Peptidase Inhibitor-Induced Antidiuresis Mediated through Angiotensin and Opioid Receptors in the Rat Hypothalamus

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ABSTRACT—We examined the effects on urine outflow rate after microinjections of thiorphan (a carboxypeptidase inhibitor) and bestatin (an aminopeptidase inhibitor) into the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei of anesthetized hydrated rats to determine the possible role of neuropeptides in the regulation of urine production. After individual microinjection of the peptidase inhibitors into the nuclei, only thiorphan at 100 nmol administered into the PVN significantly decreased the urine outflow rate. Two consecutive microinjections of the peptidase inhibitors at 100 nmol each into the nuclei induced potent antidiuresis. These effects after microinjections of the peptidase inhibitors into the PVN and SON were diminished by pretreatment with [Sar1, Ile8]angiotensin (ANG) II (an ANG II receptor antagonist) and naloxone (an opioid receptor antagonist) in the PVN and with [Sar1, Ile8]ANG II in the SON, respectively. A vasopressin (AVP) receptor antagonist, d(CH2)5-D-Tyr(Et)VAVP (i.v.), completely blocked the antidiuresis by microinjections of the peptidase inhibitors into both the nuclei. Urinary osmotic pressure was significantly increased by consecutive microinjections of the peptidase inhibitors into the PVN and SON. These results suggest that endogenously-released ANG II and opioid peptides in the PVN and ANG II in the SON regulate urine production mediated through increased AVP release.

Keywords: Antidiuresis, Peptidase inhibitor, Paraventricular nucleus, Supraoptic nucleus, Vasopressin

The hypothalamus contains many kinds of neuropeptides such as enkephalins, endorphins, corticotropin releasing factor, tachykinins and angiotensin II (ANG II) (1, 2). Immunohistochemical studies have demonstrated the presence of peptide-containing nerve terminals and fibers in the hypothalamus (1, 3, 4). The neuropeptides in the hypothalamus, as well as adrenoceptors (5–9) and cholinergic agents (10, 11) agonists, play a role in regulating vasopressin (AVP) secretion from the neurohypophysis. Bradykinin, cholecystokinin, substance P, neuropeptide Y, opioid peptides and ANG II applied into the hypothalamus increase AVP release into the circulation (1, 9, 12–21). ANG II is probably the most potent AVP releasing agent among the adrenoceptor and cholinergic agonists and neuropeptides as indicated by their relative effective doses. The mechanism for the ANG II-induced effect in increased release of norepinephrine (9, 12, 21).

Endogenously-released neuropeptides are degraded by aminopeptidases and/or carboxypeptidases, and then their effects disappear. Therefore, aminopeptidase and carboxypeptidase inhibitors are useful tools for investigating the in vivo roles of the neuropeptides. Effects of carboxypeptidase and/or aminopeptidase inhibitors applied into the central nervous system are reported to produce antinociceptive effects mediated through opioid receptors (22, 23). Moreover, the inhibitors block breakdown of the other neuropeptides, ANG II, tachykinins and so on (22–27).

The purpose of the present study is to determine the role of endogenously-released neuropeptides of the paraventricular (PVN) and supraoptic (SON) nuclei in the regulation of urine production. We examined the effects of thiorphan (a carboxypeptidase inhibitor) and bestatin (an aminopeptidase inhibitor), when microinjected directly into the PVN and SON, on the urine outflow rate and the mechanisms responsible for the effect on the urine outflow rate.

MATERIALS AND METHODS

Animals

A total of 118 male Wistar rats (290–370 g, 9- to 11-
week-old; Kitayama Labes Co., Ina) were used. The rats were housed at 22±1°C with a 12-hr light-dark cycle. They were fasted approximately 17 hr before the experiments were started, but had access to water ad libitum.

