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Pentylenetetrazol kindling alters adenine and guanine nucleotide catabolism in rat hippocampal slices and cerebrospinal fluid

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Summary Pentylenetetrazol (PTZ) is commonly used as a convulsant drug. The enhanced seizure susceptibility induced by kindling is probably attributable to plastic changes in the synaptic efficacy. Adenosine and guanosine act both as important neuromodulators and neuroprotectors with mostly inhibitory effects on neuronal activity. Adenosine and guanosine can be released *per se* or generated from released nucleotides (ATP, ADP, AMP, GTP, GDP, and GMP) that are metabolized and rapidly converted to adenosine and guanosine. The aim of this study was to evaluate nucleotide hydrolysis by ecto- and soluble nucleotidases (hippocampal slices and CSF, respectively) after PTZ-kindling (stages 3, 4, or 5 seizures) or saline treatment in rats. Additionally, the levels of purines in rat cerebrospinal fluid (CSF), as well as ecto-NTPDases (1, 2, 3, 5, 6 and 8) and ecto- 5′-nucleotidase expression were determined. Ecto-enzyme assays demonstrated that ATP, AMP, GDP, and GMP hydrolysis enhanced when compared with controls. In addition, there was an increase of ADP, GDP, and GMP hydrolysis by soluble nucleotidases in PTZ-kindling rats compared to control group. The HPLC analysis showed a marked increase in PTZ-kindled CSF concentrations of GTP, ADP, and uric acid, but GDP, AMP, and hypoxanthine

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concentrations were decreased. Such alterations indicate that the modulatory role of purines in CNS could be affected by PTZ-kindling. However, the physiological significance of these findings remains to be elucidated.

Introduction

Epilepsy is a common and chronic neurological disorder characterized by recurrent unprovoked seizures. Epileptic activity is based on broken excitatory-inhibitory balance in the brain tissue (Mody, 1999). It is a complex mechanism that leads to epileptic firing, and involves several transmitters and modulators in the brain. Epilepsy is a relatively common neurological condition, affecting about 1% of the population worldwide. There is a significant group of patients (20—30%) resistant to the currently available therapeutic agents (Morimoto et al., 2004). Chemical kindling is widely used as an experimental model of epilepsy and epileptogenesis. This phenomenon is characterized by progressive intensification of seizures activity after repeated administration of doses of different central nervous system (CNS) stimulants, including pentylenetetrazol (PTZ) (Racine, 1972). Pentylenetetrazol is commonly used as a convulsant drug, acting as a $GABA_A$ antagonist as well as by altering potassium permeability of the cell membrane via a voltage-dependent mechanism. Studies have shown that the mechanisms involved in PTZ kindling may include a decrease in central GABAergic function (Berman et al., 2000). The enhanced seizure susceptibility induced by kindling is probably attributable to plastic changes in the synaptic efficacy (Morimoto et al., 2004).

In the CNS, adenosine and guanosine act both as important neuromodulators with major inhibitory effects on neuronal activity. In the brain, the development and spread of seizures is thought to be prevented by a tonic anticonvulsant effect mediated by released adenosine via adenosine A_1 receptor (Dunwiddie and Masino, 2001; Avsar and Empson, 2004; Fedele et al., 2006; Pagonopoulou et al., 2006). Guanosine is another purine nucleoside that has only recently been shown to exert anticonvulsant properties and to increase glial glutamate uptake (Schmidt et al., 2000; Soares et al., 2004).

The nucleosides adenosine and guanosine can be released as well or generated from released nucleotides (ATP, ADP, AMP, GTP, GDP and GMP) that are metabolized and rapidly converted to adenosine and guanosine. There is evidence for the vesicular release of ATP and other nucleotides from nerves in CNS (Fields and Burnstock, 2006). After the release, ATP acts producing an excitation or an inhibition of neurotransmission (Malva et al., 2003). The inactivation of ATP signaling is mediated by the action of ecto-nucleotidases. In this group of enzymes, it has been proposed that NTPDases and a 5′-nucleotidase constitute an enzymatic cascade able to promote the hydrolysis of triphosphate and diphosphate nucleotides to the respective nucleosides. In rats, this hydrolysis can occur in the CNS (Battastini et al., 1991; Cruz Portela et al., 2002) by the action of the same set of enzymes. Eight different enzymes are described as members of the NTPDase family (Robson et al., 2006; Zimmermann, 2006). NTPDases1, 2, 3 and 8 are ecto-enzymes (E-NTPDases), with the catalytic site facing to extracellular space. On the other hand, the enzymes classified as NTPDase4, 5, 6 and 7 present intracellular localization. Among them, NTPDases5 and 6 could present secreted forms (Robson et al., 2006). Ecto- 5′-nucleotidase is a GPI-anchored enzyme which was also described in rat CNS (Sadasivudu et al., 1980; Braun et al., 1994). This ecto-enzyme was identified on mossy fibers that sprout after seizures in kainate-treated and kindled rats (Schoen et al., 1999). In previous studies, we have shown the presence of ecto-nucleotidase pathway in CNS and its involvement in several pathological conditions, including seizures and epilepsy (Battastini et al., 1991; Bruno et al., 2003; Bonan et al., 2000a,b; Cruz Portela et al., 2002; Oses et al., 2004).

