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CYSTINYL AND PYROGLUTAMYL-BETA-NAPHTHYLAMIDE HYDROLYZING ACTIVITIES ARE MODIFIED COORDINATELY BETWEEN HYPOTHALAMUS, LIVER AND PLASMA DEPENDING ON THE THYROID STATUS OF ADULT MALE RATS

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The hypothalamus determinates metabolic processes in liver through endocrine and autonomic control. Hypothalamic neuropeptides, such as thyrotropin releasing hormone or vasopressin, have been involved in liver metabolism. The thyroid status influences metabolic processes including liver metabolism in modulating those hypothalamic peptides whose functional status is regulated in part by aminopeptidase activities. In order to obtain data for a possible coordinated interaction between hypothalamus, plasma and liver, of some aminopeptidase activities that may partially reflect the hydrolysis of those peptides, pyroglutamyl- (pGluAP) and cystinyl- (CysAP) beta-naphthylamide hydrolyzing activities were determined fluorimetrically, both in their soluble and membrane-bound forms, in eu- hypo- and hyperthyroid adult male rats. Hyperthyroidism and hypothyroidism were induced with daily subcutaneous injections of tetraiodothyronine (300 μg/kg/day) or with 0.03% methimazole in drinking water for 6 weeks. Results demonstrated significant changes depending on the type of enzyme and the thyroid status. The most striking changes were observed for CysAP in liver where it was reduced in hypothyroidism and increased in hyperthyroidism. Significant intra- and inter-tissue correlations were observed. While there were positive inter-tissue correlations between liver, plasma and hypothalamus in eu-and hypothyroid rats, a negative correlation between hypothalamus and liver was observed in hyperthyroidism. These results suggest the influence of thyroid hormones and an interactive role for these activities in the control of liver metabolism. The present data also suggest a role for CysAP and pGluAP activities in liver function linked to their activities in hypothalamus.

Key words: *aminopeptidases, thyroid disorders, hyperthyroidism, hypothyroidism, hypothalamus, liver, plasma, glucose transparters, thyrotropin-releasing hormone*

INTRODUCTION

The hypothalamus through endocrine and autonomic control affects metabolic processes in liver including glucose metabolism (1, 2). Plasma thyroid hormones (triiodothyronine, T_3 and thyroxine, T_4) exert directly their influence on metabolic processes in liver but also over hypothalamus through neuronal pathways (1, 3). Therefore, the thyroid status influences metabolic processes including liver glucose metabolism (2, 4).

Various reports suggest that the hypothalamic neuropeptides thyrotropin-releasing hormone (TRH) and arginine-vasopressin (AVP) might be involved in liver metabolism as well as in glucose metabolism (5-7). Furthermore, AVP induces glycogenolysis in hepatocytes (6) and the thyroid status regulates glucose transporters (GLUT2) in liver (7). Moreover, hyperthyroidism increases insulin-stimulated glucose transport associated with an increase of GLUT4 glucose transporter in adipocytes which may be responsible for the rise of peripheral glucose utilization observed in hyperthyroidism (8).

The functional role of neuropeptides, circulatory hormones or tissue factors may be analyzed by their synthesis, the amount of peptide or receptor expression but also through the study of the activity of proteolytic enzymes which metabolize or inactivate those peptides. Enzymatic activity depends on specific conditions linked to environmental external or surrounding endogenous factors. Therefore, the analysis of enzymatic hydrolytic activities may reflect a part of the regulatory mechanism acting through changes of their endogenous substrates under certain physiological or pathological conditions (9).

Pyroglutamyl aminopeptidase activity may regulate TRH in hypothalamus (10), serum (11, 12) and liver (13) depending on the thyroid status. Two types of pyroglutamyl aminopeptidases have demonstrated to remove the N-terminal pyroglutamyl residues: the soluble form, pyroglutamyl-peptidase I (EC 3.4.19.3) and the membrane-bound, pyroglutamyl-peptidase II (EC 3.4.19.6), which is particularly abundant in liver. Pyroglutamyl-peptidase II was also purified from serum where it was identified as the same enzyme than the particulate form. It was generated by proteolytic cleavage of the particulate liver enzyme (14).

Cystinyl aminopeptidase (CysAP, EC 3.4.11.3), identified as insulin-regulated aminopeptidase (IRAP), may regulate vasopressin and oxytocin in hypothalamus and liver (15, 16). This enzyme may control cell glucose uptake through the modulation of GLUT4 transporter which co-localizes with AVP in hypothalamus (17) as well as in hippocampus, adipocytes and muscle (18). It has been demonstrated that AVP is the first physiological substrate for CysAP-IRAP (19). Although it was reported that the thyroid status affected AVP and oxytocin release from the hypothalamus-neurohypophysial system (20), no studies to our knowledge, have yet analyzed CysAP-IRAP activity in various thyroid conditions.

