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Hypothyroidism changes adenine nucleotide hydrolysis in synaptosomes from hippocampus and cerebral cortex of rats in different phases of development

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Abstract

The influence of the thyroid hormones on the normal function of the mammalian central nervous system depends on the brain region and on the developmental stage. Adenine nucleotides and their products also affect the brain function; ATP is an excitatory neurotransmitter, and adenosine has inhibitory effects on neurotransmission. Thus, this study aimed to evaluate the effects of hypothyroidism on the hydrolysis of ATP to adenosine in hippocampal and cortical synaptosomes and blood serum of rats during different phases of development. Rats aged 60 and 420 days old were divided into three groups: control, sham-operated and hypothyroid. Hypothyroidism was induced in these rats by thyroidectomy and methimazole (0.05%) added to their drinking water for 14 days. Neonatal hypothyroidism was induced by adding 0.02% methimazole in the drinking water from day 9 of gestation, and continually until 14 days old. Hypothyroidism increased the AMP hydrolysis in both hippocampus and cerebral cortex synaptosomes of rats in all aged tested. In blood serum, thyroid hormones deficiency increased the AMP hydrolysis in 14-day-old rats and the hydrolysis of ATP, ADP and AMP in 60-day-old rats; however, no alteration was observed in 420-day-old rats. Thus, our results suggest the involvement of the 5′-nucleotidase in synaptic function control in hypothyroidism throughout brain development.

Keywords: Hypothyroidism; Adenosine; Nucleotidases; Development

Disorders of the thyroid gland are among the most common endocrine maladies. Hypothyroidism is the most prevalent form of thyroid disease and symptoms may include memory and learning impairment, depression, psychotic behaviour, retarded locomotor ability, somnolence, progressive intellectual deterioration and, in extreme cases, coma (Anderson, 2001; Smith et al., 2002). Worldwide congenital hypothyroidism remains the major preventable cause of mental retardation. Since the thyroid hormones dramatically affect the maturation of specific neuronal populations, the absence of these hormones during the period of active neurogenesis leads to irreversible mental retardation and is accompanied by multiple morphological alterations in the brain (Smith et al., 2002). Thyroid hormone deficiency during foetal and neonatal periods in

Abbreviations: ADP, adenosine 5′-diphosphate; AMP, adenosine 5′-monophosphate; ATP, adenosine 5′-triphosphate; CNS, central nervous system; NMDA, *N*-methyl-D-aspartate; NTPDase, nucleotide triphosphate diphosphohydrolase; Pi, inorganic phosphate; TCA, trichloroacetic acid; T4, thyroxine

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rats produces deleterious effects, such as reduced synaptic connectivity, delayed myelination, disturbed neuronal migration, deranged axonal projections, decreased synaptogenesis and alterations in levels of neurotransmitters (Dussault and Ruel, 1987; Geel et al., 1967; Oppenheimer and Schwartz, 1997). Furthermore, adult thyroid dysfunction is also associated with both neurological and behavioural abnormalities (Calzà et al., 1997), however, the mechanisms of actions thyroid hormones in the adult central nervous system (CNS) are poorly understood.

It has been demonstrated that catecholamine levels are decreased in the neurons of hypothyroid rats (Slotkin and Slepetis, 1984). Furthermore, surgical hypothyroidism induces a decrease in glutamate release and expression of *N*-methyl-D-aspartate (NMDA) receptors in rat brain (Shuaib et al., 1994; Lee et al., 2003). The depression of brain activity observed in thyroid deficiency is also associated with a severe reduction in the number of β-adrenergic receptors (Smith et al., 1980). In addition, the sensibility to inhibitory agents, such as adenosine, is increased in the hypothyroid status (Ohisalo and Stouffer, 1979).

Adenosine is an endogenous purine associated with an important modulatory role in neuronal activity and neuroprotective actions in pathological conditions (Latini and Pedata, 2001). The neuroprotective actions of adenosine are attributed to the activation of presynaptic A_1 receptors, which reduce neurotransmitter release, depressing the neuronal activity in the CNS (Brundege and Dunwiddie, 1997; Dunwiddie and Masino, 2001).

