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# Effect of S 17092, a novel prolyl endopeptidase inhibitor, on substance P and α-melanocyte-stimulating hormone breakdown in the rat brain

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**Abstract**

In the present study, we have investigated the effects of a novel prolyl endopeptidase (EC 3.4.21.26, PEP) inhibitor, compound S 17092, on substance P (SP) and α-melanocyte-stimulating hormone (α-MSH) metabolism in the rat brain. *In vitro* experiments revealed that S 17092 inhibits in a dose-dependent manner PEP activity in rat cortical extracts ($IC_{50}$ = 8.3 nM). In addition, S 17092 totally abolished the degradation of SP and α-MSH induced by bacterial PEP. *In vivo*, a significant decrease in PEP activity was observed in the medulla oblongata after a single oral administration of S 17092 at doses of 10 and 30 mg/kg (−78% and −82%, respectively) and after chronic oral treatment with S 17092 at doses of 10 and 30 mg/kg per day (−75% and −88%, respectively). Concurrently, a single administration of S 17092 (30 mg/kg) caused a significant increase in SP- and α-MSH-like immunoreactivity (LI) in the frontal cortex (+41% and +122%, respectively) and hypothalamus (+84% and +49%, respectively). In contrast, chronic treatment with S 17092 did not significantly modify SP- and α-MSH-LI in the frontal cortex and hypothalamus. Collectively, the present results show that S 17092 elevates SP and α-MSH concentrations in the rat brain by inhibiting PEP activity. These data suggest that the effect of S 17092 on memory impairment can be accounted for, at least in part, by inhibition of catabolism of promnesic neuropeptides such as SP and α-MSH.

**Keywords:** catabolism, central nervous system, cognitive enhancer, neuropeptides, peptidase inhibitor.

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There is now clear evidence that various neuropeptides can modify behavioral activities, particularly learning and memory processes (for a review, see Kovács and De Wied 1994). Reciprocally, a number of studies have shown that peptidergic neurotransmission is altered in neurodegenerative diseases leading to memory loss, notably in Alzheimer's disease (AD) (Rossor *et al*. 1986; Husain and Nemeroff 1990). Currently, treatment of memory loss associated with AD mainly consists in preventing cholinergic deficit by inhibiting cholinesterase activity in the brain (Krall *et al*. 1999). By analogy with the anticholinesterase therapy, new strategies, aimed at protecting promnesic neuropeptides from breakdown by means of selective peptidase inhibitors, are now considered.

Substance P (SP) and α-melanocyte-stimulating hormone (α-MSH) are two of the most thoroughly studied neuropeptides in terms of modulation of memory processes. The tachykinin SP has been shown to improve consolidation of avoidance behavior in rat when given either centrally or peripherally (Huston and Oitzl 1989; Huston and Hasenöhrl 1995) and to prevent memory impairment in old rats (Hasenöhrl *et al*. 1990, 1994). It has also been found that post-trial injection of SP within the lateral hypothalamus induces facilitation of learning (Huston and Oitzl 1989; Hasenöhrl *et al*. 2000). On the other hand, α-MSH is

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*Abbreviations used*: Aβ, β amyloid protein; AD, Alzheimer's disease; DMSO, dimethylsulfoxide; LI, like immunoreactivity; α-MSH, α-melanocyte-stimulating hormone; OD, optical density; PEP, prolyl endopeptidase; RIA, radioimmunoassay; SP, substance P; TFA, trifluoroacetic acid; Z-Gly-Pro-pNA, carbobenzyloxy-glycyl-prolyl-paranitroanilide.

 

produced by a group of cells located in the hypothalamic arcuate nucleus that project to a number of brain regions involved in memory processes i.e. the lateral septum, nucleus accumbens, amygdaloid nuclei, striatum, cortex and dorsal hippocampus (Jacobowitz and O'Donohue 1978; Eskay *et al*. 1979). Moreover, it has been reported that α-MSH improves acquisition and delays extinction of avoidance behaviors (De Wied 1966; Eberle 1988; Pitsikas *et al*. 1991). Previous study have shown that SP- and α-MSH-producing neurons are impaired in AD. Indeed, a decrease in SP-like immunoreactivity (LI) has been reported in the cerebral cortex and hippocampus of AD patients as compared to control subjects (Crystal and Davies 1982; Beal and Mazurek 1987; Clevens and Beal 1989; Bouras *et al*. 1990). Concurrently, immunohistochemical studies have demonstrated that SP-containing neurons are depleted in the cerebral cortex of AD patients (Kowall *et al*. 1993; Ang and Shul 1995; Yew *et al*. 1999). A decrease in α-MSH-LI has also been found to occur in the cingulate cortex, caudate, substantia nigra and cerebrospinal fluid (CSF) of AD patients (Arai *et al*. 1986; Rainero *et al*. 1988). These observations suggest that SP and α-MSH may play an important role in AD symptomatology.

Various peptidases are involved in the degradation process of SP and α-MSH. Among these peptidases is prolyl endopeptidase, also called prolyl oligopeptidase (EC 3.4.21.26, PEP), a serine protease that hydrolyzes peptides at the carboxyl side of proline residues. PEP was first isolated in the human uterus as an oxytocin-degrading enzyme (Walter *et al*. 1971) and subsequently purified from lamb kidney (Koida and Walter 1976). PEP is widely distributed in mammalian tissues including muscle (Daly *et al*. 1985), liver (Yamakawa *et al*. 1986), testis (Yoshimoto *et al*. 1988) and brain (Kato *et al*. 1980; Kalwant and Porter 1991). *In vitro* studies have shown that PEP can actually cleave a number of neuropeptides including oxytocin, thyrotropin-releasing hormone, neurotensin and arginine-vasopressin (Taylor and Dixon 1980; Wilk 1983).

