M, Colucci R, Ghisu N, Tuccori M, Del Tacca M, Blandizzi C. Pharmacological modulation of adenosine system: novel options for treatment of inflammatory bowel diseases. Inflamm Bowel Dis 2008; 14:566-574.

Aperia A. New roles for an old enzyme: Na,K-ATPase emerges as an interesting drug target. J Intern Med 2007; 261:44–52.

Appelbaum L, Skariah G, Mourrain P, Mignot E. Comparative expression of p2x receptors and ecto-nucleoside triphosphate diphosphohydrolase 3 in hypocretin and sensory neurons in zebrafish. Brain Res 2007; 1174: 66-75.

Arvindakshan M, Sitasawad S, Debsikdar V, Ghate M, Evans D, Horrobin DF, Bennett C, Ranjekar PK, Mahadik SP. Essential polyunsaturated fatty acid and lipid peroxide

levels in never-medicated and medicated schizophrenia patients. Biol Psychiatry 2003; 53(1):56-64.

Ban TA.Neuropsychopharmacology and the genetics of schizophrenia: a history of the diagnosis of schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 2004; 28(5):753-762.

Barbazuk WB, Korf I, Kadavi C, Heyen J, Tate S, Wun E, Bedell JA, Mcpherson JD, Johnson SL. The syntenic relationship of the zebrafish and human genomes. Genome Res 2000; 10:1351-1358.

Bardgett ME, Points M, Ramsey-Faulkner C, Topmiller J, Roflow J, McDaniel T, et al. The effects of clonidine on discrete-trial delayed spatial alternation in two rat models of memory loss. Neuropsychopharmacology 2008; 33:1980–1991.

Barros DM, Pereira P, Medina J, Izquierdo I. Modulation of working memory and longbut not short-term memory by cholinergic mechanisms in the basolateral amygdala. Behav. Pharmacol 2002; 13:163-167.

Becker CG, Becker T. Adult zebrafish as a model for successful central nervous system regeneration. Restor Neurol Neurosci 2008; 26(2–3):71–80.

Becker A, Grecksch G. Ketamine-induced changes in rat behaviour: a possible animal model of schizophrenia. Test of predictive validity. Prog Neuro-Psychopharmacol Biol Psychiatry 2004; 28(8): 1267-1277.

Beis D, Stainier DY. In vivo cell biology: following the zebrafish trend. Trends Cell Biol 2006; 16(2):105–112.

Bencan Z, Sledge D, Levin ED. Buspirone, chlordiazepoxide and diazepam effects in a zebrafish model of anxiety. Pharmacol Biochem Behav 2009; 94(1):75-80.

Bertoglio LJ, Carobrez AP. Anxiolytic-like effects of NMDA/glycine-B receptor ligands are abolished during the elevated plus-maze trial 2 in rats. Psychopharmacology (Berl) 2003; 170:335–342.

Best JD, Alderton WK. Zebrafish: An in vivo model for the study of neurological diseases. Neuropsychiatr Dis Treat 2008; 4(3):567-576.

Bianchi V, Spychala J. Mammalian 5'-nucleotidases. J Biol Chem 2003; 278(47): 46195-46198.

Bigonnesse F, Lévesque SA, Kukulski F, Lecka J, Robson SC, Fernandes MJ, Sévigny J. Cloning and characterization of mouse nucleoside triphosphate diphosphohydrolase-8. Biochemistry 2004; 43(18):5511-5519.

Bilotta, J. et al. Assessing appetitive choice discrimination learning in zebrafish. Zebrafish 2005; 2(4):259-268.

Blank M, Guerim LD, Cordeiro RF, Vianna MR. A one-trial inhibitory avoidance task to zebrafish: rapid acquisition of an NMDA-dependent long-term memory. Neurobiol Learn Mem 2009; 92(4):529-534.

Blaser RE, Peñalosa YM. Stimuli affecting zebrafish (Danio rerio) behavior in the light/dark preference test. Physiol Behav 2011; 104(5):831-837.

Boehmler W, Obrecht-Pflumio S, Canfield V, Thisse C, Thisse B, Levenson R. Evolution and expression of D2 and D3 dopamine receptor genes in zebrafish. Dev Dyn 2004; 230(3):481-493.

Boehmler W, Petko J, Woll M, Frey C, Thisse B, Thisse C, Canfield VA, Levenson R. Identification of zebrafish A2 adenosine receptors and expression in developing embryos. Gene Expr Patterns 2009; 9(3):144-151.

Boison D. Adenosine and epilepsy: from therapeutic rationale to new therapeutic strategies. Neuroscientist 2005; 11 (1): 25–36.

Boison D, Chen JF, Fredholm BB. Adenosine signalling and function in glial cells. Cell Death Differ 2010; 17: 1071-1082.

Boison D, Singer P, Shen HY, Feldon J, Yee BK. Adenosine hypothesis of schizophrenia - Opportunities for pharmacotherapy. Neuropharmacology 2011*; in press.*

Boulay D, Depoortère R, Louis C, Perrault G, Griebel G, Soubrié P. SSR181507, a putative atypical antipsychotic with dopamine D2 antagonist and 5-HT1A agonist activities: improvement of social interaction deficits induced by phencyclidine in rats. Neuropharmacology 2004; 46(8): 1121–1129.

Bours MJ, Swennen EL, Di Virgilio F, Cronstein BN, Dagnelie PC. Adenosine 5'-triphosphate and adenosine as endogenous signaling molecules in immunity and inflammation. Pharmacol Ther 2006; 112:358-404.

