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Hypo-and hyperthyroidism affect the ATP, ADP and AMP hydrolysis in rat hippocampal and cortical slices

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Abstract

The presence of severe neurological symptoms in thyroid diseases has highlighted the importance of thyroid hormones in the normal functioning of the mature brain. Since, ATP is an important excitatory neurotransmitter and adenosine acts as a neuromodulatory structure inhibiting neurotransmitters release in the central nervous system (CNS), the ectonucleotidase cascade that hydrolyzes ATP to adenosine, is also involved in the control of brain functions. Thus, we investigated the influence of hyper-and hypothyroidism on the ATP, ADP and AMP hydrolysis in hippocampal and cortical slices from adult rats. Hyperthyroidism was induced by daily injections of L-thyroxine (T4) 25 μg/100 g body weight, for 14 days. Hypothyroidism was induced by thyroidectomy and methimazole (0.05%) added to their drinking water for 14 days. Hypothyroid rats were hormonally replaced by daily injections of T4 (5 μg/100 g body weight, i.p.) for 5 days. Hyperthyroidism significantly inhibited the ATP, ADP and AMP hydrolysis in hippocampal slices. In brain cortical slices, hyperthyroidism inhibited the AMP hydrolysis. In contrast, hypothyroidism increased the ATP, ADP and AMP hydrolysis in both hippocampal and cortical slices and these effects were reverted by T4 replacement. Furthermore, hypothyroidism increased the expression of NTPDase1 and 5′-nucleotidase, whereas hyperthyroidism decreased the expression of 5′-nucleotidase in hippocampus of adult rats. These findings demonstrate that thyroid disorders may influence the enzymes involved in the complete degradation of ATP to adenosine and possibly affects the responses mediated by adenine nucleotides in the CNS of adult rats.

Keywords: Hypothyroidism; Hyperthyroidism; ATP; Adenosine; 5′-Nucleotidase; Central nervous system

1. Introduction

As thyroid hormones mediate important effects on the normal function of the central nervous system (CNS), it is not surprising that the thyroid dysfunction may lead to an extensive array of neurological and behavioral clinical symptoms (Smith et al., 2002). Disorders of the thyroid gland are the most frequent endocrine diseases, but the alterations in adult condition are reversible and can be corrected by adjustment of circulatory thyroid hormones levels. Most of the current knowledge of thyroid hormones actions on the mammalian CNS has been derived from studies in neonatal rat brain. Anyway, the presence of all the isoforms of thyroid hormones, expression of enzyme deiodinase type II and receptors to the thyroid hormones in the adult CNS, have provided the biological bases for action of these hormones in mature brain (Courtin et al., 1986; Schwartz et al., 1994; Calzà et al., 1997). However, the cause of adult hypothyroidism-related memory impairment remains unknown, but there are reports of decreased excitatory transmission in central and peripheral nerves of

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patients with untreated hypothyroidism (Pollard et al., 1982).

It is well established that thyroid hormones play a significant role in the neurotransmission process by increasing the synthesis and the sensibility of central catecholamine receptors (Engstron et al., 1974) and blocking the $GABA_A$-induced Cl^- currents in adult rat brain (Martin et al., 1996). Furthermore, both adenosine transport and A_1 adenosine receptors, which are involved in neurotransmission control, can be also modulated by the thyroid hormones in rat brain (Fideu et al., 1994).