**Surgical procedure**

The rats, starved overnight, were loaded with tap water (5 ml/100 g body weight) and 45 min later, were anesthetized with the same volume of 12% ethanol, using stomach intubation. The rats were cannulated with polyethylene tubes into the urinary bladder, the trachea and the jugular vein. In some animals, an additional polyethylene cannula filled with heparinized saline was inserted into the carotid artery. Then, they were immobilized in a stereotaxic apparatus (Takahashi Co., Tokyo) and were implanted with a stainless steel cannula (outer diameter: 200 μm) into the right SON (coordinates: 6.3 mm anterior to the lambda, 1.3 mm lateral to the midline, 9.0 mm from the dura) or the right PVN (5.6 mm, 0.3 mm, 8.0 mm) according to the atlas of König and Klippel (28).

**Measurement of urine outflow rate**

Locke’s solution containing 3% ethanol was continuously infused at a rate of 0.1 ml/min into the jugular vein cannula during the experiments in order to maintain anesthesia and a measurable constant level of urine outflow rate. Urinary drops flowing from the bladder cannula were counted by a photelectric drop counter (DCT 102; Unique Medical, Inc., Tokyo) every 10 min. The volume of one urinary drop was 20 μl. Urine outflow was presented as the percentage of the control outflow (number of urinary drops for a 10-min period immediately preceding drug administration). The urine outflow rate became constant at approximately 1 hr after all surgical procedures had finished, and the constant rate was kept for 5–6 hr (29). One animal was not injected more than twice. The experiments were performed with a urine outflow rate of 40–200 μl/min.

**Administration of drugs**

After the urine outflow rate was constant for at least 20 min, drugs were injected into the nucleus or intravenously.

**Microinjection of drugs into the nuclei:** A drug solution (1 μl) was microinjected into the PVN or SON using a microsyringe connected to the stainless steel cannula inserted into the nucleus. The following drugs were employed: peptidase inhibitors (thiorphan: 30 or 100 nmol in 0.3% NaCl containing 0.1 N NaOH, bestatin: 30 or 100 nmol in saline), an opioid antagonist (naloxone: 600 nmol in saline), and an ANG II antagonist ([Sar1,Ile8]ANG II: 0.1 or 1 nmol in saline). Microinjections were carried out at a rate of 0.3 μl/min. In the case of consecutive microinjections of the peptidase inhibitors, thiorphan (1 μl) followed by bestatin (1 μl) were microinjected into the nucleus with the same procedure. To investigate the influence of the antagonists on the antidiuretic effects induced by consecutive microinjections of the two peptidase inhibitors at 100 nmol each into the nucleus, the nucleus was pretreated with the antagonist at 60 (intra-PVN injection) or 50 (intra-SON injection) min for naloxone and at 30 min for [Sar1,Ile8]ANG II before consecutive microinjections of the peptidase inhibitors into the same nucleus.

**Intravenous injection of drugs:** AVP (4 mU in 0.1 ml saline) and an AVP antagonist, d(CH2)5-D-Tyr(Et)VAVP, (50 μg/kg in 0.1 ml saline) were injected intravenously through the jugular vein cannula. In order to examine whether or not increased AVP release was involved in antidiuresis induced by consecutive microinjections of the peptidase inhibitors at 100 nmol each into the nuclei, intravenous injection of the AVP antagonist was performed at 40 min before consecutive microinjections of the peptidase inhibitors into the nuclei.

**Measurement of urinary osmotic pressure**

 Urine flowing from the urinary bladder cannula was collected for 10 min in test tubes to measure urinary osmotic pressure after consecutive microinjections of the two peptidase inhibitors (100 nmol each) into the nucleus and after i.v. injection of AVP. Urinary osmotic pressure was measured at 0 min as the control level, at 40 min after the intra-PVN injection or 30 min after intra-SON injection and i.v. injection as the peak response, and at 80 min after administration as the recovered level, using the freezing point depression method (Fiske Osmometer, Model G-62; Fiske Associates, Inc., Uxbridge, MA, USA).