Several studies have pointed out the potential therapeutic relevance of PEP inhibitors as memory promoting drugs (Yoshimoto *et al*. 1987). S 17092 is a novel, potent and specific inhibitor of PEP *in vitro* and *in vivo* (Barelli *et al*. 1999). This compound has been shown to improve spatial and working memory in young, old and amnesic mice, indicating that S 17092 possesses promnesic as well as antiamnesic properties (Lestage *et al*. 1998; Marighetto *et al*. 2000). S 17092 may therefore be of therapeutic value as a cognitive enhancer for the treatment of memory loss related either to aging or senile dementias. In the present study, we have investigated the effects of S 17092 on PEP activity *in vitro* and *in vivo*. Considering the promnesic properties of SP and α-MSH, and their possible involvement in AD symptomatology, we have studied the effects of acute and chronic administrations of S 17092 on SP and α-MSH levels in the rat frontal cortex and hypothalamus.

## Materials and methods

### Animals

Adult male Wistar rats (3 month-old, 300–400 g) were housed at constant temperature (23 ± 1°C) under a 12-h light/12-h dark schedule (lights on from 08 : 00 to 20 : 00 h). The animals had free access to standard rat chow and tap water. Animal manipulations were performed according to the recommendations of the French Ethical Committee and under the supervision of authorized investigators.

### Chemicals

(2*S*,3a*S*,7a*S*)-1-{[(*R*,*R*)-2phenylcyclopropyl]carbonyl}-2-[(thiazolidin-3-yl) carbonyl] octahydro-1H-indole (S 17092; mol wt, 384.5) was synthetized by the Institut de Recherches Internationales Servier (Courbevoie, France). Recombinant *Flavobacterium meningosepticum* PEP was purchased from ICN Biomedicals (Aurora, OH, USA). Carbobenzyloxy-glycyl-prolyl-paranitroanilide (Z-Gly-Pro-pNA) and SP were obtained from Bachem (Voisins-le-Bretonneux, France). α-MSH, SP(5–11), Tyr8-SP and polyoxyethylene-sorbitan monooleate (Tween 80) were from Sigma Aldrich (Saint-Quentin Fallavier, France).

### *In vivo* treatments

S 17092 was extemporaneously homogenized in Tween 80/distilled water (10 : 90; v/v) and the mixture was administered *per os* to rats (10 or 30 mg/kg) at 10 : 00 AM. Control groups received an equivalent volume (1 mL/kg) of vehicle. For acute treatments, the animals received a single dose of S 17092 (10 or 30 mg/kg) or vehicle. For chronic treatments, rats received a daily injection of S 17092 (10 or 30 mg/kg) or vehicle for 8 days. The animals were killed 1 h after the last administration.

### Assay of PEP activity

The inhibitory effect of S 17092 was investigated *in vitro* by using either recombinant *Flavobacterium* PEP or rat brain extracts. In the latter case, brain cortices from 10 rats were rapidly removed and homogenized in 25 mM phosphate buffer (PB; 5 mL/g tissue; pH 7.0) containing 2 mM dithiothreitol and 0.5 mM EDTA. The homogenate was centrifuged (14 000 X *g*, 4°C, 20 min), the supernatant was aliquoted and stored at −80°C until enzymatic assay. *Flavobacterium* PEP solution (0.015 UI) or 100 μL of cortex homogenate was pre-incubated in PB in the absence or presence of various concentrations of S 17092 ($10^{-12}$–$10^{-6}$ M). The reaction was initiated by adding 15 μL of Z-Gly-Pro-pNA ($10^{-4}$ M). S 17092 and Z-Gly-Pro-pNA were extemporaneously solubilized in dimethylsulfoxide (DMSO) and final solutions were made up in PB so that the concentration of DMSO was always lower than 2.5%. Incubation was carried out at 37°C for 60 min and the reaction was stopped by chilling on ice. Formation of paranitroanilide was monitored by measuring absorbance at 410 nm. The potency of the inhibitory activity of S 17092 was calculated as the IC50 value, i.e. the concentration of S 17092 that resulted in a 50% decrease of the absorbance.

The effect of S 17092 on PEP activity was also investigated *in vivo*. After oral administration of S 17092 (10 or 30 mg/kg), animals were killed by decapitation. The brain was removed and the medulla oblongata was dissected on ice within 3 min after killing.

Tissue extracts were prepared and the supernatants were frozen at 80°C until assay of PEP activity, as described above. PEP activity was expressed as nmol of paranitroanilide/min per g of tissue.

