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Distribution of peptidase activity in teleost and rat tissues

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Abstract Peptides play important roles in cell regulation and signaling in many tissues. The actions of peptides are regulated by peptidases. Although the activity of these enzymes has been thoroughly characterized in mammals, little is known about their presence or function in fish. In the present study, we compared the activity of several peptidases in selected tissues (pituitary gland, different brain areas, kidney and gills) of the gilthead sea bream and rainbow trout with that found in similar rat tissues (lungs studied in place of gills). Soluble puromycin-sensitive aminopeptidase showed the highest values in the pituitary gland of the sea bream, whereas the membrane-bound form was found to be more active in the trout kidney. Very high levels of activity of aminopeptidase N were detected in trout and sea bream plasma. In contrast, the highest levels of activity of aminopeptidase B were found in rat tissues, with the exception of the gills of the trout. Aminopeptidase N levels tended to be higher in sea bream tissues with respect to those of trout. In contrast, the level of activity of aminopeptidase B was found to be consistently much higher in trout tissues than in those of the sea bream. Prolyl endopeptidase activity was principally detected in the pituitary gland and in the brain areas of teleosts. These differences between species could be related to different mechanisms of osmoregulation in saltwater- and in freshwater-adapted fish.

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Abbreviations ANG II: Angiotensin II · APA: Acid aminopeptidase · APB: Basic aminopeptidase · APN: Aminopeptidase N · CNS: Central nervous system · PE: Prolyl endopeptidase · PSA: Puromycin-sensitive aminopeptidase · ST: Scheffé test · TRH: Thyrotropin-releasing-hormone

Introduction

Peptidases are enzymes involved in both the processing and degradation of active peptides. Thus, inactive peptides such as the decapeptide angiotensin I (ANG) may be processed into the active octapeptide ANG II by the action of peptidases (Reid et al. 1978). In addition, the hydrolysis of certain peptides converts them into fragments with partially or completely different effects. This is the case of ANG II and its metabolites ANG III and ANG (1–7) (Ferrario and Iyer 1998).

In mammals, the mechanisms of inactivation of many peptides such as enkephalins (Hersh 1982), peptide components of the kinin–kallikrein system (Casarini et al. 1999) and thyrotropin-releasing-hormone (TRH) (O'Cuinn et al. 1990) have been thoroughly characterized. In contrast, information concerning the enzymes which are involved in peptide hydrolysis in fish is sparse (Galardy et al. 1984; Goren et al. 1990), despite the fact that in teleosts, certain peptides such as vasotocin and ANG play vital roles in physiological adaptation to different salinities (Takei 2000; Warne et al. 2002).

The aim of this work was to describe several peptidase activities in different tissues involved in the osmoregulatory response (pituitary, different brain areas, kidney, gills or lung) of the gilthead sea bream (*Sparus aurata*), a euryhaline teleost, which is capable of adapting to extreme changes in environmental salinity (Mancera et al. 1993), of the rainbow trout (*Oncorhynchus mikiss*), a salmonid species capable of surviving in both fresh water and sea

water and of the Sprague-Dawley rat, a well known mammal model. Since peptides may act as hormones circulating in blood plasma, we also measured the activity of these enzymes in the plasma of the mentioned species. Enzymes whose activity was examined included: (i) puromycin-sensitive aminopeptidase (PSA) (Constam et al. 1995), (ii) aminopeptidase N (APN) (Giros et al. 1986), (iii) basic aminopeptidase (APB) (Foulon et al. 1999), (iv) acid aminopeptidase (APA) (Li et al. 1993) and (v) prolyl endopeptidase (PE) (Cunningham and O'Connor 1997). The enzymes PSA, APA and PE have been found in soluble and membrane-bound forms (Dyer et al. 1990; Li et al. 1993; O'Leary and O'Connor 1995; Wilk et al. 1998). Aminopeptidase N is membrane-bound and is also present in plasma (Giros et al. 1986; Ward et al. 1990), whereas APB is a soluble enzyme (Mantle 1992; Foulon et al. 1999).

Materials and methods

Materials

All the chemicals used in this study were obtained from The Sigma Chemical Co. (St. Louis, MO, USA) with the exception of *Z*-Gly-Pro-β-naphthylamide, purchased from Bachem (Basel, Switzerland).

Animals

Fish

Male gilthead sea breams (*S. aurata* L., 450–500 g body weight, $n = 10$) were supplied by "Planta de Cultivos Marinos" (CASEM, University of Cádiz, Puerto Real, Cádiz, Spain). Rainbow trout (*O. mykiss*, 450–550 g body weight, $n = 10$) were provided by a commercial fish culturing center (Piscifactoría "El Bosque", El Bosque, Cádiz, Spain). All blood and tissue samples were obtained (May 2002) in the same fish culturing centre according to the sampling protocol described below.

Rats

Male Sprague-Dawley rats (230–260 g body weight, $n = 10$) bred in the colony of the University of the Basque Country and maintained under controlled environmental conditions (22°C and a 12 h light–dark cycle) with free access to food and water were used in this study. Experiments were carried out in accordance with the Guidelines of the European Union Council (86/609/EU). All efforts were made to minimize animal suffering and to reduce the number of animals used.

Tissue extraction and sample preparation

Fish were anesthetized with 2-phenoxyethanol (1 ml/l water), weighed and sampled. Blood was extracted in ammonium-heparinized syringes from the caudal peduncle. Plasma samples were obtained after centrifugation of blood (1 min at 10,000g) and were immediately frozen on dry ice and stored at −80°C until required. Rats were weighed, anesthetized with equithensin (0.2 ml/100 g body weight) and sampled by dissection. Blood was extracted in heparinized syringes from the left ventricle of the heart. Plasma was separated from cells by centrifugation (1 min at 10,000g).

The following tissues were obtained by dissection: pituitary gland, selected brain areas, kidney and gills for the teleosts or lung for the rat. Fish brains were further dissected into anterior (telencephalon and olfactory bulb), middle (diencephalon, midbrain tegmentum, tectum and valvulae cerebelli) and posterior (rhombencephalon, cerebellum, vagal lobe) blocks. The brain cortex, striatum and cerebellum were dissected from the rat brain. All the samples were stored at −80°C.

Preparation of soluble and the solubilized-particulate fractions

Dissected structures were homogenized in 10 mM Tris-HCl buffer, pH 7.4, for 30 s at 800 rpm using a Heidolph PZR 50 Selecta homogenizer and ultracentrifuged in a Centrikon T-2070 Kontron Instruments apparatus at 100,000g for 35 min. The resulting supernatants were used to measure soluble enzyme activities and protein concentrations. To avoid contamination with soluble enzymes, the resulting pellets were washed three times by suspension in 10 mM Tris-HCl buffer, pH 7.4. Subsequently, pellets were homogenized in 4M NaCl and then centrifuged in order to release loosely bound proteins from the membrane. The resulting pellet was then homogenized in 10 mM Tris-HCl buffer, pH 7.4, plus 0.1% Triton X-100 for 30 s at 800 rpm and ultracentrifuged at 100,000g for 35 min. The supernatants thus obtained were used to determine the solubilized particulate enzyme activities and protein concentrations. All steps were carried out at 4°C.

