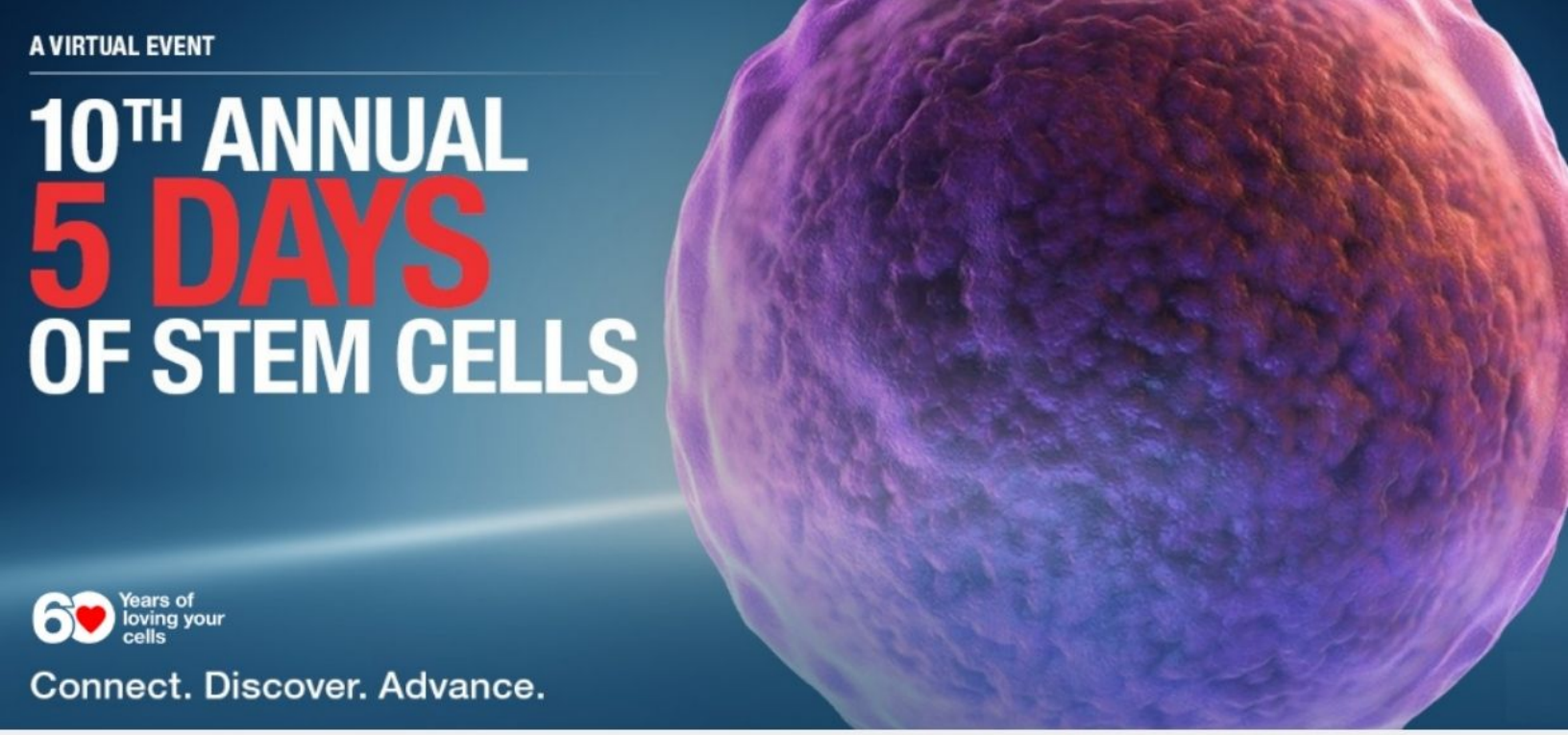

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Research Overview

Ectonucleotidases and Synaptic Plasticity: Implications in Physiological and Pathological Conditions

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ABSTRACT Several studies have suggested a role of extracellular ATP in synaptic plasticity. The signaling actions induced by extracellular ATP are directly correlated to the activity of a group of ectonucleotidases, which includes an ecto-ATPase (EC 3.6.1.3), an ATP diphosphohydrolase (apyrase, EC 3.6.1.5), and a 5′-nucleotidase (EC 3.1.3.5). These ectoenzymes trigger enzymatic conversion of ATP to adenosine, an important neuromodulator. Our studies have shown that ectonucleotidase activities are modulated in physiological and pathological situations able to induce synaptic plasticity, such as memory, epilepsy, and ischemia. Synaptosomal ectonucleotidase activities from hippocampus and entorhinal cortex were inhibited after the training session in a step-down inhibitory avoidance task in rats. Considering that adenosine has anticonvulsant effects, ectonucleotidase activities were determined after the induction of epilepsy by several animal models, such as pilocarpine, kainic acid, and kindling models. ATP diphosphohydrolase and 5′-nucleotidase activities from synaptosomes of hippocampus and cerebral cortex of rats significantly and differently increased after induction of status epilepticus by pilocarpine, kainic acid, or kindling models. Furthermore, significant changes have been observed in ATP diphosphohydrolase and 5′-nucleotidase after ischemia and reperfusion in hippocampal synaptosomes of rats. The demonstration that ectonucleotidases presented the activities altered after a memory task, or the induction of animal models of epilepsy or ischemia-reperfusion, suggests that these enzymes can act in the regulation of synaptic activity, controlling ATP and adenosine levels, depending on the synaptic plasticity developed, in physiological or pathological conditions. Drug Dev. Res. 52:57–65, 2001.

Key words: ATP diphosphohydrolase; 5′-nucleotidase; memory; epilepsy; ischemia

SYNAPTIC PLASTICITY

Plasticity is a widespread concept that involves all forms of reorganization in the mature brain. These reorganizations can concern neurons or synapses. These processes can be considered from the physiological (functional properties acquired by neurons), morphological (morphology of neurons and glia), or biochemical aspects (enzyme activities, signal transduction, and changes in gene expression) [Au Lois et al., 1997].

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After its development, the nervous system should be maintained and even modified to maintain its functions. Once mature neurons do not divide, they request repairs, through regeneration processes and migration. Recent studies demonstrate that the brain has the extraordinary capacity to develop plastic responses during life, and the functional plasticity is coupled to structural changes of long duration [Au Lois et al., 1997]. In the last few years, studies indicate that the central nervous system can exhibit subtle and specific synaptic plasticity in response to a given activity, as, for instance, the learning of a new task. Furthermore, the brain has the repair capacity after cellular loss caused by injury or a neurodegenerative disease [Cotman, 1998]. In the central nervous system of mammals, two important plastic phenomena have been described: long-term potentiation (LTP), considered as a possible mechanism involved in memory, and epilepsy-induced plasticity [Au Lois et al., 1997].