In order to obtain data for a possible coordinated interaction between hypothalamus, plasma and liver of some aminopeptidase activities, which may partially reflect the hydrolysis of TRH and AVP, pyroglutamyl- (pGluAP) and cystinyl- (CysAP) beta-naphthylamide hydrolyzing activities were determined fluorimetrically, both in their soluble (Sol) and membrane-bound (MB) forms, in eu- hypo- and hyperthyroid adult male rats.

MATERIALS AND METHODS

Animals

Twenty male Sprague-Dawley rats (Charles River Laboratories, Barcelona, Spain) weighing 180 – 200 g were used in this study. The animals were randomly divided into three groups: euthyroid (EU) (n = 7), hypothyroid (HYPO) (n = 7) and hyperthyroid (HYPER) (n = 6). The animals were kept in a temperature-controlled room (24 ± 1°C) with a 12 h/12 h light/dark schedule and housed in standard laboratory cages. Laboratory food and water were provided *ad libitum*.

The experimental procedures for animal use and care were in accordance with European Communities Council Directive 86/609/EEC.

Experimental procedures

Hyperthyroidism was induced with daily subcutaneous injections of tetraiodothyronine (Sigma-Aldrich Co., St. Louis, MO, 300 µg/kg/day) for 6 weeks. Hypothyroid rats were obtained with 0.03% methimazole (Sigma) in the drinking water for 6 weeks. Both procedures successfully increase and reduce respectively thyroid hormone levels (T_4 and T_3) in plasma (21). To keep similar experimental conditions, the group of euthyroid rats (controls) was treated with subcutaneous administration of the same solution, in the same conditions as the hyperthyroid group but without T_4. After six weeks of treatment, the animals were anesthetized with equithensin (2 ml per kg body weight) (Equithensin contained 42.5 g/l chloralhydrate dissolved in 19.76 ml ethanol, 0.396 l/l propylenglycol, 21.3 g/l magnesium sulfate and 9.72 g/l Nembutal© in distilled water) injected intraperitoneally. Depth of anesthesia was verified, by the absence of corneal reflex as well as the absence of response when pinching the tail while breathing was slow but regular. Blood samples were obtained from the left cardiac ventricle and centrifuged for 10 min at 2000 g to isolate the plasma (PL) which was stored at –20°C. Rats were then perfused with saline solution through the left cardiac ventricle and the total brain and a sample from liver (a triangular sample from the inferior border of the left lateral lobe) (22) were quickly removed (less than 60 s) and cooled in dry ice. The hypothalamus (pooled left and right) was dissected according to the stereotaxic Paxinos and Watson atlas (23). The selected area was between 7.7 mm and 3.7 mm anterior to the interaural line. From these samples, the soluble (Sol) and membrane-bound (MB) fractions were obtained as previously described (16). Shortly, in order to obtain the Sol fraction, samples from tissues were homogenized in a hypoosmolar medium (10 mM HCl-Tris buffer, pH 7.4). The homogenates were ultracentrifuged at 100,000 g for 30 min at 4°C and the obtained supernatants and PL were used to measure the protein content and enzymatic activities. To get the MB protein fraction, the pellets obtained in the above ultracentrifugation were homogenized again in HCl-Tris buffer (pH 7.4) containing 1% of detergent Triton X-100. After a new ultracentrifugation (100,000 g, 30 min, 4°C), the obtained supernatants were used to determine MB enzymatic activities and proteins in triplicate. The adsorbent polymeric Bio-beads SM-2 (Sigma, 100 mg/ml) (shaking the samples for 2 h at 4°C) was used to remove the detergent from the medium and fully recover the enzyme activity. Cystinyl aminopeptidase activity (CysAP) was measured using L-Cys-di-β-naphthylamide (Sigma-Aldrich) as substrate: 25 µl of plasma or 10 µl of each supernatant was incubated (30 min at 25°C) with 1 ml of the substrate solution: 5.53 mg/100 ml of L-cys-di-β-naphthylamide, 10 mg/100 ml dithiothreitol (DTT) and 10 mg/100 ml bovine serum albumin (BSA) in 50 mM HCl-Tris buffer, pH 6. Pyroglutamyl aminopeptidase activities were measured in a fluorometric assay with L-pGlu-β-naphthylamide (Sigma-Aldrich) as the substrate, according to the modified method of Schwabe and McDonald (24): 10 µl of each supernatant was incubated during 120 min at 37°C with 1 ml of the substrate solution (25.4 mg/l of L-pGlu-β-naphthylamide, 0.1 g/l of dithiothreitol, 378 mg/l of EDTA in 50 mmol/l of phosphate buffer, pH 7.4). The enzymes (aminopeptidases) recognize the free amino-terminal group of the arylamide and separate, by hydrolysis, the beta-naphthylamine from the adjacent amino acid. The reaction was stopped by adding 1 ml of 0.1 mol/l of acetate buffer (pH 4.2). The quantity of beta-naphthylamine released as a result of enzymatic activity was determined fluorometrically at an emission wavelength of 412 nm with an excitation wavelength of 345 nm. Proteins were quantified in triplicate by the method of Bradford (25). Specific Sol and MB CysAP, and Sol and MB pGluAP activities were expressed as nmol of L-Cys-di-β-naphthylamide or L-pGlu-β-naphthylamide hydrolyzed per min per mg of protein. The results of the fluorogenic assays were linear with respect to time of hydrolysis and protein content.