Enzyme assays

Peptidase activities were measured by incubating samples with a saturating (0.125 mM) concentration of β-naphthylamide derived substrates. Activities of APN and PSA were measured in triplicate using Ala-β-naphthylamide as a substrate following the method described by Mantle (1992) in the absence and presence of 20 μM puromycin, which is known to completely inhibit PSA (Giros et al. 1986). Acitivity of PSA was calculated by subtracting activity measured in the presence of puromycin from activity measured without the inhibitor. The enzyme activities of APB and APA were quantified with Arg-β-naphthylamide and Asp-β-naphthylamide substrates. Propyl endopeptidase activity was fluorimetrically measured using *Z*-Gly-Pro-β-naph-

thylamide as a substrate (Yoshimoto 1979). These assays are based on the fluorescence of β-naphthylamine generated from the hydrolysis of the substrate by the enzyme. Reactions were initiated by adding 10–50 µl of sample depending on the enzyme and substrate analyzed. After 30 min incubation at 20°C or 37°C, 1 ml of 0.1 M sodium acetate buffer (pH 4.2) was added to the mixture to terminate the reaction. The released β-naphthylamine was determined by measuring the intensity of fluorescence at 412 nm with excitation at 345 nm. Tubes without samples were used to determine background fluorescence. Relative fluorescence was converted into picomoles of product using a standard curve, constructed with increasing concentrations of β-naphthylamine.

Protein determination

Protein concentration was measured in triplicate by the method described by Bradford (1969). The results were recorded as units of peptidase (UP) per milligram of protein. One unit of peptidase activity is the amount of enzyme that releases 1 pmol of β-naphthylamine per minute.

Statistical analyses

Data were analyzed statistically using SPSS, version 10. One-way analysis of variance (ANOVA) was performed to detect differences between species and tissues. ANOVA was followed by the Scheffé multiple comparisons test (ST) when differences were detected. Statistically significant differences were considered at $P < 0.05$.

Results

Peptidase activities detected in the plasma of rat and different fish species (gilthead sea bream, rainbow trout) are shown in Table 1. We did not detect PSA activity in any of the studied species. At either 20°C or 37°C, the gilthead sea bream presented the highest activity of APN (the ST with other species always presented $P < 0.001$), while less than half of this activity was measured in rainbow trout plasma. Activity of APN was found to be lowest in the rat (ST trout versus rat $P < 0.01$). In contrast, APB activity was highest in rat plasma followed by sea bream plasma (ST rat versus sea bream $P < 0.05$) at 37°C. However, at 20°C this activity was similar in both species. These values were significantly higher than those obtained for the trout (ST $P < 0.001$). Activity of APA was lowest in rat plasma (ST rat versus other species $P < 0.05$), and was relatively homogeneous for the teleostean species studied. Finally, PE activity in plasma did not show significant differences between species at 37°C but at 20°C is higher in the trout than in the rat or sea bream.

The activity of soluble PSA in the pituitary gland, in a selection of different brain areas, in the lungs or gills and in the kidney of the rat, gilthead sea bream and rainbow trout is illustrated in Fig. 1. This activity is not homogeneously distributed among the different tissues (ANOVA $P < 0.001$), nor among different species for a given tissue (ANOVA $P < 0.01$), neither at 20° C (a) nor at 37° C (b). In the rat, the highest levels of activity were found in the brain areas (ST brain versus other tissues $P < 0.01$). In rainbow trout, statistically significant differences were only detected between values from the gills and the rest of the tissues (ST $P < 0.05$). In the gilthead sea bream, the highest levels of PSA activity were found in the pituitary gland, even though the differences were only significant when the gills and the kidney were compared (ST $P < 0.001$). Comparing between species, soluble PSA activity in the pituitary gland was highest in the trout at 20° C and in the gilthead sea bream at 37°C (ST sea bream or trout versus rat $P < 0.05$), whereas this activity in the kidney and gills was the highest in the rainbow trout (ST $P < 0.01$). Finally in the central nervous system (CNS) the highest activity at 37°C $P < 0.05$ was obtained in the rat (ST rat versus sea bream at least $P < 0.05$) and at 20°C in the trout (ST trout versus sea bream at least $P < 0.05$).

The activity of membrane-bound PSA was found to be heterogeneously distributed in the analyzed species and tissues (Fig. 2) either at 20°C (a) or at 37°C (b). In the rat, the highest levels of activity were found in the different areas of the CNS and in the kidney, whereas lower values were measured in the pituitary gland and in the lungs (ST $P < 0.05$ at least). In the gilthead sea bream, the highest activity was found in the kidney and

Table 1 APN, APB, APA and PE activities in the blood plasma of the rat, sea bream and rainbow trout. Values which are expressed as units of peptidase (UP)/mg protein, represent the mean ± SEM of n = 10 animals for each group

		APN	APB	APA	PE
Rat	20	505 ± 114	300 ± 36	14 ± 2[c]	25 ± 3
	37	1696 ± 222	1106 ± 121[c]	28 ± 1[c]	72 ± 5
Sea bream	20	4322 ± 522[a]	312 ± 34	69 ± 9	29 ± 4
	37	8107 ± 845[a]	824 ± 120	70 ± 9	68 ± 18
Rainbow trout	20	1606 ± 251[b]	120 ± 15[d]	60 ± 8	75 ± 7[d]
	37	3271 ± 461[b]	112 ± 14[d]	46 ± 6	72 ± 12

[a] $P < 0.001$ sea bream versus rat and trout;
[b] $P < 0.01$ trout versus rat;
[c] $P < 0.05$ rat versus sea bream and trout;
[d] $P < 0.001$ trout versus sea bream and rat