**Measurement of visceral functions**

In some experiments, visceral functions were simultaneously monitored with the urine outflow rate. Blood pressure of the carotid artery was measured through a pressure transducer (MPU-0.5-290-III; Nihon Kohden Kogyo, Co., Tokyo). Thermister probes (SR-115S and MGA III-219, Nihon Kohden Kogyo, Co.) in the trachea cannula and the rectum were used to measure respiratory rate and rectal temperature, respectively. Measurement of heart rate was by cardiography (FD-13; Fukuda, Tokyo).

**Drugs**

The following drugs were used: thiorphan (Peninsula Laboratories, Inc., Belmont, CA, USA), bestatin hydrochloride, [Sar1,Ile8]ANG II, [Arg8]vasopressin (Sigma Chemical Co., St. Louis, MO, USA), d(CH2)5-D-Tyr(Et)VAVP [1-(mercapto-β,β-cyclopentamethylene-
propionic acid)2-(O-ethyl)p-Tyr,Val⁴,Arg-vasopressin (a gift from Prof. K.G. Hofbauer, Cardiovascular Division, Ciba-Geigy, Ltd., Basel, Switzerland) and naloxone hydrochloride (a gift from Sankyo Co., Tokyo). The other chemicals used were of the highest grade available.

**Histological procedure**

After the experiments, the injection sites were histologically verified by microinjection of methylene blue. Sites stained by methylene blue were confirmed under a microscope after the brain was cut into 15-μm coronal sections with a microtome (Tissue-Tek II; Miles, Inc., Erkhart, IN, USA). For photomicrographs, the sections were stained with Hematoxylin-Eosin.

**Statistical analyses**

Values are expressed as the mean ± S.E.M. Comparison of values at each time-point was performed by Fisher's PLSD test after one-way ANOVA. A P value under 0.05 was considered to indicate a significant difference.

**RESULTS**

**Effects of bestatin and thiorphan on urine outflow rate**

Figure 1 shows some examples of identified microinjection sites for the drugs into the PVN and SON.

As shown in Fig. 2A, microinjection of thiorphan at 100 nmol into the PVN elicited weak but significant decreases in the urine outflow rate at 20 and 30 min after injection, compared with the urine outflow rate after administration of vehicle at the corresponding time points.

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**Fig. 1.** Histological verification of microinjection sites. A: Schematic representation of coronal sections of the hypothalamus. Closed circles and triangles show the locations of the tip of the cannulae in the SON and PVN, respectively. Numbers over each panel denote millimeters anterior to lambda. The effects on the urine outflow rate and the visceral functions, which were elicited by the vehicles or the peptidase inhibitors microinjected consecutively into the sites shown in these panels, are included in the results of Fig. 3 and Table 1. B: Photomicrographs of typical examples in panel A. Arrows indicate the tip of the cannula. SON: supraoptic nucleus, PVN: paraventricular nucleus, V III: third ventricle, TO: tractus opticus, CO: chiasma opticum.
After injection of bestatin at 100 nmol into the PVN, the urine outflow rate showed a decreasing tendency at 20–40 min after injection; however, this was not significant (Fig. 2). Microinjection of the two peptidase inhibitors into the SON did not change the urine outflow rate (Fig. 2).

On the other hand, consecutive microinjections of the
two peptidase inhibitors at 100 nmol each into the nuclei produced potent antidiuretic effects (Fig. 3). The time-course of the antidiuresis was relatively slow with 10-min latency, the peak response at 30 min after administration and 80-min duration. Consecutive microinjections of the peptidase inhibitors at a lower dose of 30 nmol each into the nuclei showed no effects on the urine outflow rate (Fig. 3). Consecutive microinjections of one peptidase inhibitor at 30 nmol and another at 100 nmol into the SON tended to decrease the urine outflow rate, but not significantly (30 nmol thiorphan and 100 nmol bestatin: 76±20%, n = 3; 100 nmol thiorphan and 30 nmol bestatin: 70±17% of the control level at 30 min after administration, n = 5). Also, thiorphan and bestatin at 100 nmol each, when consecutively microinjected into sites 1 mm above the SON and 1 mm toward the midline to the SON, did not show any significant decrease in the urine outflow rate (1 mm above the SON: 115±8%, n = 5; 1 mm toward the midline to the SON: 89±6% of the control level at 30 min after administration, n = 3). The urine outflow rate was not significantly changed after microinjections of saline, 0.3% NaCl containing 0.1 N NaOH and both the vehicles into the PVN and SON (Figs. 2 and 3).