### Inhibitory potency of S 17092 on SP- and α-MSH-hydrolyzing activity of *Flavobacterium* PEP

The effect of PEP on SP and α-MSH breakdown was investigated in the presence or absence of S 17092, by combining HPLC analysis with radioimmunoassay (RIA) detection. One hundred μL of recombinant *Flavobacterium* PEP (0.015 UI) was pre-incubated with S 17092 ($10^{-5}$ M) or solvent in 860 μL of PB at 37°C for 15 min. The reaction was initiated by adding 4 μg of synthetic peptide solubilized in 40 μL of distilled water. After a 60-min incubation, the solution was chilled on ice for 15 min and then evaporated in a Speed Vac Concentrator (Savant, Hiksville, NY). The dry extract was resuspended in 1 mL of a water/trifluoroacetic acid (TFA) solution (99.88 : 0.12; v/v) and injected into a Hibar Lichrosorb RP-18 column (7 μm, 0.46 × 25 cm) at a constant flow rate (1 mL/min). The mobile phase consisted of a gradient established with 0.12% TFA in water and 0.09% TFA in acetonitrile. The gradient used is shown in Fig. 2a and 3a. HPLC standards consisted of 4 μg of each synthetic peptide. Metabolites were detected using an ultraviolet detector (Perkin Elmer, Courtaboeuf, France) at a wavelength of 215 nm. All collected fractions were partially evaporated to remove acetonitrile, and the SP or α-MSH content was determined by RIA.

### Radioimmunoassay procedures

For quantification of SP and α-MSH-LI contents in the frontal cortex and hypothalamus, dissected tissues were immersed in 1 mL boiling 2 M acetic acid for 15 min. The boiled tissues were chilled on ice, homogenized with a glass Potter homogenizer and centrifuged (6000 *g*, 4°C, 30 min). The supernatants were collected, evaporated in a Speed Vac Concentrator and kept dry until peptide quantification. The concentrations of SP- and α-MSH-LI were measured in duplicate by means of double-antibody RIA methods. The production and characterization of antibodies against SP (generously provided by Dr J. M. Conlon, Creighton University, Omaha, NE, USA) and α-MSH (generously provided by Dr M. C. Tonon, INSERM U 413, Rouen, France) have been previously described (Vaudry *et al*. 1978; Conlon 1991). The SP antiserum is directed towards the COOH-terminal region of the peptide and thus SP(6–11) fully crossreacted in the SP RIA. The α-MSH antiserum is directed against the 10–13 portion of the peptide.

### Statistical analysis

Results are expressed as means ± SEM. Statistical analysis was performed using a two-way ANOVA test followed by a post hoc Newman-Keuls test.

## Results

### *In vitro* effects of S 17092 on PEP activity

The effect of S 17092 on PEP activity was monitored by using Z-Gly-Pro-pNA as a synthetic substrate. S 17092 induced a concentration-dependent inhibition of paranitroanilide formation by rat cerebral cortex extracts, with an $IC_{50}$ of 8.3 nM (Fig. 1). As a control, the effect of S 17092 on recombinant *Flavobacterium* PEP activity was also investigated, and the $IC_{50}$ value was 3.7 nM (Fig. 1).

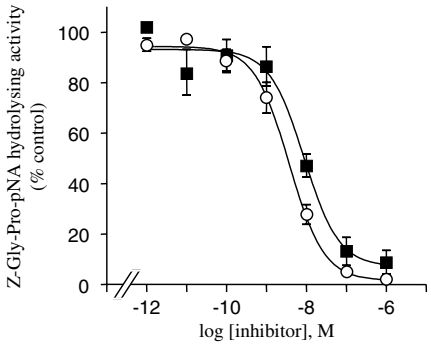


**Fig. 1** Effect of S 17092 on PEP activity. Recombinant *Flavobacterium* PEP (○) or rat cerebral cortex extract (■) was incubated with graded concentrations of S 17092 in the presence of Z-Gly-Pro-pNA at 37°C for 60 min. Data are expressed as the mean ± SEM of five independent experiments.

### *In vitro* effects of S 17092 on SP and α-MSH breakdown by *Flavobacterium* PEP

The effect of S 17092 on SP breakdown by recombinant *Flavobacterium* PEP was investigated by combining HPLC analysis with RIA detection. In control conditions, synthetic SP eluted as a single peak with a retention time of 19.8 min (Fig. 2a, left) and the amount of SP-LI contained in the corresponding fractions was approximately 3340 ng (Fig. 2a, right). Incubation of SP with *Flavobacterium* PEP at 37°C for 1 h provoked a marked decrease of the 19.8-min peak and the appearance of a more hydrophilic peptide that exhibited the same retention time as synthetic SP(5–11), i.e. 17.5 min (Fig. 2b, left). Quantification by RIA subsequently showed that both peptides were immunoreactive (Fig. 2b, right). It should be noticed that the SP antiserum, which is directed towards the COOH-terminal region of the peptide, fully cross-reacts with SP(6–11) (Conlon 1991). Quantification of SP-LI revealed that the amount of intact SP was decreased by 90% and that the amount of SP-LI contained in the 17.5-min peak was approximately 2080 ng. Addition of $10^{-5}$ M S 17092 to the incubation medium totally blocked degradation of SP induced by recombinant *Flavobacterium* PEP (Fig. 2c).

The effect of S 17092 on α-MSH breakdown by PEP was monitored by using α-MSH antibodies that are directed against α-MSH(10–13) (Vaudry *et al*. 1978; Jégou *et al*. 1981). Synthetic α-MSH eluted as a single peak with a retention time of 13.4 min (Fig. 3a). Incubation of α-MSH with *Flavobacterium* PEP did not apparently modify the amplitude of the α-MSH peak measured by optical density (OD) (Fig. 3b, left). It should be noticed