Adenosine formation may result from the sequential hydrolysis of ATP by an extracellular chain of ectonucleotidases, including the enzymes ecto-ATPase (EC 3.6.1.15), ecto-ATP diphosphohydrolase (ecto-apyrase, CD39, EC 3.6.1.5) and ecto-5′-nucleotidase (CD73, EC 3.1.3.5) (Dunwiddie et al., 1997; Zimmermann, 1996). Previous studies have demonstrated that the ATP, released as a neurotransmitter, is hydrolyzed to adenosine by the conjugated action of an ATP diphosphohydrolase (NTPDase 3) and a 5′nucleotidase in brain synaptosomes (Battasttini et al., 1991; Bonan et al., 1998). Thus, alterations in these enzymes may have an important influence on neurotransmitter functions in the CNS.

Additionally, vascular endothelial cells release endogenous ATP and soluble nucleotidases that degrade ATP to adenosine (Todorov et al., 1997; Yegutkin et al., 2000). Therefore, the adenosine generated as the final product of this enzymatic cascade may affect the synaptic excitability, as well as the local blood flow, since adenosine is a powerful vasodilator (Rongen et al., 1997).

There is some evidence to demonstrate that the thyroid hormones are involved in the modulation of the adenosinergic system in the CNS. T3 increases the transport capacity and the number of adenosine transporters in neural cells (Fideu and Miras-Portugal, 1992). On the other hand, hypothyroidism induced by thyroidectomy, alters the adenosine receptors of the A_1 subtype and reduces the adenosine transport in rat brain synaptosomes (Fideu et al., 1994). Hypothyroidism also induces a decrease in the activity of adenosine-metabolizing enzymes in different brain fractions (Mazurkiewicz and Saggerson, 1989) and an increase in plasma adenosine levels (Salin-Pascual et al., 1997).

Therefore, the objective of this study was to evaluate the presence of the possible alterations induced by hypothyroidism on the enzymes that promote the hydrolysis of ATP, ADP and AMP to adenosine in synaptosomes from hippocampus and cerebral cortex of rats in distinct developmental stages, as well as to verify whether these alterations are also present in blood serum of these rats.

1. Experimental procedures

1.1. Materials

Methimazole, nucleotides (ATP, ADP, AMP), Malachite Green Base, Coomassie Brilliant Blue G, HEPES, Trizma base, EDTA and Percoll were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Percoll was routinely filtered through Millipore AP15 prefilters to remove aggregated, incompletely coated particles. All other reagents were of the highest analytical grade.

1.2. Induction of hypothyroidism

Male Wistar rats in the following developmental phases were used throughout this study: neonatal (14-day-old rats; weighing 24–26 g) and sexually mature adult (60- and 420-day-old rats; weighing 170–570 g).

For neonatal hypothyroidism induction, male Wistar rats were mated and the day of the appearance of the vaginal plug was considered as foetal day 0. Neonatal hypothyroidism was induced by adding 0.02% methimazole in the drinking water from day 9 of gestation, and continually on each day of the experiment (Pipaón et al., 1992). Euthyroid rats with the same age were used as controls and submitted to the same environmental conditions to hypothyroid rats. Control and hypothyroid animals were decapitated when they were 14 days old. Since methimazole readily passes the placental barrier and is transmitted to the suckling pups in the mother's milk, the foetuses and neonates also became profoundly hypothyroid (Oppenheimer and Schwartz, 1997). This protocol ensures a decreased growth rate and low levels of T3 and T4, as described by Pipaón et al. (1992).

Adult rats (60 and 420 days old) were randomly divided 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 for 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.

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 7:00 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 Behaviour (SBNeC).

1.3. Subcellular fractionation

After decapitation, the hippocampus and brain cortex were removed and gently homogenized in five volumes of an ice-cold medium consisting of 0.32 M sucrose, 0.0001 M EDTA and 0.005 M HEPES, pH 7.5, with a motor-driven Teflon-glass homogenizer. The synaptosomes from hippocampus were isolated as described previously (Nagy and Delgado-Escueta, 1984). Briefly, 0.5 ml of the crude mitochondrial fraction were mixed with 4.0 ml of 8.5% Percoll solution and layered onto an isoosmotic Percoll/sucrose discontinuous gradient (10/16%). The synaptosomes that banded at the 10/16% Percoll interface were collected with wide tip disposable plastic transfer pipettes. The synaptosomal fractions were then washed twice at 12 000 × *g* for 20 min with the same ice-cold medium to remove the contaminating Percoll. The synaptosome pellet was resuspended to a final protein concentration of approximately 0.5 mg/ml. The material was prepared fresh daily and maintained at 0–4 °C throughout preparation.

