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Chronic stress effects on adenine nucleotide hydrolysis in the blood serum and brain structures of rats

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Abstract

We have previously observed that adenosine 5′-diphosphate (ADP) hydrolysis was decreased 25% in spinal cord synaptosomes of chronically stressed male rats, while no changes were observed in ATPase activity. In the present study, we investigated the effect of chronic stress on the hydrolysis of adenine nucleotides in two cerebral structures (frontal cortex and hypothalamus) and in the blood serum of male rats. Adult male Wistar rats were submitted to 1-h restraint stress/day for 45 days (chronic) and were sacrificed 24 h after the last session of stress. Adenosine 5′-triphosphate (ATP) or ADP hydrolysis was assayed in the synaptosomal fraction obtained from the frontal cortex and hypothalamus of control and chronically stressed animals. No effects on ADP or ATP hydrolysis were observed in any of the cerebral structures analyzed after chronic stress. On the other hand, reduced ADP hydrolysis was observed in the blood serum of chronic stressed rats. It is possible that the effects observed in the blood serum may represent an adaptation to chronic stress and may reflect different functions of nucleotides and/or enzymes in these tissues. It is possible that altered levels of ADPase activity in the serum may be a biochemical marker for chronic stress situations.

Keywords: Apyrase; Chronic stress; Nucleotide hydrolysis; Rat blood serum; Hypothalamus; Cerebral cortex

1. Introduction

Adenosine 5′-triphosphate (ATP) is a purine nucleotide found in millimolar concentrations in virtually all cells. In addition to its well-established role in cellular metabolism, extracellular ATP and its breakdown products, adenosine diphosphate (ADP) and adenosine, have pronounced effects in a variety of biological processes, including neurotransmission, muscle contraction, cardiac and platelet function, vasodilatation and liver glycogen metabolism (Agteresch et al., 1999).

ATP is recognized as a neurotransmitter in sympathetic, parasympathetic and sensory nerves in the periphery, as well as in the central nervous system (CNS) (Edwards et al., 1992). The receptors for ATP, P2-puriceptors, have been described in virtually every major organ and/or tissue system that has been studied (Burnstock and Williams, 2000). Apart from neuronal release as a transmitter or a cotransmitter, there are several other sources of extracellular ATP. ATP is a ubiquitous intracellular constituent, and, therefore, any cell could potentially provide it. However, the more likely role of released ATP may be to act as a neurotransmitter in both peripheral and central neurons (for a review of ATP, see Cunha and Ribeiro, 2000).

Besides ATP, its breakdown product adenosine has also several functions within the CNS, which involve an inhibitory tone of neurotransmission and neuroprotective actions in pathological conditions (Latini and Pedata, 2001). Adenosine is particularly well suited to be used as a transcellular messenger to signal metabolic imbalance. Several reports have documented an increased in the extracellular concen-

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tration of adenosine upon stressful metabolic challenges (Latini and Pedata, 2001), and the term "retaliatory metabolite" has been coined for this homeostatic role of adenosine, which occurs in virtually all cell types. (For a review on adenosine, see Cunha, 2001.)

Extracellular nucleotides can be hydrolyzed by a variety of enzymes that are located on the cell surface, or may be soluble in the interstitial medium or within body fluids (Zimmermann, 2001). In addition, soluble nucleotidases, which also break down ATP and adenosine, have also been shown to be released from sympathetic nerves (Todorov et al., 1997). Work of the past few years has demonstrated that members of several families of ectonucleotidases can contribute to the extracellular hydrolysis of nucleotides. Nucleoside 5′triphosphate (NTP) and nucleoside 5′diphosphate (NDP) may be hydrolyzed by members of the E-NTPDase (ectonucleoside triphosphate diphosphohydrolase) family, E-NPP (ectonucleoside pyrophosphatase/phosphodiesterase) family and by alkaline phophatases (Zimmermann, 2001). These ectonucleotidases, together with 5′-nucleotidase, control the availability of ligands (ATP, ADP, AMP and adenosine) for both nucleotide and nucleoside receptors, and, consequently, the duration and extent of receptor activation (Chen and Guidotti, 2001). Therefore, this cascade of ectonucleotidases is an enzymatic pathway with a double function of removing a signal (ATP) and generating a second one (adenosine). This cascade may play a role in the effective regulation of several processes, because they have considerable plasticity in different pathophysiological situations (Agteresch et al., 1999) including aging (Cunha et al., 2001). These enzymes may also have a protective function by keeping extracellular ATP/ADP and adenosine levels within physiological levels (Agteresch et al., 1999).