Blood pressure, heart rate, respiratory rate and rectal temperature were not significantly changed by consecutive microinjections of the peptidase inhibitors into the SON (Table 1). However, after consecutive injections of the peptidase inhibitors into the PVN, heart rate, respiratory rate and rectal temperature were elevated during the following time periods after the administration (injection at 0 min): 40–80 min, 30–80 min and 50–80 min, respectively (Table 1). These increases were still observed when the decreases in the urine outflow rate returned to the control rate (Fig. 3). Consecutive microinjections of the peptidase inhibitors into the PVN did not induce any effects on blood pressure. There were no significant changes in all functions after consecutive microinjections of vehicles (0.3% NaCl containing 0.1 N NaOH followed by saline) into the nuclei (Table 1).

**Effects of naloxone and [Sar^1,Ile^8]ANG II on peptidase inhibitor-induced antidiuresis**

We examined the effects of an opioid antagonist, naloxone (600 nmol), and an ANG II antagonist, [Sar^1,Ile^8]ANG II (0.1 or 1 nmol), on the antidiuresis induced by consecutive microinjections of thiorphan (100 nmol) and bestatin (100 nmol) into the nuclei (Figs. 4 and 5). Microinjection of naloxone alone into the SON showed no statistically significant decrease in the urine outflow rate; however, microinjection into the PVN elicited the

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Table 1. Effects of visceral functions of thiorphan and bestatin microinjected consecutively into the nuclei.

Thiorphan and bestatin at 100 nmol each or vehicles (0.1 N NaOH in 0.3% NaCl and saline) were consecutively microinjected into the PVN or SON. Values are the mean ± S.E.M. of 4 experiments. The effects on the urine outflow rate and urinary osmotic pressure simultaneously examined are shown in Figs. 2–7. *P<0.05 vs the corresponding 0-min values by Fisher’s PLSD test following one-way ANOVA.
Fig. 4. Effects of naloxone on antidiuresis induced by peptidase inhibitors. At the 0-min time points, the peptidase inhibitors were consecutively microinjected into the PVN (A) and SON (B). ○: 100 nmol thiorphan + 100 nmol bestatin, ●: thiorphan + bestatin after pretreatment with 600 nmol naloxone, □: vehicle, ■: naloxone alone. Pretreatment with naloxone was performed at 50 or 60 min before microinjection of the peptidase inhibitors into the SON or PVN, respectively. Values represent the mean ± S.E.M. of 6–9 experiments. *P < 0.05 vs the vehicle-injected group (○) at the corresponding time points, **P < 0.05 vs non-pretreated group (○) at the corresponding time points (one-way ANOVA followed by Fisher’s PLSD test).