The effects of adenosine within the CNS involve an inhibitory tone of neurotransmission and neuroprotective actions in pathological conditions (Latini and Pedata, 2001). These inhibitory and neuroprotective actions of adenosine are mediated by activation of presynaptic A_1 receptors, which reduce the neurotransmitter release, depressing the neuronal excitability in the CNS (Brundege and Dunwiddie, 1997; Dunwiddie and Masino, 2001). Previous studies have shown that brain slices can readily convert adenine nucleotides to adenosine (Craig and White, 1993). In the CNS, ATP is hydrolyzed to adenosine by the conjugated action of the ecto-nucleoside triphosphate diphosphohydrolase family (NTPDases), responsible for ATP and ADP hydrolysis and a 5′-nucleotidase (CD73, EC 3.1.3.5), which hydrolyzes the AMP formed to adenosine (Battasttini et al., 1991; Bonan et al., 1998; Zimmermann, 2001). The brain contains mRNA for NTPDase1 (CD39, ecto-apyrase, ecto-ATP diphosphohydrolase), NTPDase2 (CD39L1, ecto-ATPase) and NTPDase 3 (HB6) (Braun et al., 2000, 2003; Kegel et al., 1997; Smith and Kirley, 1998). NTPDase1 hydrolyses ATP and ADP equally well, NTPDase2 has a 30-fold preference for the hydrolysis of ATP over ADP and NTPDase3 hydrolyzes ATP three times better than ADP (Zimmermann, 2001). Since these enzymes contribute to maintenance of physiological levels of extracellular ATP/ADP/AMP and adenosine, a considerable effect on the effective regulation of several pathophysiological situations have been proposed for this enzymatic cascade (Agteresch et al., 1999). Hyperthyroidism, for example, is associated with a decrease in the ATP diphosphohydrolase and 5′-nucleotidase activities in synaptosomes from rat hippocampus and cerebral cortex (Bruno et al., 2003). The increased levels of the neurotransmitter ATP together with decreased adenosine levels in a synaptic fraction originated mainly from neuronal cells could explain the predominantly excitatory status found in hyperthyroidism. However, the effects of thyroid disorders on the adenine nucleotide hydrolysis-related enzymes from other brain sources, are still unknown.

Therefore, the present study intended to investigate the ATP, ADP and AMP hydrolysis in hippocampal and cortical slices after induction of both hyper- and hypothyroidism, as well as to verify whether the effects elicited by hypothyroidism are reverted by T4 replacement in adult rats.

2. Materials and methods

2.1. Materials

T4 (L-thyroxine), methimazole, nucleotides (ATP, ADP, AMP), Malachite Green Base and Coomassie Brilliant Blue were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of the highest analytical grade.

2.2. Animals

Male Wistar rats weighting 200–280 g were used in this study. All the animals were housed in cages with food and water available ad libitum. They were maintained under a 12-h light:12-h dark cycle (light on at 07:00 a.m.) at a room temperature of 25 °C. Procedures for the care and use of animals were adopted according to the regulations published by the Brazilian Society for Neuroscience and Behavior (SBNeC).

2.3. Induction of hypothyroidism

The rats were randomized into three groups: control, sham-operated and hypothyroid. Hypothyroidism was induced by the surgical ablation of the thyroid gland (thyroidectomy) under ketamine and xylazine anesthesia. After the surgery, hypothyroid rats were treated with methimazole (0.05%) added to their drinking water during 14 days. The sham-operated group was also submitted to surgery as described above, but without ablation of the thyroid gland. The control, sham-operated and hypothyroid groups were killed by decapitation 15 days after the surgery.

2.4. Induction of hyperthyroidism

Hyperthyroidism was induced by daily intraperitoneal injections of L-thyroxine (T4), 25 μg/100 g body weight, for 14 days (Pantos et al., 2000). T4 was dissolved using 0.04 M NaOH and the final solution was prepared with saline solution. Control animals received intraperitoneal injections of saline solution. Control and hyperthyroid rats were killed by decapitation 24 h after the last injection of T4.

2.5. Hormonal replacement

The hormonal replacement was started 39 days after the thyroidectomy. Rats made hypothyroid (as described above) were injected with daily intraperitoneal injections of L-thyroxine (T4), 5 μg/100 g body weight, for 5 days (Calzà et al., 1997). Sham-operated rats received intraperitoneal injections of saline solution. Sham-operated and hypothyroid rats were killed by decapitation 24 h after receiving the last injection of the T4 replacement.

2.6. Hippocampal and cortical slices

After decapitation, rats' brains were rapidly removed into a bicarbonate-buffered salt solution, containing 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. The brains were cut longitudinally, their hippocampi and cortex dissected and slices transversely cut to 400 μm thick on a Mcllwain tissue chopper.