The aim of this study was evaluate the different adenine and guanine nucleotide hydrolysis promoted by ecto- and soluble nucleotidases in rat CNS after PTZ-kindling treatment. Additionally, we determined the levels of the purines in rat cerebrospinal fluid (CSF), as well as ecto-NTPDases (1, 2, 3, 5, 6 and 8) and 5′-nucleotidase expression in hippocampi of these animals.

Material and methods

Chemicals

Reagents were obtained as follows: nucleotides, Malachite Green Base, Coomassie Brilliant Blue G, Trizma base and PTZ from Sigma (St. Louis, MO, USA); anesthetic sodium thiopental from Cristalia (São Paulo, SP, Brazil). All other reagents were of analytical grade.

Animals

Female Wistar rats (60—90 days old; 150—250 g) were used. Animals were kept on a 12 h light:12 h dark cycle (lights on at 07:00 a.m.) at constant temperature of $22 \pm 1\,^{\circ}C$. They were housed in plastic cages (five animals per cage) with water and food *ad libitum*. In all experiments, institutional protocols with animals were performed to minimize suffering and limit the number of animals sacrificed.

Pentylenetetrazol-kindling treatment

In the PTZ-kindling model, a subconvulsant dose of PTZ (35 mg/kg, i.p.) was administered every 48 h for 20 days (10 stimulations). All the injections were performed during the day, between 10:00 and 12:00 h. Animals were observed for 30 min after each injection, and seizure severity was graded according to the following scale: 1, facial clonus; 2, head nodding; 3, myoclonic jerks; 4, rearing/falling seizures; 5, running/bouncing seizures [adapted from Racine, 1972]. Seizure intensity increased with each administration until animals reached stages 3, 4 or 5 seizures, in which they are considered kindled. Animals without convulsive behavior or in stages 1 or 2 seizures at the end of treatment were not used. Behavioral response during the early stages of kindling is characterized as a freezing response during the evoked ictal discharge; and at the end of treatment, kindled animals was able to progress into a generalized seizure. Ten days after the last injection the animals were

used for experimental procedures. The control group was treated with saline using the same protocol of PTZ group.

CSF sampling

Ten days after the last injection rats were anesthetized with 40 mg/kg of sodium thiopental, i.p., and the cerebrospinal fluid (CSF) was drawn (60—80 μL per rat), by direct puncture of the *cisterna magna* with an insulin syringe (27 gauge × 1/2 in. length). It was performed only one CSF sampling from each rat in the morning, and the samples were pooled in a single tube and stored for 1 h on ice (CSF volume pooled was approximately 200 μL). Individual samples that presented visible blood contamination were discarded. Before performing the assays, the pool was centrifuged at 4500 × *g* at 5°C for 5 min, to obtain cell-free supernatants. The supernatant was added to the reaction medium, and the enzymatic assay carried out within 2 h of the sampling (Cruz Portela et al., 2002).

Hippocampal slices

The rats were killed by decapitation and the brains were rapidly removed into a bicarbonate-buffered salt solution with the following composition: 115 mM NaCl, 3.0 mM KCl, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 10 mM glucose, 2.0 mM $CaCl_2$, pH 7.4, and gassed with 95% O_2 and 5% CO_2 mixture (incubation medium). The brains were cut longitudinally, their hippocampi dissected and slices transversely cut to 400 μm thick on a Mcllwain tissue chopper.

Assay for nucleotides hydrolysis by CSF

The reaction medium contained 2.0 mM $CaCl_2$, and 50 mM Tris—HCl buffer, pH 7.5, in a final volume of 200 μL. CSF (40 μL) was added to the reaction medium (4 μg of protein per tube), and pre-incubated for 10 min at 37°C. The reaction started by addition of ADP, GDP, AMP and GMP to a final concentration of 1.0 mM and stopped, after 300 min, by the addition of 200 μL of trichloroacetic acid 10%. The amount of released Pi was measured by the method described by Chan et al., 1986. Controls to correct for non-enzymatic hydrolysis were performed by adding CSF after stopping the reaction. Enzymatic activity was expressed as nmol of Pi/min/mg.