1.4. Isolation of blood serum fraction

Blood was drawn after the decapitation of male Wistar rats of different ages. Blood samples were centrifuged in plastic tubes for 5 min at 5000 × *g* at 20 °C and the serum was maintained on ice at 4 °C throughout the experiments.

1.5. Enzyme assays

The reaction medium used to assay ATP and ADP hydrolysis in the synaptosomal preparation was essentially as described previously (Battasttini et al., 1991). The reaction medium contained 0.005 M KCl, 0.0015 M $CaCl_2$, 0.0001 M EDTA, 0.01 M glucose, 0.225 M sucrose and 0.045 M Tris–HCl buffer, pH 8.0, in a final volume of 200 μl.

The reaction medium used to assay 5′-nucleotidase activity contained 0.01 M $MgCl_2$, 0.1 M Tris–HCl, pH 7.5 and 0.15 M sucrose in a final volume of 200 μl (Heymann et al., 1984).

The synaptosomal fraction (10–20 μg protein) was added to the reaction mixture, pre-incubated for 10 min and incubated for 20 min at 37 °C. The reaction was initiated by the addition of ATP, ADP or AMP to a final concentration of 0.001 M and stopped by the addition of 0.2 ml 10% trichloroacetic acid (TCA). 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).

The enzymatic assays in rat blood serum were determined using the method described by Bruno et al. (2002). The samples were incubated for 40 min at 37 °C in a final volume of 0.2 ml containing serum protein in the range 1.0–1.5 mg, 0.1125 M Tris–HCl, pH 8.0 for ATP and ADP hydrolysis and 0.1 M Tris–HCl, pH 7.5 for AMP hydrolysis. The reaction was started by adding ATP, ADP or AMP to a final concentration of 0.003 M and stopped by the addition of 0.2 ml 10% trichloroacetic acid.

For both assays, the incubation times and protein concentrations were chosen to ensure the linearity of the reaction (results not shown) and absorbance was measured at 630 nm. Inorganic phosphate released was determined as previously described by Chan et al. (1986). Controls with the addition of the enzyme preparation after addition of trichloroacetic acid were used to correct nonenzymatic hydrolysis of the substrates. All samples were assayed in triplicate. Enzyme activities were generally expressed as nanomoles of Pi released per minute per milligram of protein.

1.6. Protein determination

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

1.7. Statistical analysis

The data obtained are expressed as mean ± S.D. values of at least six experiments. Neonatal hypothyroidism results were statistically analysed by Student's *t*-test. Statistical analysis of data obtained for other ages was performed by one-way analysis of variance (ANOVA), with Duncan's test as post hoc. Values of $P < 0.05$ were considered significant.

2. Results

The hydrolysis of ATP, ADP and AMP was evaluated in rat blood serum and synaptosomal fractions from hippocampus and cerebral cortex after the induction of hypothyroidism during different phases of development.

The results obtained for the 60- and 420-day-old rats submitted to thyroidectomy surgery were compared to sham-operated rats of the same age. Sham-operated rats were compared to control rats in order to verify the effects of the surgery on the adenine nucleotide hydrolysis in studied fractions. Our results demonstrated that the ATP, ADP and AMP hydrolysis measured in the hippocampus, cerebral cortex, or blood serum of sham-operated rats was not significantly altered when compared to control animals at

any age tested. This result excludes the influence of thyroidectomy surgery on the hydrolysis of the adenine nucleotides in 60- and 420-day-old rats.

Sham-operated rats were not made for the 14-day-old rats, since they were not submitted to surgery. For this reason, the data obtained for 14-day-old rats were compared only to their respective control animals.