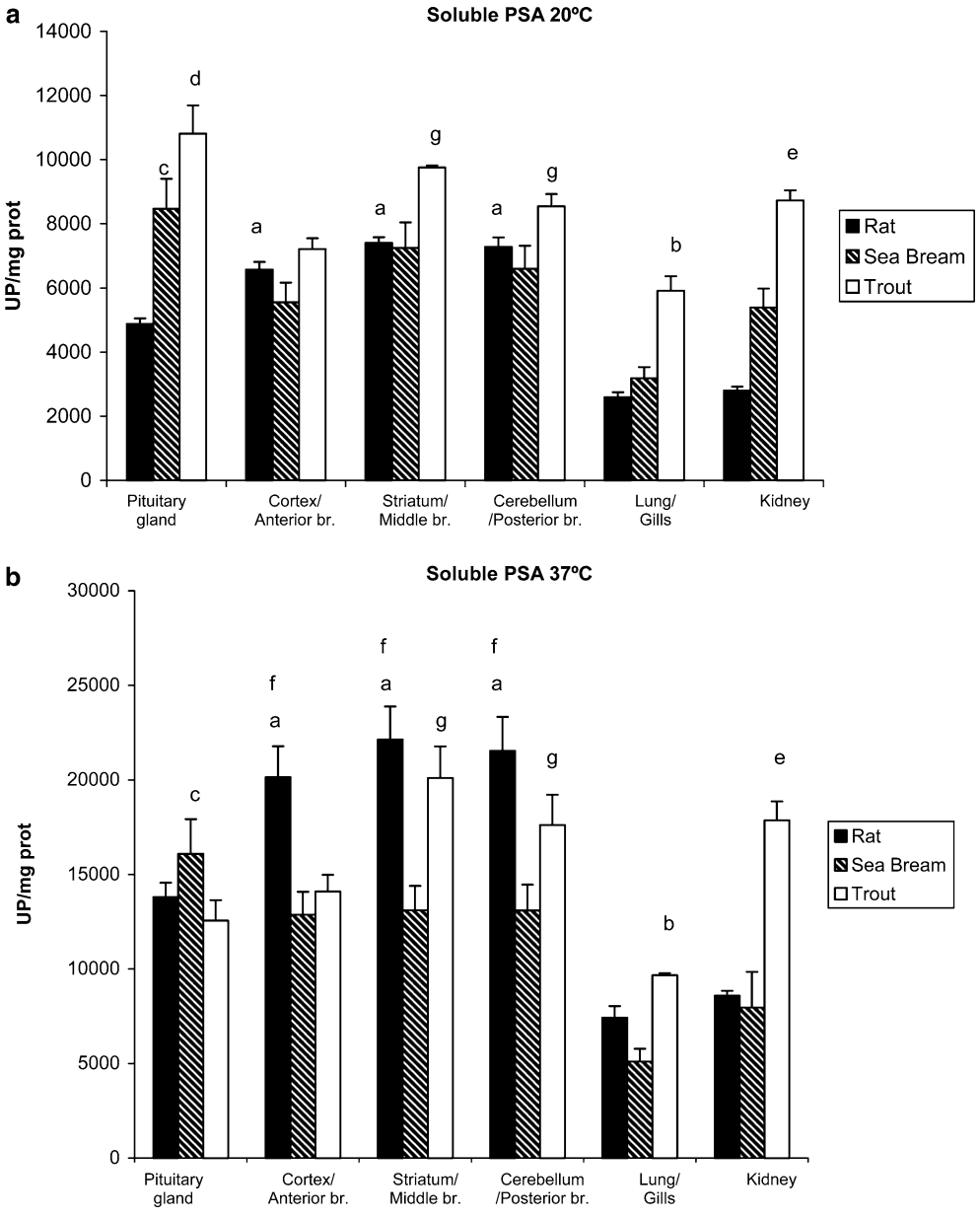

Fig. 1 Soluble PSA activity at 20°C (**a**) and 37°C (**b**) in different tissues from the rat, sea bream and rainbow trout. Values which are expressed as units of peptidase (UP)/mg protein, represent the mean ± SEM of $n = 10$ animals for each group. *a*: $P < 0.01$ CNS areas versus other rat tissues; *b*: $P < 0.01$ gills versus other trout tissues; *c*: $P < 0.001$ pituitary gland versus gills and kidney of sea bream; *d*: $P < 0.05$ pituitary gland of sea bream and trout versus pituitary gland of rat; *e*: $P < 0.01$ kidney of trout versus kidney of rat and sea bream; *f*: $P < 0.01$ CNS of rat versus CNS of sea bream. *g*: $P < 0.05$ middle and posterior brain of trout versus middle and posterior brain of sea bream

the gills. Finally in the trout, the highest level of activity of membrane-bound PSA was found in the kidney (ST kidney versus other tissues $P < 0.001$); moderate activity was found in the brain areas, with the exception of the activity in the anterior brain at 20°C, while the pituitary gland and the gills presented the lowest activities (ST pituitary gland and gills versus medium and posterior brain $P < 0.05$). Comparing between species, membrane-bound PSA activity in the kidney and gills was found to be highest in the trout (ST $P < 0.001$) and in the sea bream. respectively. Temperature did not affect the species- or tissue-related distribution of this enzyme.

Values of APN activity at 20°C (a) and 37°C (b) detected in the different animals and tissues are represented in Fig. 3. At both temperatures, the highest levels of activity of APN in the rat were found in the kidney (ST kidney versus other areas $P < 0.001$), while the lowest were found in the brain areas and in the pituitary gland (ST gills versus brain areas and pituitary gland $P < 0.01$). In the gilthead sea bream, we found similar activity in the kidney and gills and lower activity in the rest of the studied tissues (ST $P < 0.05$). In the trout, we found the highest activity in the kidney. Comparing among species, at 37°C we found highest activity of this enzyme in the rat, moderate activity in the sea bream and the lowest level of activity of APN in all analyzed tissues was found in the rainbow trout (ST rat tissues versus trout tisues at least $P < 0.05$). However, at 20°C the activity is slightly and non-significantly higher in the sea bream brain than in the rat brain areas.

Results of determinations of APB activity at 20°C (a) and 37°C (b) are shown in Fig. 4. The activity in sea bream tissues was found to be much lower than that in

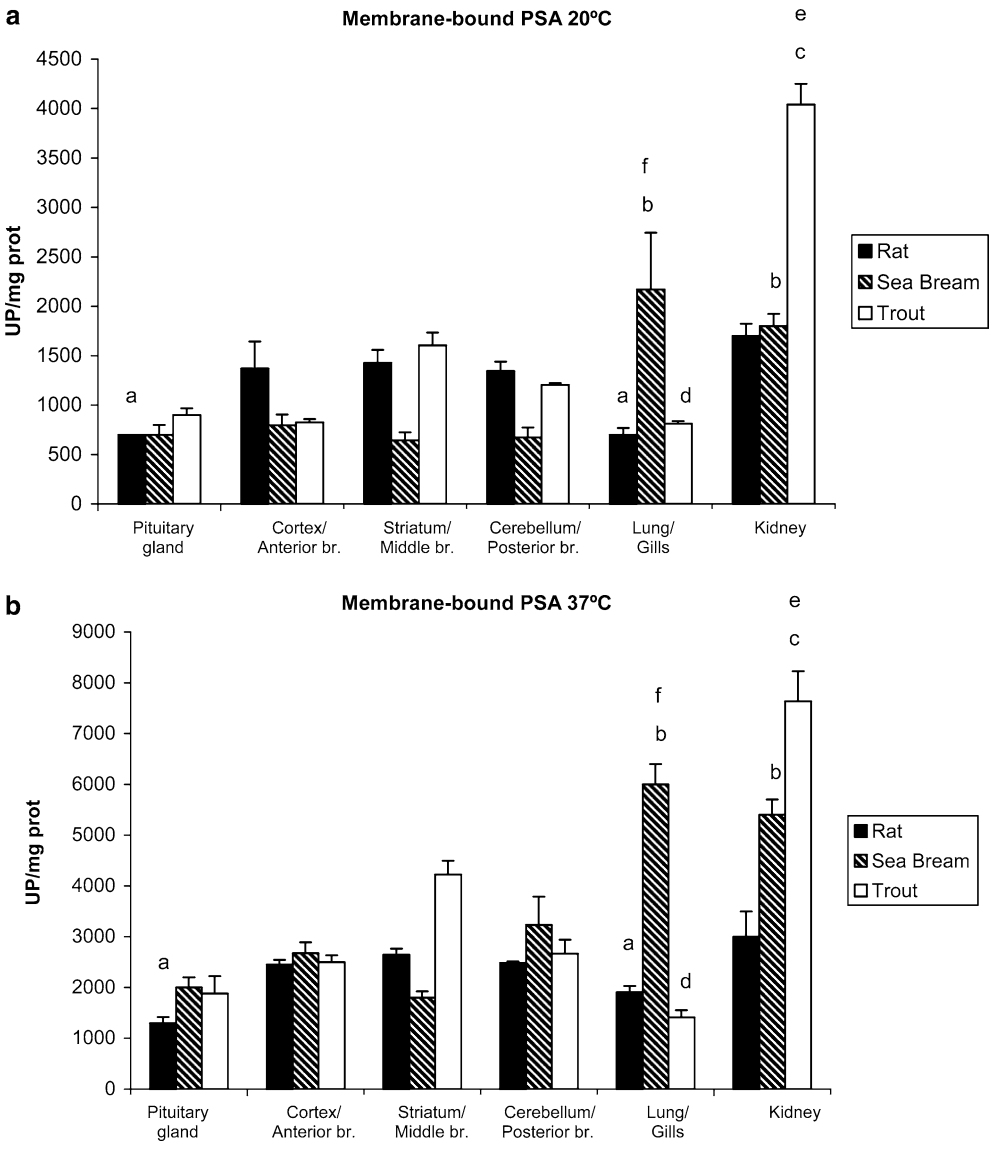

Fig. 2 Membrane-bound PSA activity at 20°C (**a**) and 37°C (**b**) in different tissues from the rat, sea bream and rainbow trout. Values which are expressed as units of peptidase (UP)/mg protein, represent the mean ± SEM of $n = 10$ animals for each group *a*: $P < 0.05$ lungs and pituitary gland versus kidney and CNS of rat; *b*: $P < 0.05$ gills and kidney versus other areas of sea bream; *c*: $P < 0.001$ kidney versus other tissues of trout; *d*: $P < 0.05$ gills versu CNS of trout; *e*: $P < 0.001$ kidney of trout versus kidney of rat and sea bream; *f*: $P < 0.05$ gills of sea bream versus gills of trout

