オピオイドペプチドによるバゾプレシン分泌調節に関する研究

課題番号　01570633

平成２年度科学研究費補助金一般研究（C）
研究成果報告書

平成３年３月

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謝辞

本研究は文部省科学研究費補助金（一般研究(C)）に基づき行われ、また下記の研究分担者の協力を得てなされたものであることを巻頭に記し、感謝の意を表わすものである。

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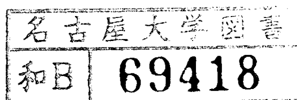
研究経費

平成元年度	１，０００千円
平成２年度	４００千円
計	１，４００千円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和B 69418

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大竹千生、近藤国和、伊藤雅史、大磯ユタカ、高槻健介;

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研究成果

今回の研究目的は下垂体後葉ホルモンである arginine vasopressin (AVP)の分泌制御機構における中枢性調節物質であるオピオイドペプチドの役割についての検討およびAVP分泌経路に対する他の神経伝達物質とオピオイドペプチドの作用上の関連を明らかとすることにあった。我々のこれまでの検討結果において、オピオイドペプチド受容体のサブクラスの相違によりAVP分泌調節上の作用点が異なる可能性が示唆されていた。この結果はオピオイドペプチドのδ受容体に比較的高い特異性をもつ拮抗剤は容量受容体系により賦活化されたAVP分泌を抑制するが、他のサブクラス受容体に特異性の高い拮抗剤は全く影響を与えなかったという成績に基づいている。この事実をさらに検討するために今回はオピオイド受容体の各種サブクラス作動薬を用いて、浸透圧系および容量受容体系の各々を活性化した場合の作用態度について観察した。結果の詳細は英文の報告書部分に示す通りであるが、今回の検討においてもオピオイドペプチドのδ受容体系作動薬は他のμあるいはκ受容体系作動薬とは異なった作用を示し、AVP分泌調節機構におけるオピオイドペプチドの作用効果は浸透圧系と容量受容体系とではそのサブクラスにより異なったものである可能性が示された。

一方、AVP分泌制御におけるオピオイドペプチドの作用と他の神経伝達物質の相互作用についても検討を行った。今回の研究では抑制性神経伝達物質であるγ-アミノ酪酸(GABA)とオピオイドペプチドの相互作用について、浸透圧性のAVP分泌亢進下において検討した。この研究結果の詳細も本報告書の英文部分に記載されているが、GABAのAVP分泌に対する影響を観察すると共に、一部のラットにおいてはGABA投与前にオピオイドペプチド拮抗剤であるnaloxone処置を行いAVP分泌の変化を観察した。高張食塩水負荷ラットにおいてはGABA脳室内投与により血漿AVPは有意に低下しその反応は用量依存性であった。 GABAおよびnaloxoneのAVP分泌に与える影響の検討では、高張食塩水投与により上昇した血漿AVPは前述したようにGABAにより有意の抑制をうけ低下を示したが、この抑制はnaloxoneの前処置により阻害された。このようにGABAによるAVP分泌抑制がnaloxoneの前処置により部分的に解除された事実から、GABAのAVP分泌調節系には内因性オピオイドペプチドを介する経路が介在する可能性が考えられた。

今回の研究の結果からAVPの分泌制御機構には内因性オピオイドペプチドが神経性調節物質として強くまた特異的に関与すること、さらにAVP分泌制御に関する複数の神経伝達物質間に相互関係が存在し纖細な調節作用をもつことが強く示唆された。

COMPARISON OF SUPPRESSIVE EFFECTS OF OPIOID KAPPA-, MU- AND DELTA-RECEPTOR AGONISTS ON ARGININE VASOPRESSIN SECRETION

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ABSTRACT

The role of opioid kappa-, mu- and delta-receptor in the secretion of arginine vasopressin (AVP) was studied. Opioid kappa- and mu-receptor agonist significantly suppressed plasma AVP increased by hyperosmolar or hypovolemic stimulation. Opioid delta-receptor agonist did not suppress plasma AVP induced by hypovolemia. These results suggest that suppressive activity of opioid receptor agonist for AVP secretion depends on its biological subclass.

INTRODUCTION

Pharmacological evidences support the existence of multiple opioid receptors (Paterson et al. 1983). These receptors independently affect several important biological activities (Pleuvry 1983). Although contradictory effects of opioid receptor agonist on arginine vasopressin (AVP) secretion were

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mainly reported using in vitro technique (Bicknell et al. 1985, Nordmann et al. 1986), we previously indicated that secretion of AVP was remarkably suppressed by opioid kappa-receptor agonist in the hyperosmolar state or hypovolemic state in the conscious rats (Oiso et al. 1988).