A significant increase in AMP hydrolysis was observed in hippocampal synaptosomes from animals of different ages, indicating a role for this pathology on the 5′-nucleotidase activity during the hippocampal development (Fig. 1).

In the hippocampi of 14-day-old rats submitted to congenital hypothyroidism, the AMP hydrolysis (19.83 ± 4.3 nmol Pi min^{-1} mg^{-1}, $P < 0.05$) was increased by 97% in relation to control animals (10.05 ± 2.4 nmol Pi min^{-1} mg^{-1}) (Fig. 1C). Conversely, the ATP and ADP hydrolysis (132.02 ± 30.9 nmol Pi min^{-1} mg^{-1}; 47.06 ± 12 nmol Pi min^{-1} mg^{-1}) was not altered in relation to the hydrolysis observed in control animals (133.85 ± 27.3 nmol Pi min^{-1} mg^{-1}; 43.9 ± 9.9 nmol Pi min^{-1} mg^{-1}) (Fig. 1A and B).

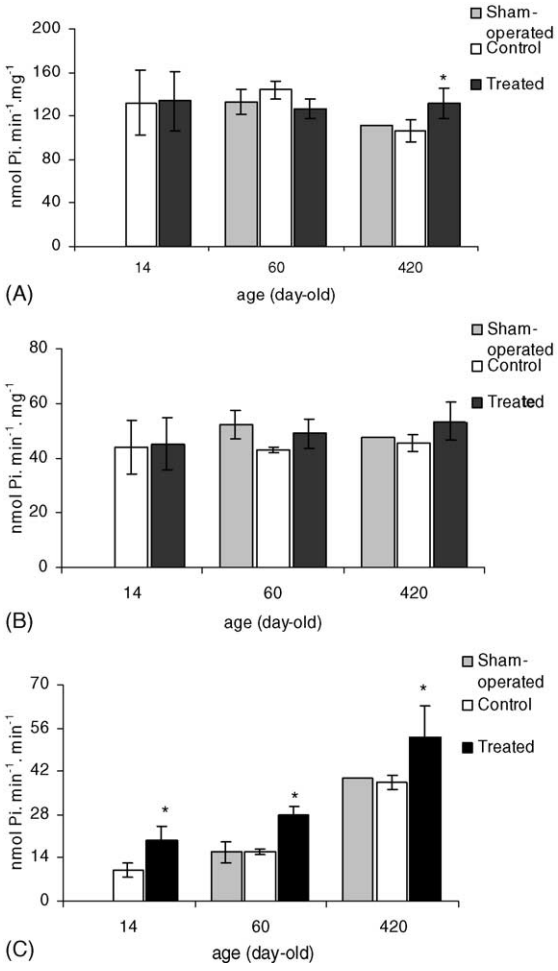

Fig. 1. Effects of hypothyroidism on ATP (A), ADP (B) and AMP (C) hydrolysis in synaptosomes from hippocampus of rats during different phases of development (14, 60 and 420 days old). Bars represent mean ± S.D. of at least six animals. *Significant difference between control group and rats submitted to neonatal hypothyroidism (14-day-old rats) ($P < 0.05$, Student's t-test) and significant difference between sham-operated group and 60- and 420-day-old rats submitted to thyroidectomy ($P < 0.05$, one-way ANOVA test).

In hippocampal synaptosomes from 60-day-old rats, the ATP and ADP hydrolysis was not altered by hypothyroidism (135.7 ± 15.6 nmol Pi min^{-1} mg^{-1}; 45.9 ± 2.9 nmol Pi min^{-1} mg^{-1}) in comparison with sham-operated rats (115.2. ± 7.6 nmol Pi min^{-1} mg^{-1}; 41.31 ± 5.9 nmol Pi min^{-1} mg^{-1}) (Fig. 1A and B). In contrast, the AMP hydrolysis was increased by 76% in 60-day-old hypothyroid rats (27.88 ± 2.7 nmol Pi min^{-1} mg^{-1}, $P < 0.05$) when compared with the sham-operated rats (15.82 ± 3.6 nmol Pi min^{-1} mg^{-1}) (Fig. 1C).