Bowie CR, Harvey PD. Schizophrenia from a neuropsychiatric perspective. Mt Sinai J Med 2006; 73(7):993-998.

Breier A, Berg PH, Thakore JH, Naber D, Gattaz WF, Cavazzoni P, Walker DJ, Roychowdhury SM, Kane JM. Olanzapine versus ziprasidone: results of a 28-week double-blind study in patients with schizophrenia. Am J Psychiatry 2005; 162(10):1879-1887.

Brunstein MG, Silveira Jr, EM, Chaves LS, Machado H, Schenkel O, Belmonte- de-Abreu P, Souza DO, Lara DR. Q2 Increased serum adenosine deaminase activity in schizophrenic receiving antipsychotic treatment. Neurosci Lett 2007; 414 (1):61– 64.

Bubenikova-Valesova V, Svoboda J, Horacek J, Vales K. The effect of a full agonist/antagonist of the D1 receptor on locomotor activity, sensorimotor gating and cognitive function in dizocilpine-treated rats. Int J Neuropsychopharmacol 2009; 12(7): 873-883.

Buchanan RW, Carpenter WT. Concept of Schizophrenia. In: Sadock BJ, Sadock VA, editors. Kaplan & Sadock's comprehensive textbook of psychiatri. 8th ed. Philadelphia: Lippincott Williams & Wilkins, 2005.

Burnstock G. Purinergic nerves. Pharmacol Rev. 1972; 24(3): 509-81. Review.

Burnstock G. Cotransmission. Curr Opin Pharmacol 2004; 4: 47-52.

Burnstock G. Purine and pyrimidine receptors. Cell Mol Life Sci 2007; 12:1471-1483.

Burnstock G. Purinergic signalling and disorders of the central nervous system. Nat Rev Drug Discov 2008; 7(7): 575-590.

Carageorgiou H, Zarros A, Tsakiris S. Selegiline long-term effects on brain acetylcholinesterase, (Na+,K+)-ATPase activities, antioxidant status and learning performance of aged rats. Pharmacol Res 2003; 48(3):245-251.

Chadwick BP, Frischauf AM. The CD39-like gene family: identification of three new human members (CD39L2, CD39L3, and CD39L4), their murine homologues, and a member of the gene family from Drosophila melanogaster. Genomics 1998; 50(3):357-367.

Chakraborty H, Sen P, Sur A, Chatterjee U, Chakrabarti S. Age-related oxidative inactivation of Na+, K+-ATPase in rat brain crude synaptosomes. Exp Gerontol 2003; 38:705-710.

Champagne DL, Hoefnagels CC, de Kloet RE, Richardson MK. Translating rodent behavioral repertoire to zebrafish (Danio rerio): relevance for stress research. Behav Brain Res 2010; 214(2):332-342.

Chartoff EH, Heusner CL, Palmiter RD. Dopamine is not required for the hyperlocomotor response to NMDA receptor antagonists. Neuropsychopharmacology 2005; 30(1):324–333.

Colwill RM, Raymond MP, Ferreira L, Escudero H. Visual discrimination learning in zebrafish (Danio rerio). Behav Processes 2005; 70(1):19-31.

Coyle JT, Tsai G, Goff D. Converging evidence of NMDA receptor hypofuncion in the pathophysiology of schizophrenia. Ann N Y Aad Sci 2003; 1003: 318-327.

Cunha RA. Adenosine as a neuromodulator and as a homeostatic regulator in the nervous system: different roles, different sources and different receptors. Neurochem Int 2001; 38(2): 107-125.

Dadheeck G, Mishra S, Gautam S, Sharma P. Evaluation of antioxidant deficit in schizophrenia. Indian J Psychiatry 2008; 50(1):16-20.

Darland T, Dowling JE. Behavioral screening for cocaine sensitivity in mutagenized zebrafish. Proc Natl Acad Sci USA 2001; 98 (20): 11691–11696.

Dawe GS, Hwang EH, Tan CH. Pathophysiology and animal models of schizophrenia. Ann Acad Med Singapore 2009; 38(5):425-426.

Delanty M, Dichter NA. Oxidative injury in nervous system. Acta Neurol Scand 1998; 98:145-153.

Dencher NA, Frenzel M, Reifschneider NH, Sugawa M, Krause F. Proteome alterations in rat mitochondria caused by aging. Ann. N. Y. Acad Sci 2007; 1100: 291–298.

de Moura Linck V, Herrmann AP, Goerck GC, Iwu MM, Okunji CO, Leal MB, et al. The putative antipsychotic alstonine reverses social interaction withdrawal in mice. Prog Neuro-Psychopharmacol Biol Psychiatry 2008; 32:1449–1452.

Diaz-Hernandez M, Cox JA, Migita K, Haines W, Egan TM, Voigt MM. Cloning and characterization of two novel zebrafish P2X receptor subunits. Biochem Biophys Res Com 2002; 295 (4): 849-853.

Didriksen M, Skarsfeldt T, Arnt J. Reversal of PCP-induced learning and memory deficits in the Morris' water maze by sertindole and other antipsychotics. Psychopharmacology (Berl) 2007; 193:225-233.

Dietrich-Muszalska A, Olas B, Rabe-Jablonska J. Oxidative stress in blood platelets from schizophrenic patients. Platelets 2005; 16: 386–391.