In this study, we examined the role of each opioid receptor in the secretion of AVP with the use of opioid mu-, delta- and kappa-receptor agonists in the rats given hypertonic saline or polyethylene glycol which reduces plasma volume without altering plasma osmolality (Dunn et al. 1973).

MATERIALS AND METHODS

Conscious male Sprague-Dawley rats weighing 250-300g were used in the all experiments. All rats were maintained on standard rat chow and given tap water ad libitum before experiment.

The activity of each opioid agonist was examined in the rats elevated AVP secretion by hyperosmolar or hypovolemic stimulation.

In the hyperosmolar stimulation, the rats were injected subcutaneously with various doses of opioid agonists, subsequently hypertonic saline (900 mOsm/kg) was administered intraperitoneally (20 ml/kg) 30 min later and blood collection was done by decapitation after an additional 30 min.

In the hypovolemic stimulation, the rats were injected intraperitoneally with polyethylene glycol (MW 3,000, 20% w/v; 20 ml/kg). Thirty minutes later, various doses of opioid agonists were injected subcutaneously, and blood collection was performed by decapitation after an additional 60 min.

Following opioid agonists were used in this experiment; FK33-824 (Sandoz Ltd., Basel, Switzerland; MW 604) as relatively high agonist for opioid mu-receptor (Barnard and Demoliou-Mason, 1983), leucine enkephalin (Peptide Institute, Inc., Osaka, Japan; MW 556) as relatively high agonist for opioid delta-receptor (Paterson et al. 1983) and U50488H (Upjohn, Kalamazoo, Mich., USA; MW 465) as relatively high agonist for opioid kappa-receptor (Voigtlander et al. 1983). All opioid agonists were dissolved in isotonic saline and injected in a volume of 1 ml/kg.

Blood samples were collected in chilled tubes with EDTA-2K and AVP was extracted from 1 ml plasma using Sep-Pak C18 (Waters Associates, Milford, MA, USA). Plasma AVP was measured by radioimmunoassay kit provided by Mitsubishi Petrochemical Co. Ltd. (Tokyo, Japan) (Oiso et al. 1988).

RESULTS

Fig. 1 indicated the effects of opioid mu-, delta- and kappa-receptor agonists on plasma AVP in rats given hypertonic saline intraperitoneally. Plasma AVP was elevated by hypertonic saline injection from basal level (0.94 ± 0.08 pg/ml; M ± SE) to 9.42 ± 0.67 pg/ml in the control rats. U50488H, an opioid kappa-receptor agonist, strongly and significantly suppressed this elevation to 0.90 ± 0.17 pg/ml at a dose of 5 mg/kg and to 0.53 ± 0.14 pg/ml at a dose of 10 mg/kg. FK33-824, an opioid mu-receptor

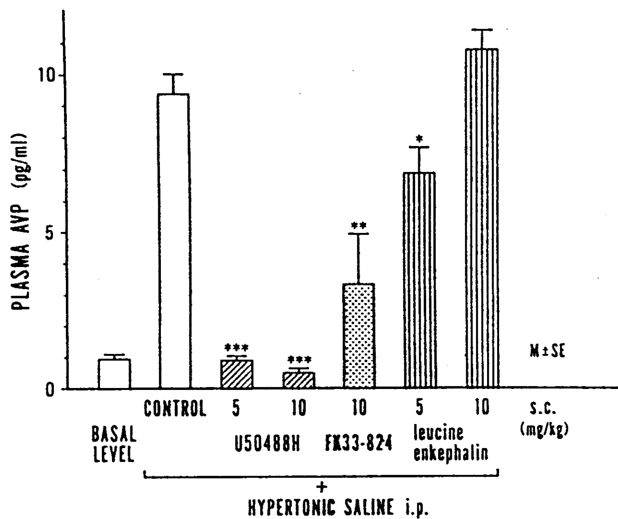

Fig. 1. Effects of opioid agonists injected subcutaneously (s.c.) on plasma arginine vasopressin (AVP) in rats given hypertonic saline intraperitoneally (i.p.).
*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$ vs control.

agonist, showed moderate suppression of plasma AVP (3.29 ± 1.6pg/ml) at a dose of 10 mg/kg. However, leucine enkephalin which is an opioid delta-receptor agonist indicated a weak or no definite suppression of plasma AVP. Although 5 mg/kg of leucine enkephalin showed weak but significant suppression of plasma AVP (6.88 ± 0.82 pg/ml), higher dose (10 mg/kg) of leucine enkephalin did not show any effect on plasma AVP.