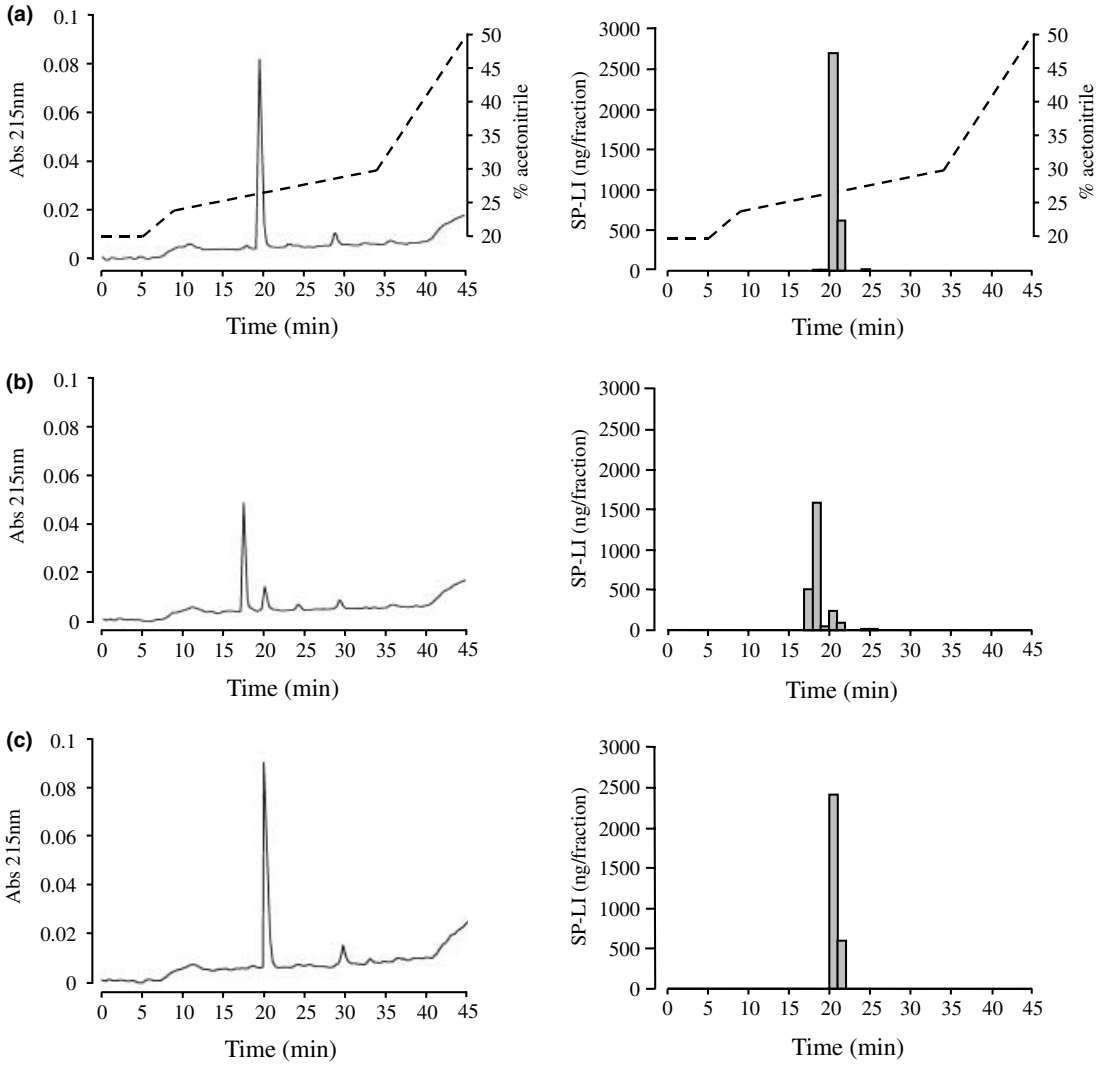


**Fig. 2** Effect of S 17092 on substance P (SP) breakdown by recombinant *Flavobacterium* PEP. Synthetic SP (4 μg/mL) was incubated at 37°C for 60 min without (a) or with (b and c) *Flavobacterium* PEP in the absence (a and b) or presence (c) of S 17092. The medium was analyzed by HPLC and the resolved peptides were monitored by UV detection (left) and by radioimmunoassay (right). The dashed lines show the concentration of acetonitrile in the eluting solvent.

however, that the HPLC gradient that was used did not allow to discriminate authentic α-MSH from the α-MSH(1–12) peptide. In contrast, quantification of α-MSH-LI by RIA revealed that the amount of intact α-MSH was actually decreased by 82% (Fig. 3b, right). Addition of $10^{-5}$ M S 17092 almost completely blocked the disappearance of immunoreactive α-MSH induced by PEP (Fig. 3c).

### *In vivo* effects of S 17092 on brain PEP activity

PEP activity was measured in rat medulla oblongata by monitoring the absorbance of paranitroanilide at 410 nm. Administration of a single dose of S 17092 (10 or 30 mg/kg) induced a significant reduction of PEP activity within 60 min after treatment (−78% and − 82%, respectively) (Fig. 4a). Similarly, repeated administration of S 17092 (10 or 30 mg/kg) for 8 days markedly reduced PEP activity (−75% and 88%, respectively) (Fig. 4b).