The physiological response to emotional or physical stress consists of an integration of endocrine and autonomic changes. In this situation, adrenomedullary epinephrine is released, and hormones such as CRH, ACTH and glucocorticoids are released by the hypothalamic–pituitary–adrenocortical (HPA) axis (Sapolsky, 1992). Besides controlling the HPA axis, the hypothalamus is also responsible for the integration of autonomic endocrine and even behavioral responses to stress (for a review, see Herman and Cullinan, 1997). Inputs from hypothalamic homeostasis get high priority, as the HPA system contributes to redistribution of bodily resources in times of physiologic need. HPA responses are affected by the cortex function. Restraint, fear conditioning or exposure to a novel environment, for example, are affected by lesions of the prefrontal cortex (for a review, see Herman and Cullinan, 1997). Exposure to a wide variety of stressors causes marked increase in neuronal and genomic activation of cortical neurons (for a review, see Sullivan and Gratton, 2002).

As mentioned above, extracellular adenosine concentrations have been observed to be increased upon stressful challenges (Latini and Pedata, 2001), including exposure to inescapable shock (Minor et al., 2001). Alterations of enzyme activities involved in nucleotide hydrolysis have also been reported in the spinal cord after repeated restraint stress (Torres et al., 2002). In this study, we investigated the effect of chronic restraint stress on the ATP, ADP and AMP hydrolysis in the blood serum, as well as ATP, ADP hydrolysis in synaptosomal fractions from the hypothalamus and cerebral cortex (two structures involved in the regulation of the HPA axis) of adult male Wistar rats.

2. Method

2.1. Animals

Adult male Wistar rats (60 days at the beginning of the treatment), weighing 150–230 g, were used. Experimentally naive animals were housed in groups of five in home cages made of Plexiglas material (65 × 25 × 15 cm) with the floor covered with sawdust. They were maintained under a standard dark–light cycle (light on between 07:00 and 19:00 h) at a room temperature of 22 ± 2 °C. The rats had free access to food (standard lab rat chow) and water, except during the period when restraint stress was applied. The restraint procedure was performed between 10:00 and 12:00 h.

2.2. Chronic-restraint stress procedure

The animals were divided in two groups: stressed and control. Restraint was applied by placing the animals in a 25 × 7-cm plastic bottle and fixing it with plaster tape on the outside, so that the animal was unable to move. There was a 1-cm hole at one far end for breathing. The animals were stressed 1 h/day, 5 days/week for 45 days (Ely et al., 1997). After the stress procedure, the animals were returned to the home cages. Control animals were kept in their home cages during the period of treatment.

2.3. Subcellular fractionation

The animals were killed by decapitation 24 h after the last stress session. The brain was rapidly removed and the hypothalamus and cerebral cortex were dissected and gently homogenized in 10 vol of ice-cold medium consisting of 320 mM sucrose, 0.1 mM EDTA and 5.0 mM HEPES, pH = 7.5, with a motor-driven Teflon-glass homogenizer. The synaptosomes were isolated as described previously (Nagy et al., 1984). Briefly, 0.5 ml of the crude mitochondria fraction was mixed with 4.0 ml 8.5% Percoll solution and was layered onto an isosmotic Percoll sucrose discontinuous gradient (10%/20%). The synaptosomes that banded at the 10%/20% Percoll interface were collected with wide tip disposable plastic transfer pipettes. The material was prepared fresh daily and maintained at 0–4 °C throughout the preparation.