ECTONUCLEOTIDASES

The ATP released by synapses can be hydrolyzed by ectonucleotidases in a highly sophisticated pathway composed by ectoenzymes that includes ecto-ATPase (EC 3.6.1.3) and ecto-apyrase (EC 3.6.1.5), both belonging to the E-type ATPase family; ectonucleotide pyrophosphatase (EC 3.6.1.9); ectoadenylate kinase (EC 2.7.4.3); and ectoalkaline phosphatase (EC 3.1.3.1), which can transform ATP and ADP to AMP. There was a controversy about the first step of the ectonucleotidase pathway in regards to the existence of multiple enzymes that can hydrolyze nucleosides di- or triphosphates in the extracellular space. However, the biochemical properties, cellular localization, and functional properties of these enzymes have now been well demonstrated [Zimmermann, 1996]. Moreover, the molecular identity of these proteins, recently established, demonstrated definitively the existence and certainly of their participation in the nucleotide degradation.

The AMP produced is subsequently hydrolysed to adenosine by ecto-5′-nucleotidase (EC 3.1.3.5), a key enzyme in this pathway [Zimmermann, 1992]. In contrast to the E-type ATPases, ecto-5′-nucleotidase is a membrane protein anchored to the cell surface by a phosphatidylinositol glycan. Cytosolic and secreted forms of this enzyme have also been described [Zimmermann, 1992]. The molecular properties and physiological roles of ecto-5′-nucleotidase have been extensively studied [for review see Zimmermann, 1992]. The role of this enzyme in neuromodulation mediated by adenosine as well as the alterations of 5′-nucleotidase levels in a considerable number of pathological conditions have also been reviewed recently [Zimmermann, 1996; Zimermann et al., 1998].

During the last decade, we have been focused our investigations on ATP diphosphohydrolase (ecto-apyrase) activity in synaptosomes from central nervous system of rats in physiological and pathological conditions [Schadeck et al., 1989; Rocha et al., 1990; Battastini et al., 1991; Müller et al., 1993; Wyse et al., 1994; Schetinger et al., 1994; Bonan et al., 1997, 1998, 1999, 2000a; Bonan et al., 2000b]. We have also solubilized the enzyme from rat brain synaptic plasma membranes [Battastini et al., 1995], demonstrated that ecto-apyrase from rat brain is a transmembrane-polypeptide-anchored protein [Battastini et al., 1998], and investigated the amino acids involved in ATP and ADP hydrolysis [Wink et al., 2000].

ATP diphosphohydrolase (apyrase, ATPDase, EC 3.6.1.5) is an enzyme that catalyses the conversion of nucleoside tri- and diphosphates to monophosphate and inorganic phosphate in the presence of Ca^{2+} or Mg^{2+}. It is insensitive to inhibitors of various ATPase (P-type, F-type, and V-type) and has an optimum pH of approximately 8.0.

We have shown that the enzyme ATP diphosphohydrolase is firmly associated with synaptic plasma membrane [Battastini et al., 1998]. This can be explained by the presence of two transmembrane domains at N- and C-terminus [Kaczmarek et al., 1996], as was recently confirmed by the amino acid sequence analysis [Wang et al., 1997; Kegel et al., 1997]. Recently, Wang et al. [1998] have shown that the sensitivity of the enzyme to different detergents as observed by us [Battastini et al., 1998] is caused by the dissociation of the tetrameric structure of the protein. This feature of the enzyme makes the solubilization and purification process difficult [Battastini et al., 1995].

Recently, considerable progress has been made in the study of the molecular structure of ATP diphosphohydrolase of several sources [Kegel et al., 1997; Wang et al., 1997; Smith and Kirley, 1998]. Wang et al. [1997] isolated rat and mouse brain ecto-apyrase cDNAs and suggested that there might be only a single copy of the ecto-apyrase (APY/CD39) gene. Moreover, it was shown that ATP diphosphohydrolase is a highly glycosylated protein with six potential N-linked glycosylation sites [Maliszewski et al., 1994; Christoforidis et al., 1995; Kaczmarek et al., 1996; Kegel et al., 1997; Wang et al., 1997]. Analysis of the amino acid sequences of apyrases from different sources has shown several potential phosphorylation sites [Kegel et al., 1997; Wang et al., 1997; Smith and Kirley, 1998], indicating that ecto-apyrase could be regulated by phosphorylation. We have recently shown that ecto-apyrase present in different brain tissue preparations and cell culture can be detected as a phosphoprotein, with possible implications for the regulation of the enzyme [Wink et al., 2000b]. In addition, the existence of alternative splicing in the ecto-ATPase family may provide a regulatory mechanism that controls the extracellular ATP hydrolysis, contributing to the diversity of purinergic transmission [Vlajkovic et al., 1999].

Thus, the participation of this enzyme in the "enzyme chain" together with a 5′-nucleotidase for the complete hydrolysis of ATP to adenosine in the synaptic cleft during neurotransmission is well established [Battastini et al., 1991; Zimmermann, 1996; Kegel et al., 1997]. These two enzymes have a dual function controlling the availability of ligands (ATP, ADP, AMP, and adenosine] for either nucleotide or nucleoside receptors. By the hydrolysis of the nucleotide, they might be involved in controlling activation of receptor [Zimmermann, 1996]. Therefore, it will be the purpose of this review to present findings with regard to the involvement of ecto-apyrase and ecto-5′-nucleotidase in some physiological and pathological conditions potentially related to synaptic plasticity.

ATP, ADENOSINE, AND MEMORY

It is accepted that the neurophysiological basis for memory and learning should involve alteration in the synaptic efficiency between neurons integrated in a network. Although the activation of glutamate receptors and intracellular cascades controlled by protein kinases are events considered to be necessary for the induction of LTP and formation of memory in the hippocampus, other factors, such as arachidonic acid [Bliss et al., 1991], NO, CO [Zhuo et al., 1993], and extracellular ATP [Wieraszko and Ehrlich, 1994], may be involved in the synaptic plasticity induced by learning.

In the central nervous system, ATP induces fast synaptic currents in cultured neurons from the hippocampus and in slices from the medial habenula [Inoue et al., 1992; Edwards et al., 1992]. Furthermore, ATP is able to induce LTP, recording in hippocampal slices from mouse and guinea pig [Wieraszko and Seyfried, 1989; Fujii et al., 1999]. These results suggested that extracellular ATP might be involved in the modulation of synaptic efficiency. Studies have shown that the release of ATP was greater at a brief, high-frequency stimulation (LTP stimulation paradigm), whereas the release of adenosine was slightly greater at a more prolonged low-frequency stimulation (long-term depression stimulation paradigm) [Cunha et al., 1996].