### *In vivo* effects of S 17092 on SP-LI in the frontal cortex and hypothalamus

A single administration of S 17092, at a dose of 10 mg/kg, did not affect the content of SP-LI in the frontal cortex (Fig. 5a) and in the hypothalamus (Fig. 5b). At a dose of 30 mg/kg, acute administration of S 17092 provoked a significant increase in the content of SP-LI in the frontal cortex (+41%; Fig. 5a) and in the hypothalamus (+84%;

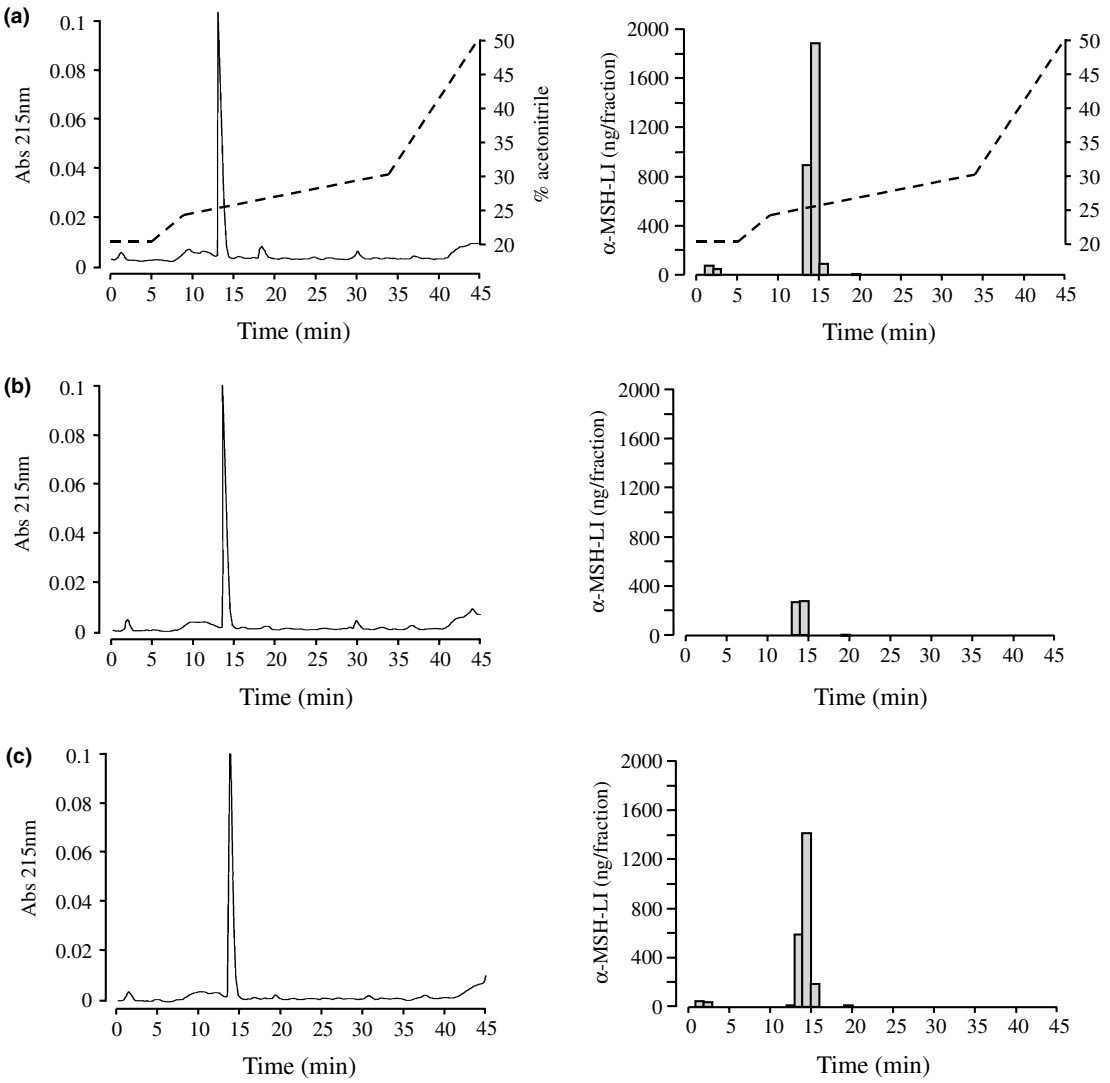


**Fig. 3** Effect of S 17092 on α-melanocyte-stimulating hormone (α-MSH) breakdown by recombinant *Flavobacterium* PEP. Synthetic α-MSH (4 μg/mL) was incubated at 37°C for 60 min without (a) or with (b and c) *Flavobacterium* PEP in the absence (a and b) or presence (c) of S 17092. The medium was analyzed by HPLC and the resolved peptides were monitored by UV detection (left) and by radioimmunoassay (right). The dashed lines show the concentration of acetonitrile in the eluting solvent.

Fig. 5b). In contrast, chronic administration of S 17092 at doses of 10 or 30 mg/kg for 8 days did not modify SP-LI in these two brain regions (Fig. 6).

### *In vivo* effects of S 17092 on α-MSH-LI in the frontal cortex and hypothalamus

A single administration of S 17092 at doses of 10 and 30 mg/kg induced a significant increase in the content of α-MSH-LI in the frontal cortex (+164% and +122%, respectively) (Fig. 7a). Similarly, acute administration of S 17092 caused a significant increase in α-MSH-LI in the hypothalamus (+50% and +49%, respectively) (Fig. 7b). Conversely, chronic administration of S 17092 did not affect α-MSH content in the frontal cortex (Fig. 8a) and in the hypothalamus (Fig. 8b).