trout or rat tissues at both temperatures (ST at least $P < 0.05$). At 37°C, this activity was found to be higher in the rat than in the gilthead sea bream and rainbow trout (ST at least $P < 0.05$). This was not the case at 20°C, because at this temperature the activity is similar in the rat and in the trout. The tissue distribution of APB activity in the gilthead sea bream and the trout (with the exception of the pituitary gland) was quite homogeneous. In the rat, APB activity was found to be higher in the CNS than in the peripheral tissues (ST at least $P < 0.05$). The activity of APB in the trout was very similar at 20 and 37°C. However, in the rat and in the sea bream higher activities were found at 37°C (ST at least $P < 0.05$).

Species-related differences in the activity of soluble APA in the different analyzed tissues were not found (Fig. 5). The highest levels of APA activity were found in the pituitary gland and the kidney of all the three studied species (kidney and pituitary gland versus other tissues, ST at least $P < 0.05$).

The activity of the membrane-bound fraction of APA (Fig. 6) in the gilthead sea bream was quite homogeneous. However, in the rainbow trout and the rat, the kidney presented the highest levels of activity of membrane-bound APA (ST at least $P < 0.05$). In addition, high levels of activity were also found in the rat lung and pituitary gland (ST lung and pituitary gland versus brain areas $P < 0.01$). Acitivity of APA was lower in the CNS than in the rest of the tissues and without species-related differences. However, in the kidney and in the pituitary gland the activity was the highest in the rat and the lowest in the sea bream. Temperature did not appear to affect the regional distribution of the activity of this enzyme.

A heterogeneous distribution of soluble PE activity was observed when comparing both species (ANOVA $P < 0.001$) and tissues (ANOVA $P < 0.001$) (Fig. 7). At 20° C (a), soluble PE activity was higher in the tissues of the teleosts than in those of the rat (ST at least $P < 0.05$), with the trout presenting generally higher

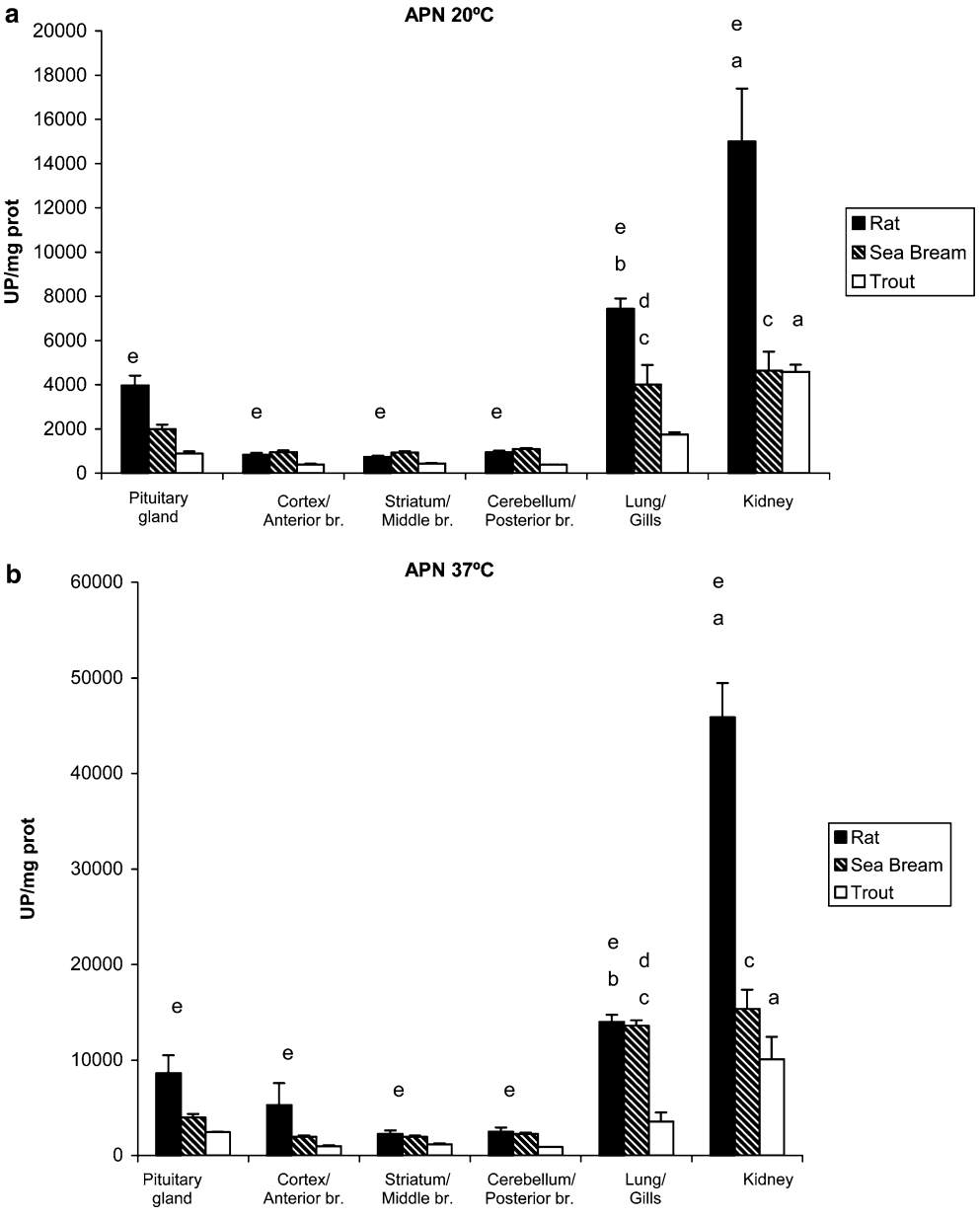

Fig. 3 The levels of activity of APN at 20°C (**a**) and 37°C (**b**) in different tissues from the rat, sea bream and rainbow trout. Values which are expressed as units of peptidase (UP)/mg protein, represent the mean ± SEM of $n = 10$ animals for each group. *a*: $P < 0.001$ kidney versus other tissues of rat and trout; *b*: $P < 0.05$ lungs versus CNS areas and pituitary gland of rat; *c*: $P < 0.05$ kidney and gills versus other tissues of sea bream; *d*: $P < 0.05$ gills of sea bream versus gills of trout. *e*: $P < 0.05$ rat tissues versus trout tissues

activity than the sea bream. However, at 37°C the activity is higher (ST at least $P < 0.05$) in the sea bream than in the trout. Rat enzyme activity at this temperature is lower than trout's soluble PE activity in peripheral tissues (ST at least $P < 0.05$) and very similar in the areas of the CNS. The high activity found in the pituitary gland and, to a lesser degree, in the brain areas of sea bream (ST pituitary gland and brain areas versus gills and kidney at least $P < 0.05$) is noteworthy. In the rat, the highest levels of activity were found in the different areas of the CNS (ST brain areas versus other tissues at least $P < 0.05$).