2.7. Enzyme assay

Two slices per tube (approximately 0.16 mg protein to hippocampus and 0.22 mg to cortex) were preincubated for 10 min at 37 °C with 500 μl of the medium described above and gassed directly with 95% O_2 and 5% CO_2. The reaction was started by adding ATP, ADP or AMP to a final concentration of 2.0 mM, incubated for 20 min and stopped by the addition of 10% trichloroacetic acid (TCA). Non-enzymatic inorganic phosphate (Pi) released from nucleotides into assay medium without slices, and Pi released from slices without nucleotide were used as controls. Pi released was determined as previously described by Chan et al. (1986) and the enzymatic activity was expressed as nmol of Pi released per minute per milligram of protein. All enzyme conditions of this assay were previously described (Bruno et al., 2002) in order to ensure the linearity of reaction.

2.8. RT-PCR

Total RNA from hippocampus from male rats was isolated with Trizol reagent (Life Technologies) in accordance with the manufacturer's instructions. The cDNA species were synthesized with SuperScript II (Life Technologies) from 2 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 1 h at 42 °C and stopped by boiling for 5 min. Two μl of cDNA was used as a template for PCR with primers specific for 5′-nucleotidase (CD73) and NTPDase1. As a control for cDNA synthesis, β-actin-PCR was performed. Two microliters of the RT reaction mix was used for PCR in a total volume of 25 μl using a concentration of 0.5 μM of each primer indicated below and 50 μM of dNTP and 1 U Taq polymerase (Life Technologies) in the supplied reaction buffer.

The PCR cycling conditions were as follows: for CD73 an initial 3 min denaturation step at 95 °C, 1 min 30 s at 95 °C, 1 min 30 s at 64.3 °C, 1 min at 72 °C for 39 cycles (amplification product 403 bp); for β-actin an initial 2 min denaturation step at 94 °C, 1 min at 94 °C, 1 min at 59.8 °C, 1 min at 72 °C for 34 cycles (amplification product 210 bp); for NTPDase 1 an initial 3 min denaturation step at 92 °C, 30 s at 92 °C, 1 min 30 s at 64 °C, 1 min at 72 °C for 32 cycles (amplification product 543 bp). All PCRs were included a final 10 min extension at 72 °C. Ten microliters of the PCR reaction was analyzed on a 1.5% agarose gel. The following set of primers were used: for CD73: 5′CCC GGG GGC CAC TAG CAC CTC A3′ and 5′GCC TGG ACC ACG GGA ACC TT3′ (from Invitrogen); for β-actin: 5′TAT GCC AAC ACA GTG CTG TCT GG3′ and 5′TAC TCC TGC TTC CTG ATC CAC AT3′ (from Invitrogen); for NTPDase 1: 5′GAT CAT CAC TGG GCA GGA GGA AGG3′ and 5′AAG ACA CCG TTG AAG GCA CAC TGG3′ (from Imprint).

2.9. Protein determination

Slices were homogenized using a motor-driven Teflon-glass homogenizer and the protein concentration was measured by the Coomassie Blue method using bovine serum albumin as standard (Bradford, 1976).

2.10. Statistical analysis

The data obtained are expressed as mean ± S.D. values of at least six experiments with slices from different animals. The results of hyperthyroidism were analyzed by Student's *t* test. Statistical analysis of the results of hypothyroidism was performed by one-way analysis of variance (ANOVA). Values of $P < 0.05$ were considered significant.

3. Results

The results obtained with the rats rendered hypothyroid by thyroidectomy surgery were compared to those of sham-operated rats. At the same time, sham-operated rats were compared to control rats in order to verify the effects of the surgery on the adenine nucleotide hydrolysis. The hydrolysis of ATP, ADP and AMP measured in hippocampal and cortical slices from sham-operated rats was not significantly changed in comparison to control animals. This result excludes the influence of thyroidectomy surgery on the hydrolysis of the adenine nucleotides in slices from hippocampus and cerebral cortex. Thus, the effects demonstrated to ATP, ADP and AMP hydrolysis in thyroidectomized rats can be attributed exclusively to hypothyroidism.

ATP, ADP and AMP hydrolysis in hippocampal and cortical slices was assayed in the presence of specific inhibitors of ATPases and alkaline phosphatase (data not shown). The following inhibitors were ineffective as inhibitors of ATP, ADP and AMP hydrolysis by hippocampal and cortical slices: (a) inhibitor of Na^+, K^+-ATPase: ouabain (1 mM), (b) inhibitor of Ca^{2+}, Mg^{2+}-ATPase: *N*-ethylmaleimide -NEM (1 mM), (c) mitochondrial inhibitor: sodium azide (1 mM) and (d) levamisole, a specific inhibitor of alkaline phosphatase (1 mM) (data not shown).