Statistical analysis

The difference between groups was evaluated by one-way analysis of variance. LSD-tests were used for post-hoc comparisons. We considered significant P-values below 0.05. To analyze the relationship between enzymatic activities into- and inter-tissues in EU, HYPO and HYPER, the Pearson's coefficient of correlation was used. Calculations were performed using SPSS 13.0 and STATA 9.0. Correlations with P-values under 0.05 were considered significant.

RESULTS

Results are shown in *Figs. 1-3* and *Table 1*. For MB activities (*Fig. 1*) in hypothalamus, CysAP did not significantly differ between the three groups. In contrast, MB pGluAP was higher ($P < 0.05$) in HYPER than in EU but did not differ between EU and HYPO nor between HYPER and HYPO. In liver, CysAP was higher ($P < 0.001$) in HYPER in comparison with EU and HYPO and HYPO was lower ($P < 0.05$) than EU. MB pGluAP in liver showed no difference between groups.

For Sol activities (*Fig. 2*), CysAP was significantly higher ($P < 0.01$) in HYPER than EU in hypothalamus, in contrast to the MB activities. Also CysAP did not significantly differ between

EU and HYPO nor between HYPO and HYPER. On the contrary, Sol pGluAP was lower ($P < 0.05$) in HYPER than in HYPO but there was no significant difference between EU and HYPO nor between EU and HYPER. In liver, the behavior of Sol activities was identical to that in MB: CysAP was higher ($P < 0.001$) in HYPER in comparison with EU and HYPO and HYPO was lower ($P < 0.05$) than EU. MB pGluAP did not differ between groups in liver. Surprisingly, Sol pGluAP was one order of magnitude higher than MB pGluAP in liver.

In plasma (*Fig. 3*), CysAP activity was lower ($P < 0.001$) in HYPER compared to EU or HYPO. However, CysAP did not differ between EU and HYPO. pGluAP was also lower in HYPER in comparison with EU ($P < 0.001$) and HYPO ($P < 0.01$) and no differences between EU and HYPO were observed.

Significant intra-tissue and inter-tissue correlations between MB and soluble enzymatic activities were found in EU, HYPO and HYPER (*Table 1*). Whereas inter-tissue correlations between HT and LI and between HT and PL of pGluAP versus CysAP were positive in EU and HYPO, the observed correlation between HT and LI of MB CysAP versus MB pGluAP was negative and highly significant ($r = –0.928$, $P = 0.002$) in HYPER.

DISCUSSION

The main enzymatic changes were clearly depending on the thyroid status: in liver, Sol and MB CysAP activities showed the lowest levels in hypothyroid and the highest in hyperthyroid rats. This is particularly interesting because of the proposed role for CysAP/IRAP in glucose metabolism (26). In addition, highly significant intra-tissue correlations between CysAP and pGluAP were observed in HT, LI and PL. Remarkably, while positive inter-tissue correlations were found in EU and HYPO, a negative one was obtained in HYPER between liver MB pGluAP and MB CysAP (vasopressinase) in hypothalamus (*Table 1*).