Membrane-bound PE activity is illustrated in Fig. 8. The profile of activity is similar to that found for the soluble enzyme; thus enzymatic activity was highest in trout tissues at 20°C (a) with the exception of the gills (ST at least $P < 0.05$), and in sea bream tissues at 37°C with the exception of the kidney (ST at least $P < 0.05$). The lowest activity of membrane-bound PE was detected in rat tissues (ST $P < 0.005$ rat versus gilthead sea bream at 37°C and $P < 0.005$ rat versus rainbow trout at 20°C), with the exception of activity in the anterior brain region of the rainbow trout at 37°C. Membrane-bound PE activity in the gilthead sea bream was especially high in the CNS areas and in the pituitary gland (ST pituitary gland and brain areas versus gills and kidney at least $P < 0.05$).

Discussion

Peptidases are responsible for controlling the activity of many peptides. Although information abounds regarding the role of these enzymes in mammals (Gil

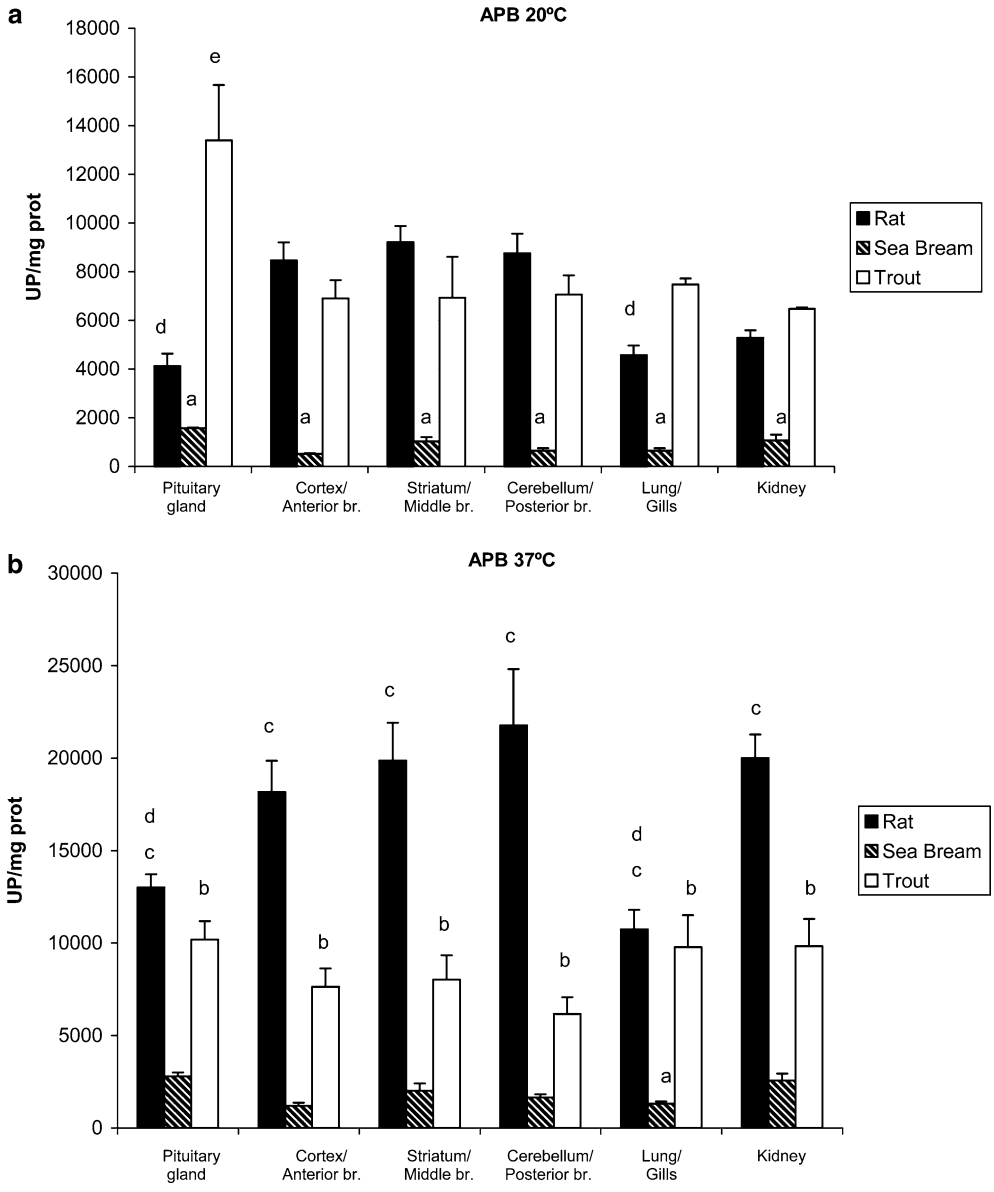

Fig. 4 The levels of activity of soluble basic APB at 20°C (**a**) and 37°C (**b**) in different tissues from the rat, sea bream and rainbow trout comprising. Values which are expressed as units of peptidase (UP)/mg protein, represent the mean ± SEM of $n = 10$ animals for each group. *a*: $P < 0.05$ sea bream tissues versus trout and rat tissues; *b*: $P < 0.05$ trout tissues versus sea bream tissues; *c*: $P < 0.05$ rat tissues versus trout and sea bream tissues; *d*: $P < 0.05$ gills and pituitary gland versus CNS of rat; *e*: $P < 0.05$ pituitary gland of the rat versus pituitary gland of the trout

et al. 2001; Irazusta et al. 2002; Varona et al. 2003), similar studies in fish are scarce (Galardy et al. 1984; Goren et al. 1990). Therefore, the objective of the present study was to compare the activity of a panel of peptidases in tissues (gills, kidney and different brain areas including the pituitary gland) of two teleosts (the marine gilthead sea bream and the freshwater rainbow trout) with the rat, a well-studied mammalian model. Significant differences in the activity of the studied enzymes were found between rat and gilthead sea bream or rainbow trout tissues. Enzymatic activity in the rat and sea bream at 37°C was always 2–3-fold higher than that measured at 20°C. However, in the trout APB and PE activities are very similar at both temperatures. Therefore, the activities of these two peptidases are comparatively higher in the trout. These results are consistent with the fact that the gilthead sea bream is more adaptable to higher water temperatures than the rainbow trout. Differences in the measured levels of activity of enzymes are not necessarily indicative of a difference in the quantity of enzymes in each species, because Kcat values may not be the same in rat and teleosts. However, these measured differences do provide information about the relative importance of each enzyme activity in each species and tissue.

One of the most interesting findings of the present study is the high level of activity of APN in the plasma of the gilthead sea bream and to a lesser extent in the rainbow trout. Thus, in the gilthead sea bream, this activity is 5-fold higher than in rat plasma (8107 UP versus 1696 UP) and it is very close to that found in the tissues of this species. However, high plasma APN activity is not a general feature of all teleosts because low APN activity has been found in other teleosts such as the sole or the Lusitanian toad fish (unpublished observations). Thus, this enzyme may be important for the clearance of circulatory peptides in specific types of fish.