2.4. Determination of ATP and ADP hydrolysis in synaptosomes from distinct brain structures

For brain structures, the reaction medium used to assay ATP and ADP hydrolysis was described previously (Battastini et al., 1991) and contained 5.0 mM KCl, 1.5 mM CaCl2, 0.1 mM EDTA, 10 mM glucose, 225 mM sucrose and 45 mM Tris–HCl buffer, pH 8.0, in a final volume of 200 μl. The enzyme preparation (10–20 μg protein) was added to the reaction mixture and preincubated for 10 min at 37 °C. For the evaluation of synaptosomes from the hypothalamus, a pool of hypothalamus from three rats was used in each assay. The reaction was initiated by the addition of ATP or ADP to a final concentration of 1.0 mM and was stopped by the addition of 200 μl 10% trichloroacetic acid. The samples were chilled on ice for 10 min, and 100-μl samples were taken for the assay of released inorganic phosphate (Pi) (Chan et al., 1986). Enzyme activities were expressed as nanomoles of phosphate released per minute per milligram of protein.

2.5. Isolation of the blood serum fraction

Trunk blood was drawn after decapitation of the animals, 24 h after the last stress session. Blood samples were centrifuged in plastic tubes for 5 min at 3000 × *g* at room temperature. The serum was separated, and it was used in the enzyme assay immediately.

2.6. Determination of ATP, ADP and AMP hydrolysis in the blood serum

ATP and ADP hydrolysis was performed using a modification of the method described by Yegutkin (1997). The reaction mixture containing ADP or ATP as substrate, 112.5 mM Tris–HCl, pH 8.0, was incubated with 1.0–1.5 mg protein serum at 37 °C in a final volume of 200 μl. The reaction was stopped by the addition of 200 μl 10% TCA. The amount of Pi liberated was measured by the method of Chan et al. (1986).

The reaction mixture containing AMP as a substrate in 100 mM Tris–HCl, pH 7.5, was incubated with 1.0–1.5 mg protein serum at 37 °C in a final volume of 200 μl. All other procedures were the same as for ATP and ADP hydrolysis, as described above.

For all enzyme assays, incubation times and protein concentration were chosen in pilot studies to ensure the linearity of the reactions. Controls with the addition of the enzyme preparation after the reaction was stopped with trichloroacetic acid were used to correct nonenzymatic hydrolysis of the substrates. All samples were run in duplicate. Enzyme activities were expressed as nanomoles of phosphate released per minute per milligram of protein.

2.7. Protein determination

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

2.8. Statistical analysis

Data were expressed by means ± standard error of the mean and analyzed by Student's *t* test.

3. Results

3.1. Experiment 1. Effect of chronic stress on hydrolysis of ATP and ADP in brain structures of rats

The effects of chronic stress on hydrolysis of ATP and ADP were assessed in brain structures 24 h after the last stress session. The hydrolysis of these nucleotides was not affected by chronic restraint stress in synaptosomes of hypothalamus and cerebral cortex (Student's *t* test, $P > .05$) (Table 1). The ATPase/ADPase ratio also showed no differences between groups (Student's *t* test, $P > .05$) (Table 1).