Dietrich-Muszalska A, Olas B. Isoprostanes as indicators of oxidative stress in schizophrenia. World J Biol Psychiatry 2007; 4: 1–6.

Di Iorio P, Ballerini P, Caciagli F, Ciccarelli R. Purinoceptor-mediated modulation of purine and neurotransmitter release from nervous tissue. Pharmacol Res 1998; 37:169–178.

Dobrota D, Matejovicova M, Kurrela EG, Boldyrev AA. Na/K-ATPase under oxidative stress: molecular mechanisms of injury. Cell Mol Neurobiol 1999; 19(1):141-149.

Domino EF, Mirzoyan D, Tsukada H. N-methyl-D-aspartate antagonsits as drug model of schizophrenia: a surprising link to tabacco smoking. Prog Neuropsychopharmacol Biol Psychiatry 2004; 28:801-811.

DSM-IV-TR. Manual diagnóstico e estatístico de transtornos mentais. Trad. Cláudia Dornelles. 4. ed. Rev. Porto Alegre: Artmed, 2002.

Dunwiddie TV, Masino SA. The role and regulation of adenosine in the central nervous system. Annu Rev Neurosci 2011; 24: 31-55.

Edwards JG, Michel WC. Odor-Stimulated glutamatergic neurotransmission in the zebrafish olfactory bulb. J Comp Neurol 2002; 454(3): 294-309.

Egan R, Bergner CL, Hart PC, Cachat JM, Canavello PR, Elegante MF, et al. Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. Behav Brain Res 2009; 205(1):38-44.

Egan TM, Cox JA, Voigt MM. Molecular cloning and functional characterization of the zebrafish ATP-gated ionotropic receptor P2X3 subunit. FEBS Lett 2000; 475: 287-290.

Ellenbroek, B. A., & Cools, A. R. Animal models for the negative symptoms of schizophrenia. Behav Pharmacol 2000; 11(3–4): 223−233.

Erb L, Liao Z, Seye CI, Weisman GA. P2 receptors: intracellular signaling. Pflugers Arch 2006; 452 (5):552-562.

Erecinska M, Cherian S, Silver IA. Energy metabolism in mammalian brain during development. Prog Neurobiol 2004; 73:397-345.

Farber NB, Wozniak DF, Price MT, Labruyere J, Huss J, St Peter H, Olney JW. Age-specific neurotoxicity in the rat associated with NMDA receptor blockade: potential relevance to schizophrenia? Biol Psychiatry 1995; 38(12):788-796.

Franco R, Casado V, Ciruela F, Saura C, Mallol J, Canela EI, Lluis C. Cell surface adenosine deaminase: much more than an ectoenzyme. Prog Neurobiol 1997; 52 (4): 283-294.

Fredduzzi S, Moratalla R, Monopoli A, Cuellar B, Xu K, Ongini E, Impagnatiello F, Schwarzschild MA, Chen JF. Persistent behavioral sensitization to chronic L-DOPA requires A2A adenosine receptors. J Neurosci 2002; 22(3):1054-1062.

Fredholm BB. Adenosine and neuroprotection. Int Rev Neurobiol 1997; 40:259-280.

Fredholm BB. Adenosine receptors as targets for drug development. Drug News Perspect 2003; 16:283-289.

Fredholm BB, Ijzerman AP, Jacobson KA, Klotz KN, Linden J. International union of pharmacology. XXV. Nomenclature and classification of adenosine receptors. Pharmacol Rev 2001; 53(4): 527-552.

Gardner DM, Baldessarini RJ, Waraich P. Modern antipsychotic drugs: a critical overview. CMAJ 2005; 172(13):1703-1711.

Gerlai R, Lahav M, Guo S, Rosenthal A. Drinks like a fish: zebra fish (Danio rerio) as a behavior genetic model to study alcohol effects. Pharmacol Biochem Behav 2000; 67(4):773–782.

Gerlai R. Zebra fish: an uncharted behavior genetic model. Behav Genet 2003; 33(5):461-468.

Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology (Berl) 2001; 156:117–154.

Geyer MA, Ellenbroek B. Animal behavior models of the mechanisms underlying antipsychotic atypicality. Prog Neuropsychopharmacol Biol Psychiatry. 2003; 27(7): 1071-1079.

Goff DC, Coyle JT. The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. Am J Psychiatry 2001; 158:1367-1377.

Goldberg TE, Gold JM. Neurocognitive Deficits in Schizophrenia. London, UK: Blackwell, 1995.

Goldsmith P. Zebrafish as a pharmacological tool: the how, why and when. Curr Opin Pharmacol 2004; 4(5): 504-512.

Gomes CV, Kaster MP, Tomé AR, Agostinho PM, Cunha RA. Adenosine receptors and brain diseases: neuroprotection and neurodegeneration. Biochim Biophys Acta 2011; 1808(5):1380-1399.

Gordon J.A. Testing the glutamate hypothesis of schizophrenia. Nat Neurosci 2010; 13: 2–4.

Gregory M, Jagadeeswaran P. Selective labeling of zebrafish thrombocytes: quantitation of thrombocytes function and delection during development. Blood Cells Mol Dis 2002; 29: 286-295.

Grignon S, Chianetta JM. Assessment of malondialdehyde levels in schizophrenia: a meta-analysis and some methodological considerations. Prog Neuropsychopharmacol Biol Psychiatry 2007; 31(2):365-369.