Fig. 1 depicts the results obtained for ATP (Fig. 1A), ADP (Fig. 1B) and AMP (Fig. 1C) hydrolysis in hippocampal

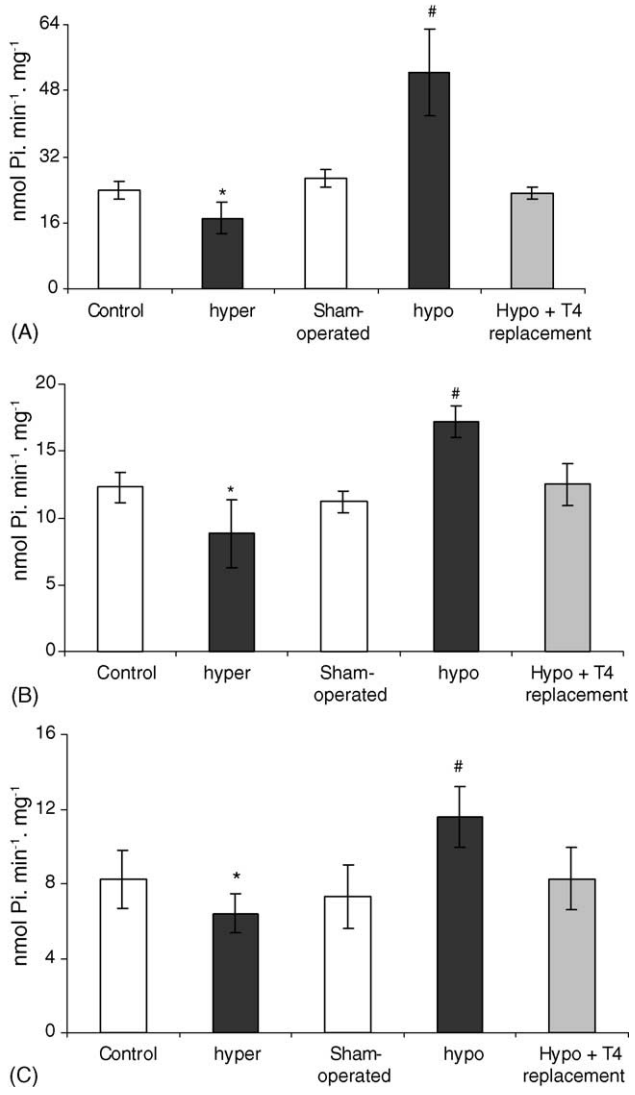

Fig. 1. Effects of hyperthyroidism, hypothyroidism, and hypothyroidism with T4 replacement on ATP (A), ADP (B) and AMP (C) hydrolysis in hippocampal slices of adult rats. Bars represent mean ± S.D. of at least six animals of each group. *Significantly different from control group ($P < 0.05$). #Significantly different from sham-operated group ($P < 0.05$).

slices after induction of hyper-, and hypothyroidism with or without T4 replacement.

When the ATP hydrolysis was measured in hippocampal slices, it was possible to observe a significant decrease of 29% in hyperthyroid rats ($P < 0.05$) in relation to their respective control rats. On the other hand, the ATP hydrolysis was increased by 95% in hypothyroid rats ($P < 0.05$) when compared to sham-operated rats. This effect was completely abolished when the thyroidectomized rats were submitted to T4 replacement. The ATP hydrolysis after the hormonal replacement treatment ($P < 0.05$) was statistically different from hypothyroid rats and equal to sham-operated and control rats (Fig. 1A).

Correspondingly to effects, observed with ATP in hippocampal slices, the ADP hydrolysis was inhibited by 28% in hyperthyroid rats ($P < 0.05$) in comparison to their respective control rats. The parallel profile of inhibition observed for ATP and ADP hydrolysis (29% and 28%, respectively) is characteristic of NTPDase1, previously described by us in rat hippocampal slices (Bruno et al., 2002). In contrast, hypothyroidism increased the ADP hydrolysis by 53% ($P < 0.05$) in relation to sham-operated rats. However, this activation was reverted in hypothyroid rats submitted to T4 replacement ($P < 0.05$) (Fig. 1B).