## Discussion

The present study has shown that compound S 17092 inhibits, *in vitro*, the activities of recombinant *Flavobacterium* PEP and rat PEP, with $IC_{50}$ values in the nanomolar range. Our data also indicate that acute and chronic oral administration of S 17092 at doses of 10 and 30 mg/kg provokes strong reduction of PEP activity in the rat brain. These results are consistent with previous studies which have demonstrated that the $ID_{50}$ value of S 17092 is 8 mg/kg (Portevin *et al*. 1996). It has been reported that S 17092 is effective in enhancing memory processes when administered per os at doses of 10 and 30 mg/kg (Lestage *et al*. 1998). Collectively, these data suggest that this novel antiamnesic

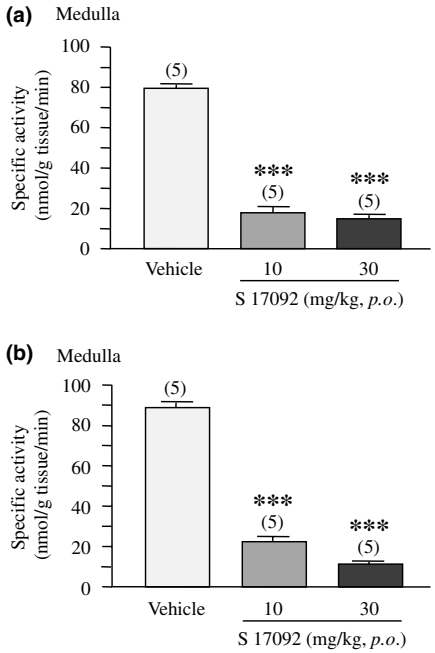


**Fig. 4** Effect of acute (a) or chronic (b) administration of S 17092 on PEP activity in the rat medulla oblongata. (a) Rats received a single dose of S 17092 (10 or 30 mg/kg per os) and were killed 60 min later. (b) Rats were treated daily with S 17092 (10 or 30 mg/kg per os) and were killed 60 min after the last administration. Data are expressed as the mean ± SEM of five rats. ***$p < 0.001$ vs. vehicle-treated rats (Newman-Keuls multiple comparison test).

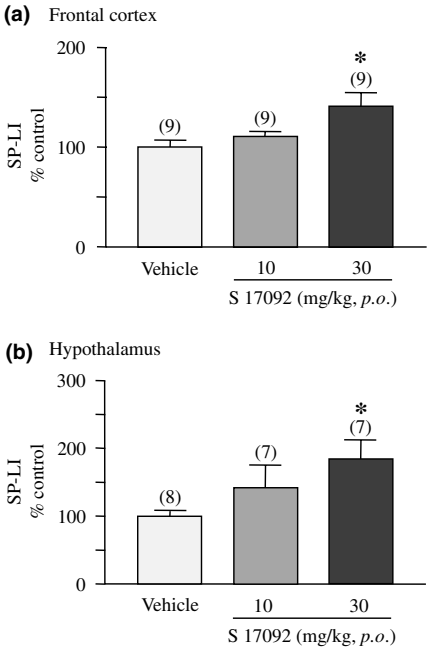


**Fig. 5** Effect of acute administration of S 17092 on substance P-like immunoreactivity (SP-LI) in the rat frontal cortex (a) and hypothalamus (b). Rats received a single dose of S 17092 (10 or 30 mg/kg per os) and were killed 60 min later. Data are expressed as the mean ± SEM from seven to nine rats. *$p < 0.05$ vs. vehicle-treated rats (Newman–Keuls multiple comparison test).

drug may act via inhibition of promnesic neuropeptide catabolism. We have thus investigated the effects of S 17092 on SP and α-MSH degradation by *Flavobacterium* PEP *in vitro*. We have also studied the effects of S 17092 at doses of 10 and 30 mg/kg *in vivo* on the metabolism of SP and α-MSH in the rat cerebral cortex and hypothalamus.

### Involvement of PEP in SP and α-MSH catabolism in the rat brain

Various peptidases have been shown to hydrolyze SP *in vitro*, i.e. endopeptidases 24.15 and 24.16 (Dahms and Mentlein 1992), dipeptidyl aminopeptidase IV and aminopeptidase M (Ahmad *et al*. 1992). It has been reported that degradation of SP by rat and human synaptic membranes is inhibited by phosphoramidon and thiorphan, indicating that neutral endopeptidase 24.11 is actually involved in the inactivation of SP *in vivo* (Matsas *et al*. 1985; Sakurada *et al*. 1999). The present study reveals that SP is cleaved by recombinant *Flavobacterium* PEP *in vitro* at the Pro4-Gln5 bond and that administration of a single dose of S 17092 provokes a significant increase in the content of SP-LI in the frontal cortex and the hypothalamus. These data provide strong evidence that PEP may be implicated in the catabolism of SP in the rat brain.

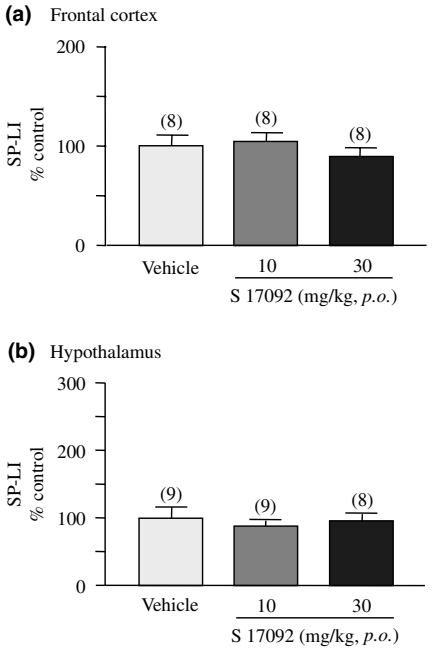


**Fig. 6** Effect of chronic administration of S 17092 on substance P-like immunoreactivity (SP-LI) in the rat frontal cortex (a) and hypothalamus (b). Rats were treated daily with S 17092 (10 or 30 mg/kg per os) and were killed 60 min after the last administration. Data are expressed as the mean ± SEM from eight to nine rats.