Grossman L, Utterback E, Stewart A, Gaikwad S, Chung KM, Suciu C, Wong K, Elegante M, Elkhayat S, Tan J, Gilder T, Wu N, Dileo J, Cachat J, Kalueff AV. Characterization of behavioral and endocrine effects of LSD on zebrafish. Behav Brain Res 2010; 214(2): 277-284.

Guido B, Keiichi E, Yan W, Lindsay M, Yara B, Xiaofeng S, Robson S. The rose of purinergic signaling in the liver and in transplantation: effects of extracellular nucleotides on hepatic graft vascular injury, rejection and metabolism. Front Biosci 2008; 13: 2588-2603.

Guo S. Linking genes to brain, behaviour and neurological diseases: what can we learn from zebrafish? Genes Brain Behav 2004; 3: 63-74.

Häfner H, an der Heiden W, Behrens S, Gattaz WF, Hambrecht M, Löffler W, Maurer K, Munk-Jørgensen P, Nowotny B, Riecher-Rössler A, Stein A. Causes and consequences of the gender difference in age at onset of schizophrenia. Schizophr Bull 1998; 24(1): 99-113.

Halliwell B. Oxidative stress and neurodegeneration: are we now? J. Neurochem 2006; 97: 1634-1658.

Halliwell B, Gutteridge JM. Free radicals in biology and medicine. New York: Oxford University Press, 2007.

Hattori N, Kitagawa K, Higashida T, Yagyu K, Shimohama S, Wataya T, Perry G, Smith MA, Inagaki C. Cl-ATPase and Na+/K+-ATPase activities in Alzheimers disease brains. Neurosci Lett 1998; 254(3):141-144.

Heine P, Braun N, Heilbronn A, Zimmermann H. Functional characterization of rat ecto-ATPase and ecto-ATP diphosphohydrolase after heterologous expression in CHO cells. Eur J Biochem 1999; 262(1):102-107.

Heinz A, Schlagenhauf F. Dopaminergic dysfunction in schizophrenia: salience attribution revisited. Schizophr Bull 2010; 36: 472–485.

Heresco-Levy U. Glutamatergic neurotransmission modulation and the mechanisms of antipsychotic atypicality. Prog Neuropsychopharmacol Biol Psychiatry 2003; 27(7):1113-1123.

Herken H, Uz E, Ozyurt H, Söğüt S, Virit O, Akyol O. Evidence that the activities of erythrocyte free radical scavenging enzymes and the products of lipid peroxidation are increased in different forms of schizophrenia. Mol Psychiatry 2001; 6(1):66-73.

Hunsucker S, Mitchell B, Spychala J. The 5-nucleotidase as regulators of nucleotide and drug metabolism. Pharmacol Ther 2005; 107:1-30.

Illarionova NB, Gunnarson E, Li Y, Brismar H, Bondar A, Zelenin S, Aperia A. Functional and molecular interactions between aquaporins and Na,K-ATPase. Neuroscience 2010; 168 (4):915-925.

Illes P, Ribeiro JA. Molecular physiology of P2 receptor in the central nervous system. Eur J Pharmacol 2004; 483, 5-17.

Iwaki-Egawa S, Namiki C, Watanabe Y. Adenosine deaminase 2 from chicken liver: purification, characterization, and N-terminal amino acid sequence. Comp Biochem Physiol B Biochem Mol Biol 2004; 137(2), 247-254.

Izquierdo I, Bevilaqua L, Rossato JI, Bonini JS, Medina JS, Cammarota M. Different molecular cascades in different sites of the brain control memory consolidation. Trends in Neurosci 2006; 29: 496-505.

Izquierdo I, Medina JH. Correlation between the pharmacology of long-term potentiation and the pharmacology of memory. Neurobiol Learn Mem 1995; 63: 19-32.

Jentsch JD, Roth RH. The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 1999; 20: 201–225.

Jorgensen, PL, Hakansson KO, Karlish SJD. Structure and mechanism of Na, K-ATPase: funcional sites and their interactions. Annu Rev Physiol 2003; 65: 817-849.

Kabashi E, Brustein E, Champagne N, Drapeau P. Zebrafish models for the functional genomics of neurogenetic disorders. Biochim Biophys Acta 2011; 1812(3): 335-345.

Kane J, Honigfeld G, Singer J, Meltzer H. Clozapine for the treatment-resistant schizophrenic. A double-bind comparison with chlorpromazine. Arch Gen Psychiatry 1988; 45: 789-796.

Kane JM, Carson WH, Saha AR, McQuade RD, Ingenito GG, Zimbroff DL, Ali MW. Efficacy and safety of aripiprazole and haloperidol versus placebo in patients with schizophrenia and schizoaffective disorder. J Clin Psychiatry 2002; 63(9):763-771.

Kaplan JH. Biochemistry of Na, K-ATPse. Annu. Rev. Biochem. 2002; 71:511-535.

Kapur S, Mamo D. Half a century of antipsychotics and still a central role for dopamine D2 receptors. Prog Neuropsychopharmacol Biol Psychiatry 2003; 27:1081-1090.

Karcz-Kubicha M, Jessa M, Nazar M, Plaznik A, Hartmann S, Parsons CG, et al. Anxiolytic activity of glycine-B antagonists and partial agonists—no relationto intrinsic activity in the patch clamp. Neuropharmacology 1997; 36:1355–1367.

Kaslin J, Panula P. Comparative anatomy of the histaminergic and other aminergic system in zebrafish (Danio rerio). J Comp Neurol 2001; 440: 342-377.