Assay for nucleotides hydrolysis by hippocampal slices

Two slices per tube (approx. 0.16 mg protein) were preincubated for 10 min at 37°C with 500 μL of the incubation medium (described above) and gassed directly with 95% O_2 and 5% CO_2. Adding ATP, ADP, AMP, GTP, GDP or GMP started the reaction, to a final concentration of 2.0 mM. The reaction was stopped by the addition of 100 μl of 10% trichloroacetic acid. Non-enzymatic Pi released from nucleotides into assay medium without slices and Pi released from slices without nucleotide were subtracted from total Pi released during incubation. All assays were performed in duplicate or triplicate. Pi was measured by the method of Chan et al., 1986 and the enzymatic activity was expressed as nmol of Pi/min/mg.

HPLC analysis of nucleotides in CSF

High-performance liquid chromatography (HPLC) was done to measure CSF concentrations of the following nucleotides and nucleosides: adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), adenosine (Ado), guanosine triphosphate (GTP), guanosine diphosphate (GDP), guanosine monophosphate (GMP), guanosine (Guo), inosine monophosphate (IMP), inosine (Ino), hypoxanthine (Hypo), xanthine (Xan), and uric acid (UA). Analyses were performed with Shimadzu Class-VP chromatography system consisting of a quaternary gradient pump with vacuum degassing and piston desalting modules, Shimadzu SIL-10AF auto injector valve with 50 μL loop, UV—vis detector. Separations were achieved on Discovery C18 250 mm × 4.6 mm, 5 μm particle size column (Supelco). The mobile phase flowed at a rate of 1.2 mL/min and column temperature was 24°C. Buffer composition remained unchanged (A: 150 mmol/L phosphate buffer, pH 6.0, containing 150 mmol/L potassium chloride; B: 15% acetonitrile in buffer A). The gradient profile was modified to the following content of buffer B in the mobile phase: 0% at 0.00 min, 2% at 0.05 min, 7% at 2.45 min, 50% at 10.00 min, 100% at 11.00 min, 100% at 12.30 min, 0% at 12.40 min. Samples of 5 μL were injected every 18 min into the injection valve loop. Absorbance was read at 254 nm. Concentrations were expressed as median in picomol/mg protein (Domanski et al., 2006).

Semi-quantitative RT-PCR

Total RNA from hippocampus was isolated with Trizol™ reagent (Invitrogen) in accordance with the manufacturer's instructions. The cDNA species were synthesized with SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen) from 3 μg of total RNA in a total volume of 20 μl with an oligo (dT) primer in accordance with the manufacturer's instructions. cDNA reactions were performed for 50 min at 42°C and stopped by boiling for 5 min. Two microliters of cDNA were used as a template for PCR with primers specific for E-NTPDases1, 2, 3, 5, 6, 8 and 5′-nucleotidase. As a control for cDNA synthesis, β-actin-PCR was performed. Two microliters of the cDNA were used for PCR in a total volume of 25 μl using a concentration of 0.4 μM of each primer indicated below, 200 μM of dNTPs and 1 U Taq polymerase (Invitrogen) in the supplied reaction buffer.

The PCR cycling conditions were as follows: initial 1 min denaturation step at 94°C, 1 min at 94°C, 1 min annealing step (NTPDase 1—3, 5, 6 and 5′nucleotidase: 65°C; NTPDase2: 66°C; β-actin: 58.5°C), 1 min extension step at 72°C for 35 cycles and a final 10 min extention at 72°C. The amplification products were: NTPDase1, 543bp; NTPDase2, 331bp; NTPDase3, 267 bp; 5′-nucleotidase, 405bp; β-actin, 210 bp. Primers for NTPDase8 (394 bp) were also used in this study. Seven microliters of the PCR reaction was analyzed on a 1% agarose gel. The following set of primers were used for NTPDase1: 5′-GAT CAT CAC TGG GCA GGA GGA AGG-3′ and 5′-AAG ACA CCG TTG AAG GCA CAC TGG-3′; for NTPDase2: 5′-GCT GGG TGG GCC GGT GGA TAC G-3′ and 5′-ATT GAA GGC CCG GGG ACG CTG AC-3′; for NTPDase3: 5′-CGG GAT CCT TGC TGT GCG TGG CAT TTC TT-3′ and 5′-TCT AGA GGT GCT CTG GCA GGA ATC AGT-3′; for NTPDase5: 5′-TGG TGG TAA CCA AGA AGG GGA GAT GG-3′ and 5′-GCA GGT GAA AGG TGG CTC CCA AGG-3’; for NTPDase6: 5′-GGC CTC TAC GAG CTG TGT GCC AGC AG-3′ and 5′-TCA GTA CCT TGT CCC CGG GAA AAC C-3′; for NTPDase8: 5′-CCA CAC TGT CAC TGG CTT CCT TG-3′ and 5′-ACG AGG ATG TAT AGG CCT GAG G-3′; for 5′-nucleotidase (CD73): 5′-CCC GGG GGC CAC TAG CAC CTC A-3′ and 5′-GCC TGG ACC ACG GGA ACC TT-3′; for β-actin: 5′-TAT GCC AAC ACA GTG CTG TCT GG-3′ and 5′-TAC TCC TGC TTC CTG ATC CAC AT-3′.