Fig. 2 showed the effects of various opioid receptor agonists on the change of plasma AVP in hypovolemic rats. Hypovolemic stimulation increased plasma AVP from basal level (1.40 ± 0.17 pg/ml) to 4.69 ± 0.56 pg/ml in the control rats. Again, U50488H strongly and significantly suppressed this increment to 0.68 ± 0.10 pg/ml at a dose of 5 mg/ml and to 0.15 ± 0.04 pg/ml at a dose of 10 mg/kg. FK33-824 (10 mg/kg) moderately suppressed plasma AVP to 1.37 ± 0.30 pg/ml. Leucine enkephalin (5 and 10 mg/kg), however, did not show any suppressive effects on the elevated plasma AVP.

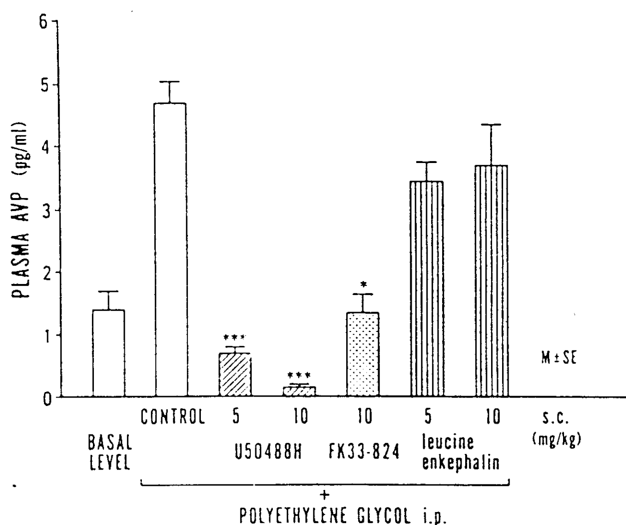

Fig. 2. Effects of opioid agonists injected subcutaneously (s.c.) on plasma arginine vasopressin (AVP) in rats given polyethylene glycol intraperitoneally (i.p.).
*: $p<0.05$, ***: $p<0.001$ vs control.

DISCUSSION

This study indicates that suppressive activity of opioid receptor agonist for AVP secretion depends on its biological subclass such as mu-, delta- and kappa-receptors. The strongest effect among them was induced by opioid kappa-receptor agonist. We already reported that opioid kappa-receptor agonist shows a strong inhibitory activity for AVP secretion in the rats (Oiso et al. 1988). Opioid mu-receptor agonist, FK33-824, had moderate but definite inhibitory activity for AVP secretion elevated by hyperosmolar or hypovolemic stimulation. On the other hand, opioid delta-receptor agonist, leucine enkephalin, had different effect compared with these two opioid receptor agonists. Firstly, the suppressive activity per se was very weak and secondly this agonist did not possess an inhibitory effect on plasma AVP increased by hypovolemic stimulation. These findings suggest that the difference of opioid receptor subclass induces independent modulation at the action sites of the pathway in AVP regulation system. It seems that opioid kappa- and mu-receptor agonists mainly affect on the integrated efferent pathway for the secretion of AVP since these agonists inhibited AVP secretion elevated by both hyperosmolar and hypovolemic stimuli. On the contrary, opioid delta-receptor agonist showed a weak effect on the pathway in osmolar regulation system of AVP secretion and volume regulation system for AVP secretion was not modulated by opioid delta-receptor agonist, indicating that the site at which such result could occur is different from that of other receptor agonists. Our previous observation (Robertson et al. 1985) also indicated that AVP secretion due to hypovolemic stimulation is differently influenced by the action of each opioid receptor. The reason for no suppressive activity of leucine enkephalin at a dose of 10 mg/kg in the hyperosmolar state is unclear, however, hypotension which is well known to activate AVP secretion might be occurred by such high dose of leucine enkephalin.

To clarify more precise roles of each opioid receptor in the osmolar and volume regulation systems for AVP secretion, further studies using more specific agonist or antagonist of the each

opioid receptor will be needed.