These results may suggest a complex mechanism of action implying plasma and both the Sol and MB CysAP as well as Sol and MB pGluAP activities in liver and hypothalamus in an

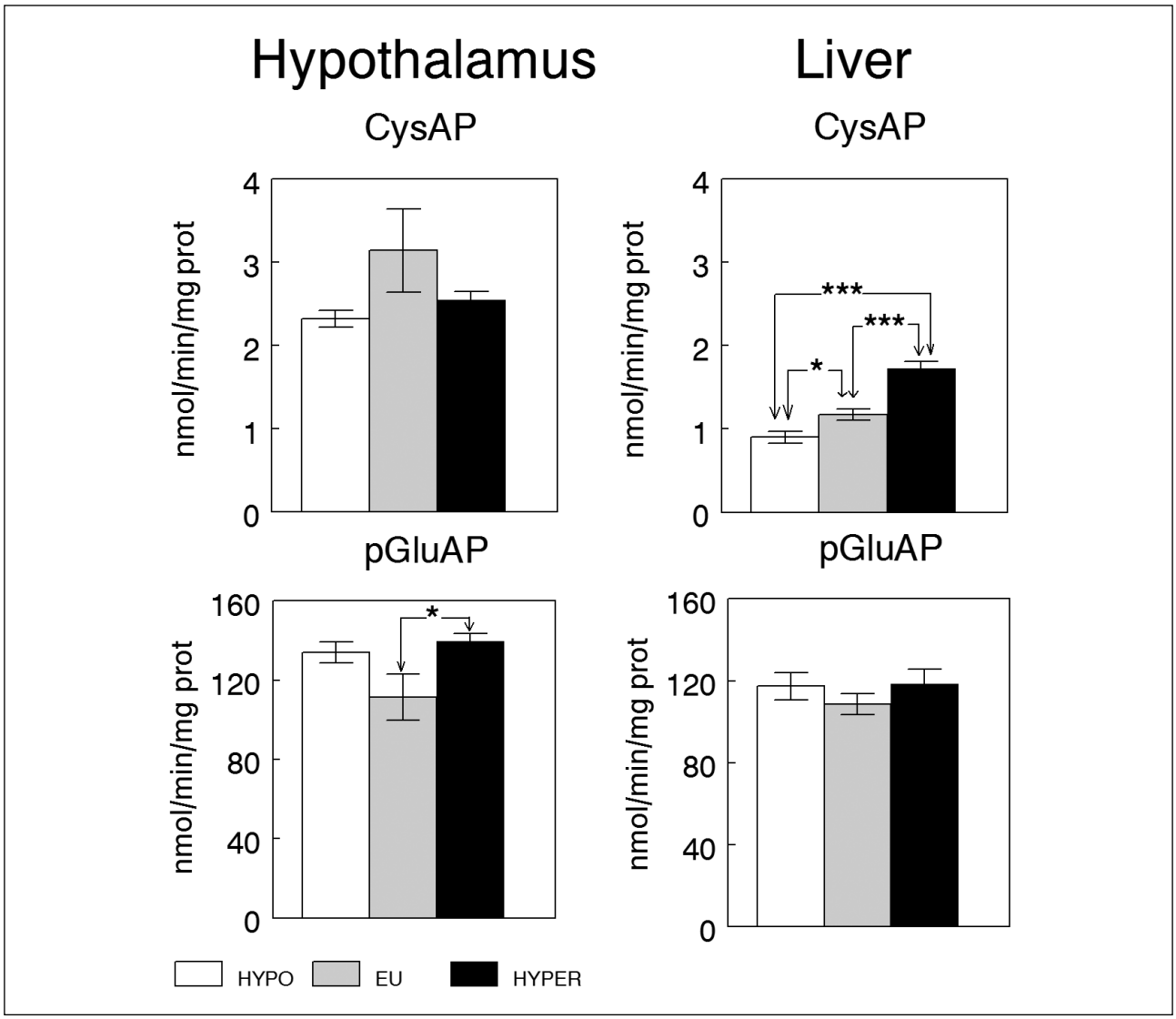

Fig. 1. Membrane-bound Cystinyl-(CysAP) and pyroglutamyl-(pGluAP) aminopeptidase activities in hypothalamus and liver of hypothyroid (n=7) (HYPO), euthyroid (n=7) (EU) and hyperthyroid (n = 6) (HYPER) adult male rats. Values represent mean ± SEM for levels in the groups of 6 or 7 animals assayed individually. Aminopeptidase activities are expressed as nmol of cystinyl- or pyroglutamyl-β-naphthylamide hydrolyzed per min per mg of protein; *$P < 0.05$; ***$P < 0.001$.