The ATP and AMP hydrolysis in hippocampi from 420-day-old rats submitted to hypothyroidism (131.34 ± 13.8 nmol Pi min^{-1} mg^{-1}; 56.06 ± 9.9 nmol Pi min^{-1} mg^{-1}, $P < 0.05$) was significantly increased (17 and 33%, respectively) in relation to sham-operated rats (112.02 ± 11 nmol Pi min^{-1} mg^{-1}; 39.7 ± 3.3 nmol Pi min^{-1} mg^{-1}) (Fig. 1A and B). Conversely, no change was observed in ADP hydrolysis in hippocampus from hypothyroid (53.4 ± 7 nmol Pi min^{-1} mg^{-1}) and sham-operated rats (47.25 ± 5.27 nmol Pi min^{-1} mg^{-1}) (Fig. 1B).

Fig. 2 shows the effect of hypothyroidism on the adenine nucleotide hydrolysis in synaptosomes from cerebral cortex. In cerebral cortex from 14-day-old rats, the AMP hydrolysis was enhanced 68% by congenital hypothyroidism treatment (15.81 ± 3.1 nmol Pi min^{-1} mg^{-1}, $P < 0.05$) in comparison with their respective control animals (9.37 ± 2 nmol Pi min^{-1} mg^{-1}) (Fig. 2C); however, ATP (106.38 ± 26.8 nmol Pi min^{-1} mg^{-1}) and ADP (49.56 ± 9.9 nmol Pi min^{-1} mg^{-1}) hydrolysis were not altered when compared to control rats (110 ± 12.7 nmol Pi min^{-1} mg^{-1}; 47.75 ± 10.7 nmol Pi min^{-1} mg^{-1}) (Fig. 2A and B). In cerebral cortex from 60-day-old rats, ATP and ADP hydrolysis were not modified by hypothyroidism (128.81 ± 8.8 nmol Pi min^{-1} mg^{-1}; 48.92 ± 5.33 nmol Pi min^{-1} mg^{-1}) in relation to sham-operated rats (133 ± 14.3 nmol Pi min^{-1} mg^{-1}; 50.4 ± 3.16 nmol Pi min^{-1} mg^{-1}) (Fig. 2A and B). However, AMP hydrolysis was activated by 24% in cerebral cortex from hypothyroid rats (20.42 ± 1.65 nmol Pi min^{-1} mg^{-1}, $P < 0.05$) compared to their respective sham-operated rats (16.4 ± 2.39 nmol Pi min^{-1} mg^{-1}, $P < 0.05$) (Fig. 2C). ATP and ADP hydrolysis was not modified by hypothyroidism in 420-day-old rats (145.87 ± 14.2 nmol Pi min^{-1} mg^{-1}; 53.86 ± 10.22 nmol Pi min^{-1} mg^{-1}) when compared to sham-operated rats (134.27 ± 12.17 nmol Pi min^{-1} mg^{-1}; 48.46 ± 3.9 nmol Pi min^{-1} mg^{-1}) (Fig. 2A and B). In contrast, the AMP hydrolysis was activated by 46% in the brain cortex of 420-day-old hypothyroid rats (38.9 ± 5.5 nmol Pi min^{-1} mg^{-1}, $P < 0.05$) in comparison with sham-operated rats (27.46 ± 6.2 nmol Pi min^{-1} mg^{-1}) (Fig. 2C). Moreover, there are no significant differences in the ATP and ADP hydrolysis in synaptosomal fractions from hippocampus and