Protein determination

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

Statistical analysis

The data are represented as mean ± S.D. and the statistical analysis was performed with Student's *t*-test for NTPDase results. The data are shown as mean ± S.E. for HPLC results and the statistical analysis was performed using a non-parametric Mann—Whitney test. For all results, $P \leq 0.05$ was considered a significant difference between groups.

Results

PTZ-kindling produced a progressive increase in the seizure susceptibility of the treated rats. In the first injection the animals began with an average of 1.92 ± 0.26 in the kindling stage and after the 10th stimulation, the mean kindling stage was 4.45 ± 0.10. At the end of 20 days of treatment 11 animals reached stage 3, 51 stage 4 and 42 stage 5 of seizure ($N = 104$ animals) and were considered kindled. The convulsive behaviour was evaluated during 30 min each day after PTZ injection. It was observed that each animal experienced at least seven seizures during the treatment.

The study of the ecto-nucleotidase activities may be an interesting approach to understanding the hippocampal functional response in epileptic events. We determined the enzymatic activities for PTZ-kindled rats in stages 3, 4, and 5. These three stages were not different among them but were statistically different from the control group. For this reason PTZ-kindled rats were included in a single group namely kindling. In this investigation, hippocampal slices demonstrated an enhancement of ATP (151.00 ± 25.90 nmol Pi/min/mg), AMP (46.46 ± 5.90 nmol Pi/min/mg), GDP (4.43 ± 0.96 nmol Pi/min/mg), and GMP (4.3 ± 1.08 nmol Pi/min/mg) hydrolysis when compared with controls (115.43 ± 21.90; 29.41 ± 1.97; 2.10 ± 0.42 and 2.04 ± 0.50 nmol Pi/min/mg, for ATP; AMP; GDP; and GMP, respectively). For ADP and GTP hydrolysis, there were no significant differences between controls and PTZ-kindled animals (ADP: 66.53 ± 5.58 and 61.47 ± 8.60; GTP 8.73 ± 0.50 and 10.09 ± 1.98 nmol Pi/min/mg for control and PTZ-kindled animals, respectively) (Fig. 1).

CSF analysis is a basic tool to study a number of parameters in the CNS, including the ongoing changes in the brain during acute and chronic diseases. In this sense we evaluated the hydrolysis of monophosphate and diphosphate nucleotides by soluble nucleotidases present in CSF. Soluble nucleotidases from PTZ-kindled animals promoted an increase of ADP (9.59 ± 2.79 nmol Pi/min/mg), GDP (46.34 ± 2.93 nmol Pi/min/mg), and GMP (6.87 ± 3.07 nmol Pi/min/mg) hydrolysis when compared with controls (6.12 ± 2.07; 40.72 ± 3.39; and 3.81 ± 1.75 nmol Pi/min/mg for ADP, GDP and GMP, respectively). AMP hydrolysis did not change after kindling treatment (control: 6.05 ± 0.8; PTZ-kindled rat: 5.35 ± 1.6 nmol Pi/min/mg) (Fig. 2). Additionally, we evaluated the levels of purine derivatives present in CSF. CSF levels for the purines GTP, ADP and the oxypurine uric acid were increased in PTZ-kindled animals in comparison with control rats (Table 1, Fig. 3). Moreover, the