The involvement of purinergic system in LTP induced electrically and by ATP was investigated [Wierazsko and Ehrlich, 1994]. This study showed that extracellular ATP and its analogue, ATP-γ-S, amplify the magnitude of population spike. However, this effect was not observed in the presence of other ATP analogues, such as AMPPNP, 2MeSATP, α-β-methylene ATP, or a purinergic receptor antagonist Cibacron Blue 3G [Wieraszko and Ehrlich, 1994]. It has been proposed that the removal of the γ-phosphate of ATP by an ecto-protein kinase or by ecto-ATPase could be necessary for the facilitatory effect on LTP [Wieraszko and Ehrlich, 1994].

Among ligands of P2 receptors, suramin has been used as a nonselective antagonist of P2 receptors [Ralevic and Burnstock, 1998], presenting an inhibitory effect on the enzyme activities involved in ATP degradation [Bonan et al., 1999]. Despite its antagonist action, suramin produced a facilitatory effect on the synaptic responses recorded in hippocampal slices [Wieraszko, 1995], using mechanisms that also participate in the induction and maintenance of LTP. However, it has been shown that suramin impairs fear-conditioned responses in rats [Zou et al., 1998]. Recently, our laboratory has shown that suramin, a noncompetitive inhibitor of ecto-apyrase, produced an amnesic effect in the step-down inhibitory avoidance task, when administered intrahippocampally [Bonan et al., 1999]. Such an effect probably occurs because suramin can act as an antagonist of *N*-methyl-D-aspartate (NMDA) receptors [Ong et al., 1997]. The dissociation observed between the effects of suramin on LTP and behavioral data is probably because of the broad spectrum of biological effects promoted by this drug.

Adenosine, a product of extracellular ATP metabolism, is an endogenous nucleoside that also exerts an important role in the regulation of neuronal excitability. It has previously been shown that adenosine may modulate synaptic plasticity in rats [De Mendonça and Ribeiro, 1997]. The endogenous adenosine is able to modulate LTP, because this phenomenon was facilitated in the presence of the selective A_1 adenosine receptor antagonist, 1,3-dipropyl-8-cyclopentylxanthine, and it was reduced in the presence of the adenosine uptake blocker, nitrobenzylthioinosine, suggesting that the endogenous adenosine exerts an inhibitory effect on LTP [De Mendonça and Ribeiro, 1994]. The inhibitory effects of adenosine on LTP are mediated by the activation of A_1 receptors, and this effect is observed during or a few seconds after high-frequency stimulation, suggesting that adenosine affects the LTP induction [De Mendonça and Ribeiro, 1997]. Furthermore, it has been shown that the activation of A_1 and A_2 adenosine receptors can modulate the performance in the retention of responses, controlling mechanisms of memory and learning [Hooper et al., 1996; Kopf et al., 1999].

Because LTP is generally recognized as a possible mechanism of memory and evidence indicate that ATP and adenosine may play a role in the modulation of LTP, a study of the possible effect of memory tasks on ATP-metabolizing enzymes would be interesting. Recently, our laboratory observed that one-trial inhibitory avoidance training is associated with a learning-specific, time-dependent decrease in hippocampal ectonucleotidase activities [Bonan et al., 1998]. The results showed a significant decrease in ATP diphosphohydrolase and 5′-nucleotidase activities immediately after training session in a step-down inhibitory avoidance task. In the test session, no significant changes were observed in the enzyme activities studied [Bonan et al., 1998].

To further explore the participation of the ectonucleotidase pathway in different brain structures involved in the formation of inhibitory avoidance memory, our laboratory evaluated the effect of training session on ectonucleotidase activities from hippocampus, entorhinal cortex, and parietal cortex of rats. ATP diphosphohydrolase presented a significant decrease in hippocampal synaptosomes of rats killed at 180 min, but not at 360 min after training [Bonan et al., 2000a]. In synaptosomes from entorhinal cortex of rats, a decrease in ATP diphosphohydrolase activity was observed immediately, but not at 180 and 360 min after training [Bonan et al., 2000a].

Extracellular ATP is associated with memory formation, probably by interaction with purinergic receptors [Wieraszko and Ehrlich, 1994], ectonucleotidases [Bonan et al., 1998; 2000a] and ecto-protein phosphorylation [Fujii et al., 1995; Chen et al., 1996]. The significant inhibition observed on ectonucleotidase pathway in hippocampus and in entorhinal cortex after training could represent an important biochemical mechanism related to memory acquisition and consolidation, but has no influence in the retrieval [Bonan et al., 1998; 2000a].

Among several biochemical mechanisms triggered immediately after training, it is possible to observe an enhanced activity of protein kinase G, calcium-calmodulin-dependent protein kinase II, protein kinase C cascades, and an increase in protein kinase A activity at 0 and 3 h after training [Izquierdo and Medina, 1997]. Recently, our laboratory has presented evidence pointing to a possible modulation of rat brain ecto-apyrase by phosphorylation [Wink et al., 2000b]. Although the mechanism by which ecto-apyrase decreases its activity after training has not been elucidated, it is possible that its phosphorylation represents an important regulatory mechanism, controlling extracellular ATP levels, which could be used as substrate in ecto-protein phosphorylation. Because ATP and activation of NMDA receptors are necessary for LTP induction, it is possible that extracellular ATP levels reach a suprathreshold concentration that triggers the biological process that results in LTP by phosphorylation of extracellular domains of NMDA receptor/channels [Fujii et al., 1999]. Furthermore, it has been proposed that the maintenance of LTP involve the activity of an ecto-protein kinase, using extracellular ATP as substrate [Chen et al., 1996]. These findings constitute the first evidence on the involvement of ectonucleotidases in memory acquisition and consolidation and raise questions about the importance of these enzymes in biochemical events related to memory formation.