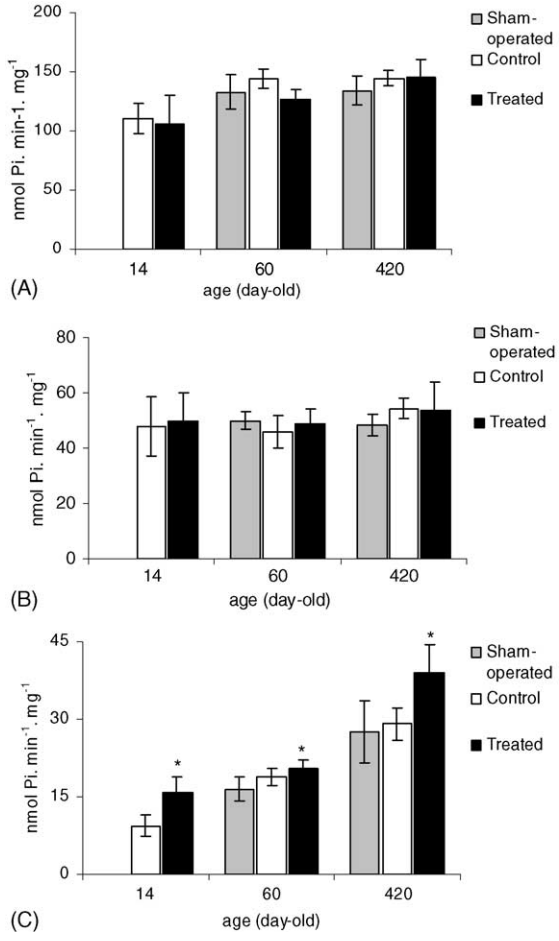

Fig. 2. Effects of hypothyroidism on ATP (A), ADP (B) and AMP (C) hydrolysis in synaptosomes from cerebral cortex of rats during different phases of development (14, 60 and 420 days old). Bars represent mean ± S.D. of at least six animals. *Significant difference between control group and rats submitted to neonatal hypothyroidism (14-day-old rats) ($P < 0.05$, Student's t-test) and significant difference between sham-operated group and 60- and 420-day-old rats submitted to thyroidectomy ($P < 0.05$, one-way ANOVA test).

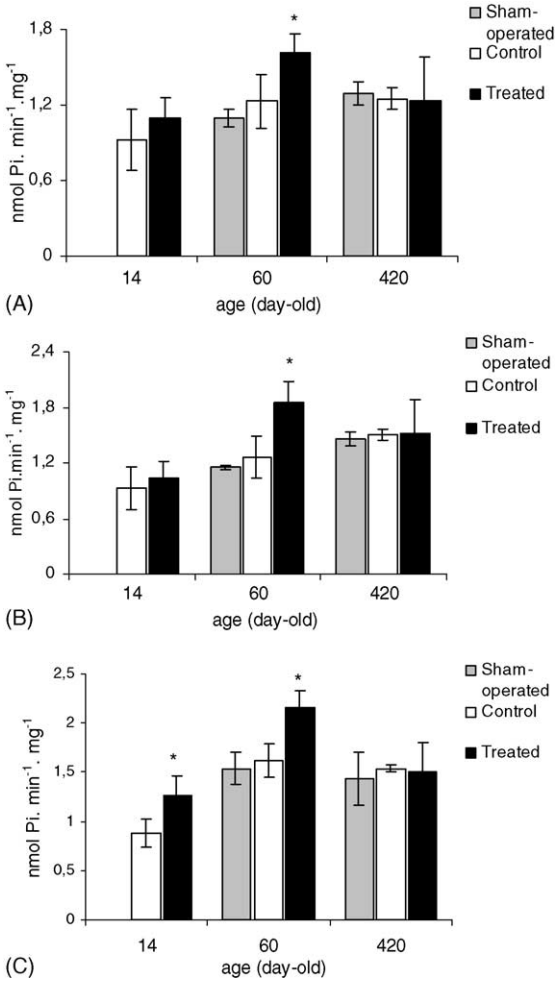

Fig. 3. Effects of hypothyroidism on ATP (A), ADP (B) and AMP (C) hydrolysis in blood serum of rats during different phases of development (14, 60 and 420 days old). Bars represent mean ± S.D. of at least six animals. *Significant difference between control group and rats submitted to neonatal hypothyroidism (14-day-old rats) ($P < 0.05$, Student's t-test) and significant difference between sham-operated group and 60- and 420-day-old rats submitted to thyroidectomy ($P < 0.05$, one-way ANOVA test).

cerebral cortex of control rats throughout development (Figs. 1 and 2). However, AMP hydrolysis was significantly increased in synaptosomal fractions from hippocampus and cerebral cortex of control rats throughout development (Figs. 1 and 2), in agreement with previous studies (Smith et al., 1980).