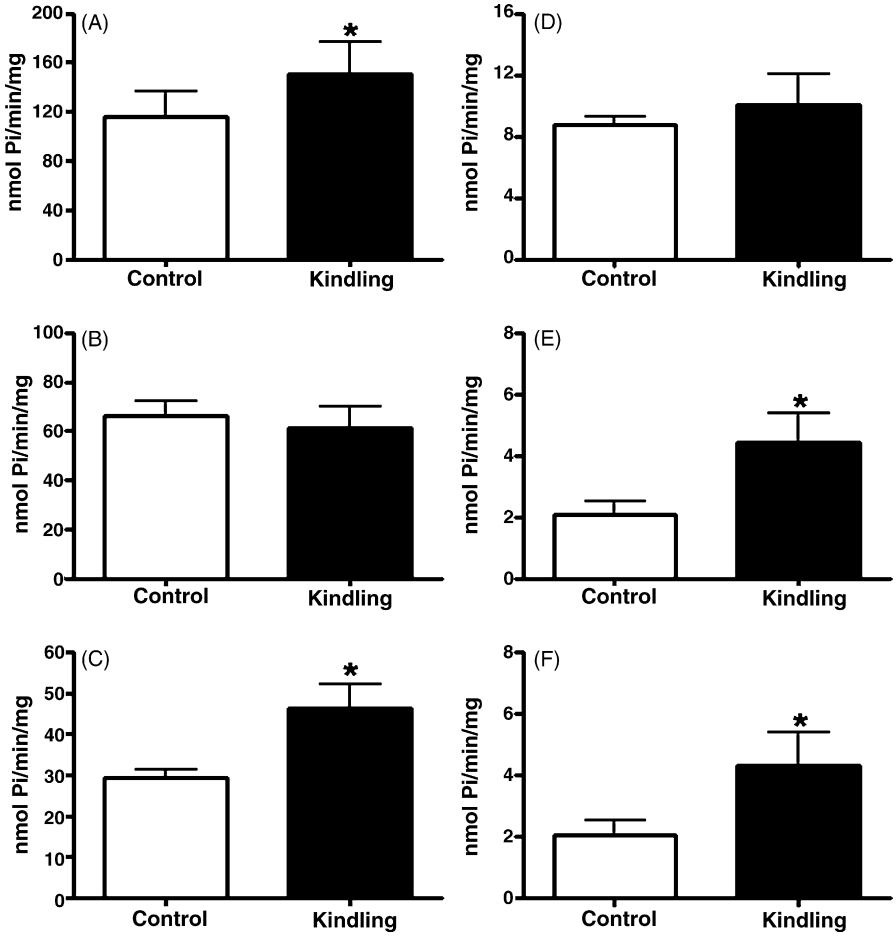

Figure 1 Effects of PTZ-kindling (stages 3, 4, or 5; black bars) when compared with control animals (white bars) on ATP (A), ADP (B), AMP (C), GTP (D), GDP (E) and GMP (F) hydrolysis in rat hippocampal slices 10 days after last injection. Bars represent mean $\pm$ S.D. of at least four animals. *PTZ-treated group significantly different from control group ($P \leq 0.05$, Student's t-test).

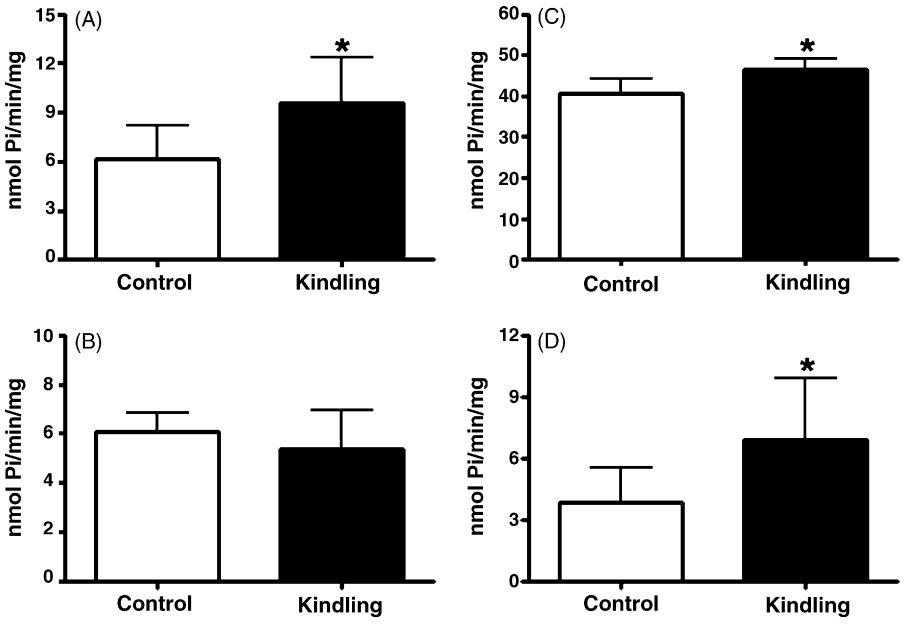

Figure 2 Effects of PTZ-kindling (stages 3, 4, or 5; black bars) when compared with control animals (white bars) on ADP (A), AMP (B), GDP (C) and GMP (D) hydrolysis in rat CSF 10 days after last injection. Bars represent mean ± S.D. of at least four animals. *PTZ-treated group significantly different from control group ($P \leq 0.05$, Student's t-test).