3.2. Experiment 2. Effect of chronic stress on hydrolysis of adenine nucleotides in the blood serum of rats

The effects of chronic stress on hydrolysis of ATP, ADP and AMP were evaluated in the blood serum. As shown in Table 2, ATPase and 5′-nucleotidase activities remained unaltered after chronic stress (Student's *t* test, $P > .05$). ADPase activity, however, was significantly decreased after

Table 1
Effect of chronic stress on ATP and ADP hydrolysis in synaptosomes from hypothalamus and cerebral cortex

	Hypothalamus		Cerebral cortex	
	Control	Stressed	Control	Stressed
ATPase	156.65 ± 20.56 (3)	166.06 ± 36.37 (3)	122.36 ± 9.89 (7)	121.99 ± 10.78 (7)
ADPase	27.41 ± 2.41 (3)	27.65 ± 1.78 (3)	23.47 ± 1.99 (7)	27.41 ± 2.41 (7)
ATPase/ADPase ratio	5.87 ± 0.25 (3)	5.88 ± 0.97 (3)	5.32 ± 0.40 (7)	4.14 ± 0.54 (7)

Values are means ± S.E.M. specific activity (picomoles of phosphate released per minute per milligram of protein). The number of assays per group is enclosed in parentheses. There were no differences between the groups (Student's *t* test, $P > .05$).

Table 2
Effect of chronic stress on the hydrolysis of ATP (ATPase), ADP (ADPase) and AMP (5′-nucleotidase), and on ATPase/ADPase ratio in the blood serum of rats

	Control	Stressed
ATPase	1.55 ± 0.17 (12)	1.47 ± 0.17 (11)
ADPase	1.76 ± 0.17 (12)	1.28 ± 0.12 (11) *
5′-Nucleotidase	1.73 ± 0.11 (17)	1.73 ± 0.06 (17)
ATPase/ADPase ratio	0.88 ± 0.04 (12)	1.15 ± 0.11 (11) **

The number of assays per group is enclosed in parentheses. Values are means ± S.E.M. specific activity (nanomoles of phosphate released per minute per milligram of protein).

* Significantly different from the control group (Student's t test, $P < .05$).

** Indicates significant difference from the ratio in the control group (Student's t test, $P < .05$).

chronic stress, remaining at 70% of control (Student's t test, $P < 0.01$). Accordingly, when these results are expressed as ATPase/ADPase ratios, it was observed an increased ratio in the blood serum of chronically stressed rats, consistent with the decreased ADPase activity (Table 2).

4. Discussion

In the present study, among the parameters studied, only the ADP hydrolysis in the blood serum was altered by chronic stress. When analyzing enzyme activities in the synaptosomal fraction of brain regions thought to be involved in the stress response, no effects were observed in chronically stressed rats, either on ATP or ADP hydrolysis. This was a surprising result, because adenosine is one of the main neuromodulators associated with cell stress (Cunha, 2001). Indeed, it is known that adenosine A1 receptors are induced by glucocorticoids (Svenningsson and Fredholm, 1997). Furthermore, the expression of mRNA for adenosine A1 receptors is significantly decreased after adrenalectomy (Svenningsson and Fredholm, 1997). Enhanced release of adenosine has also been demonstrated in brain tissue after exposure to stress. For example, inescapable shocks, as well as metabolic stress (glucoprivation by 2-deoxy-D-glucose administration), have been suggested to alter extracellular adenosine levels in the brain (Minor et al., 2001). In our work, no effect was observed in brain structures in the enzymes analyzed. Two points should be considered—that different stressors were used and, especially, that repeated restraint could lead to a process of adaptation that may cause different effects compared to those observed with acute stress. Chronically stressed animals do not experience all the hormonal consequences that animals exposed to one single stress episode do (Hashiguchi et al., 1997; Torres et al., 2001), and this phenomenon of adaptation to chronic stress may be reflected in several biochemical and physiological processes. A previous work showed that chronically stressed male rats present a decreased ADP hydrolysis in synaptosomes from the spinal cord (Torres et al., 2002). Therefore, alterations that can be observed in particular tissues or structures may reflect the different functions of the nucleotides and enzymes in different regions.