In the same way, the AMP hydrolysis was decreased by 23% in hippocampal slices from hyperthyroid rats ($P < 0.05$) and increased by 58% in hypothyroid rats ($P < 0.05$) when compared to their respective control and sham-operated rats. The T4 replacement effectively reversed the effect on the AMP hydrolysis ($P < 0.05$) in hippocampal slices from hypothyroid rats (Fig. 1C).

Therefore, the results obtained in hippocampal slices show that both hyper- and hypothyroidism influence the enzymatic chain involved in complete hydrolysis of ATP to adenosine.

The effects of hyper-, hypo-, and hypothyroidism after T4 replacement on the ATP (Fig. 2A), ADP (Fig. 2B) and AMP (Fig. 2C) hydrolysis in slices from rat brain cortex are showed in Fig. 2. Hyperthyroidism did not induce significant changes in the ATP ($P > 0.05$) and ADP hydrolysis ($P > 0.05$) in comparison to control animals (Fig. 2A and B). However, the AMP hydrolysis was decreased by 21% in hyperthyroid rats ($P < 0.05$) in relation to control rats (Fig. 2C). These results indicate that hyperthyroidism affects only the 5′-nucleotidase activity in slices from rat cerebral cortex.

In contrast, we observed increases of 33% and 34% in hydrolysis of the ATP and ADP, respectively, in cortical slices from hypothyroid rats ($P < 0.05$) when compared to sham-operated rats. A significant reversal in the ATP and ADP hydrolysis was found after T4 replacement ($P < 0.05$) (Fig. 2A and B). The parallel profiles for ATP and ADP hydrolysis, described for hippocampal slices, was also observed in slices from cerebral cortex.

Furthermore, the AMP hydrolysis was increased 38% by hypothyroidism ($P < 0.05$) in comparison to sham-operated rats and this effect was abolished by replacement with T4 ($P < 0.05$) (Fig. 2C), demonstrating an effect of hypothyroidism on the 5′-nucleotidase activity in slices from adult rat brain cortex. In addition, the effects observed on adenine nucleotides hydrolysis observed in hypothyroid rats may be considered reversible.

Since the effects of thyroid disorders on the adenine nucleotide hydrolysis were more pronounced in hippocampal slices and were parallel to ATP and ADP hydrolysis, we analyzed by RT-PCR, the expression of NTPDase1 (Fig. 3) and 5′-nucleotidase (Fig. 4) in hippocampus of adult rats. Fig. 3 shows a significant increase in expression of NTPDase1 in hippocampus of hypothyroid (78%), but not in hyperthyroid rats. In addition, the expression of the