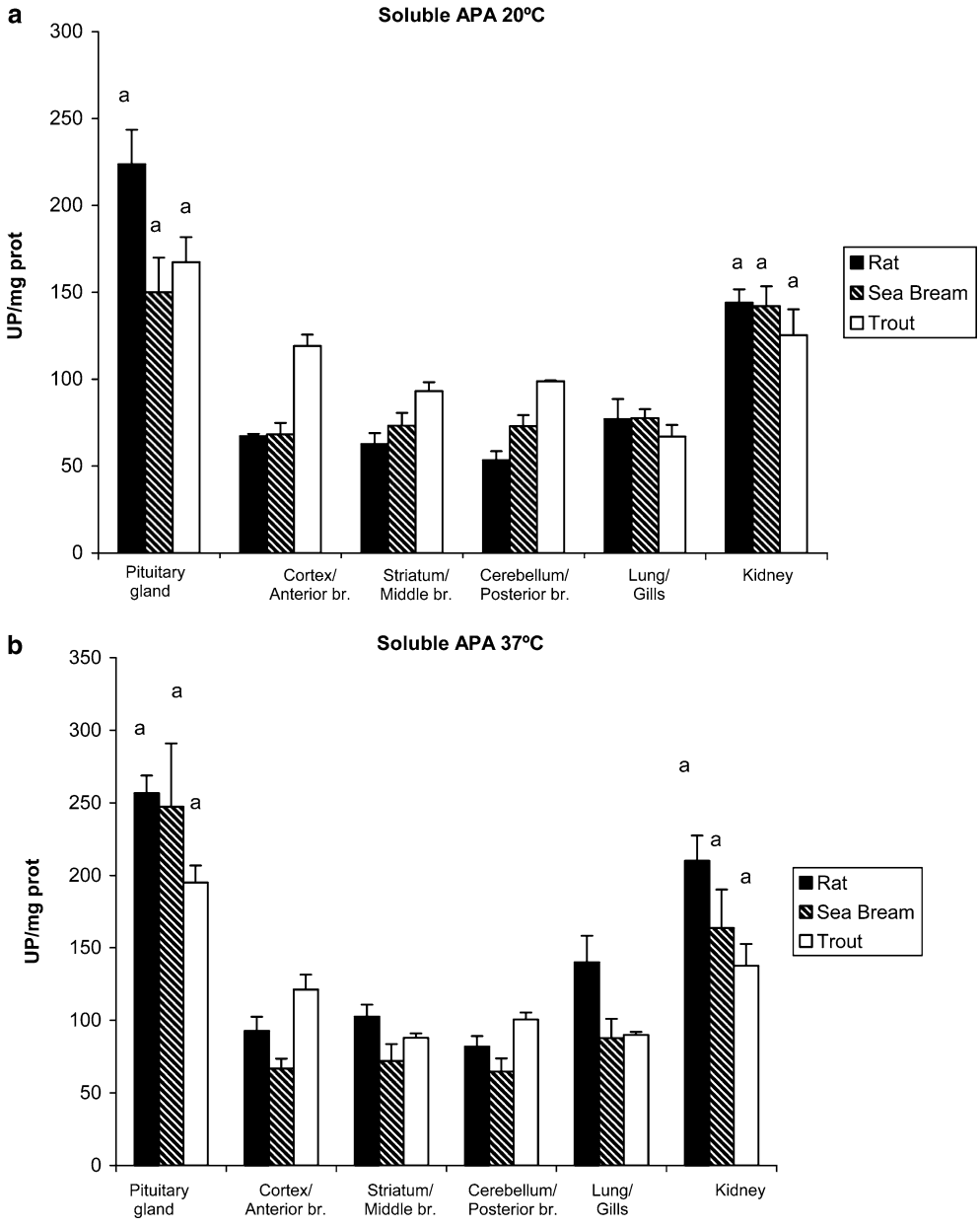

Fig. 5 Soluble acid APA activities at 20°C (**a**) and 37°C (**b**) in different tissues of the rat, sea bream and rainbow trout. Values which are expressed as units of peptidase (UP)/mg protein, represent the mean ± SEM of $n = 10$ animals for each group. *a*: $P < 0.05$ pituitary gland and kidney versus other tissues of rat, sea bream and trout

Since APN is a broad spectrum aminopeptidase capable of catalyzing the hydrolysis of many different peptides (Ward et al. 1990), it is difficult to correlate the high activity found in the plasma of the gilthead sea bream and rainbow trout with the levels of a concrete peptide. Nevertheless, candidate peptides which may be degraded by APN in fish serum include fish ANG II and vasotocin. The former differs from mammalian ANG II in that its N-terminal residue is Asn instead of Asp (Balment et al. 2003) and as a consequence, APN rather than APA is responsible for degrading ANG II in fish (Ward et al. 1990). The mammalian analogue of fish vasotocin is vasopressin. This is degraded by means of a step-wise degradation which is catalyzed also by neutral aminopeptidases (Mizutani et al. 1995). In teleosts, ANG peptides and vasotocin play an important osmoregulatory role in the adaptation of the fish to environments with different salinities (Takei 2000; Warne et al. 2002). Therefore, the high APN activity found in the plasma of the gilthead sea bream and rainbow trout may be related to the degradation of osmoregulatory peptides in these species. In this regard, it is interesting to note that the gilthead sea bream is a euryhaline teleost, which is capable of adapting to extreme changes in environmental salinity (Mancera et al. 1993), while the rainbow trout is a salmonid species capable of surviving in both freshwater and sea water (Shoji et al. 1996). It is conceivable that the high levels of activity of aminopeptidase detected in fish plasma may facilitate these teleosts to change their peptide levels in plasma quickly in order to adapt to changing conditions of environmental osmolarity. This possibility can be verified in the future by comparing plasma APN activity in gilthead sea bream and rainbow trout adapted to different environmental salinities.