Kilts CD. The changing roles and targets for animal models of schizophrenia. Biol Psychiatry 2001; 50: 845–55.

Kim JS., Kornhuber HH., Schmid-Burgk W., Hollzmuller B. Low cerebrospinal fluid glutamate in schizophrenia patients and a new hypothesis on schizophrenia. Neurosci Lett 1980; 20(3): 379-382.

Krystal JH, Anand A, Moghaddam B. Effects of NMDA receptor antagonists: implications for the pathophysiology of schizophrenia. Arch Gen Psychiatry 2002; 59: 663-664.

Kucenas S, Li Z, Cox JA, Egan TM, Voigt MM. Molecular characterization of the zebrafish P2X receptor subunit gene family. Neurosci 2003; 121: 935-945.

Kurella EG, Tyulina OV, Voldyrev AA. Oxidative resistance of Na/K-ATPase. Cell. Mol. Neurobiol 1999; 19: 133-140.

Kurta A, Palestis BG. Effects of ethanol on the shoaling behavior of zebrafish (Danio rerio). Dose Response 2010; 8(4): 527-533.

Kurup AR, Kurup PA. Membrane Na+, K+-ATPase mediated cascade in bipolar mood disorder, major depressive disorder, and schizophrenia-relationship to hemispheric dominance. Int J Neurosci 2002; 112: 965–982.

Lara DR, Belmonte-de-Abreu P, Souza DO. Allopurinol for refractory aggression and self-inflicted behaviour. J Psychopharmacol 2000;14(1): 81-3.

Lara DR, Dall'Igna OP, Ghisolfi ES, Brunstein MG. Involvement of adenosine in the neurobiology of schizophrenia and its therapeutic implications. Progress in Neuro-Psychopharmacology & Biological Psychiatri 2006; 30: 617-629.

Lara DR, Souza DO. Schizophrenia: a purinergic hypothesis. Med Hypotheses 2000; 54: 157-166.

Lara DR, Vianna MR, de Paris F, Quevedo J, Oses JP, Battastini AM, Sarkis JJ, Souza DO. Chronic treatment with clozapine, but not haloperidol, increases striatal ecto-5´-nucleotidase activity in rats. Neuropsychobiology 2001; 44(2): 99-102.

Large CH. Do NMDA receptor antagonist models of schizophrenia predict the clinical efficacy of antipsychotic drugs? J Psychopharmacol 2007; 21:283– 301.

Latini S, Pedata F. Adenosine in the central nervous system: Release mechanisms and extracellular concentrations. J Neurochem 2001; 79: 463–484.

Lazarowski ER, Boucher RC, Harden TK. Mechanism of release of nucleotides and integration of their action as P2X – and – P2Y receptor activating molecules. Mol Pharmacol 2003; 64: 785-795.

Lees GJ. Contributory mechanisms in the causation of neurodegenerative disorders. Neuroscience 1993; 54(2): 287-322.

Levin ED, Bencan Z, Cerutti DT. Anxiolytic effects of nicotine in zebrafish. Physiol Behav 2007; 90(1): 54-58.

Levin ED, Chen E. Nicotinic involvement in memory function in zebrafish. Neurotoxicol Teratol 2004; 26(6): 731-735.

Lewis DA, Lieberman JA. Catching up on schizophrenia: natural history and neurobiology. Neuron 2000; 28(2): 325-334.

Lieschke GJ, Currie PD. Animal models of human disease: zebrafish swim into view. Nat Rev Genet 2007; 8(5): 353-367.

Maier SA, Galellis JR, McDermid HE. Phylogenetic analysis reveals a novel protein family closely related to adenosine deaminase. J Mol Evol 2005; 61(6): 776-794.

Maj M, Sartorius N. Esquizofrenia. 2ed. Porto Alegre: Artemed, 2005.

Mathur P, Guo S. Use of zebrafish as a model to understand mechanisms of addiction and complex neurobehavioral phenotypes. Neurobiol Dis 2010; 40(1): 66–72.

Maximino C, de Brito TM, Dias CA, Gouvea Jr A, Morato S. Scototaxis as anxiety-like behavior in fish. Nat Protocol. 2010; 5(2): 221-228.

Meltzer HY, Li Z, Kaneda Y, Ichikawa J. Serotonin receptors: their key role in drugs to treat schizophrenia. Prog. Neuropsychopharmacol Biol Psychiatry 2003; 27: 1159-1172.

Meltzer HY, Park S, Kessler R. Cognition, schizophrenia, and the atypical antipsychotic drugs. Proc Natl Acad Sci U S A 1999; 96(24): 13591-13593.

Mico JÁ, Rojas-Corrales MO, Gibert-Rahola J, Parellada M, Moreno D, Fráguas D et al. Reduced antioxidant defense in early onset first-episode psychosis: a case control study. BMC Psychiatry 2011; 11: 26.

Miklósi A, Andrew R. The zebrafish as a model for behavioral studies. Zebrafish. 2006; 3(2): 227-234.

Miyamoto S, Duncan GE, Marx CE, Lieberman JA. Treatments for schizophrenia: a critical review of pharmacology and mechanisms of action of antipsychotic drugs. Mol Psychiatry 2005; 10 (1): 79-104.

Mobasheri, J. Avila, I. Cozar-Castellano, M.D. Brownleader, M. Trevan, M.J. Francis, J.F. Lamb and P. Martin-Vasallo, Na+,K+-ATPase isozyme diversity; comparative biochemistry and physiological implications of novel functional interactions. Biosci Rep 2000; 20: 51–91.