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POSSIBLE INVOLVEMENT OF ENDOGENOUS OPIOID PEPTIDES IN THE INHIBITION OF ARGININE VASOPRESSIN RELEASE BY γ-AMINOBUTYRIC ACID IN CONSCIOUS RATS.

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A short title: Naloxone reverses GABAergic inhibition of vasopressin release.

Key words: GABA, Arginine vasopressin, Naloxone, Opioid peptides.

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This work was supported by Grant #01570633 from the Japanese Ministry of Education.

Abstract

We examined the effects of γ-aminobutyric acid (GABA) and naloxone, a potent opioid antagonist, on arginine vasopressin (AVP) secretion in conscious rats in order to study the relationship of GABA and endogenous opioid peptides in the regulation of AVP secretion. Intracerebroventricular administration of GABA caused a time and dose dependent decrease in the plasma concentration of AVP that was elevated by hypertonic saline injection, whereas it did not affect the basal AVP. Pretreatment with naloxone (10 mg/kg) significantly attenuated the inhibitory effect of GABA (100 μg) on AVP release. These results suggest that GABA produces an inhibition of AVP release stimulated by hypertonic saline, and that this inhibitory effect may be mediated at least in part by the endogenous opioid systems.

Introduction

It is well accepted that γ-aminobutyric acid (GABA) is an inhibitory neurotransmitter in the central nervous system, and some studies have demonstrated that GABA plays an important role in pituitary hormone secretion [1,2,3,4]. GABA seems to possess an inhibitory effect upon arginine vasopressin (AVP) release. Several investigators have suggested that the administration of GABA or muscimol, a GABA agonist, attenuated the pressor responses or the release of AVP stimulated by hypertonic saline, angiotensin II, carbachol, or polyethylene glycol-induced hypovolemia [5,6,7,8,9,10,11]. Endogenous opioid peptides, such as dynorphin, are present in the neurohypophysis [12] and opioid receptors also exist in the hypothalamus and neural lobe [13]. Previous studies

from this and other laboratories demonstrated that opioid peptides or opioid agonists suppress the release of AVP [14,15,16,17,18]. GABA and opioid peptides which are reported to be colocalized in the same striatal neurons or ventral pallidal cell bodies and dendrites [19,20,21], appear to interact as neuromodulators. GABA regulates the action of the opioid peptides in the central nervous system [22,23]. Recent studies have reported the interaction between GABA and opioid peptides in the regulation of pituitary hormones, for example: GH and LH [24,25]. The relationship between GABA and opioid peptides in regard to the inhibitory effect on AVP release has not been clarified. This study was undertaken to investigate whether the opioid system is involved in the regulation of GABA on AVP release in conscious rats.

Materials and Methods

Male Sprague-Dawley rats (body weight 250-300 g) obtained from the Chubu Science Materials Co. (Nagoya, Japan) were used. They were kept in cages with a light-dark cycle of 12 hr at 22 ℃.

A cannula was placed for intracerebroventricular (i.c.v.) injection before any experimentation was done. The rats were anesthetized with sodium pentobarbital (50 mg/kg body weight) by intraperitoneal (i.p.) injection and positioned in the stereotaxic apparatus. A 21 gauge stainless steal guide cannula was inserted into the lateral ventricle in accord with the following coordinates: 0.8 mm posterior to bregma, 1.4 mm lateral to midline, and 4.0 mm below the surface of the skull [26]. The cannula was fixed with dental cement and anchored to the skull with two jeweler's screws.

A 25 gauge obturator of equal length remained in place until the experiments began. The animals were housed individually with free access to tap water and food for a week after surgery.

These rats moving freely, received the following drugs in the ensuing experiments: GABA (Sigma, St. Louis, U. S. A.) was given i. c. v. in a volume of 5 μl for 1 min with a Hamilton microsyringe. Naloxone hydrochloride (10 mg/kg body weight, 1.0 ml/kg body weight; Sankyo Co., Tokyo, Japan) was administered by subcutaneous (s. c.) injection. All these substances were dissolved in an isotonic saline solution (290 mOsm/kg). Hypertonic saline (600 mOsm/kg, 20.0 ml/kg body weight) was injected i. p. The control rats received the same volume of isotonic saline. To verify the position of the i. c. v. injection site, Pontamine Sky Blue was administered through the cannula at the end of each experiment, and the resulting data were confirmed by the staining of the rat's lateral ventricle.