purines GMP, AMP and the oxypurine hypoxantine levels were decreased in the same animals when compared with control rats (Table 1, Fig. 3). PTZ-kindling treatment did not promote significant changes in the levels of other nucleosides analyzed (Table 1, Fig. 3).

Considering that the changes observed on ecto-nucleotidase activities could be promoted by transcriptional control, the relative expression of hippocampal ecto-enzymes (NTPDase1, 2, 3, 5, 6, 8, and 5′-nucleotidase) of control and PTZ-kindled rats has also been analyzed by semi-quantitative RT-PCR. We observed a significant decrease (50%) in the expression of NTPDase1 (Fig. 4A), but there were no changes in the transcript mRNA levels for the enzymes NTPDase2, 3, 5, 6 and 5′-nucleotidase in hippocampus from PTZ-kindled rats (Fig. 4B—F). NTPDase8 was not expressed in hippocampus of rats, which was in accordance to previous studies (data not show).

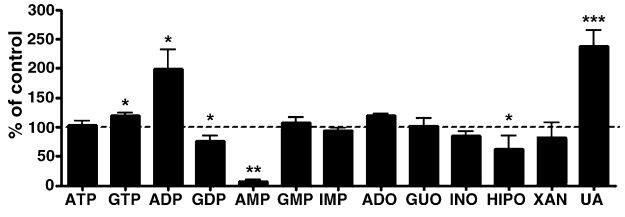

Figure 3 Relative nucleotides, nucleosides and oxypurines levels, when compared with control in rat CSF after PTZ-kindling (stages 3, 4, or 5). ATP, GTP, ADP, GDP, AMP, GMP, IMP, adenosine (ADO), guanosine (GUO), inosine (INO), xantine (XAN), hypoxantine (HIPO) and uric acid (UA) were measured 10 days after the last injection. Data represent mean ± S.E. ($n \geq 4$) *$P \leq 0.05$, **$P \leq 0.01$ and ***$P \leq 0.001$, as compared to control, using a non-parametric Mann—Whitney test.

Discussion

Previous studies have described ATP, ADP, and AMP hydrolysis by slices from the CNS of rats (Bruno et al., 2002). For ATP and ADP hydrolysis (Fig. 2A and B), our results suggests a possible participation of NTPDase3 or NTPDase8, since ATP hydrolysis is approximately two times higher than ADP hydrolysis (Fig. 1A and B) (Robson et al., 2006). We excluded the presence of NTPDase8, since this enzyme was not detected in rat hippocampus as previously described (Bigonnesse et al., 2004). Our results have shown that PTZ-kindling was able to alter ATP and AMP hydrolysis, but not ADP hydrolysis (Fig. 1A—C). ATP may influence several P1 and P2 receptors-mediated processes by controlling extracellular concentrations of ATP and adenosine (Bruno et al., 2002b). ATP may modulate the release and/or influence other neurotransmitters either by acting through its own receptors or by altering the neurotransmitter receptors (Fields and Burnstock, 2006). Several works have demonstrated an involvement of ATP hydrolysis in other animal models of seizures and epilepsies (Anderson et al., 1994; Fernandes et al., 1996; Nagy et al., 1997; Bruno et al., 2002a; Bruno et al., 2003; Nicolaidis et al., 2005; Oses et al., 2004). Adaptive plasticity in chronic epilepsy could involve nucleotide homeostasis, and this balance can be achieved through a delicate regulation of the amount of ATP released and of the rate of ATP hydrolysis by ecto-enzymes.