It was also shown that hydrolysis of ADP was altered in the blood serum of chronically stressed male rats. It has been demonstrated that stimulation of endothelial cells from umbilical vein by shear stress induces the release of endogenous adenosine triphosphate (ATP) (Bodin and Burnstock, 2001). It was suggested that the release of ATP from these cells, like that of nerve cells, is probably by vesicular exocytosis (Bodin and Burnstock, 2001). This phenomenon is accompanied by an extracellular increase in the activity of enzymes degrading both ATP (ATPases) and AMP (5′-nucleotidase) (Yegutkin and Burnstock, 2000). In addition, it was suggested that the enzymes, which are released during shear stress, are not released from an intracellular compartment together with ATP, but have an extracellular origin. In the present work, different results were observed, which could be determined by differences between in vitro and in vivo studies and between chronic and acute stress exposure.

In this study, it is important to note that the apparent dissociation between the two substrates (ATP and ADP) by the serum enzymes may be due to the simultaneous presence of two different enzymes involved in ATP hydrolysis, as described in other tissues, named an E-NTPDase and an ecto-ATPase (Zimmermann, 1996). However, just one of these two enzymes, the E-NTPDase, is implicated in ATP–ADP hydrolysis until AMP (Zimmermann, 1996). Inhibition of extracellular ADP hydrolysis, as observed in chronically stressed male rats, could induce decrease in adenosine production from extracellular ATP breakdown, because in this condition, there is a decrease in AMP levels and AMP is a substrate for 5′-nucleotidase.

Since we observed a difference in the activity of E-NTPDase in the serum of chronic stressed animals, and because there are different forms for this group of enzymes, probably, we are dealing with its soluble form, E-NTPDase 5 and/or E-NTPDase-6 (for a review, see Zimmermann, 2001). E-NTPDase-5 (or CD39-L4), for example, is secreted from mammalian cells and is soluble once secreted. It has specificity for NDPs over NTPs as substrates (Mulero et al., 1999). Its presence in macrophages indicates that this enzyme might be present in the blood and might have a role in modulating the levels of circulating ADP (Mulero et al., 1999). In addition, another nucleotide hydrolyzing enzyme has been reported to be present in the serum, the 5′-nucleotide phosphodiesterase (PDEase), which is able to promote the hydrolysis of both nucleotides, ADP and ATP (Sakura et al., 1998). The physiological function of this enzyme in the serum is still unclear, but it has been used as a marker of hepatoma (Tsou et al., 1982). The possible involvement of this enzyme in the hydrolysis of ADP cannot be completely discarded in our experimental conditions.

Considering time course determination for the inhibition of ADP hydrolysis, we have investigated the effect of acute stress on this parameter (to be published). No significant difference in ADP hydrolysis was observed immediately after 1 h restraint or 24 afterwards. Therefore, it is the repetition of the stress experience (chronic stress) that causes the decreases in ADP hydrolysis in the blood serum of rats at 24 h after the end of chronic stress treatment. It is not known, however, at what point of the 45 days of stress regimen this effect begins to manifest itself.

The ATPase/ADPase ratio may have an important role on the signaling properties of ATP (Chen and Guidotti, 2001). When an E-NTPDase is active, extracellular ATP is converted to AMP and then to adenosine by the action of a 5′-nucleotidase, and ADP is not an appreciable product. However, when E-NTPDase activity is reduced, as is the case in the blood serum from chronically stressed males, ATP could be converted to ADP by other ATPases, and this ADP would be relatively stable. Conversely, the ATPase activity did not change, probably due to an up-regulation of an ATPase that is coexpressed with the ATP diphosphohydrolase (Zimmermann, 1996; Sakura et al., 1998).