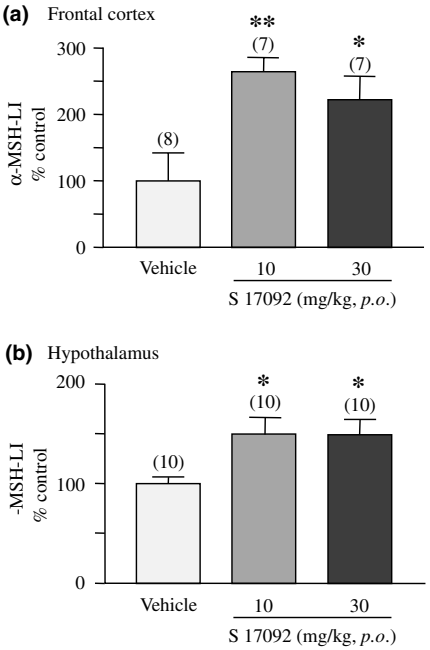


**Fig. 7** Effect of acute administration of S 17092 on α-melanocyte-stimulating hormone-like immunoreactivity (α-MSH-LI) in the rat frontal cortex (a) and hypothalamus (b). Rats received a single dose of S 17092 (10 or 30 mg/kg per os) and were killed 60 min later. Data are expressed as the mean ± SEM from seven to 10 rats. $^{*}p < 0.05$; $^{**}p < 0.01$ vs. vehicle-treated rats (Newman–Keuls multiple comparison test).

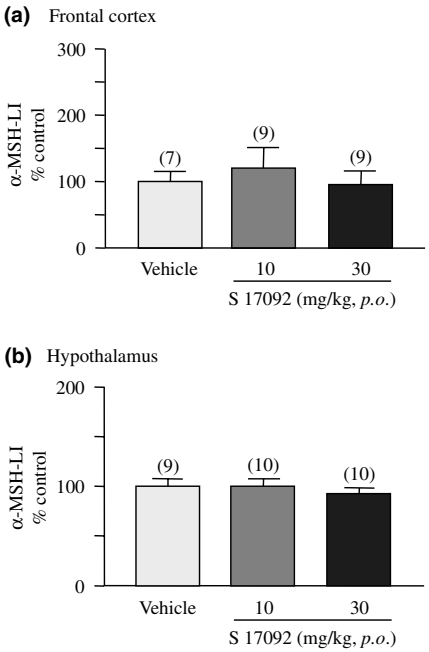


**Fig. 8** Effect of chronic administration of S 17092 on α-melanocyte-stimulating hormone-like immunoreactivity (α-MSH-LI) in the rat frontal cortex (a) and hypothalamus (b). Rats were treated daily with S 17092 (10 or 30 mg/kg per os) and were killed 60 min after the last administration. Data are expressed as the mean ± SEM from seven to 10 rats.

Our knowledge on the biodegradation of α-MSH is still fragmentary. Early studies have shown that the presence of an acetylated N-terminal residue and an amidated C-terminal residue confers to α-MSH resistance to amino and carboxypeptidases (Marks *et al*. 1976). It has been suggested that alanyl aminopeptidase (EC 3.4.11.14) and angiotensin-converting enzyme (EC 3.4.15.1) play a role in α-MSH(4–10) catabolism by rat serum (Potaman *et al*. 1993). The fact that [D-Phe7]α-MSH analogs are more resistant to inactivation by rat serum enzymes than native α-MSH (Akiyama *et al*. 1984; Castrucci *et al*. 1984; Eberle 1988) suggests that the His6-Phe7 and/or Phe7-Arg8 bonds are susceptible to proteolysis. Until now, the contribution of PEP in the inactivation of α-MSH in the brain had not been investigated. The present data show that α-MSH is cleaved by recombinant PEP *in vitro* at the Pro12-Val13 bond. Our study also shows that acute administration of S 17092, at doses of 10 or 30 mg/kg, induces a significant increase in α-MSH-LI content in the frontal cortex and the hypothalamus. These observations indicate that PEP may also play a role in the catabolism of α-MSH in the brain.

Although PEP is predominantly a cytosolic enzyme (Dresdner *et al*. 1982; Mentlein *et al*. 1990), the presence of PEP activity has been detected in particulate fractions of brush border cells (Sudo and Tanabe 1985), N1E 115 cells (Checler *et al*. 1986), NG108-15 cells (Chappell *et al*. 1990), and human and rat brain neurons (Irazusta *et al*. 2002). A novel membrane-bound form of PEP has even been purified and characterized in bovine neurons (O'Leary and O'Connor 1995) and this particulate PEP form was found to possess a higher affinity for certain peptide substrates than the cytosolic enzyme (O'Leary *et al*. 1996). Since neuropeptide catabolism may predominantly occur at the cell surface or in the extracellular space, these observations strongly suggest that PEP may actually play a significant role in neuropeptide catabolism.