Experimental protocols

Protocol for GABA i. c. v. time-course study

Each rat was administered 100 μg of GABA 30 min after hypertonic saline i. p. Then these rats were decapitated 10, 20, and 30 min after GABA i. c. v. Control rats were decapitated 30, and 60 min after hypertonic saline i. p.

Protocol for GABA i. c. v. dose-response study

Hypertonic saline i. p. was administered 30 min before GABA injection in each rat. The rats were decapitated 30 min after

random administration of 25, 50, 100, and 200 μg of GABA.

Protocol for effect of naloxone on GABA-induced suppression of AVP secretion

Isotonic or hypertonic saline-injected rats were simultaneously treated with either naloxone (10 mg/kg) or isotonic saline s.c. Rats received 100 μg of GABA or isotonic saline 30 min after these administrations. Then rats were decapitated 30 min after i.c.v. injection.

AVP and sodium measurements

Trunk blood was collected by decapitation into chilled tubes containing EDTA-2K. Plasma sodium was measured by flame photometry. Plasma samples (1.0 ml) were extracted with Sep-pak C18 (Waters Associates, Inc., Milford, MA, USA), placed in a water bath (37.0 ℃), and dried under a stream by air. Plasma AVP was measured by a radioimmunoassay kit provided by Mitsubishi Petrochemical Co. (Tokyo, Japan), as previously described [14].

Statistics

Data were expressed as the mean ± standard error of the mean. Statistical evaluation was performed by one-way analysis of variance (one-way ANOVA) followed by Tukey's multiple comparisons with the Bonferroni correction (GABA time-course study), one-way ANOVA followed by Williams' test (GABA dose-response study), or Student's t-test modified for multiple comparisons with the Bonferroni correction (effect of naloxone on GABA-induced suppression of AVP secretion).

Results

Effect of GABA on AVP release induced by hypertonic saline

GABA time-course

Plasma AVP was elevated to 3.75 ± 0.82 pg/ml and administration of 100 μg of GABA significantly suppressed plasma AVP ($F[3, 14]=5.68$, $P<0.01$, by one-way ANOVA) in the hypertonic saline-injected rats. Maximum suppression occurred 30 min after GABA injection. At this time, plasma AVP was significantly suppressed to 1.17 ± 0.14 pg/ml compared with the control rats' (3.37 ± 0.42 pg/ml) ($P<0.05$, by Tukey's multiple comparisons with the Bonferroni correction) (Fig. 1).

GABA dose-response

Plasma AVP was 4.10 ± 0.28 pg/ml at control and decreased significantly to 1.96 ± 0.25, 1.43 ± 0.21 and 1.35 ± 0.09 pg/ml in accordance with doses of 50, 100, and 200 μg of GABA in a dose-dependent manner ($F[4, 18]=15.80$, $P<0.001$, by one-way ANOVA: $P<0.05$ in each dose, by Williams' test) in rats treated with hypertonic saline. Doses of 25 μg did not significantly decrease plasma AVP levels (Fig. 2).

Effect of naloxone pretreatment on the inhibition of AVP release by GABA i.c.v.

I.c.v. administration of significantly ($P<0.01$, by t-test modified for multiple comparisons with the Bonferroni correction)

reduced the elevated plasma AVP from 4.44 ± 0.53 pg/ml to 1.43 ± 0.22 pg/ml 30 min later in rats treated with hypertonic saline. Pretreatment with naloxone significantly ($P<0.05$, by t-test modified for multiple comparisons with the Bonferroni correction) reversed the inhibitory effect of GABA on AVP release (2.88 ± 0.36 pg/ml), whereas naloxone alone did not significantly alter the plasma AVP level stimulated by hypertonic saline i.p. (3.25 ± 0.54 pg/ml) (Fig. 3). No significant differences in plasma sodium were observed among the four groups of rats, however, these values of plasma sodium were higher than in the basal state (data not shown).

In rats given isotonic saline i.p., 100 μg of GABA i.c.v. with or without naloxone did not significantly alter the AVP levels (Table 1).

Discussion

GABA has been reported to play a physiological role in regulating the release of AVP and the AVP-mediated pressor response [4,5,6,7,8,9,10,11,27]. We confirmed that GABA inhibits AVP release under the stimulated condition in this study. In the time-course study, plasma AVP decreased at 10, 20 and 30 min intervals after administration of GABA. As observed in the dose-response study, i.c.v. injections of 50, 100, and 200 μg of GABA significantly reduced plasma AVP levels elevated by hypertonic saline in a dose-related manner.