A cascade of nucleotidases may play a role in the effective regulation of several processes and may also have a protective function by keeping extracellular ATP and adenosine values within physiological levels (Agteresch et al., 1999). In the blood serum, adenine nucleotides have been implicated in several functions. ATP has been suggested to play a role in vascular tone, cardiac function and renal epithelial transport (Ravelic, 2000). In addition, other functions of extracellular adenine nucleotides include ADP-induced platelet aggregation (Hoylaerts et al., 2000). ADP is a potent platelet-recruiting factor and induces platelet aggregation via interaction with two P2 platelet receptors; a $P2Y_1$ receptor linked to phospholipase C pathways. ADP induces not only platelet shape change, exposure of fibrinogen binding sites and aggregation, but also the influx and intracellular mobilization of Ca^{2+}. The $P2Y_T/P2Y_{12}$ receptor is negatively coupled to adenylate cyclase, which mediates degranulation and sustained aggregation (for a review, see Puri, 1999). Hydrolysis of ADP by nucleotidases present in the serum inhibits platelet aggregation by removing ADP and by forming adenosine, which also inhibits aggregation (Zimmermann, 1999). In this context, the change of the ADP hydrolysis observed in the present study in the blood serum is very interesting. Since stress is one of the factors involved in atherosclerosis, and being ADP a signaling molecule, which activates platelet aggregation, the increase in ADP concentration in the serum of chronically stressed animals, as suggested here, may indicate the role of this factor in the etiology of atherosclerosis.

In conclusion, ADPase activity in the blood serum was altered in chronically stressed male rats. It is tempting to propose that the altered levels of ADPase activity in the serum may be a biochemical marker for chronic stress situations. Resolution of the possibility that it could be also a promoter of the stress-induced atherosclerosis will require additional work to further comprehend the involvement of nucleotide hydrolyzing enzymes in the blood of chronically stressed male rats.

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References

Agteresch HJ, Dagnelie PC, van den Berg JW, Wilson JL. Adenosine triphosphate: established and potential clinical applications. Drugs 1999;58:211–32.

Battastini AMO, da Rocha JBT, Barcellos CK, Dias RD, Sarkis JJF. Characterization of an ATP diphosphohydrolase (EC 3.6.1.5) in synaptosomes from cerebral cortex of adult rats. Neurochem Res 1991;16:1303–10.

Bodin P, Burnstock G. Evidence that of adenosine triphosphate from endothelial cells during increased shear stress is vesicular. J Cardiovasc Pharmacol 2001;38:900–8.

Bradford MMA. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem 1976;72:218–51.

Bunstook G, Willians M. P2 purinergic receptors: modulation of cell function and therapeutic potential. J Pharmacol Exp Ther 2000;295:862–9.

Chan K, Delfert D, Junger KD. A direct colorimetric assay for Ca^{+2}-ATPase activity. Anal Biochem 1986;157:375–80.

Chen W, Guidotti G. Soluble apyrases release ADP during ATP hydrolysis. Biochem Biophys Res Commun 2001;282:90–5.

Cunha RA. Adenosine as a neuromodulator and as a homeostatic regulator in nervous system: different roles, different sources and different receptors. Neurochem Int 2001;38:107–25.

Cunha RA, Ribeiro JA. ATP as a presynaptic modulator. Life Sci 2000;68:119–37.

Cunha RA, Almeida T, Ribeiro JA. Parallel modification of adenosine extracellular metabolism and modulatory action in the hippocampus of aged rats. J Neurochem 2001;76:371–82.

Edwards FA, Gibb AJ, Colquhoun D. ATP receptor-mediated synaptic currents in the central nervous system. Nature 1992;359:144–7.

Ely DR, Dapper V, Marasca J, Correa JB, Gamaro GD, Xavier MH, et al. Effects of restraint stress on feeding behavior of rats. Physiol Behav 1997;61:395–8.

Hashiguchi H, Ye HSH, Morris M, Alexander N. Single and repeated environmental stress: effect on plasma oxytocin, corticosterone, catecholamines and behavior. Physiol Behav 1997;61:731–6.

Herman JP, Cullinan W. Neurocircuitry of stress: central control of the hypothalamo-pituitary–adrenocortical axis. TINS 1997;20:78–84.