Fig. 5. Effects of [Sar¹,Ile⁸]ANG II on peptidase inhibitor-induced antidiuresis. At the 0-min time points, the peptidase inhibitors were consecutively microinjected into the PVN (A) and SON (B). ○: 100 nmol thiorphan + 100 nmol bestatin, ●: thiorphan + bestatin after pretreatment with 0.1 nmol [Sar¹,Ile⁸]ANG II, ▲: thiorphan + bestatin after pretreatment with 1 nmol [Sar¹,Ile⁸]ANG II, □: vehicle, ■: 1 nmol [Sar¹,Ile⁸]ANG II alone. The pretreatment with [Sar¹,Ile⁸]ANG II was performed at 30 min before consecutive microinjections of the peptidase inhibitors into the nuclei. Values represent the mean ± S.E.M. of 4–9 experiments. *P < 0.05 vs the vehicle-injected group (○) at the corresponding time points, **P < 0.05 vs the non-pretreated group (○) at the corresponding time points (one-way ANOVA followed by Fisher’s PLSD test).
transient antidiuretic effects. Naloxone inhibited the antidiuretic effects of the peptidase inhibitors microinjected into the PVN, but not those microinjected into the SON. The inhibitory effect in the PVN was partial and a weak antidiuretic effect remained.

On the other hand, microinjection of \([\text{Sar}^1,\text{Ile}^8]\text{ANG II}\) alone into the nuclei did not have any effects on the urine outflow rate. The antidiuresis in both the nuclei were partially antagonized by only the higher dose of the antagonist.

Effects of \(d(\text{CH}_2)_5\text{-d-Tyr(Et)}\text{VAVP}\) on peptidase inhibitor-induced antidiuresis

We examined changes in urinary osmotic pressure at the peak antidiuresis (at 30 or 40 min) after consecutive microinjections of the peptidase inhibitors (100 nmol thiorphan and 100 nmol bestatin) into the nuclei and effects of \(d(\text{CH}_2)_5\text{-d-Tyr(Et)}\text{VAVP}\) (an AVP receptor antagonist; 50 \(\mu\)g/kg, i.v.; ref. 30) on the peptidase inhibitor (100 nmol thiorphan and 100 nmol bestatin)-induced antidiuresis. Figure 6A shows significant increases in urinary osmotic pressure after consecutive microinjections of the peptidase inhibitors into the PVN and SON. These increases in urinary osmotic pressure were similar in extent to that after intravenous injection of 4 mU AVP. Also, no significant differences in the urine outflow rate were found among the three groups. The urinary osmotic pressure returned to the control level in all experiments when the urine outflow rate regained the control level. Consecutive microinjections into the nuclei and intravenous injection of the vehicles did not significantly alter the urine outflow rate and urinary osmotic pressure (Fig. 6B).

The AVP receptor antagonist \(d(\text{CH}_2)_5\text{-d-Tyr(Et)}\text{VAVP}\) alone did not show any effects on the urine outflow rate. Statistically significant differences were shown between the AVP antagonist-pretreated and non-pretreated groups in both the nuclei. The pretreatment with \(d(\text{CH}_2)_5\text{-d-Tyr(Et)}\text{VAVP}\) blocked antidiuresis induced by consecutive microinjections of the peptidase inhibitors into the nuclei, as shown in Fig. 7. In the SON, the urine outflow rate at 20 min in the \(d(\text{CH}_2)_5\text{-d-Tyr(Et)}\text{VAVP}\)-

![Figure 6](image_url)

Fig. 6. Effects of peptidase inhibitors on urinary osmotic pressure and urine outflow rate. Values represent the mean ± S.E.M. of 4–6 experiments. Urinary osmotic pressure was examined at 0 min (basal level), at 30 or 40 min (at the peak antidiuretic effects) and at 80 min (recovery level) after consecutive microinjections or intravenous injection of drugs (A) or vehicle (B). *P < 0.05 vs corresponding control level and recovery level (one-way ANOVA followed by Fisher’s PLSD test).
pretreated group seemed to increase compared with that in the vehicle group; however, this was not statistically significant.