In addition, we evaluated the GTP, GDP, and GMP hydrolysis in PTZ-kindled slices, but just GDP and GMP hydrolysis was affected (Fig. 1D—F). The guanosine, generated by hydrolysis of these nucleotides, stimulates the glutamate uptake and protect against seizures induced by glutamatergic agents (Lara et al., 2001; Soares et al., 2004). Therefore, these results suggest an involvement of NTPDase5 and

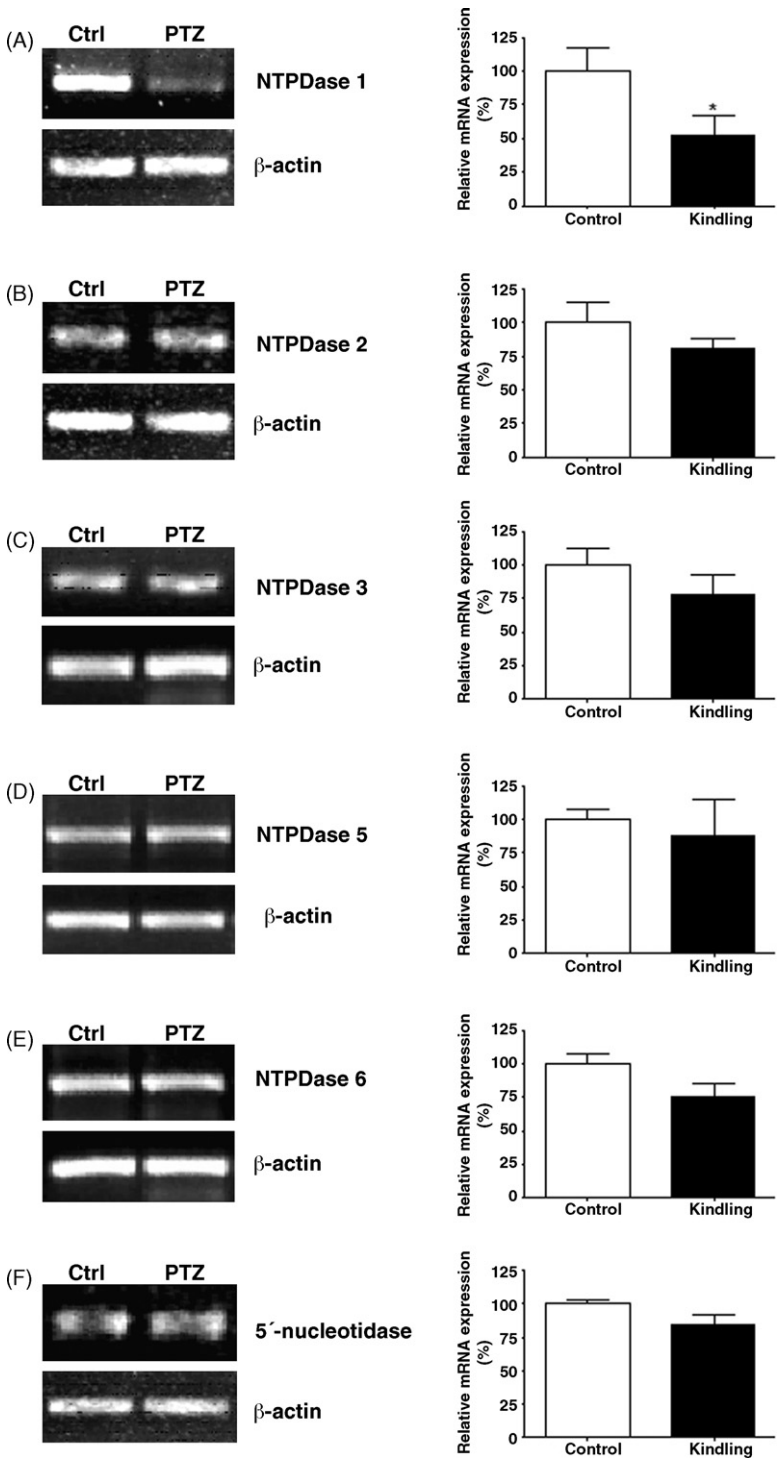

Figure 4 Representative semi-quantitative RT-PCR mRNA for NTPDase1, 2, 3, 5, 6 and 5′-nucleotidase from hippocampus of saline (Ctrl) and PTZ-kindled (stages 3, 4, or 5; PTZ) rats. The animals were sacrificed 10 days after the last injection and the samples were stored at −70 °C until PCR-analysis. The expression was evaluated for NTPDases to β-actin mRNA ratio. Three independent experiments were performed with entirely consistent results.

6, since these enzymes present a preference for guanine nucleotides.