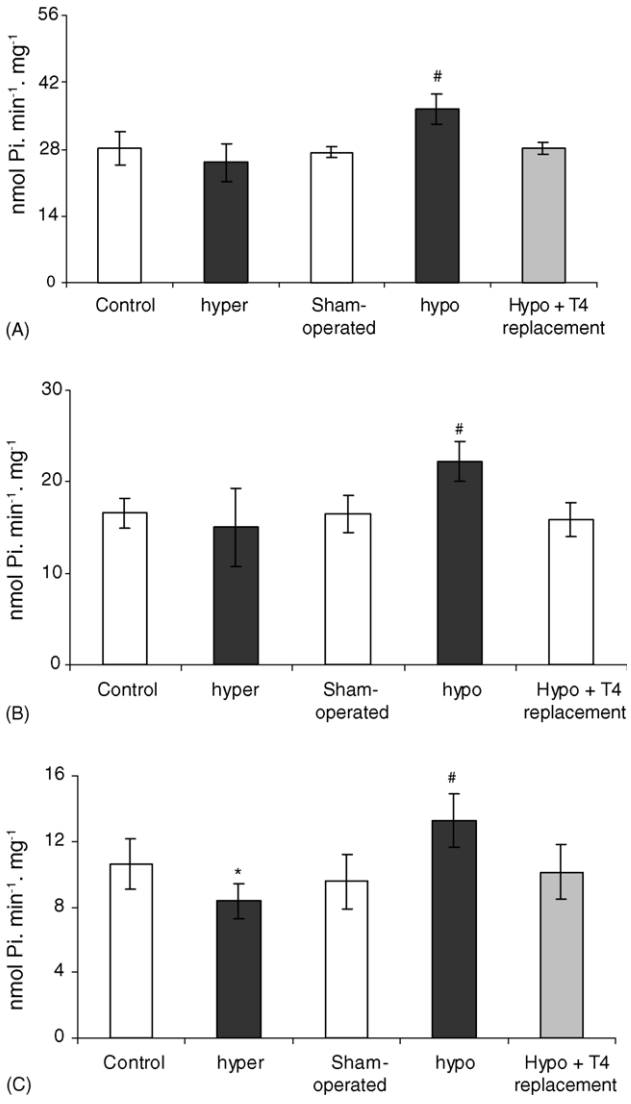

Fig. 2. Effects of hyperthyroidism, hypothyroidism, and hypothyroidism with T4 replacement on ATP (A), ADP (B) and AMP (C) hydrolysis in cortical slices of adult rats. Bars represent mean ± S.D. of at least six animals of each group. *Significantly different from control group ($P < 0.05$). #Significantly different from sham-operated group ($P < 0.05$).

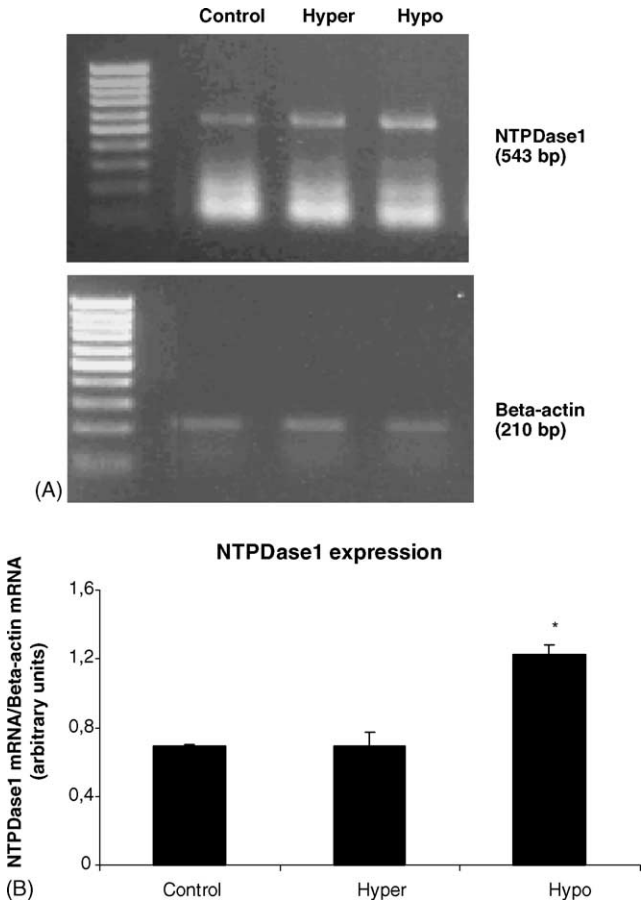

Fig. 3. Representative semi-quantitative RT-PCR mRNA for NTPDase1 from hippocampus of hyperthyroid and hypothyroid rats. The expression was evaluated by NTPDase1 to β-actin mRNA ratio (A). Bars represent arbitrary units of densitometry and are relative to mean ± S.D. of NTPDase1 mRNA/β-actin mRNA ratio of at least three animals of each group (B). *Significantly different from the control group ($P < 0.05$).

5′-nucleotidase also coincided with the measurement of AMP hydrolysis in hippocampus of rats submitted to pathologies studied (Fig. 4). Hypothyroidism increased by 94% the expression of the 5′-nucleotidase, while hyperthyroidism decreased by 60% the expression of this enzyme in hippocampus of adult rats (Fig. 4).

4. Discussion

A strong relationship between the CNS and the thyroid hormones has been recognized not to be exclusive in neurons, but has also been seen in glial cells. In the present study, we investigated the influence of the thyroid disorders on the enzymes that metabolize the adenine nucleotides in an intact fraction from hippocampus and cerebral cortex, which contain both neuronal and glial cells.