ATP, ADENOSINE AND EPILEPSY

Several studies have shown that adenosine, a ubiquitous neuromodulator, has potent anticonvulsant effects [Chin, 1989; Young and Dragunow, 1994]. Adenosine and adenosine analogues administered either centrally or peripherally reduce seizure activity in a dose-dependent manner in electrically kindled rats [Barraco et al., 1984; Rosen and Berman, 1987]. In 1985, Turski et al. demonstrated that the analogue 2-cloroadenosine blocked the appearance of seizures induced by pilocarpine and prevented the occurrence of neuronal damage in mice, with these effects probably being mediated by A_1 receptors. The A_1 receptor antagonist, 8-cyclopentyl-1,3-dimethylxanthine, produced status epilepticus (SE) in rats when administered intraperitoneally, but their agonists, N^6-cyclopentyladenosine or N^6-cyclohexyladenosine, suppressed the development of SE [Young and Dragunow, 1994]. Studies have shown that adenosine A_1 and A_{2A} receptors are involved in the suppression of seizures in audiogenic seizure-sensible DBA/2 mice [De Sarro et al., 1999].

Single or repeated seizures induced by pentylenetetrazol (PTZ) were associated with an increase of A_1 receptors in cerebral cortex, hippocampus, and cerebellum [Angelatou et al., 1990]. Furthermore, an increased affinity for adenosine A_1 receptors has been observed in the hippocampus of kindled rats, suggesting that these receptors might play a role in the anticonvulsant effects of adenosine analogues [Simonato et al., 1994]. However, a lower density of A_1 receptors was found in nucleus reticularis thalami in rats with genetic absence epilepsy [Ekonomou et al., 1998]. Immunoreactivity studies have shown a reduced expression of A_1 receptors in the CA2/CA3 regions in rats after kainate and kindling treatments [Ochiishi et al., 1999]. Ekonomou et al. [2000] have also observed a progressive decrease in A_1 receptor density in CA1 and CA3 regions of rat hippocampus after kainic acid–induced limbic seizures.

Studies developed in humans observed that adenosine levels significantly increased (30 times) during seizures in patients with temporal lobe epilepsy [During and Spencer, 1992]. Furthermore, a decreased binding to A_1 adenosine receptors was observed in temporal cortex of patients with epilepsy [Glass et al., 1996]. Evidence suggests that a decrease in the adenosine levels or a perturbation in adenosine system may play an important role in the etiology of epilepsy [Young and Dragunow, 1994].

Besides the release of adenosine as such, biochemical studies have established that a potential source of adenosine is its formation in the extracellular space from adenine nucleotides [Dunwiddie et al., 1997]. Once adenine nucleotides reach the extracellular space, they are subsequently converted to adenosine through the action of ectonucleotidases [Zimmermann, 1996, Bonan et al., 1998]. Several studies have shown the involvement of ectonucleotidases in several pathological conditions, such as epilepsy. A deficiency of ecto-ATPase activity is seen in cultured glia cells raised from neonatal, seizure-prone mice [Trams and Lauter, 1978]. Nagy et al. [1990] showed

a significant decrease in ecto-ATPase activity in temporal cortex of humans with epilepsy. However, a substantially increased ecto-ATPase activity was observed in the posterior part of epileptic hippocampus [Nagy et al., 1990]. It is interesting to observe that the chromosomal position of human CD39/ecto-apyrase (10q23.1 to q24.1) [Maliszewski et al., 1994] is co-located with the gene involved in the human partial epilepsy with audiogenic symptom (10q 22 to 24) [Ottman et al., 1995]. The localization of these genes led to the hypothesis that ecto-apyrase is probably involved in the epilepsy. Furthermore, studies have shown a reduction in the ecto-ATPase activity in cerebral cortex of rats during prolonged SE induced by sequential administration of lithium and pilocarpine [Nagy et al., 1997].

The distribution of the 5′-nucleotidase in patients with temporal lobe epilepsy showed that the enzyme is significantly increased in the dentate gyrus and in the mossy fiber endings in CA4 and CA3 areas, when compared with the activity in normal hippocampus of humans [Lie et al., 1999]. Recently, the presence of the 5′-nucleotidase was observed in mossy fibers of rat dentate gyrus after systemic kainate injection and induction of kindling, being less detected in normal hippocampus [Schoen et al., 1999].

Recently, our laboratory has investigated ectonucleotidase activities after the induction of epilepsy by several chronic animal models, such as pilocarpine, kainic acid, and kindling models. ATP diphosphohydrolase and 5′-nucleotidase activities from synaptosomes of hippocampus and cerebral cortex of rats significantly increased at 48–52 h, 7–9 days, and 45–50 days after induction of SE by pilocarpine or kainic acid models [Bonan et al., 2000b]. However, only 5′-nucleotidase activity remains elevated at 100–110 days after the treatment with kainic acid [Bonan et al., 2000b]. Our findings lead us to the hypothesis that an increase in ectonucleotidase activities could modulate the seizure activity in a time window (48 h–110 days) after SE, contributing to production of extracellular adenosine, a known endogenous neuromodulator [During and Spencer, 1992]. If ATP is released in large amounts and for a long time, it may promote a dramatic increase in intracellular calcium levels mediated by P_{2X} receptors, which could represent significant damage, as that induced by excess of glutamate [Edwards et al., 1992]. If all members of the ectonucleotidase pathway work at an elevated rate, an efficient removal of extracellular ATP and enhanced adenosine production could occur in this condition. Then, adenosine could modulate the release of a variety of neurotransmitters, including glutamate, acetylcholine, noradrenaline, and dopamine [Di Iorio et al., 1998]. In summary, after SE, an important adaptive plasticity of the ectonucleotidase pathway could occur to decrease ATP levels, an excitatory neurotransmitter, and to increase adenosine levels, a neuroprotective compound. Our results suggest that SE can induce late and prolonged changes in ectonucleotidase activities. The regulation of the ectonucleotidase pathway may play a modulatory role during the evolution of behavioral and pathophysiological changes induced by SE [Bonan et al., 2000b].

Considering that adenosine has potent anticonvulsant effects on various models of epilepsy, such as PTZ kindling, we evaluated ectonucleotidase activities after the induction of this model. Our results have indicated that rats showing greater resistance to PTZ kindling presented an increase in ATP hydrolysis in synaptosomes from hippocampus and cerebral cortex [Bonan et al., 2000c]. To examine whether the altered ATP hydrolysis was due to the chronic, long-lasting changes induced by, kindling or by the drug, we investigated enzyme activities after a single acute seizure induced by PTZ. Changes in ectonucleotidase activities were not seen at different times (immediately, 1 h, 24 h, and 5 days) after a single convulsant PTZ injection [Bonan et al., 2000c]. These alterations seems to be related to the chronic, long-lasting synaptic plasticity induced by kindling, because such changes are not seen in acute seizures, which are insufficient to activate these mechanisms. When these results are considered together, they support the hypothesis that changes in nucleotide hydrolysis may represent an important mechanism in the modulation of epileptogenesis [Bonan et al., 2000c].