**DISCUSSION**

A number of studies have demonstrated that a carboxypeptidase inhibitor, thiorphan and bestatin, an aminopeptidase inhibitor, potentiate antinociceptive effects of opioid peptides (22, 23, 31). Moreover, the peptidase inhibitors can produce antinociception by themselves (22, 23). The peptidase inhibitor-induced antinociception is antagonized by opioid antagonists, suggesting that these effects were produced by inhibiting the degradation of endogenously-released opioid peptides after administration of the peptidase inhibitors. In the present study, we showed that consecutive microinjections of the peptidase inhibitors into the PVN induced antidiuretic effects that were diminished by pretreatment with naloxone in the nucleus. Therefore, it is suggested that antidiuresis induced by consecutive microinjections of the peptidase inhibitors into the PVN involves an increase in the opioid peptide concentration in the nucleus by administration of the peptidase inhibitors in the nucleus. This strongly supports the idea that endogenous opioid peptides in the PVN play a role in regulating urine production. The subtype of opioid receptors involved in the regulation is probably the δ- or κ-subtype, because the dose of naloxone used in this study was relatively high. Naloxone is reported to have slightly higher affinity to the κ-subtype than the other opioid receptor subtypes. A tenfold higher dose of naloxone is needed to block the δ- or κ-subtype, compared to the dose blocking the μ-subtype (32). Our earlier studies under the same experimental conditions have demonstrated that D-Ala²-D-Leu⁵-enkephalin (a δ-agonist) and dynorphin (a κ-agonist) applied into the PVN increase the plasma AVP level and result in antidiuresis that was partially inhibited by the dose of naloxone used in this study (19, 20), and morphine- and [D-Ala²,N-Me-Phe⁴,Gly⁵-ol]-enkephalin (μ-agonists)-induced antidiuretic effects were completely inhibited by a lower dose of naloxone (ref. 33 and H. Tsushima et al., unpublished data).

On the other hand, in the SON, since naloxone does not diminish the effects, the opioid receptors in the nucleus may not have an important role in the regulation. However, histochemical studies show terminals of enkephalinergic neurons and opioid receptors in the SON (34–36). Moreover, microinjection into the SON of opioid peptides, except dynorphin, could cause anti-

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![Figure 7](https://via.placeholder.com/150)

**Fig. 7.** Inhibitory effects of d(CH₂)₅-D-Tyr(Et)VAVP on peptidase inhibitor-induced antidiuresis. ○: 100 nmol thiorphan + 100 nmol bestatin, ●: thiorphan + bestatin after pretreatment with the AVP antagonist, △: d(CH₂)₅-D-Tyr(Et)VAVP alone, □: vehicle for the drugs microinjected, ■: vehicle for the drug injected intravenously. Values represent the mean ± S.E.M. of 5–9 experiments. At the 0-min time points, the peptidase inhibitors were consecutively microinjected into the PVN (A) and SON (B). d(CH₂)₅-D-Tyr(Et)VAVP was intravenously injected 40 min before consecutive microinjections of the peptidase inhibitors into the nuclei. *P<0.05 vs the vehicle-injected group (○) at the corresponding time points, *₂P<0.05 vs the non-pretreated group (□) at the corresponding time points (one-way ANOVA followed by Fisher's PLSD test).
diuretic effects through opioid receptors, as well as in the PVN (19, 20, 33, 37). Therefore, it is speculated that opioid mechanisms in the SON may have a role in the regulation under specific conditions.

Thiorphan and bestatin also block degradation of ANG II (23–25), which is well-known to produce potent antidiuresis through increased AVP secretion in the central nervous system (9, 12, 18, 21). The inhibitory effect of the ANG II antagonist [Sar^1,Ile^8]ANG II on the antidiuresis provides evidence that ANG II receptors in both nuclei contribute to the regulation of urine production. Our previous report using the same experimental methods showed that potent antidiuresis of ANG II at 1 nmol microinjected into the nuclei was completely blocked by pretreatment with 1 nmol of the antagonist into the same nuclei (21). On the other hand, thiorphan and bestatin suppress degradation of the other peptides such as tachykinins, cholecystokinin and bradykinin (22–27). Therefore, involvement of these peptides can not be completely excluded in the antidiuresis. These peptides are reported to increase secretion of AVP (1, 13–15). Also, it has been demonstrated that thiorphan decreases cleavage of atrial natriuretic factor (38–41). However, this peptide suppresses the secretion of AVP (42).