The results reported here demonstrated that both excess and deficiency of thyroid hormone are capable of inducing changes in the enzymatic cascade responsible for the hydrolysis of ATP to adenosine, depending on the brain region studied.

Hyperthyroidism inhibited the ATP and ADP hydrolysis very similarly (29% and 28%, respectively) in hippocampal slices. A parallel behavior for ATP and ADP hydrolysis is a characteristic of the ATP diphosphohydrolase (NTPDase1) previously described in this same preparation (Bruno et al., 2002). In addition, specific inhibitors of ATPases and alkaline phosphatase were ineffective as inhibitors of ATP, ADP and AMP hydrolysis by hippocampal and cortical slices (data not shown). These results suggest an effect on the NTPDase1, however the expression of this enzyme was not altered in hippocampus of hyperthyroid rats (Fig. 3). Thus, the results obtained to ATP and ADP hydrolysis in

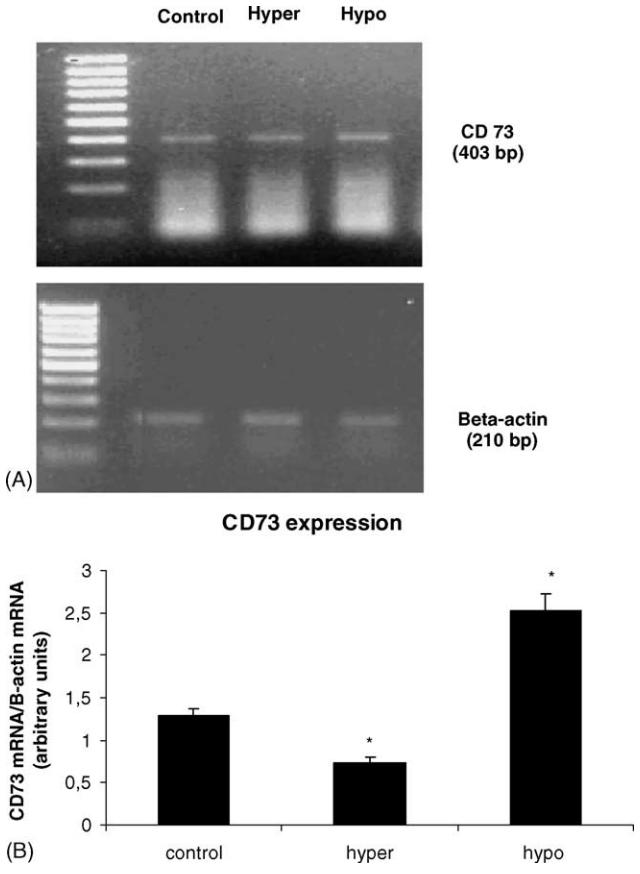

Fig. 4. Representative semi-quantitative RT-PCR mRNA for 5′-nucleotidase (CD73) from hippocampus of hyperthyroid and hypothyroid rats. The expression was evaluated by 5′-nucleotidase (CD73) to β-actin mRNA ratio (A). Bars represent arbitrary units of densitometry and are relative to mean ± S.D. of CD73 mRNA/β-actin mRNA ratio of at least three animals of each group (B). *Significantly different from the control group ($P < 0.05$).

hyperthyroid rats could be due to a non-genomic effect of this pathology on the NTPDase1 in hippocampal slices. In contrast to results obtained in hippocampal slices, the ATP and ADP hydrolysis was not altered by hyperthyroidism in slices from rat cerebral cortex. We recently showed that ATP and ADP hydrolysis are also inhibited after hyperthyroidism induction in the synaptosomal fraction obtained from hippocampus and cerebral cortex (Bruno et al., 2003). Since the synaptosomal preparation is predominantly comprised of neuronal cells, the presence of glial cells in cortical slices may be hiding the effect of thyroid hormones on the neuronal ATP diphosphohydrolase activity, observed earlier in cortical synaptosomes.