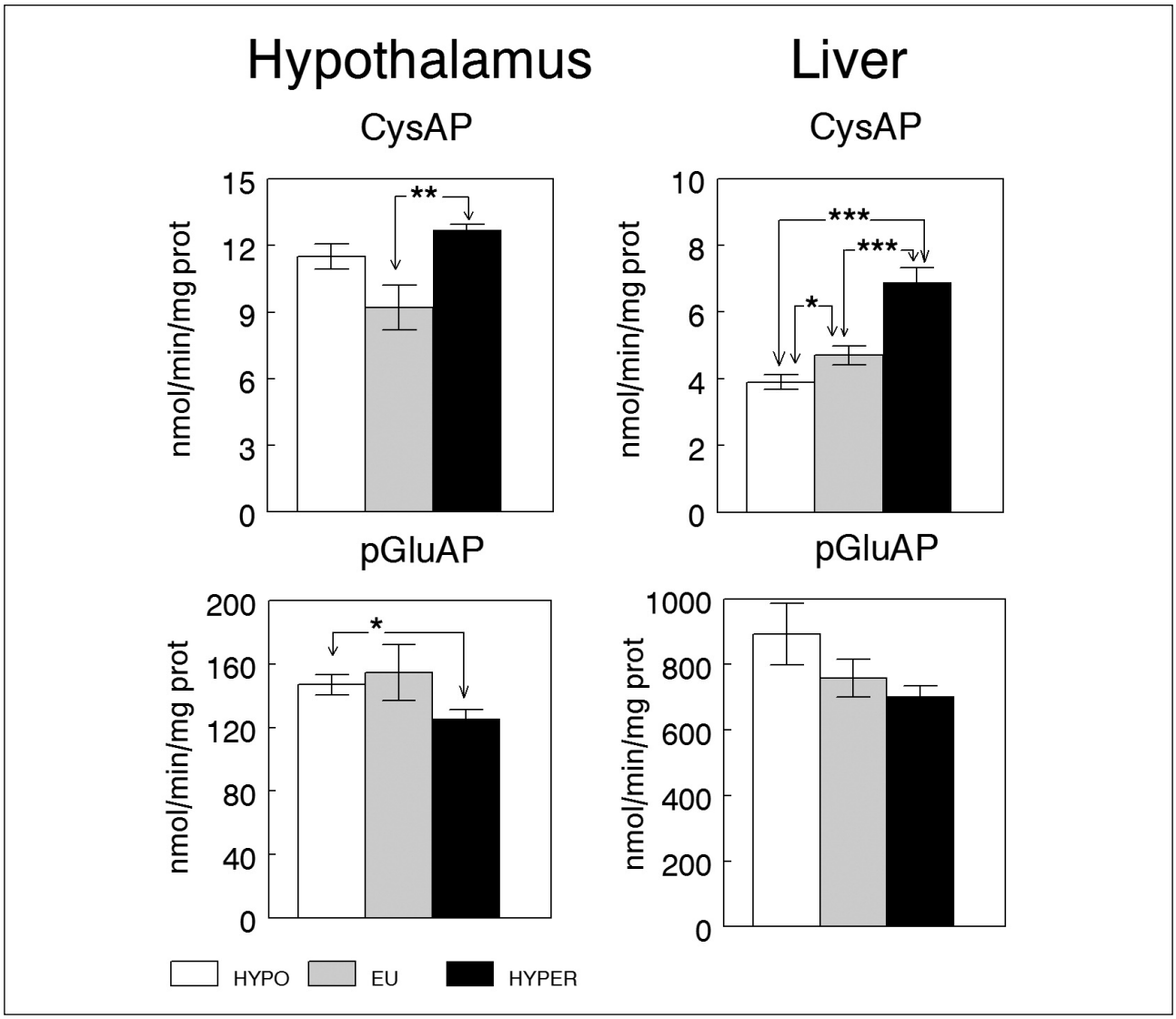

Fig. 2. Soluble Cystinyl-(CysAP) and pyroglutamyl-(pGluAP) aminopeptidase activities in hypothalamus and liver of hypothyroid (n = 7) (HYPO), euthyroid (n = 7) (EU) and hyperthyroid (n = 6) (HYPER) adult male rats. Values represent mean ± SEM for levels in the groups of 6 or 7 animals assayed individually. Aminopeptidase activities are expressed as nmol of cystinyl- or pyroglutamyl-β-naphthylamide hydrolyzed per min per mg of protein; *P < 0.05, **P < 0.01, ***P < 0.001.

independent or coordinated manner. Although these aminopeptidase activities, in their Sol or MB forms, are able to hydrolyze identical substrates, the processes that regulate each form of the enzymes may be different and, as a consequence, exert different functions as discussed below (16).