Morgan CJ, Mofeez A, Brandner B, Bromley L, Curran HV. Acute effects of ketamine on memory systems and psychotic symptoms in healthy volunteers. Neuropsychopharmacology 2004; 29(1): 208-218.

Müller CE, Jacobson KA. Recent developments in adenosine receptor ligands and their potential as novel drugs. Biochim Biophys Acta 2011; 1808(5): 1290–1308.

Murphy BP, Chung YC, Park TW, McGorry PD. Pharmacological treatment of primary negative symptoms in schizophrenia: a systematic review. Schizophr Res 2006; 88 (1-3): 5-25.

Ng F, Berk M, Dean O, Bush AI. Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. Int J Neuropsychopharmacol 2008; 11(6): 851–876.

Ninan I, Kulkarni SK. Preferential inhibition of dizocilpine-induced hyperlocomotion by olanzapine. Eur J Pharmacol 1999; 368: 1-7.

Ninkovic J, Bally-Cuif L. The zebrafish as a model system for assessing the reinforcing properties of drugs of abuse. Methods 2006; 39(3): 262-274.

North RA. Molecular physiology of P2X receptors. Physiol Rev 2002; 82: 1013–1067.

Obata T. Dopamine efflux by MPTP and hydroxyl radical generation. J Neural Transm 2002; 109(9): 1159-1180.

O'Neill MF, Shaw G. Comparison of dopamine receptor antagonists on hyperlocomotion induced by cocaine, amphetamine, MK-801 and the dopamine D1 agonist C-APB in mice. Psychopharmacology (Berl) 1999; 145: 237– 250.

Owe-Larsson B, Ekdahl K, Edbom T, Osby U, Karlsson H, Lundberg C, Lundberg M. Increased plasma levels of thioredoxin-1 in patients with first episode psychosis and long-term schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 2011; 35(4): 1117-1121.

Padilla S, Hunter DL, Padnos B, Frady S, Macphail RC. Assessing locomotor activity in larval zebrafish: Influence of extrinsic and intrinsic variables. Neurotoxicol Teratol 2011*; in press.*

Paraskevas GP, Kapaki E, Petropoulou O, Anagnostouli M, Vagenas V, Papageorgiou C. Plasma levels of antioxidant vitamins C and E are decreased in vascular parkinsonism. J Neurol Sci 2003; 215(1-2): 51-55.

Piato AL, Capiotti KM, Tamborski AR, Oses JP, Barcellos LJ, Bogo MR, Lara DR, Vianna MR, Bonan CD. Unpredictable chronic stress model in zebrafish (Danio rerio): Behavioral and physiological responses. Prog Neuropsychopharmacol Biol Psychiatry. 2011; 35(2): 561-567.

Pinkham AM, Penn DL, Perkins DO, Lieberman J. Implications for the neural basis of social cognition for the study of schizophrenia. Am J Psychiatry 2003; 169: 815-824.

Potts MB, Koh SE, Whetstone WD, Walker BA, Yoneyama T, Claus CP, Manvelyan HM, Noble-Haeusslein LJ. Traumatic injury to the immature brain: inflammation, oxidative injury, and iron-mediated damage as potential therapeutic targets. NeuroRx 2006; 3(2): 143–153.

Powell CM, Miyakawa T. Schizophrenia-relevant behavioral testing in rodent models: a uniquely human disorder? Biol Psychiatry 2006; 59(12):1198-1207.

Rajarao JR, Canfield VA, Loppin B, Thisse B, Thisse C, Yan YL, Postlethwait JH, Levenson R. Dev Dyn. Two Na,K-ATPase beta 2 subunit isoforms are differentially expressed within the central nervous system and sensory organs during zebrafish embryogenesis. Dev Dyn 2002; 223: 254-261.

Ralevic V, Burnstock G. Receptors for purines and pyrimidines. Pharmacol. Rev. 1998; 50: 413-492.

Reddy R, Keshavan M, Yao JK. Reduced plasma antioxidants in first-episode patients with schizophrenia. Schizophr Res 2003; 62(3): 205-212.

Reddy RD, Yao JK. Free radical pathology in schizophrenia: a review. Prostaglandins Leukot Essent Fatty Acids 1996; 55 (1-2): 33-43.

Ribeiro JA, Sebastião AM, Mendonça A. Participation of adenosine receptors in neuroprotection. Drug News Pers 2003; 16: 80-86.

Ricatti MJ, Alfie LD, Lavoie EG, Sévigny J, Schwarzbaum PJ, Faillace MP. Immunocytochemical localization of NTPDases1 and 2 in the neural retina of mouse and zebrafish. Synapse 2009; 63(4): 291-307.

Rico EP, Rosemberg DB, Senger MR, Arizi MB, Bernardi GF, Dias RD, Bogo MR, Bonan CD. Methanol alters ecto-nucleotidases and acetylcholinesterase in zebrafish brain. Neurotoxicol Teratol. 2006; 28: 489–496.

Rico EP, Senger MR, Fauth MG, Dias RD, Bogo MR, Bonan CD. ATP and ADP hydrolysis in brain membranes of zebrafish (Danio rerio). Life Sci 2003; 73: 2071-2082.

Riegel RE. Radicais Livres. In:_____ Bioquímica. 3. ed. São Leopoldo: Unisinos, p.507-536, 2002.