ECTONUCLEOTIDASES AND CEREBRAL ISCHEMIA

Brain ischemia and hypoxia are the main causes of neuronal damage resulting in human neurological disability [Ginsberg, 1995]. The molecular mechanisms underlying the neuropathological events involved in these processes have been the subject of intense experimental investigation. The main molecular consequences of brain ischemia include depletion of ATP, acidosis, massive release of excitatory amino acids, increase in intracellular calcium, and free radical formation that may lead to cell death [Rudolphi et al., 1992; Pulsinelli, 1992; Ginsberg, 1995].

Ischemic injury evokes cellular stress response, which involves the activation or inhibition of several mechanisms. It is known that brief ischemic episodes, not enough to produce cell death, induce tolerance to longer episodes of ischemia [Kirino et al., 1991].

Although the molecular mechanisms for tolerance is poorly understood, a variety of cellular and molecular changes have been implicated in the induced tolerance phenomenon, such as increase in specific heat-shock protein synthesis [Nakamura et al., 1992] and increase in ATP, ADP, and AMP hydrolysis and adenosine production [Schetinger et al., 1998a,b]. Recently, Reshef et al. [2000] reported that the opening of K_{ATP} channels is mandatory for acquisition of ischemic tolerance by adenosine in neu-

ronal cultures, because glibenclamide, a K_{ATP} channel blocker, abolished the protection conferred by the preconditioning substances N^6-(R)-phenylisopropyladenosine, 1,2 dioctanoyl-rac-glycerol, and cromakalim. In another study, it was reported that chemical preconditioning with 3-nitroproprionate that mediate hypoxic tolerance is associated with a transient A_1 receptor mRNA upregulation that was observed at 1 h but not at 24 h after preconditioning, whereas receptor mRNA levels remained unchanged [Von Arnim et al., 2000]. These changes may be related to the role played by purines in the central nervous system that includes both immediate effects, such as neurotransmission, and trophic effects, which induce changes in cell metabolism, structure, and function [Rathbone et al., 1999].

During ischemia, ATP can be released from cells and stimulate specific P2-purinoreceptors. In fact, the activation of P2-purinoreceptors triggers a sequence of events that could potentiate the excitotoxic effects of glutamate that increases considerably in the synaptic cleft after ischemia [DiVirgilio et al., 1990; Edwards, 1996]. Consequently, a sustained increase in extracellular ATP can contribute to exacerbate the deleterious effects of ischemia to neuronal cells. Thus, a rapid mechanism for ATP inactivation is crucial for adequate cell functioning.

The extracellular concentration of adenosine can be increased during in vitro and in vivo ischemic episodes, and it is believed to confer cytoprotection by the activation of specific P_1-purinoreceptors [Rudolphi et al., 1992; Latini et al., 1999a,b]. Several studies demonstrated that 1) the activation of A_1 adenosine receptors depressed synaptic transmission with subsequent reduction in glutamate release [Rudolphi et al., 1992]; 2) the stimulation of A_{2A} adenosine receptors attenuate the A_1-mediated depression [Cunha et al., 1995; Latini et al., 1999b]; 3) the stimulation of the A_3 receptor produces a delayed desensitization of the A_1 receptor [Dunwiddie et al., 1997] and activates the antioxidant defense system [Maggirwar et al., 1994]. It is plausible to suppose that these distinct receptor-mediated adenosine effects represent a compensatory mechanism that can protect the neural cells against ischemic damage.

As described earlier in this review, adenosine can be produced extracellularly by the conjugated action of ATP diphosphohydrolase and 5′-nucleotidase. Consequently, we hypothesised that ischemia could change the enzymes activities involved in adenosine formation in the extracellular space [Schetinger et al., 1994]. In fact, we observed that there exists a link between changes in ATP, ADP, and AMP hydrolysis and the induced-tolerance phenomenon after ischemia (four-vessel occlusion method) and reperfusion. Results from our laboratory provided evidence for the first time that changes in ATP diphosphohydrolase activity could be involved in such events [Schetinger et al., 1994]. We observed that 2 and 10 min of ischemia increase ATP diphosphohydrolase activity from hippocampal synaptosomes immediately after the ischemic episode. In a more detailed study, Schetinger et al. [1997] reported that the response of synaptosomal ATP diphosphohydrolase and 5′-nucleotidase from rat hippocampus to different ischemic episodes was complex, depending on whether the animals were made tolerant to ischemia by exposure to a short ischemic episode of 2 min. In fact, the activity of ATP diphosphohydrolase from tolerant animals did not vary after 10 min of ischemia, whereas the activity of animals submitted to a single ischemic episode increased immediately after ischemia and decreased from 24 to 48 h after the ischemic insult. Also, another important finding was that after 24 h of reperfusion, the activity of 5′-nucleotidase was clearly enhanced in the tolerant animals. These data demonstrate a lasting modulation of ATP diphosphohydrolase and 5′-nucleotidase activities in preconditioned rats. Subsequently, other studies confirmed that the enzymes that participate in ATP, ADP, and AMP degradation were modulated by different models of ischemia in rats [Braun et al., 1997, Braun et al., 1998; Schetinger et al., 1998a].

The effects of ischemia on the enzymes that produce adenosine are consistent with a protective role proposed for this purine. In fact, a considerable number of studies have indicated a cerebroprotective role for adenosine against ischemic and excitotoxic amino acid–induced damage [Rudolphi et al., 1992; Parkinson et al., 1994; Schubert et al., 1996; Parkinson et al., 2000]. Thus, after ischemic insult, the ectonucleotidase activities are modulated in a such way that facilitates the production of adenosine, a neuroprotective agent.

CONCLUSIONS

The demonstration that ectonucleotidases present their activities differently altered after the acquisition of a memory task, or the induction of different animal models of epilepsy or ischemia /reperfusion makes possible the suggestion that these enzymes may act in the regulation of synaptic activity. These enzymes can control extracellular ATP and adenosine levels and, consequently, modulate the activation of P2 and P1 receptors, depending on the type of synaptic plasticity developed, in physiological or pathological situations. Future studies will contribute significantly to the identification of the mechanism involved in modulation of these enzymes in different conditions, as well as increase our understanding on their functional role in synaptic plasticity.

REFERENCES

Angelatou F, Pagonopoulou O, Kostopoulos G. 1990. Alterations of the A_1 receptors in different mouse brain areas after pentylenetetrazol induced seizures, but not in the epileptic mutant mouse 'tottering'. Brain Res 534:251–256.

Au Lois NC, Niquet J, Ben-Ari Y, Represa A. 1997. Cellular plasticity. In: Engel J Jr, Pedley TA, editors. Epilepsy: a comprehensive textbook. Lippincott-Raven Publishers. p 387–396.