It was hypothesized that surrounding biochemical conditions induced by pathologic or physiologic circumstances may regulate aminopeptidase activities (9). Therefore, although the regulation of the enzymatic activity involves necessarily the control of the enzyme synthesis at the nuclear level, other mechanisms must also be implicated. For example, intra-tissue secretory processes from nerve terminals of the autonomic nervous system could also be involved (27). It is well-known that enzymatic activities are modified by biophysical and biochemical conditions such as temperature, pH, endogenous or exogenous inhibitors or activators in the medium such as changes in the levels of nitric oxide (28), catecholamines (29), steroids (9) or thyroid hormones (30). In this sense it is interesting to indicate that the thyroid function is regulated in part by melatonin which, in addition to pineal gland, is also synthesized by thyroid gland (31). In addition, hyperthyroidism increases lipid metabolism, especially in skeletal muscles with high capacity for fatty acid oxidation (32). These results emphasize the multiple physiological modifications that the thyroid status can produce, thus creating certain environmental conditions that can influence the enzymatic activities. The heterogeneity of the environment may have a different influence on the Sol (encompassing both interstitial and intracellular) or MB form of the enzyme (16). In particular, it was suggested that MB CysAP activity acts in a more tissue-specific manner than Sol CysAP which may be more subject to environmental changes and therefore be less specific on its possible endogenous substrates (16). In addition, neurovisceral integrative mechanism involving proteolytic activities between hypothalamus and several organs, through endocrine and autonomic pathways have been proposed (27).

Cystinyl aminopeptidase activity, which exhibits broad substrate specificity, hydrolyzes amino-terminal cysteine residues of various peptides and polypeptides. CysAP has been reported

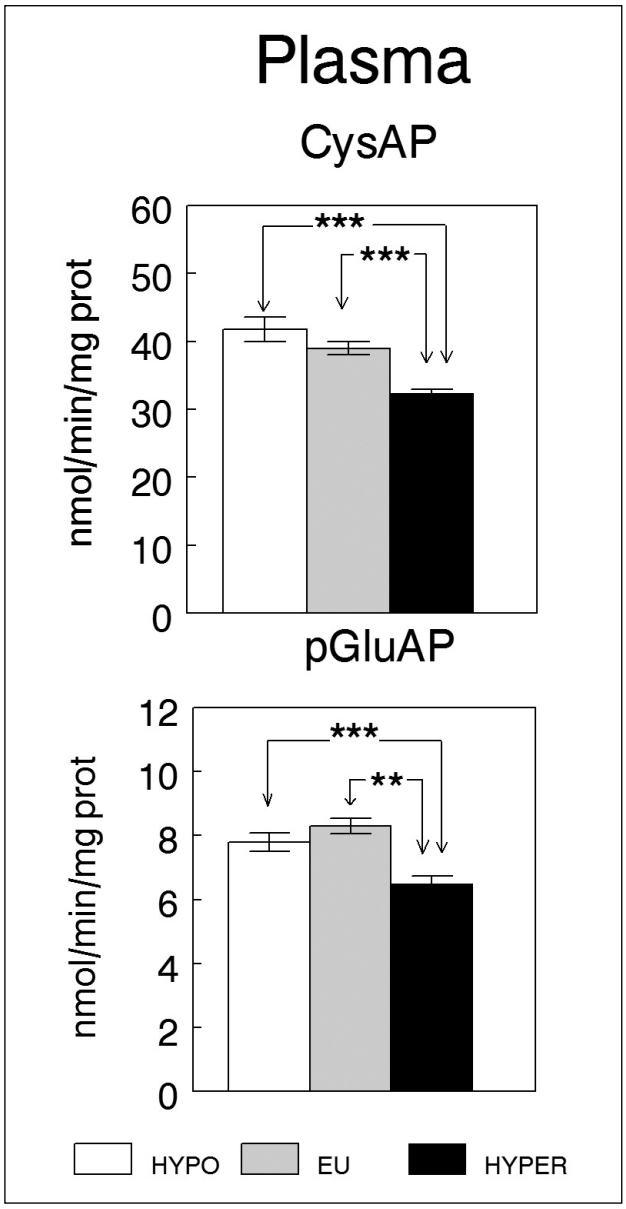

Fig. 3. Cystinyl-(CysAP) and pyroglutamyl-(pGluAP) aminopeptidase activities in plasma of hypothyroid (n = 7) (HYPO), euthyroid (n = 7) (EU) and hyperthyroid (n = 6) (HYPER) adult male rats. Values represent mean ± SEM for levels in the groups of 6 or 7 animals assayed individually. Aminopeptidase activities are expressed as nmol of cystinyl- or pyroglutamyl-β-naphthylamide hydrolyzed per min per mg of protein; **P < 0.01; ***P < 0.001.