Robson SC, Sévigny J, Zimmermann H. The E-NTPDase family of ectonucleotidases: Structure function relationships and pathophysiological significance. Purinergic Signal 2006; 2: 409–430.

Rosemberg DB, Rico EP, Guidoti MR, Dias RD, Souza DO, Bonan CD, Bogo MR.Adenosine deaminase-related genes: molecular identification, tissue expression pattern and truncated alternative splice isoform in adult zebrafish (Danio rerio). Life Sci 2007; 81 (21-22): 1526-1534.

Rosemberg DB, Rico EP, Senger MR, Dias RD, Bogo MR, Bonan CD, Souza DO. Kinetic characterization of adenosine deaminase activity in zebrafish (Danio rerio) brain. Comp Biochem Physiol B Biochem Mol Biol 2008; 151(1): 96-101.

Ross CA, Margolis RL, Reading SA, Pletnikov M, Coyle JT. Neurobiology of schizophrenia. Neuron 2006; 52: 139-153.

Rung JP, Carlsson A, Markinhuhta KR, Carlsson ML. (+)-MK-801 induced social withdrawal in rats; a model for negative symptoms of schizophrenia. Progr Neuropsychopharmacol Biol Psychiatry 2005; 29: 827-832.

Rybakowski JK, Lehmann W. Decreased activity of erythrocyte membrane ATPases in depression and schizophrenia. Biol Psychiatry 1994; 30: 11–14.

Salvador M, Henriques JAP. Radicais livre e a resposta celular ao estresse oxidativo. 1º edição. Canoas: Editora da Ulbra, 2004.

Saverino C, Gerlai R. The social zebrafish: Behavioral responses to conspecific, heterospecific, and computer animated fish. Behav Brain Res 2008; 191(1): 77-87.

Schanaider A. Radicais livres: vilões ainda em estudo. In:_____. Ciência Hoje. 2000; 27(158): 60-62.

Schetinger MR, Morsch VM, Bonan CD, Wyse AT. NTPDase and 5'- nucleotidase activities in physiological and disease conditions: new perspectives for human health. Biofactors 2007; 31 (2): 77-98.

Seeman P, Tallerico T. Antipsychotic drugs which elicit little or no parkinsonism bind more loosely than dopamine to brain D2 receptors, yet occupy high levels of these receptors. Mol Psychiatry 1998; 3(2): 123-134.

Senger MR, Rico EP, Dias RD, Bogo MR, Bonan CD. Ecto-5'-nucleotidase activity in brain membranes of zebrafish (Danio rerio). Comp Biochem Physiol 2004; 139B: 203-207.

Serra EL, Medalha CC, Mattioli R. Natural preference of zebrafish (Danio rerio) for a dark environment. Braz J Med Biol Res 1999; 32(12): 1551-1553.

Serrano F, Klann E. Reactive oxygen species and synaptic plasticity in the aging hippocampus. Ageing Res Rev 2004; 3: 431-443.

Siems WG, Hapner SJ, van Kuijk F. 4-hydroxynonenal inhibits Na(+)–K(+)-ATPase, Free Radic Biol Med 1996; 20: 215–223.

Simpson GM, Weiden P, Pigott T, Murray S, Siu CO, Romano SJ. Six-month, blinded, multicenter continuation study of ziprasidone versus olanzapine in schizophrenia. Am J Psychiatry 2005; 162:1535-1538.

Singh OP, Chakraborty I, Dasgupta A, Datta S. A comparative study of oxidative tress and interrelationship of important antioxidants in haloperidol and olanzapine treated patients suffering from schizophrenia. Indian J Psychiatry 2008; 50(3): 171-176.

Sison M, Gerlai R. Associative learning in zebrafish (Danio rerio) in the plus maze. Behav Brain Res 2010; 207(1): 99-104.

Spence R, Gerlach G, Lawrence C, Smith C. The behaviour and ecology of the zebrafish, Danio rerio. Biol Rev Camb Philos Soc 2008; 83(1): 13-34.

Stern HM, Zon LI. Cancer genetics and drug discovery in the zebrafish. Nat Rev Cancer 2003; 3(7): 533-539.

Stewart A, Wu N, Cachat J, Hart P, Gaikwad S, Wong K, Utterback E, Gilder T, Kyzar E, Newman A, Carlos D, Chang K, Hook M, Rhymes C, Caffery M, Greenberg M, Zadina J, Kalueff AV. Pharmacological modulation of anxiety-like phenotypes in adult zebrafish behavioral models. Prog Neuropsychopharmacol Biol Psychiatry 2011; 35(6): 1421-1431.

Sun T, Hu G, Li M. Repeated antipsychotic treatment progressively potentiates inhibition on phencyclidine-induced hyperlocomotion, but attenuates inhibition on amphetamine-induced hyperlocomotion: Relevance to animal models of antipsychotic drugs. Eur J Pharmacol 2009; 602: 334-342.

Sun T, He W, Hu G, Li M. Anxiolytic-like property of risperidone and olanzapine as examined in multiple measures of fear in rats. Pharmacol Biochem Behav 2010; 95(3): 298-307.

Su YA, Si TM, Zhou DF, Guo CM, Wang XD, Yang Y, et al. Risperidone attenuates MK-801-induced hyperlocomotion in mice via the blockade of serotonin 5-HT 2A/2C receptors. Eur J Pharmacol 2007; 564: 123–130.