Since ATP is recognized as an important excitatory neurotransmitter in the CNS (Di Iorio et al., 1998), the inhibition in ATP hydrolysis observed in hippocampal slices from hyperthyroid rats can disturb a number of processes related to brain excitability. Because the massive release of extracellular ATP leads to excitotoxicity and cell death via activation of P2X receptors in neuronal and glial cells, studies have included this nucleotide among the causes of neuropathological events targeting the CNS (Volonté et al., 1999; Amadio et al., 2002; Ryu et al., 2002). Furthermore, the effects of ATP as a cell death mediator are more prominent in fully differentiated brain (Amadio et al., 2002). ATP can also stimulate astrocyte proliferation, contributing to the processes of reactive astrogliosis, a hypertrophic response that is associated with some neurodegenerative disorders (Burnstock and Williams, 2000).

Our results also showed that hyper- and hypothyroidism modified the AMP hydrolysis in hippocampal and cerebral cortical slices, indicating a role of these hormonal disorders on the 5′-nucleotidase activity, the rate-limiting enzyme of extracellular adenosine production. The effect of thyroid hormones on activity and expression of 5′-nucleotidase has also been demonstrated in other tissues (Carneiro-Ramos et al., 2004).

The inhibition of excitatory neurotransmission mediated by adenosine, is associated with processes, such as neuroprotection, decrease of motor activity, sedation, anticonvulsant actions, regulation of sleep and modulation of anxiety (Brundege and Dunwiddie, 1997; Florio et al., 1998; Dunwiddie and Masino, 2001). Our results demonstrated that hyperthyroidism significantly inhibited the activity of 5′-nucleotidase in hippocampal and cortical slices of adult rats. In addition, the expression of 5′-nucleotidase was also inhibited in hippocampus of these rats. Thus, a possible decrease in the extracellular adenosine levels in brain of hyperthyroid rats could be related to some excitatory features observed in this pathology.

In contrast, the thyroid hormones deficiency increased the adenine nucleotide hydrolysis in slices from hippocampus and cerebral cortex. In addition, this increase was reverted by the T4 replacement treatment. ATP and ADP hydrolysis increased by 33% and 34%, respectively, in cerebral cortex from hypothyroid rats, indicating the same parallel effect mentioned above. Furthermore, the increase of ATP and ADP hydrolysis in hippocampal slices, may be attributed to NTPDase1, since the expression of this enzyme was also increased in hippocampus of hypothyroid rats.

Additionally, the activation of the activity and expression of the 5′-nucleotidase by hypothyroidism and the potential increase of the neuromodulation mediated by adenosine may contribute to the genesis of some clinical characteristics elicited by changes in neurotransmission and previously described in hypothyroid brain (Slotkin and Slepetis, 1984; Shuaib et al., 1994). Moreover, the T4 replacement treatment was also efficient in reverting the activation of the 5′-nucleotidase activity in hypothyroid rats, emphasizing the reversibility of some hypothyroidism effects, which could be attributed to an imbalance in adenosine levels in the CNS.

It was also demonstrated that hyperthyroidism inhibits the 5′-nucleotidase activity in hippocampal and cortical synaptosomes from adult rats (Bruno et al., 2003), whilst hypothyroidism increases the 5′-nucleotidase activity in

these same preparations (Mazurkiewicz and Saggerson, 1989). Previous studies have described the ecto-5′-nucleotidase in the neuron surface, however the most common localization of this enzyme in the brain is in the glial cells (Kreutzberg et al., 1986).

Moreover, the hypothyroidism effect on adenine nucleotide hydrolysis in hippocampus was greater than in cerebral cortex. This result can be attributed to the raised vulnerability of hippocampus to postnatal and adult hypothyroidism (Madeira et al., 1992). Hippocampus is crucially involved in mental processes, such as learning and memory, but it is extremely sensitive to toxic events, such as a massive glutamate release and to microenvironment signals including hormones (Calzà et al., 1997). Therefore, the hippocampal ecto-nucleotidases may also be more vulnerable to hormonal variations or to brain injury produced by these variations.

In conclusion, these findings demonstrate the actions induced by hyper- and hypothyroidism on the enzyme cascade that hydrolyzes ATP to adenosine in fractions from CNS of adult rats. These effects are in agreement with the clinical manifestations ascribed to hyper- and hypothyroidism, and then, may help us to understand some of the features related to these disorders. Moreover, the reversion of the effects in adenine nucleotide hydrolysis in mature brain by the hormonal replacement treatment in hypothyroid rats is compatible with the reversibility of some symptoms of this disorder after the administration of an adequate treatment based on T4 replacement.

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