### Neurochemical mechanisms underlying the promnesic effects of S 17092

Several reports indicate that compound S 17092 is a potent cognitive enhancer. In particular, S 17092 has been found to improve learning and memory in aged mice and to alleviate scopolamine-induced amnesia in young mice (Lestage *et al*. 1998). Several lines of evidence support the view that the beneficial effects of S 17092 on memory could be ascribed to its ability to inhibit breakdown of SP and α-MSH. For instance, it has been shown that SP prevents memory impairment in old rats (Hasenöhrl *et al*. 1994, 2000) and stimulates cholinergic neurotransmission in the septohippocampal pathway which is involved in memory processes (Lamour *et al*. 1988; Kouznetsova and Nistri 2000). The fact that the N-terminal portion of SP, notably fragment (1–7),

bears the memory-promoting effects while the C-terminal region mediates the reinforcing properties of SP (Gerhardt *et al*. 1992; Huston and Hasenöhrl 1995) suggests that the cleavage of SP elicited by PEP at the Pro4-Gln5 bond may directly modulate the mnemocognitive activity of the neuropeptide. Therefore, the promnesic effects of S 17092 may be mediated through protection of SP against enzymatic degradation by PEP. On the other hand, the effects of α-MSH on memory processes have been demonstrated in active and passive avoidance paradigms (De Wied 1966, 1999; Eberle 1988). Interestingly, it has been shown that the C-terminal region of α-MSH is implicated in the binding of the peptide to the MC3 and MC4 melanocortin receptors (Schiöth *et al*. 1998) which mediate the behavioral effects of α-MSH (Adan *et al*. 1994), indicating that cleavage of α-MSH by PEP at the Pro12-Val13 bond should impair the behavioral activity of the peptide. Collectively, these data suggest that the cognitive effects of S 17092 might be accounted for, at least in part, by the inhibition of the catabolism of the promnesic neuropeptides SP and α-MSH.

While acute administration of S 17092 provoked rapid inhibition of SP and α-MSH breakdown, previous studies have shown that chronic treatment with S 17092 is required to improve cognitive task performances (Lestage *et al*. 1998; Marighetto *et al*. 2000; Schneider *et al*. 2002). A similar delayed therapeutic efficacy has been described for a number of antidepressant drugs that are known to have an immediate inhibitory effect on serotonin and noradrenaline reuptake while their beneficial effect on mood disorder is only achieved after long-term treatment (Mongeau *et al*. 1997). The delayed onset of the therapeutic efficacy of antidepressants has been ascribed to their effects on Fos expression (Lino-de-Oliveira *et al*. 2001), DARPP-32 protein phosphorylation (Svenningsson *et al*. 2002), and cAMP response element-binding protein activation (Nibuya *et al*. 1996). Similar mechanisms may account for the delayed efficacy of S 17092 on memory impairment inspite of its immediate effect on SP and α-MSH catabolism. In support of this hypothesis, it has been shown that a single injection of SP in the nucleus basalis magnocellularis induces promnesic effects and provokes a long-lasting increase in the extracellular concentration of dopamine in the nucleus accumbens (Boix *et al*. 1995). The transient increase in SP and α-MSH concentrations induced by S 17092 may therefore provoke long-term adaptative changes of other neurotransmitter systems that may be responsible for its mnemocognitive effects.

### Pharmacological implications

Owing to their beneficial effects on learning and memory, it has been proposed that PEP inhibitors could have a therapeutic value for the treatment of AD (Toide *et al*. 1997, 1998). Consistent with this notion, it has been shown that selective PEP inhibitors suppress *in vitro* β amyloid protein (Aβ) formation in NG108-15 cells (Shinoda *et al*. 1997) and prevent *in vivo* Aβ deposition in the hippocampus of senescence-accelerated mice (Kato *et al*. 1997). However, it has been clearly demonstrated that PEP is unable to degrade Aβ and does not contribute to the formation of Aβ40 and Aβ42 in human cells overexpressing the β amyloid precursor protein (Petit *et al*. 2000) indicating that the effect of PEP inhibitors on Aβ formation occurs indirectly.

It has been proposed that the action of PEP inhibitors on AD symptomatology may be mediated through protection of mnemocognitive neuropeptides (Petit *et al*. 2000). The fact that the neurotoxic effects of Aβ peptides is mimicked by tachykinin antagonists and reversed by SP *in vitro* and *in vivo* (Yankner *et al*. 1990; Kowall *et al*. 1991) suggests that blockade of SP degradation could account for the effect of PEP inhibitors on Aβ accumulation. Interestingly, it has been found that SP content is significantly reduced in the cerebral cortex of AD patients (Crystal and Davies 1982; Beal and Mazureck 1987; Clevens and Beal 1989; Bouras *et al*. 1990) and that SP-containing neurons are depleted in AD brains (Kowall *et al*. 1993; Ang and Shul 1995; Yew *et al*. 1999). Similarly, α-MSH concentrations are decreased in the brain and CSF of AD patients (Arai *et al*. 1986; Rainero *et al*. 1988). It has also been reported that the fragment (11–13) of α-MSH, that contains the scessile prolyl bond, inhibits nitric oxide and tumor necrosis factor-α production by murine microglial cells activated by Aβ (Galimberti *et al*. 1999). Taken together, these observations suggest that the protective effects of PEP inhibitors against Aβ neurotoxicity could be mediated, at least in part, through inhibition of SP and/or α-MSH catabolism.

In conclusion, the present study has shown that inhibition of PEP activity by S 17092 treatment is associated with an increase in SP and α-MSH levels in the rat brain. Owing to the promnesic activity of SP and α-MSH, these data suggest that the beneficial effects of S 17092 on memory impairment may be attributable to the protection of these peptides against degradation by PEP.

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