Barraco R, Swanson T, Phillis J, Berman R. 1984. Anticonvulsant effects of adenosine analogues on amygdaloid-kindled seizures in rats. Neurosci Lett 46:317–322.

Battastini AMO, Rocha JBT, Barcellos CK, Dias RD, Sarkis JJF. 1991. Characterization of an ATP diphosphohydrolase (EC 3.6.1.5) in synaptosomes from cerebral cortex of adult rats. Neurochem Res 16:1303–1310.

Battastini AMO, Oliveira EM, Moreira CM, Bonan CD, Sarkis JJF, Dias RD. 1995. Solubilization and characterization of an ATP diphosphohydrolase (EC 3.6.1.5.) from rat brain synaptic plasma membranes. Biochem Mol Biol Int 37:209–219.

Battastini AMO, Emanuelli T, Koester L, Wink MR, Bonan CD, Dias RD, Sarkis JJF. 1998. Studies on the anchorage of ATP diphosphohydrolase in synaptic plasma membranes from rat brain. Int J Biochem Cell Biol 30:669–678.

Bliss TVP, Clements MP, Errington ML, Lynch MA, Williams JH. 1991. Presynaptic changes associated with long-term potentiation in the dentate gyrus. In: Baudry M, Davis JL, editors. Long-term potentiation. Cambridge, MA: MIT Press. p 3–18.

Bonan CD, Battastini AMO, Schetinger MRC, Moreira CM, Frassetto SS, Dias RD, Sarkis JJF. 1997. Effects of 9-amino-1,2,3,4-tetrahydroacridine (THA) on ATP diphosphohydrolase (EC 3.6.1.5) from rat brain synaptosomes. Gen Pharmacol 28:761–766.

Bonan CD, Dias MM, Battastini AMO, Dias RD, Sarkis JJF. 1998. Inhibitory avoidance learning inhibits ectonucleotidase activities in hippocampal synaptosomes of adult rats. Neurochem Res 23: 979–984.

Bonan CD, Roesler R, Quevedo J, Battastini AMO, Izquierdo I, Sarkis JJF. 1999. Effects of suramin on hippocampal apyrase activity and inhibitory avoidance learning of rats. Pharmacol Biochem Behav 63:153–158.

Bonan CD, Roesler R, Pereira GS, Battastini AMO, Izquierdo I, Sarkis JJF. 2000a. Learning-specific decrease in synaptosomal ATP diphosphohydrolase activity from hippocampus and entorhinal cortex of adult rats. Brain Res 854:253–256.

Bonan CD, Walz R, Pereira GS, Worm PV, Battastini AMO, Cavalheiro EA, Izquierdo I, Sarkis JJF. 2000b. Changes in synaptosomal ectonucleotidase activities in two rat models of temporal lobe epilepsy. Epilepsy Res 39:229–238.

Bonan CD, Amaral OB, Rockenbach IC, Walz R, Battastini AMO, Izquierdo I, Sarkis JJF. 2000c. Altered ATP hydrolysis induced by pentylenetetrazol kindling in rat brain synaptosomes. Neurochem Res 25:775–779.

Braun N, Lenz C, Gillardon F, Zimmermann M, Zimmermann H. 1997. Focal ischemia enhances glial expression of ecto-5′-nucleotidase. Brain Res 766:213–226.

Braun N, Zhu Y, Krieglstein J, Culmsee C, Zimmermann H. 1998. Upregulation of the enzyme chain hydrolyzing extracellular ATP after transient forebrain ischemia in the rat. J Neurosci 18:4891–4900.

Chen W, Wieraszko A, Hogan MV, Yang HA, Kornecki E, Ehrlich YH. 1996. Surface protein phosphorylation by ecto-protein kinase is required for the maintenance of hippocampal long-term potentiation. Proc Natl Acad Sci USA 93:8688–8693.

Chin JH. 1989. Adenosine receptors in brain: neuromodulation and role in epilepsy. Ann Neurol 26:695–698.

Christoforidis S, Papamarcaki T, Galaris D, Kellner R, Tsolas O. 1995. Purification and properties of human placental ATP diphosphohydrolase. Eur J Biochem 234:66–74.

Cotman CW. 1998. Axonal sprouting. In: Siegel GJ, Agranoff BW, Albers RW, Fischer SK, Uhler MD, editors. Basic neurochemistry: molecular, cellular and medical aspects. Lippincott-Raven Publishers, p 589–612.

Cunha RA, Johansson B, Fredholm BB, Ribeiro JA, Sebastião AM. 1995. Adenosine A2A receptor stimulate acetylcholine release from nerve terminals of the rat hippocampus. Neurosci Lett 196:41–44.

Cunha RA, Vizi ES, Ribeiro JÁ, Sebastião AM. 1996. Preferential release of ATP and its extracellular catabolism as a source of adenosine upon high- but not low-frequency stimulation of rat hippocampal slices. J Neurochem 67:2180–2187.

De Mendonça A, Ribeiro JA. 1994. Endogenous adenosine modulates long-term potentiation in the hippocampus. Neuroscience 62:385–390.

De Mendonça A, Ribeiro JA. 1997. Adenosine and neuronal plasticity. Life Sci 60:245–251.

De Sarro G, de Sarro A, Di Paola ED, Bertorelli R. 1999. Effects of adenosine receptor agonists and antagonists on audiogenic seizure-sensitive DBA/2 mice. Eur J Pharmacol 371:137–145.

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

DiVirgilio F, Pizzo P, Zanovello P, Bronte V, Collavo D. 1990. Extracellular ATP as a possible mediator of cell-mediated cytotoxicity. Immunol Today 11:274–277.

Dunwiddie TW, Diao L, Proctor WR. 1997. Adenine nucleotides undergo rapid, quantitative conversion to adenosine in the extracellular space in rat hippocampus. J Neurosci 17:7673–7682.

During MJ, Spencer DD. 1992. Adenosine: a mediator of seizure arrest and postictal refractoriness. Ann Neurol 32:618–624.

Edwards FA. 1996. Features of P2X receptor-mediated synapses in the rat brain: why doesn't ATP kill the postsynaptic cells? In: Chadwick DJ, editor. P2 purinoreceptors: localization, function, and transduction mechanisms. Chichester: p 278–289.

Edwards FA, Gibb AJ, Colquhoun D. 1992. ATP receptor-mediated synaptic currents in the central nervous system. Nature 359:144–147.

Ekonomou A, Angelatou F, Vergnes M, Kostopoulos G. 1998. Lower density of A_1 adenosine receptors in nucleus reticularis thalami in rats with genetic absence epilepsy. Neuroreport 9:2135–2140.