The kinetic effects observed on adenine and guanine nucleotide hydrolysis after PTZ-kindling could be a consequence of transcriptional control. To assess the effects of PTZ-kindling on the expression of NTPDase1, 2, 3, 5, 6 and 5′-nucleotidase in the hippocampi of rats, we evaluated the mRNA levels of these enzymes with the use of RT-PCR. For NTPDase 2, 3, 5, 6 and 5′-nucleotidase, the results have shown that PTZ-kindling did not alter the enzyme expression (Fig. 4B—F), suggesting that the effects observed were not due to a decreased synthesis of these

Table 1 Nucleotides, nucleosides and oxypurines levels in control and treated rats CSF

Compound	Treatment	Compound level (picomol/μL) (mean ± S.E.)
ATP	Control	244 ± 24
	Kindling	252 ± 18
GTP	Control	0.97 ± 0.09
	Kindling	1.15 ± 0.05*
ADP	Control	317 ± 20
	Kindling	633 ± 104*
GDP	Control	257 ± 134
	Kindling	197 ± 22*
AMP	Control	150 ± 67
	Kindling	10 ± 4**
GMP	Control	172 ± 13
	Kindling	186 ± 25
IMP	Control	970 ± 43
	Kindling	912 ± 44
ADO	Control	851 ± 155
	Kindling	1016 ± 23
GUO	Control	263 ± 37
	Kindling	269 ± 34
INO	Control	870 ± 127
	Kindling	737 ± 73
HYPO	Control	436 ± 92
	Kindling	273 ± 98*
XAN	Control	1995 ± 614
	Kindling	1648 ± 504
URIC ACID	Control	1075 ± 140
	Kindling	2560 ± 291***

$^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ and as compared to control, using a non-parametric Mann–Whitney test.

proteins. Despite the ATP and GDP hydrolysis increased in PTZ-kindled slices, the levels of the NTPDase1 mRNA have been decreased (Fig. 4A). The mechanism that could explain the up-regulation of NTPDase1 activity and at the same time down-regulation of transcriptional levels is known as negative feedback autoregulatory loop. This mechanism allows for genes that are not transcription factors to negatively regulate their own synthesis (Krishna et al., 2006). Several works have proposed putative mechanisms that involve a negative feedback control associated with long-term synaptic changes in epilepsies (Simonato, 1993; Qian et al., 1994; Vreugdenhil et al., 1999).

Other NTPDases members can contribute to extracellular nucleotide metabolism. Soluble NTPDases were described in CSF and there is an increased activity during seizures induced by PTZ (Cruz Portela et al., 2002; Oses et al., 2004). Thus, we evaluated the nucleotidase activities present in CSF, demonstrating an increase in ADP, GDP and GMP hydrolysis (Fig. 2A, C and D). The increment in hydrolysis of purine nucleotides after epileptic episode suggests that this CSF enzymatic system may have a biological relevance in the regulation of purine levels and also could be a putative marker of brain injuries (Bonan et al., 2000a,b; Bruno et al., 2002b and 2003; Oses et al., 2004).

The analysis of nucleotides, nucleosides and oxypurines levels in CSF by HPLC showed a marked increase in PTZ-kindled CSF concentrations of GTP, ADP and uric acid when compared with controls (Fig. 3). By the other hand, CSF concentrations of GDP, AMP and hypoxantine appear decreased (Fig. 3). Increase in ADP levels agree with the increment of ATPase activity (Fig. 1A), but not change ATP levels (Fig. 3). It could be explained by previous studies showing that the ATP levels increase followed by a concomitant enhancement of ATPase activity after electrical stimulation (Wieraszko and Seyfried, 1989). The marked decrease in AMP levels are in agreement with the data obtained for 5′-nucleotidase activities (Fig. 1C). It has been observed an increase of GTP levels and a decrease of GDP levels, which corroborate with results obtained in nucleotidase assays (Figs. 1 and 2). The lack of nucleosides accumulation could be the result of extracellular mechanisms that are responsible for the control of their concentrations, such as the nucleoside uptake by a specialized transport system. The enhanced uric acid and the decreased hypoxantine found in the CSF of these animals may not be solely due to increased hemato-encephalic barrier permeability, but may be the result of the catalysis by xanthine oxidase (Rodriguez-Nunez et al., 2003). Moreover, these results can be reflecting a glutamate-mediated excitotoxicity, since the uric acid levels were increased after neurological damage (Stover et al., 1997; Rodriguez-Nunez et al., 2003). Therefore, measurements of nucleotide and nucleoside levels can constitute an important contribution to the knowledge of the role of purines in epilepsy.

In summary, our results demonstrated an enhancement of ecto- and soluble nucleotidase activities in hippocampal brain slices and CSF after PTZ-kindling, with distinct influence on the levels of nucleotides, nucleosides and oxypurines and also hippocampal NTPDases mRNA levels. Such alterations indicate that the modulatory role of purines could be affected by PTZ-kindling. However, the physiological significance of these findings remains to be elucidated.

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