Fig. 3 demonstrates the ATP (Fig. 3A), ADP (Fig. 3B) and AMP (Fig. 3C) hydrolysis after the induction of hypothyroidism in rat blood serum at different developmental stages. Hypothyroidism elicited a significant increase of 43% only in the AMP hydrolysis in blood serum from 14-day-old hypothyroid rats (1.26 ± 0.19 nmol Pi min^{-1} mg^{-1}, $P < 0.05$) in relation to their respective control rats (0.88 ± 0.14 nmol Pi min^{-1} mg^{-1}). However, in 60-day-old rats, hypothyroidism increased the ATP (35%), ADP (61%) and AMP (39%) hydrolysis (1.61 ± 0.16 nmol Pi min^{-1} mg^{-1}; 1.86 ± 0.22 nmol Pi min^{-1} mg^{-1}; 2.16 ± 0.17 nmol Pi min^{-1} mg^{-1}, $P < 0.05$) when compared with their respective sham-operated rats hydrolysis (1.19 ± 0.07 nmol Pi min^{-1} mg^{-1}; 1.15 ± 0.02 nmol Pi min^{-1} mg^{-1}; 1.54 ± 0.16 nmol Pi min^{-1} mg^{-1}, $P < 0.05$). In contrast, the adenine nucleotide hydrolysis in the blood serum of 420-day-old rats was not significantly altered by hypothyroidism.

3. Discussion

A number of studies have emphasized the susceptibility of the CNS to thyroid hormone effects during different developmental stages (Anderson, 2001; Calzà et al., 1997; Dussault and Ruel, 1987; Leitolf et al., 2002). In the present study, we observed alterations predominantly in synaptosomal 5′-nucleotidase activity, the rate-limiting enzyme to

the extracellular adenosine formation via AMP hydrolysis (Zimmermann, 1996). The developmental phase of rats submitted to hypothyroidism crucially affected the activities of the enzymes studied.

Changes due to developmental stage have been demonstrated in adenine nucleotides hydrolysis-related enzymes activity in the CNS (Bruno et al., 2002; Muller et al., 1990; Smith et al., 1980). Furthermore, the brain receptors for ATP (P2) and adenosine (A_1 and A_2) are also modified in function of rat age (Amadio et al., 2002; Sperlágh et al., 1997; Weaver, 1996).

The activation of the 5′-nucleotidase activity observed in the brain of rats submitted to neonatal hypothyroidism may represent modifications in adenosine production and possibly in expression of A_1-adenosine receptors, which are detected in the brain on gestational day 14. The expression of the excitatory receptor A_{2A} is much more restricted in foetal rats than the expression of the inhibitory receptor A_1 (Weaver, 1996). Since adenosine inhibits the neuronal excitability and the release of neurotransmitters via A_1-receptors (Brundege and Dunwiddie, 1997), changes in the adenosine levels may disturb these processes. This consideration is critic during brain development and may explain some cognitive disturbances that are observed in congenital hypothyroidism. Moreover, recent data demonstrate that the activation of the A_1-adenosine receptors potently impairs the brain formation, since agonists of the A_1-receptor decreased the cortical and hippocampal white matter volume, reflecting axonal losses (Rivkees et al., 2001; Turner et al., 2002). Thus, the increase in adenosine levels may activate the A_1-receptors and trigger a cascade of events leading to impaired neural growth during development.

In addition to the well-established role of hypothyroidism in neonatal brain development, the mechanisms that involve the effects of thyroid hormone deficiency in mature brain are not fully understood. In this study, the 5′-nucleotidase activity measured in hippocampal and brain cortical synaptosomes from 60-and 420-day-old rats was increased after hypothyroidism induction. Previous studies have demonstrated that adenosine transport and adenosine kinase activity are decreased in synaptosomal preparations in brain regions such as hippocampus and cerebral cortex from adult hypothyroid rats (Fideu et al., 1994; Mazurkiewicz and Saggerson, 1989). These previous results, taken together with the data showing increased 5′-nucleotidase activity reported here, may result in a substantial increase in the brain adenosine levels, in turn, disturbing the hippocampal and cortical neurotransmission in adult hypothyroidism. Hypothyroidism-related memory impairment is frequently associated with decreased excitatory transmission, mainly with glutamatergic transmission at the NMDA receptors (Lee et al., 2003). Since neuromodulation exerted by adenosine includes the inhibition of the release of excitatory neurotransmitters, such as glutamate, our results may also further our understanding regarding impaired memory performance in hypothyroid condition. Other recent study has shown the influence of thyroid hormones on the 5′-nucleotidase activity in glioma cells (Wink et al., 2003). To these cells, the ecto-5′-nucleotidase is important to the cellular proliferation and differentiation, and enhanced levels of extracellular adenosine, could also be an important proliferation signal (Wink et al., 2003).