Ekonomou A, Sperk G, Kostopoulos G, Angelatou F. 2000. Reduction of A1 adenosine receptor in rat hippocampus after kainic acid-induced limbic seizures. Neurosci Lett 284:49–52.

Fujii S, Kato H, Furuse H, Ito K-I, Osada H, Hamaguchi T, Kuroda Y. 1995. The mechanisms of ATP-induced long-term potentiation involves extracellular phosphorylation of membrane proteins in guinea—pig hippocampal CA1 neurons. Neurosci Lett 187:130–132.

Fujii S, Kato H, Kuroda Y. 1999. Extracellular adenosine 5′-triphosphate plus activation of glutamatergic receptors induces long-term potentiation in CA1 neurons of guinea pig hippocampal slices. Neurosci Lett 276:21–24.

Ginsberg MD. 1995. Neuroprotection in brain ischemia: an update. Neuroscientist 1:95–103.

Glass M, Faull RLM, Bullock JY, Jansen K, Mee EW, Walker EB, Synek BJL, Dragunow M. 1996. Loss of A_1 adenosine receptors in human temporal lobe epilepsy. Brain Res 710:56–68.

Hooper N, Fraser C, Stone TW. 1996. Effects of purine analogues on spontaneous alternation in mice. Psychopharmacology (Berl) 23: 250–257.

Inoue K, Nakazawa K, Fujimori K, Watano W, Takanaka A. 1992. Ex-

tracellular adenosine 5′-triphosphate-evoked glutamate release in cultured rat hippocampal neurons. Neurosci Lett 134:215–218.

Izquierdo I, Medina JH. 1997. Memory formation: the sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. Neurobiol Learn Mem 68:285–316.

Kaczmarek E, Koziak K, Sevigny J, Siegel JB, Anrather J, Beaudoin AR, Bach FH, Robson SC. 1996. Identification and characterization of CD39 vascular ATP diphosphohydrolase. J Biol Chem 271: 33116–33122.

Kegel B, Braun N, Heine P, Maliszewski CR, Zimmermann H.1997. An ecto-ATPase and an ecto-ATP diphosphohydrolase are expressed in rat brain. Neuropharmacology 36:1189–1200.

Kirino T, Tsujita Y, Tamura A. 1991. Induced tolerance in gerbil hippocampal neurons. J Cereb Blood Flow Metab 11:299–307.

Kopf SR, Melani A, Pedata F, Pepeu G. 1999. Effect of A(1) and A(2) receptor antagonists. Psychopharmacology 146:214–219.

Latini S, Bordoni F, Pedata F, Corradetti R. 1999a. Extracellular adenosine concentrations during in vitro ischaemia in rat hippocampal slices. Br J Pharmacol 127:729–739.

Latini S, Bordoni F, Corradetti R, Pepeu G, Pedata F. 1999b. Effect of A2A adenosine receptor stimulation and antagonism on synaptic depression induced by in vitro ischaemia in rat hippocampal slices. Br J Pharmacol 128:1035–1044.

Lie AA, Blumcke I, Beck H, Wiestler OD, Elger CE, Schoen SW.1999. 5′-Nucleotidase activity indicates sites of synaptic plasticity and reactive synaptogenesis in the human brain. J Neuropathol Exp Neurol 58:451–458.

Maggirwar SB, Dhanraj DN, Somani SM, Ramkumar V. 1994. Adenosine acts as an endogenous activator of the cellular antioxidant defense system. Biochem Biophys Res Commun 210:508–515.

Maliszewski CR, Delespesse GJT, Schoenborn MA, Armitage RJ, Fanslow WC, Nakakima T, Baker E, Sutherland GR, Poindexter K, Birks C, Alpert A, Friend D, Gimpel SD, Gayle III RB. 1994. The CD39 lymphoid cell activation antigen. Molecular cloning and structural characterization. J Immunol 149:3574–3583.

Müller J, Rocha JBT, Battastini AMO, Sarkis JJF, Dias RD. 1993. Postnatal development of ATPase-ADPase activities in synaptosomes fraction from cerebral cortex of rats. Neurochem Int 23:471–477.

Nagy AK, Houser RC, Delgado-Escueta AV. 1990. Synaptosomal ATPase activities in temporal cortex and hippocampal formation of humans with focal epilepsy. Brain Res 529:192–201.

Nagy AK, Walton NY, Treiman DM. 1997. Reduced cortical ecto-ATPase activity in rat brains during prolonged status epilepticus induced by sequential administration by lithium and pilocarpine. Mol Chem Neuropathol 31:135–147.

Nakamura M, Araki M, Oguro K. 1992. Differential distribution of 68 Kd and 200 Kd neurofilament proteins in the gerbil hippocampus and their early distributional changes following transient forebrain ischemia. Exp Brain Res 89:31–39.

Ochiishi T, Takita M, Ikemoto M, Nakata H, Suzuki S. 1999. Immunohistochemical analysis on role of adenosine A1 receptors in epilepsy. Neuroreport 10:3535–3541.

Ong WY, Motin LG, Hansen MA, Dias LS, Ayrout C, Bennet MR, Balcar VJ. 1997. P2 purinoceptor blocker suramin antagonizes NMDA receptors and protects against excitatory behaviour caused by NMDA receptor agonist (RS)-(tetrazol-5-yl)-glycine in rats. J Neurosci Res 49:627–638.

Ottman R, Risch N, Hauser WA, Pedley TA, Lee JH, Barker-Cummings C, Lustenberg A, Nagle KJ, Lee SK, Seheur ML, Neystat M, Susser M, Wilhelmsen KC. 1995. Localization of a gene for partial epilepsy to chromosome 10q. Nature Gen 10:56–60.

Parkinson FE, Rudolphi KA, Fredholm BB. 1994. Propentofylline: a nucleoside transport inhibitor with neuroprotective effects in cerebral ischemia. Gen Pharmacol 25:1053–1058.

Parkinson FE, Zhang YW, Shepel PN, Greenway SC, Peeling J, Geiger JD. 2000. Effects of nitrobenzylthioinosine on neuronal injury, adenosine levels, and adenosine receptor activity in rat forebrain ischemia. J Neurochem 75:795–802.

Pulsinelli W. 1992. Pathophysiology of acute ischaemic stroke. Lancet 339:533–536.

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

Rathbone MP, Middlemiss PJ, Gysbers JW, Andrew C, Herman MA, Reed JK, Ciccarelli R, Di Iorio P, Caciagli F. 1999. Trophic effects of purines in neurons and glial cells. Prog Neurobiol 59:663–690.