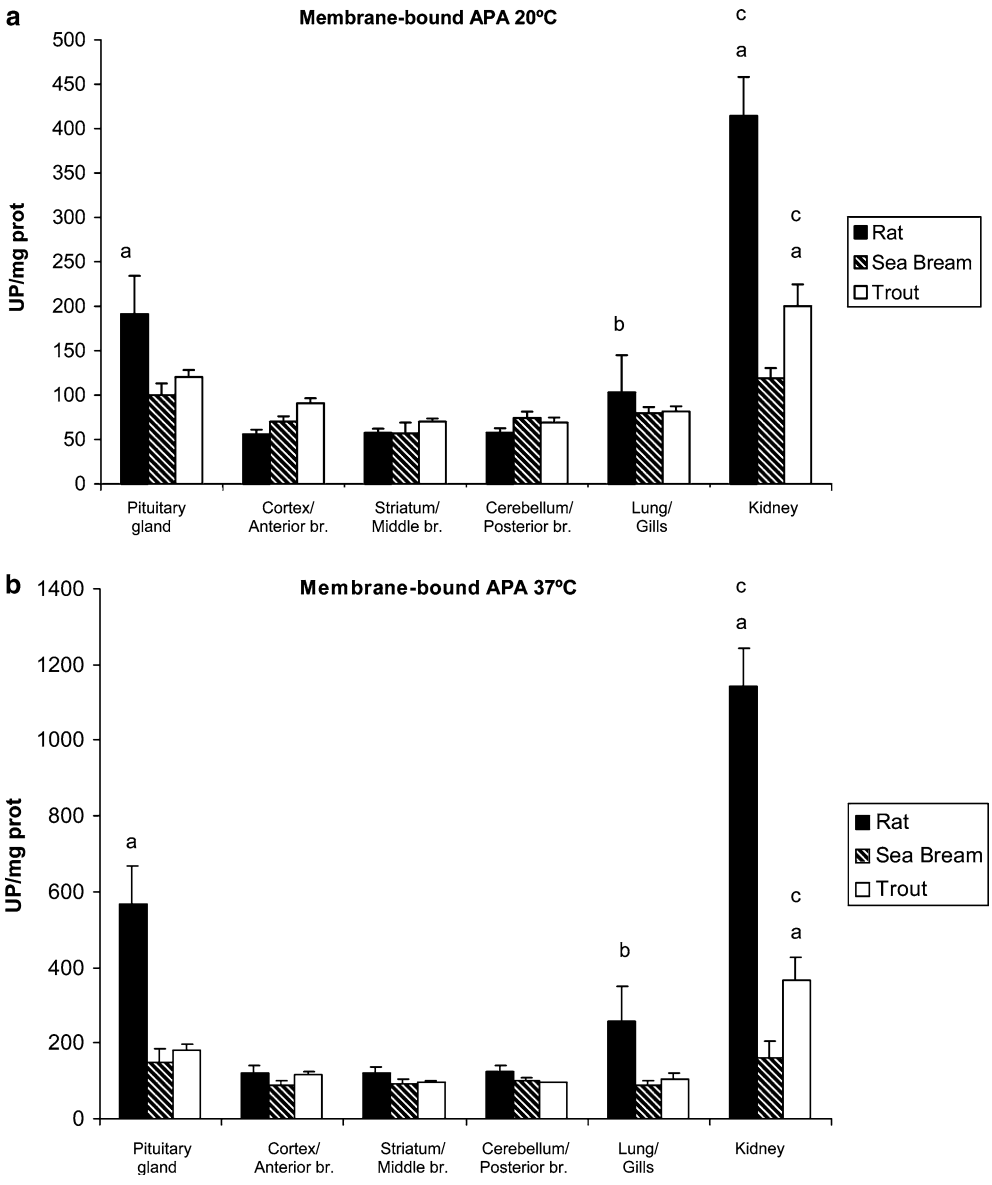

Fig. 6 Membrane-bound acid APA activities at 20°C (**a**) and 37°C (**b**) in different tissues of the rat, sea bream and rainbow trout. Values which are expressed as units of peptidase (UP)/mg protein, represent the mean ± SEM of $n = 10$ animals for each group. *a*: $P < 0.01$ kidney and pituitary gland of the rat and kidney of the trout versus other tissues of rat and trout; *b*: $P < 0.01$ lungs versus brain areas of rat; *c*: $P < 0.001$ kidney of rat and trout versus kidney of sea bream

High APN activity was also found in the kidneys of the three studied species. High levels in the rat kidney reported in this and other (Fernandez et al. 2002) studies may be related to the fact that the mammalian kidney is one of the principal clearance organs for neurohypophysial peptides (Itoh and Nagamatsu 1995). The fact that APN activity is also high in the kidneys of the rainbow trout and gilthead sea bream, suggests that the teleost kidney may also play a relevant role in the degradation of circulatory peptides. In addition, APN activity found in the gills of the gilthead sea bream was similar to that found in the kidney. Because the gills receive the entire cardiac output they are, like mammalian lungs, ideally situated to regulate plasma hormone levels (Galardy et al. 1984). Indeed, the pulmonary circulation is known to be the principal site for inactivation of kinins (Orawski et al. 1989).

Basic aminopeptidase is an enzyme which is capable of hydrolyzing Arg- or Lys-residues of peptides. It was originally identified in several rat tissues and plays a role in transforming kallidin (Lys-bradykinin) to bradykinin (Hopsu et al. 1966). The activity of this enzyme is 5–10-fold lower in the different tissues of the gilthead sea bream than in those of the rat. Intermediate levels of activity of this enzyme were found in rainbow trout tissues at 37°C, However, at 20°C the activity in the trout was similar to that observed in rat tissues. This elevated level of activity of APB in trout may be related to the metabolism of kinin peptides (Lipke et al. 1990). The existence of a kinin system in mammals has been extensively described (Casarini et al. 1999; Hayashi et al. 2000). However, its function in fish is not well characterized (Seki et al. 1973; Dunn and Perks 1975). Thus, some authors have proposed that kallidin is generated in rainbow trout plasma only under non-physiological conditions (Conlon and Olson 1993). However, others have reported that the components of the kinin system are present in certain tissues of the trout, and

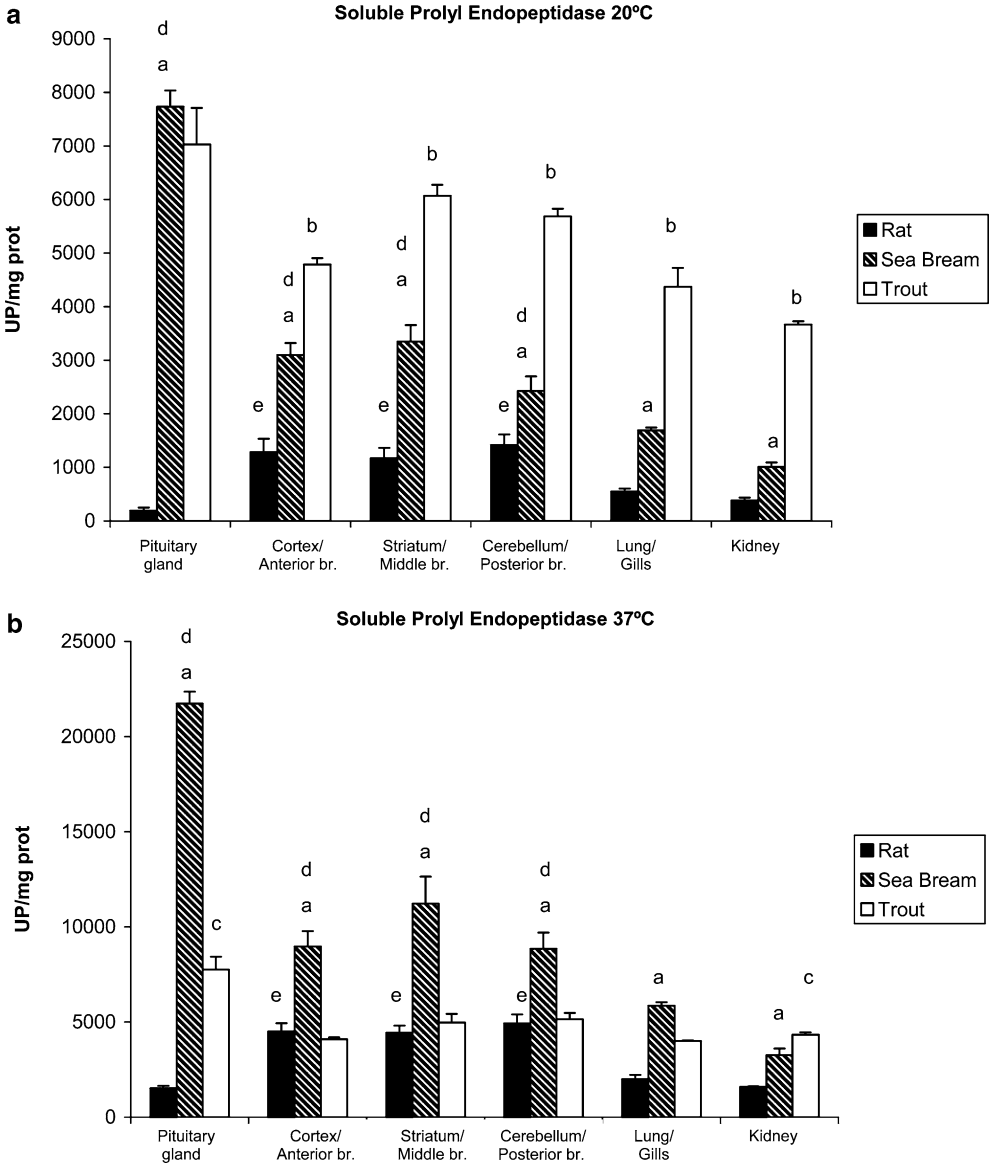

Fig. 7 Soluble PE activity at 20°C (**a**) and 37°C (**b**) in different tissues of the rat, sea bream and rainbow trout. Values which are expressed as units of peptidase (UP)/mg protein, represent the mean ± SEM of $n = 10$ animals for each group. *a*: $P < 0.05$ tissues of sea bream versus tissues of rat; *b*: $P < 0.05$ tissues of trout versus tissues of rat and sea bream (with the exception of the pituitary gland); *c*: $P < 0.05$ pituitary gland and kidney of trout versus pituitary gland and kidney of rat; *d*: $P < 0.05$ pituitary gland and brain areas versus gills and kidney of sea bream; *e*: $P < 0.05$ brain areas versus other tissues of rat

that [Arg^0, Trp^5, Leu^8]-bradykinin is the active kinin peptide (Jensen et al. 2000). Since bradykinin is a potent antidipsogenic hormone in some fish (Takei 2000), higher APB activity in the trout in comparison to the sea bream may be indicative of a more developed bradykinin system in the freshwater-adapted trout than in the gilthead sea bream, in order to avoid overhydration.