Furthermore, the ATP hydrolysis was significantly increased in hippocampal synaptosomes from 420-day-old rats submitted to hypothyroidism, indicating that the thyroid hormones deficiency may be activating the hippocampal ecto-ATPase (NTPDase2) activity during this developmental stage. It is known that the thyroid function undergoes a decrease in hypothalamic stimulation with the ageing process (Leitolf et al., 2002). Therefore, the decreased thyroid function, as well as a potential increase in the adenosine levels and a lower availability of ATP as an excitatory neurotransmitter, could be contributing to the severity of hypothyroidism during ageing. Secondly, the data demonstrating that hypothyroidism increased the ATP and AMP hydrolysis in 420-day-old rats is particularly important in the hippocampus synaptosomal fraction, since this brain region plays a key role in memory and learning and both hypothyroidism and ageing are related to memory deficit and cognitive disturbances.

We have recently demonstrated that hyperthyroidism inhibits the ATP, ADP and AMP hydrolysis in synaptosomes from hippocampus and cerebral cortex of rats during different phases of development (Bruno et al., 2003). Thus, hyperthyroidism affects the complete enzyme cascade responsible for the hydrolysis of ATP to adenosine, whereas hypothyroidism seems to directly interfere in adenosine production in the synaptosomal fraction. Although hyper- and hypothyroidism affect distinct biochemical events, both thyroid diseases are able to influence the adenosine production in brain synaptosomes.

The changes observed in the brain, on the other hand, are partially accomplished in rat blood serum, where the deficiency in thyroid hormones increased the AMP hydrolysis in 14- and 60-day-old rats and the hydrolysis of ATP, ADP and AMP in 60-day-old rats. It is possible that the increase in ATP, ADP and AMP hydrolysis, observed in the blood serum of 60-day-old rats, may reflect the influence of hypothyroidism on the soluble ATP diphosphohydrolase and 5′-nucleotidase activities, recently described in rat blood serum (Oses et al., 2004).

With respect to the vascular system, studies have established the importance of the enzymatic control on the ATP, ADP, AMP and adenosine levels in the process of haemostasis and thrombus formation (Marcus et al., 1997; Ralevic and Burnstock, 2003). From this point of view, the potential increase in the soluble ATP diphosphohydrolase activity in 60-day-old rats, following hypothyroidism, may contribute to decrease the amount of ATP, a nucleotide involved in tonus vascular maintenance (Soslau and

Youngprapakorn, 1997) and decline of the ADP levels, known to induce platelet aggregation (Puri and Colman, 1997). Furthermore, the activation of the 5′-nucleotidase activity in the blood serum of 14- and 60-day-old rats after hypothyroidism may represent an attempt to enhance the serum adenosine levels, a nucleoside that, as well as inhibiting platelet aggregation (Kawashima et al., 2000), is also capable of acting as a vasodilator (Rongen et al., 1997). This enzyme response to hypothyroidism in the rat blood serum may signify a compensatory mechanism in order to restrain some vascular irregularities frequently described in hypothyroidism (Honda et al., 2000; Masunaga et al., 1997).

In conclusion, our results demonstrate that the 5′-nucleotidase activity is increased in synaptosomes from hippocampus and cerebral cortex of hypothyroid rats at all ages studied. The potential enhances in adenosine levels in brain synaptosomes may trigger the inhibitory effects mediated by this nucleoside. Thus, the imbalance in adenosine production may be associated with disturbances related to hippocampal and cortical neurotransmission and consequently with the behaviour and cognitive disorders found in hypothyroidism.

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