Swain HA, Sigstad C, Scalzo FM. Effects of dizocilpine (MK-801) on circling behavior, swimming activity, and place preference in zebrafish (Danio rerio). Neurotoxicol Teratol 2004; 26(6): 725-729.

Szymanski S, Lieberman JA, Alvir JM, Mayerhoff D, Loebel A, Geisler S, Chakos M, Koreen A, Jody D, Kane J, et al. Gender differences in onset of illness, treatment response, course, and biologic indexes in first-episode schizophrenic patients. Am J Psychiatry 1995; 152(5): 698-703.

Taguchi K, Kumanogoh H, Nakamura S, Maekawa S. Ouabain-induced isoform-specific localization change of the Na+,K+-ATPase subunit in the synaptic plasma membrane of rat brain. Neurosci Lett 2007; 413: 42–45.

Tessier C, Nuss P, Staneva G, Wolf C. Modification of membrane heterogeneity by antipsychotic drugs: an X-ray diffraction comparative study. J. Colloid Interface Sci 2008; 320: 469–475.

Whitaker PM, Crow TJ, Ferrier IN. Tritiated LSD binding in frontal cortex in schizophrenia. Arch Gen Psychiatry 1981; 38(3): 278-280.

Williams FE, White D, Messer WS. A simple spatial alternation task for assessing memory function in zebrafish. Behav Process 2002; 58(3):125–132.

Wong K, Elegante M, Bartels B, Elkhayat S, Tien D, Roy S, Goodspeed J, Suciu C, Tan J, Grimes C, Chung A, Rosenberg M, Gaikwad S, Denmark A, Jackson A, Kadri F, Chung KM, Stewart A, Gilder T, Beeson E, Zapolsky I, Wu N, Cachat J, Kalueff AV. Analyzing habituation responses to novelty in zebrafish (Danio rerio). Behav Brain Res 2010; 208(2): 450-457.

Wood SJ, Yücel M, Pantelis C, Berk M. Neurobiology of schizophrenia spectrum disorders: the role of oxidative stress. Ann Acad Med Singapore. 1989; 38(5): 396-406.

Wyse ATS, Bavaresco CS, Reis EA, Zugno AI, Tagliari B, Calcagnotto T, Netto CA. Training in inhibitory avoidance causes a reduction of Na+, K+-ATPase activity in rat hippocampus. Physiol Behav 2004; 80: 475-479.

Wyse ATS, Streck EL, Worm P, Wajner A, Ritter F, Netto CA. Preconditioning prevents the inhibition of Na^+, K^+-ATPase activity after brain ischemia. Neurochem Res 2000; 25:971-975.

van Os J, Kapur S. Schizophrenia. Lancet 2009; 374: 635-645.

Vianna EPM, Ferreira AT, Dona F, Cavalheiro EA, Fernandes MJ. Modulation of seizures and synaptic plasticity by adenosinergic receptors in an experimental model of temporal lobe epilepsy induced by pilocarpine in rats. Epilepsia 2005; 46(5):166-173.

Vulink NC, Figee M, Denys D. Review of atypical antipsychotics in anxiety. Eur Neuropsychopharmacol 2011; 21(6): 429-449.

Yang L, Ho NY, Alshut R, Legradi J, Weiss C, Reischl M, et al. Zebrafish embryos as models for embryotoxic and teratological effects of chemicals. Reprod Toxicol 2009; 28(2): 245–253.

Yao JK, Reddy R. Oxidative stress in schizophrenia: pathogenetic and therapeutic implications. Antioxid Redox Signal 2011; 15(7): 1999-2002.

Yegutkin GG. Nucleotide- and nucleoside-converting ectoenzymes: important modulators of purinergic signalling cascade. Biochim Biophys Acta 2008; 1783(5): 673-694.

Yu SP. Na^+,K^+-ATPase: the new face of an old player in pathogenesis and apoptotic/hybrid cell death. Biochem Pharmacol 2003; 66: 1601–1609.

Zajaczkowski W, Czyrak A, Wedzony K. A competitive antagonist of NMDA receptors CGP 40116 attenuates experimental symptoms of schizophrenia evoked by MK-801. Pol J Pharmacol 2003; 55: 703–711.

Zanatta LM, Nascimento FC, Barros SV, Silva GR, Zugno AI, Netto CA, Wyse AT. In vivo and in vitro effect of imipramine and fluoxetine on Na+,K+-ATPase activity in synaptic plasma membranes from the cerebral cortex of rats. Braz J Med Biol Res 2001; 34(10): 1265-1269.

Zavialov AV, Engstrom A. Human ADA2 belongs to a new family of growth factors with adenosine deaminase activity. Biochem J 2005; 391(1): 51-57.

Zimmermann H. Extracellular metabolism of ATP and other nucleotides. Arch Pharmacol 2000; 362:299-309.

Zimmermann H. Ecto-nucleotidases: some recent developments and a note on nomenclature. Drug Dev Res 2001; 52: 44-56.

Zimmermann H. ATP and acetylcholine: equal brethren. Neurochem Int 2008; 52:634-648.

Zimmermann H, Braun N, Kegel B, Heine P. New insights into molecular structure and function of ectonucleotidases in the nervous system. Neurochem Int 1998; 32(5-6):421-425.

Zugno AI, Valvassori SS, Scherer EB, Mattos C, Matté C, Ferreira CL, Rezin GT, Wyse AT, Quevedo J, Streck EL. Na^+,K^+-ATPase activity in an animal model of mania. J Neural Transm 2009; 116(4): 431-436.