Hoylaerts MF, Oury C, Toth-Zsamboki E, Vermylen J. ADP receptors in platelet activation and aggregation. Platelets 2000;11:307–9.

Latini S, Pedata F. Adenosine in the central nervous system: release mechanisms and extracellular concentrations. J Neurochem 2001;79:463–84.

Minnor TR, Rowe MK, Soames Job RF, Ferguson EC. Escape deficits induced by inescapable shock and metabolic stress are reversed by adenosine receptor antagonists. Behav Brain Res 2001;120:203–12.

Mulero JJ, Yeung G, Nelken ST, Ford JE. CD39-L4 is a secreted human apyrase, specific for the hydrolysis of nucleoside diphosphates. J Biol Chem 1999;274:20064–7.

Nagy AK, Houser C, Delgado-Escueta AV. Rapid preparation of synaptosomes from mammalian brain using a nontoxic isosmotic gradient (Percoll). J Neurochem 1984;43:1114–23.

Puri RN. ADP-induced platelet aggregation and inhibition of adenylyl ciclase activity stimulated by prostaglandins. Biochem Pharmacol 1999;57:851–9.

Ravelic V. P2 receptors in the central and peripheral nervous systems moduling sympathetic vasomotor tone. J Auton Nerv Syst 2000;81: 205–11.

Sakura H, Nagashima S, Nagashima A, Maeda M. Characterization of fetal serum 5′nucleotide phosphodiesterase: a novel function as a platelet aggregation inhibitor in fetal circulation. Thromb Res 1998;91:83–9.

Sapolsky RM. An introduction to the adrenocortical axis. In: Sapolsky RM, editor. Stress, the Aging Brain, and the Mechanisms of Neuron Death. Bradford: Cambridge; 1992. p. 11–27.

Sullivan RM, Gratton A. Prefrontal cortical regulation of hypothalamic–pituitary–adrenal function in the rat and implications for psychopathology: side matters. Psychoneuroendocrinology 2002;27:99–114.

Svenningsson P, Fredholm BB. Glucocorticoids regulate the expression of A_1 but not A_{2A} receptors in rat brain. J Pharmacol Exp Ther 1997;280: 1094–101.

Todorov LD, Mihaylova-Todorov S, Westfall TD, Sneddon O, Kennedy C, Bjur RA, et al. Neuronal release of soluble and their role in neurotransmitter inactivation. Nature 1997;387:76–9.

Torres ILS, Gamaro GD, Silveira-Cucco SN, Michalowski MB, Corrêa JB, Perry MLS, et al. Effect of acute and repeated restraint stress on glucose oxidation to CO2 in hippocampal and cerebral cortex slices. Bras J Med Biol Res 2001;34:111–6.

Torres ILS, Buffon A, Silveira PP, Duarte MSZ, Bassani MG, Oliveira SS, et al. Effect of chronic and acute stress on ecto-nucleotidase activities in spinal cord. Physiol Behav 2002;75:1–5.

Tsou KC, Lo KW, Rosato EF, Yuk A, Enterline H, Schwegman C. Evaluation of 5′-nucleotide phosphodiesterase isozyme-V as a predictor for liver metastasis in breast cancer patients. Cancer 1982;50:191–6.

Yegutkin GG. Kinetic analysis of enzymatic hydrolysis of ATP in human and rat blood serum. Biochemistry (Moscow) 1997;62:619–22.

Yegutkin GG, Burnstock G. Effect of shear stress on the release of soluble ecto-enzymes ATPase and 5′-nucleotidase along with endogenous ATP from vascular endothelial cells. Br J Pharmacol 2000;129:921–6.

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

Zimmermann H. Nucleotides and CD39: principal modulatory players and thrombosis. Nat Med 1999;5:987–8.

Zimmermann H. Ectonucleotidases: some recent developments and note on nomenclature. Drug Dev Res 2001;52:44–56.