under various terms such as insulin-regulated aminopeptidase (IRAP), placental-leucyl aminopeptidase, vasopressinase, oxytocinase, and even Ang IV receptor (AT_4) but all refer to the same protein (26, 33). This enzyme hydrolyzes oxytocin, vasopressin, angiotensins and opioid peptides (34, 35). However, the assumption that the AT_4 receptor is IRAP was also challenged and it was proposed that this molecule is the c-Met tyrosine kinase receptor (36). Cystinyl aminopeptidase is a protein involved in a large variety of functions including parturition, milk ejection, blood pressure, water balance, local blood flows, glucose homeostasis, and cognitive functions (16). Regarding the involvement of CysAP in glucose homeostasis, it was proposed that the binding of Ang IV to its receptor, AT_4 (CysAP/IRAP), results in the inhibition of the receptor's metabolic activity, reducing the catabolism of its substrates and consequently increasing their availability and extending their action (26). Ang IV could therefore regulate glucose uptake in modulating CysAP activity: CysAP/IRAP is indeed co-localized with the glucose transporter GLUT4. In the presence of insulin, CysAP and GLUT4 are expressed in the plasma membrane, where GLUT4 induces glucose uptake. It was suggested that the inhibition of CysAP, following binding of AngIV to the AT_4 receptor could increase glucose uptake in neurons (26). Increased Ang IV inhibits IRAP, increases ADH and affects glucose uptake (37). Insulin also stimulates and translocates IRAP (38). However, it has been recently reported that acute inhibition of IRAP aminopeptidase activity does not affect glucose homeostasis in normal and diabetic rats (39). It should be taken into account that the above mentioned proposed functions for CysAP refer to its MB form (IRAP). Currently, no specific endogenous substrates have been yet reported for Sol CysAP.

The physiological role of pGluP I activity remains poorly understood. A distinctive biochemical feature of pGluP I activity is its broad pyroglutamyl substrate specificity which include biologically active peptides such as TRH, gonadotropin-releasing hormone, neurotensin, bombesin and anorexigenic peptide (40). Because of this broad substrate specificity, it is difficult to discuss a physiological role for the results obtained in the present study that could be applied to this activity. However, based on its relatively ubiquitous distribution, it was proposed that this enzyme could be involved in regulating the cellular pool of free pGlu. Pyroglutamic acid is known to have pharmacological properties improving the age-associated memory impairment (41) and elevated levels of free pGlu have been established in certain diseases (42, 43). Interestingly, pyroglutamic acid improves glucose tolerance and serum insulin levels in diabetic rodents (44). In marked contrast with pGluP I, the particulate pGluP II activity has substrate specificity restricted to TRH (40). pGluP II activity has also been observed in mammalian serum which displays biochemical characteristics remarkably similar to those of the tissue type II form including its narrow substrate specificity also restricted to TRH (40). Subsequently, as indicated above, this serum TRH degrading enzyme has been identified as a product of liver origin, generated by proteolytic cleavage of the particulate liver pGluP II (14). Several authors have reported a large diversity of results on the influence of the thyroid status on these enzymatic activities in tissues or serum, some even conflicting although under the same experimental conditions (10-13). In the hyperthyroid animals, blood levels of thyroid hormone are chronically increased which, could condition the response by feedback mechanisms. This would imply a reduction of TRH, by these feedback regulatory mechanisms. Considering the relation 'high enzymatic activity/low level of the substrate', our results would be in agreement with the high hypothalamic MB pGluAP activity observed in the group of hyperthyroid rats but disagree with the low levels measured in the plasma of this group. Interestingly, Sue and Wilk (10) reported important differences in their results depending on the duration of the administration of triiodothyronine to the animals: the acute administration did not affect at all pGluP I, whereas pGluP II was increased significantly in frontal cortex and pituitary. However, the treatment during 10 or 14 days significantly increased pGluP I (but not pGluP II) in pituitary, hypothalamus, olfactory bulb, hippocampus, and thalamus. Finally, the chronic treatment increased only pGluP II just in frontal cortex and in serum. As these authors also suggest, feedback mechanisms of control may be involved and possible differences in the feedback control may account for the variability in the results.

A reduction in CysAP activity may reflect an increased glucose uptake and vice versa an increased CysAP activity mirrors

Table 1. Significant correlations between soluble (Sol) and/or membrane-bound (MB) CysAP and pGluAP activities measured in hypothalamus (HT), liver (LI) and plasma (PL) of euthyroid, hypothyroid and hyperthyroid rats. Pearson's correlation coefficients (r) and p-values are indicated and specify the significance of the differences between these correlations. Negative correlations are in italics. Asterisks highlight the inter-tissue correlations.