To examine the possibility that the antidiuretic effect was produced by the peptidase inhibitors diffused outside the nuclei, the drugs were consecutively microinjected into the two sites at 1 mm from the SON. The urine outflow rate was not significantly changed by the peptidase inhibitors applied into these sites. The sites 1 mm above the SON and 1 mm toward the midline to the SON are also near the PVN, and lie at a distance of 1.2 mm diagonally anterior and 1.2 mm diagonally ventral to the PVN, respectively. Moreover, we have already shown that microinjection of norepinephrine and oxotremorine into the sites outside the nuclei do not influence the urine outflow rate (6, 7, 43). Taken together, the peptidase inhibitor-induced antidiuresis probably resulted from increased concentration of the peptides in the nuclei by microinjection of the drugs into the nuclei.

Consecutive microinjections of bestatin and thiorphan into the nuclei produced antidiuresis through increased secretion of AVP, because intravenous injection of the AVP antagonist, d(CH2)3-d-Tyr(Et)VAVP, definitely blocked the effects. Also, increases in urinary osmotic pressure after microinjection of the peptidase inhibitors into the nuclei were similar to those after intravenous injection of AVP. It was previously reported that antidiuretic effects of ANG II, substance P, Met-enkephalin (α-opioid receptor), D-Ala²-Met⁵-enkephalaminde (a µ-opioid receptor) and dynorphin (a δ-opioid receptor) microinjected into the SON and/or PVN resulted from AVP secretion (14, 15, 19, 20). Under the ethanol-anesthetized water-loaded condition in this study, the basal secretion of AVP is suppressed (20), and the urine outflow rate seems to increase compared with that under the conscious non-hydrated condition. However, anesthetized hydrated rats can still produce diuretic effects after central injection of timolol, Leu- and Met-enkephalin, prostaglandins E₂ and F₂α (44–46).

We measured blood pressure, heart rate, respiratory rate and rectal temperature as the factors that might be responsible for change in the urine outflow rate. These indexes, except for blood pressure, increased after microinjection of the peptidase inhibitors into only the PVN. The PVN seems to contribute to visceral functions more than the SON. The increases were significant from 30 min for respiratory rate, 40 min for heart rate and 50 min for rectal temperature after the administration and continued after the decrease in the urine outflow rate recovered to the control level. The findings showed that the antidiuretic effects did not result from these changes.

Individual microinjection of bestatin and thiorphan into the SON and that of bestatin into the PVN did not show any significant antidiuresis, however, consecutive microinjections of both the inhibitors into the nuclei produced powerful effects. This is the same phenomenon as the inhibitor-induced antinociception (22). Both inhibition of aminopeptidase and carboxypeptidase is needed to obtain significant effects. For peptides of small molecular weight like enkephalins and ANG II, both the C- and N-terminals seem to be important for the interaction with the receptors and then produce the effects.

The antidiuretic effects after individual and consecutive microinjections of the peptidase inhibitors into the PVN appeared to be slightly more marked than those in the SON. The most likely explanation for this phenomenon is that peptides, whose degradation is inhibited by thiorphan and bestatin, regulate AVP release in the PVN more powerfully than in the SON. In our previous study, the effects of ANG II applied in the PVN were more marked than that in the SON (21). There remains a possibility that the activities of peptidases in the SON may be more potent than those in the PVN. No reports to date have presented measurements of peptidase activities in the SON and PVN.

In conclusion, consecutive microinjections of thiorphan and bestatin into the SON and PVN decrease urine production through increased secretion of AVP, which is regulated by endogenously-released ANG II and opioid peptides in the PVN and ANG II in the SON. However, other mechanisms may be involved in the antidiuretic effects. The regulation of urine production by the peptides in the PVN may be more marked than that in the SON.
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