Reshef A, Sperling O, Zoref-Shani E. 2000. Opening of K_{ATP} channels is mandatory for acquisition of ischemic tolerance by adenosine. Neuroreport 11:463–465.

Rocha JBT, Mello CF, Sarkis JJF, Dias RD. 1990. Undernutrition during the preweaning period changes calcium ATPase and ADPase activities of synaptosomal fractions of weaning rats. Br J Nutr 63: 274–284.

Rosen JB, Berman RF. 1987. Differential effects of adenosine analogs on amygdala, hippocampus and caudate nucleus kindled seizures. Epilepsia 28:658–666.

Rudolphi KA, Schubert P, Parkinson FE, Fredholm BB. 1992. Neuroprotective role of adenosine in cerebral ischaemia. TIPS 13:439–445.

Schadeck RJG, Sarkis JJF, Dias RD, Araújo HMM, Souza DOG. 1989. Synaptosomal apyrase in the hypothalamus of adult rats. Braz J Med Biol Res 22:303–314.

Schetinger MRC, Barcellos CK, Barlem A, Zwestch G, Gubert A, Bertuol C, Arteni N, Dias RD, Sarkis JJF, Netto CA. 1994. Activity of synaptosomal ATP diphosphohydrolase from hippocampus of rats tolerant to forebrain ischemia. Braz J Med Biol Res 27:1123–1128.

Schetinger MRC, Bonan CD, Schierholt RC, Webber A, Sarkis JJF, Dias RD, Netto CA. 1997. Ecto-ATPases. In: Plesner et al., editor. ATP diphosphohydrolase and 5′-nucleotidase activities from hippocampal synaptosomes after brain ischemia. New York: Plenum Press. p 213–219.

Schetinger MRC, Bonan CD, Schierholt RC, Webber A, Arteni N, Emanuelli T, Dias RD, Sarkis JJF, Netto CA. 1998a. Nucleotide hydrolysis in rats submitted to global cerebral ischemia: a possible link between preconditioning and adenosine production. J Stroke Cerebrovasc Dis 7:281–286.

Schetinger MRC, Falquembach F, Michelot F, Mezzomo A, Rocha JBT. 1998b. Heparin modulates adenine nucleotide hydrolysis by synaptosomes from cerebral cortex. Neurochem Int 33:243–249.

Schoen SW, Ebert U, Loscher W. 1999. 5′-Nucleotidase activity of mossy fibers in the dentate gyrus of normal and epileptic rats. Neuroscience 93: 519–526.

Schubert P, Ogata T, Ferroni S, McRae A, Nakamura Y, Rudolphi K. 1996. Modulation of glial cell signalling by adenosine and pharmacological reinforcement. A neuroprotective strategy? Mol Chem Neuropathol 28:185–190.

Simonato M, Varani K, Muzzolini A, Bianchi C, Beani L, Borea PA. 1994. Adenosine A_1 receptors in the rat brain in the kindling model of epilepsy. Eur J Pharmacol 265:121–124.

Smith TM, Kirley TL.1998. Cloning, sequencing and expression of a

human brain ecto-apyrase related to both ecto-ATPases and CD39 ecto-apyrases. Biochim Biophys Acta 1386:65–78.

Trams EG, Lauter CJ. 1978. Ecto-ATPase deficiency in glia of seizure-prone mice. Nature 271:270–271.

Turski WA, Cavalheiro EA, Ikonomidou C, Mello LEAM, Bortolotto ZA, Turski L. 1985. Effects of aminophylline and 2-chloroadenosine on seizures produced by pilocarpine in rats: morphological and electroencephalographic correlates. Brain Res 361:309–323.

Vlajkovic SM, Housley GD, Greenwood D, Thorne PR. 1999. Evidence for alternative splicing of ecto-ATPase associated with termination of purinergic transmission. Brain Res Mol Brain Res 73:85–92.

Von Arnim CA, Timmler M, Ludolph AC, Riepe MW. 2000. Adenosine receptor up-regulation initiated upon preconditioning but not upheld. Neuroreport 27:1223–1226.

Wang T-F, Rosenberg PA, Guidotti G. 1997.Characterization of brain ecto-apyrase: evidence for only one ecto-apyrase (CD39) gene. Mol Brain Res 47:295–302.

Wang T-F, Ou Y, Guidotti G. 1998. The transmembrane domains of ecto-apyrase (CD39) affect its enzymatic activity and quaternary structure. J Biol Chem 273:24814–24821.

Wieraszko A, Seyfried TN. 1989. ATP-induced synaptic potentiation in hippocampal slices. Brain Res 491:356–359.

Wieraszko A, Ehrlich YH. 1994. On the role of extracellular ATP in the induction of long-term potentiation in the hippocampus. J Neurochem 63:1731–1738.

Wieraszko A. 1995. Facilitation of hippocampal potentials by suramin. J Neurochem 64:1097–1101.

Wink MR, Buffon A, Bonan CD, Valenzuela MA, Sarkis JJF, Battastini AMO. 2000a. Effect of protein-modifying reagents on ecto-apyrase from rat brain. Int J Biochem Cell Biol 32:105–113.

Wink MR, Lenz G, Rodnight R, Sarkis JJF, Battastini AMO. 2000b. Identification of brain ecto-apyrase as a phosphoprotein. Mol Cell Biochem 213:11–16.

Wyse AT, Sarkis JJ, Cunha-Filho JS, Teiseira MV, Schetinger MR, Wajner M, Wannmacher C. 1994. Effect of phenylalanine and its metabolites on ATP diphosphohydrolase activity in synaptosomes from rat cerebral cortex. Neurochem Res 19:1175–1180.

Young D, Dragunow M. 1994. Status epilepticus may be caused by loss of adenosine anticonvulsant mechanisms. Neuroscience 58: 245–261.

Zhuo M, Small AS, Kandel ER, Hawkins RD.1993. Nitric oxide and carbon monoxide produce activity-dependent long-term synaptic enhancement in hippocampus. Science 260:1946–1950.

Zimmermann H. 1992. 5′-Nucleotidase: molecular structure and functional aspects. Biochem J 285:345–365.

Zimmermann H. 1996. Biochemistry, localization and functional roles of ecto-nucleotidases in the nervous system. Prog Neurobiol 49: 589–618.

Zimmermann H, Braun N, Kegel B, Heine P. 1998. New insights into molecular structure and function of ecto-nucleotidases in the nervous system. Neurochem Int 32:421–425.

Zou CJ, Onaka TO, Yagi K. 1998. Effects of suramin on neuroendocrine and behavioural responses to conditioned fear stimuli. Neuroreport 9:997–999.