Correlation	r	P
Hypothyroid		
HT MB CysAP vs. HT MB pGluAP	+0.895	0.006
*HT Sol pGluAP vs. LI MB pGluAP	+0.771	0.04
Euthyroid		
HT MB CysAP vs. *HT MB pGluAP*	*–0.742*	*0.05*
HT MB CysAP vs. *HT Sol CysAP*	*–0.834*	*0.01*
HT MB pGluAP vs. HT Sol CysAP	+0.956	0.0008
LI MB CysAP vs. LI MB pGluAP	+0.777	0.03
*HT MB pGluAP vs. LI MB CysAP	+0.786	0.03
*HT Sol pGluAP vs. PL CysAP	+0.925	0.002
Hyperthyroid		
HT MB pGluAP vs. HT Sol CysAP	+0.910	0.004
PL CysAP vs. PL pGluAP	+0.957	0.0007
**HT MB CysAP* vs. *LI MB pGluAP*	*–0.928*	*0.002*

a reduction in glucose uptake. Therefore, the negative correlation observed in HYPER between liver MB pGluAP and MB CysAP in hypothalamus could suggest that glucose uptake in hypothalamus, modulated by CysAP/IRAP activity, could be related to the availability of TRH in liver and vice versa. Increased thyroid hormones could therefore play a regulatory role.

Although the expression of GLUT4 in liver is not currently clearly established, some authors do assign a significant role to this transporter in porcine hepatic glucose metabolism (45). The presence of GLUT4 in mice hepatocytes has been detected (46), raising the possibility that the expression of GLUT4 in liver is species dependent. In liver of hyperthyroid rats there was an increased CysAP/IRAP activity. This would imply a reduction in glucose uptake and consequently an increased glycemia. This may be linked to the reduced levels of pGluAP in plasma of hyperthyroid rats which suggests low plasmatic levels of pyroglutamic acid that would worsen glucose tolerance and insulin levels (44). In contrast, there was a reduction in CysAP/IRAP activity in hypothyroid rats, suggesting an increase in glucose uptake and therefore a reduction in blood glucose. Both data could be in part responsible for the reported levels of glycemia, which depend on the thyroid status (47).

Inactivation of AVP by IRAP activity, exocytic translocation of vesicles containing GLUT4 and glucose uptake are interrelated in muscle cells (48). These data do not agree with our observation showing increased CysAP activity in liver of hyperthyroid rats. However, it has also been reported that AVP stimulates glycogenolysis in liver (6). Therefore, considering the vasopressinase activity of CysAP, our results would suggest a reduction of AVP and therefore, reduced glycogenolysis in liver of hyperthyroid rats which contrast with the previously suggested decrease in glucose uptake. In addition, glucose homeostasis may be regulated to some extent by vasopressin in liver through the action of the vasopressin receptor (V1a) which is predominantly expressed in liver. Hepatic glucose production is indeed higher in V1a vasopressin receptor-deficient mice than in wild-type mice (49). The highly significant changes of CysAP in liver, which is depending on the thyroid disorder, may support a role for this aminopeptidase on glucose homeostasis through its possible action on both GLUT4 and/or on its vasopressinase activity. Hypothalamic CysAP/IRAP may also play a major role in glucose metabolism (18). It is clearly established that hyperthyroidism involves sympathetic activation and that beta-adrenergic blockade improves significantly the symptoms (50). In addition, hyperthyroidism increases hepatic glucose production and insulin resistance. While sympathetic hepatic innervation increases glucose production, the parasympathetic one restrains it (51). Therefore, the highly significant correlation observed between hypothalamic CysAP (IRAP activity) and liver MB pGluAP in hyperthyroid rats, suggests an interactive role for these enzymatic activities in the control of liver metabolism, probably mediated by the autonomic nervous system.

Our present study is limited to the analysis of pGluAP and CysAP activities in the soluble and membrane-bound fractions of hypothalamus and liver as well as in plasma, using aminoacyl-beta-naphthylamides as substrates in the specific conditions we have established. Therefore, the interpretation of the results is restricted to the analysis of both fractions in general terms and no discussion about the cellular and subcellular compartmentalization could be performed. Since no identification of the present activities could be clearly assigned to the enzymes pGluP I and pGluP II, our interpretations should be considered as suggestive and not conclusive.

In conclusion, the present are preliminary results that may suggest an influence of thyroid hormones on pGluAP and CysAP activities and that may indicate an interactive role for these enzymes in the control of liver metabolism. In addition, these results suggest a role for CysAP and pGluAP activities in liver function which could be linked to their role in hypothalamus through endocrine or autonomic mechanisms. Clearly, these results warrants additional research to further analyze the possible enzymatic interaction between hypothalamus, liver and plasma and its consequences on biological parameters.

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Conflicts of interests: None declared.

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