Soluble APA activity did not show significant tissue-related differences in the studied animals, suggesting that this enzyme may not have characteristic, tissue-specific functions in the studied tissues. In contrast, the levels of activity of membrane-bound APA were higher in peripheral tissues of the rat in comparison to the teleost species. As mentioned previously, APA is involved in the degradation of ANG II in mammals, but not in teleosts, in which Asn is the N-terminal residue of ANG II (Balment et al. 2003). The expression of APA in sites in which the renin–angiotensin system is active, such as the rat kidney and lungs, indicates that APA may regulate ANG II systems in this animal. However, in teleosts, other enzymes such as APN are likely to play a more important role in the degradation of this osmoregulatory octapeptide.

Propyl endopeptidase activity was found to be higher in both teleost species than in rat. Among teleostean tissues studied, the pituitary gland showed the highest activity. Among the different brain regions, the activity of this enzyme was found to be highest in the middle brain. In the mammalian brain, PE can hydrolyze several peptides such as vasopressin, ANG peptides or TRH, which are related to osmoregulation and the control of body fluids (Cunningham and O'Connor 1997). Indeed, this enzyme is responsible for the formation of ANG (1–7) (Ferrario and Iyer 1998), which is known to have a long lasting hypotensive effect in trout (Russell et al. 2001). In rat, changes in the activity of this enzyme associated with hydrosaline challenges have been described (Irazusta et al. 2001).

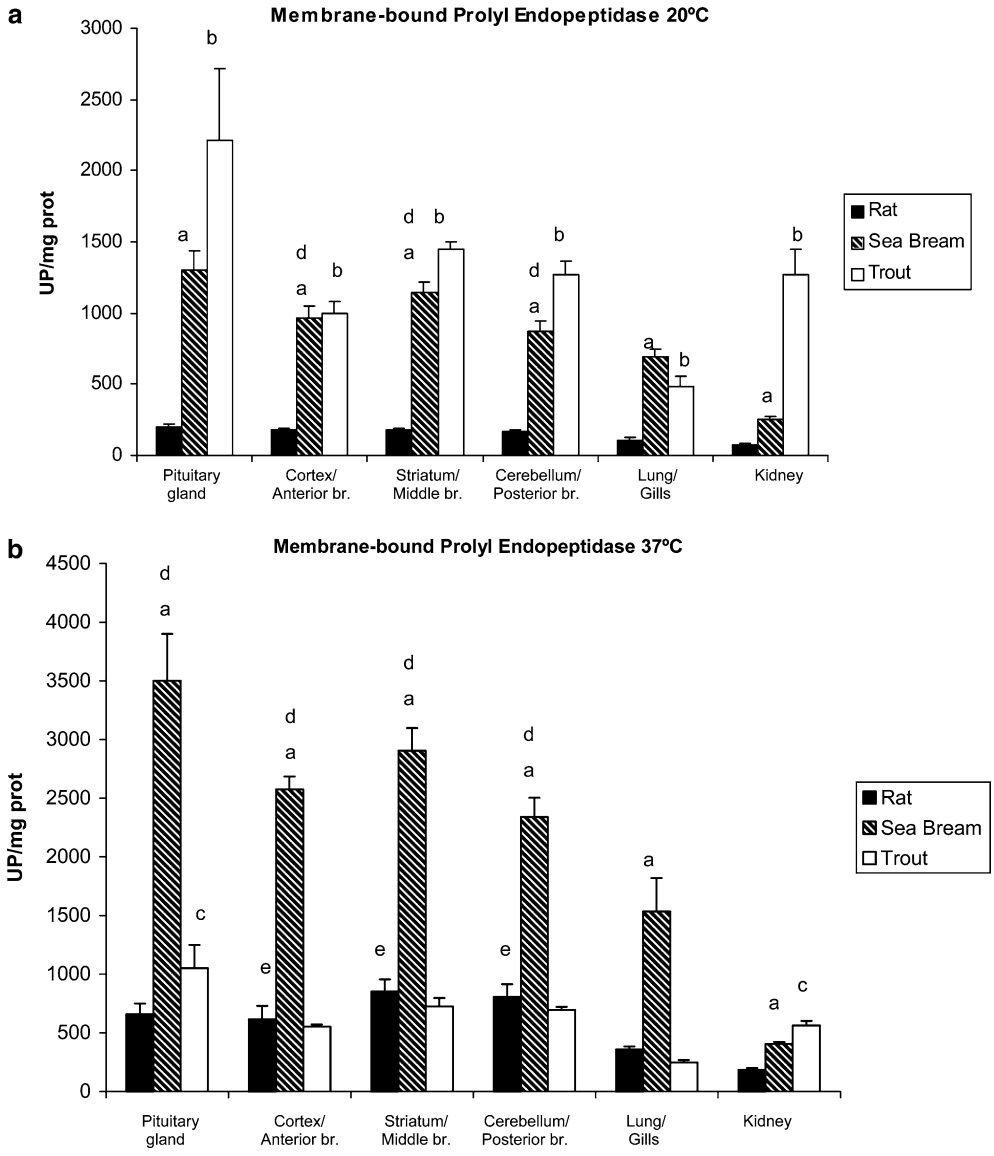

Fig. 8 Membrane-bound PE activity at 20°C (**a**) and 37°C (**b**) in different tissues of the rat, sea bream and rainbow trout. Values which are expressed as units of peptidase (UP)/mg protein, represent the mean ± SEM of $n = 10$ animals for each group. *a*: $P < 0.05$ tissues of sea bream versus tissues of rat; *b*: $P < 0.05$ tissues of trout versus tissues of rat and sea bream (with the exception of the anterior brain and the gills); *c*: $P < 0.05$ pituitary gland and kidney of trout versus pituitary gland and kidney of rat; *d*: $P < 0.05$ pituitary gland and brain areas versus gills and kidney of sea bream; *e*: $P < 0.05$ brain areas versus lungs and kidney of rat

The high level of activity of PE in the brain areas and in the pituitary gland of the gilthead sea bream and rainbow trout may facilitate the degradation of such osmoregulatory peptides and thus, may represent one of the mechanisms which allow both teleostean species to adapt to changing conditions of environmental osmolarity.

An overall analysis of the results from both teleosts under study reveals that in the kidney, analyzed peptidase activities are predominantly higher in the trout than in the sea bream. There is at least a significant exception to this general rule: APN, the enzyme responsible for degrading dipsogenic ANG II (Ward et al. 1990), presented similar activity in the kidney of the sea bream and the trout. On the other hand, APB, the enzyme responsible for degrading the antidipsogenic hormones kallidin and bradykinin (Hopsu et al. 1966), presented higher levels of activity in the freshwater-adapted trout. These differences between the two species may well be related to the different mechanisms of osmoregulation in salt water and in freshwater-adapted fish.

In summary, we report here distinct distributions of peptidase activities in teleosts which are both tissue- and species-related. The differences between species could be related to different mechanisms of osmoregulation in saltwater- and in freshwater- adapted fish. These results provide a starting point for the study of the involvement of peptidases in the adaptation of fish to different salinities.

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