GABA receptors were found in the supraoptic nucleus (SON) by autoradiographical study [28], and GABAergic innervation was demonstrated in the neural lobe [29], SON, and paraventricular

nucleus (PVN) [30,31] by immunocytochemical and ultrastructural studies. Electrophysiological studies further demonstrated GABA inhibited the electrical excitatory response of SON neurons [17, 32] and PVN neurons [33,34]. Experiments with cultured hypothalamo-neurohypophyseal explants revealed GABA antagonists stimulated AVP release [35], and GABA inhibited the release of AVP in the rat hypothalamus in vitro [36]. Thus, GABA has the potential to act as a local inhibitory regulator of AVP release in the hypothalamo-neurohypophyseal system.

On the other hand, opioid peptides are well known to affect physiological AVP release. Although the reported effects of opioid peptides on AVP release have varied [37], there is now increasing evidence that opioid peptides (especially κ-agonists) exert a potent inhibitory effect on AVP release [14,18]. Opioid peptides have been demonstrated in the neural lobe, SON, and PVN [38], and they have been shown to be colocalized with AVP in some magnocellular neurons [39]. Moreover, receptors of dynorphin have been demonstrated in the neurohypophysis [12]. These findings support that opioid peptides also appear to exert an inhibitory effect on AVP release in the neurohypophysis and the hypothalamus.

As mentioned above, both GABA and opioid peptides have been indicated as inhibitory neuromodulators of AVP release, and they may concomitantly modulate the function of the hypothalamo-neurohypophyseal system. Moreover, colocalization of GABA and opioid peptides have also been reported in various brain regions in immunocytochemical and autoradiographical studies [19,20,21]. Therefore we propose the hypothesis that opioid peptides affect the GABAergic action on AVP release. This study demonstrated that the

inhibitory effect of GABA on AVP release stimulated by hypertonic saline was reversed by the opioid antagonist naloxone. Namely, GABAergic inhibition of AVP release was mediated at least in part by activating endogenous opioids. In addition, it has been reported that chronic activation of the GABA system induces a sustained release of met-enkephalin and, in order to replenish the depletion, the biosynthesis of met-enkephalin is augmented in rat striatum [23]. This evidence indicates a possible action of GABAergic regulation on opioid peptides and supports our findings that GABA activates the endogenous opioid system in the regulation of AVP release.

This study showed that GABA did not alter the basal AVP in rats. Previous studies in vivo also demonstrated that central administration of GABA or the elevation of endogenous GABA only inhibited AVP response to various stimuli that normally increase plasma AVP levels [5,6,7,8,9,11]. These observations suggest that GABA exerts inhibitory control on AVP secretion in stimulated conditions rather than in a basal state.

In conclusion, GABAergic inhibition of AVP release was mediated partially by endogenous opioid systems in rats treated with hypertonic saline.

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The legends to figures

Fig. 1. Effect of GABA 100 μg on plasma AVP elevated by hypertonic saline (600 mOsm/kg). Conscious rats were administered intraperitoneally with hypertonic saline 30 min prior to intracerebroventricular (i.c.v.) injection. Each point represents the mean ± SEM. $F[3, 14] = 5.68$, $P<0.01$, by one-way ANOVA. * $P<0.05$, by Tukey's multiple comparisons with the Bonferroni correction. (●) indicates rats treated with GABA 100 μg. (○) indicates control.

Fig. 2. Effect of different doses of GABA on plasma AVP elevated by hypertonic saline (600 mOsm/kg). Conscious rats were administered intraperitoneally with hypertonic saline 30 min prior to intracerebroventricular (i.c.v.) injection of GABA. Vehicle is isotonic saline (SAL). Data represent the mean ± SEM. $F[4, 18] = 15.80$, $P<0.001$, by one-way ANOVA. * $P<0.05$ vs. control, by Williams' test.

Fig. 3. Effect of naloxone (NAL) on GABA-induced suppression of plasma AVP elevated by hypertonic saline (600 mOsm/kg). Conscious rats were administered with hypertonic saline intraperitoneally and 10 mg/kg naloxone or vehicle subcutaneously (s.c.) 30 min prior to intracerebroventricular (i.c.v.) injection of GABA 100 μg. Vehicle is isotonic saline (SAL). Data represent the mean ± SEM. * $P<0.05$, ** $P<0.01$, by Student's t-test modified for multiple comparisons with the Bonferroni correction. NS: not significant.

Fig.1

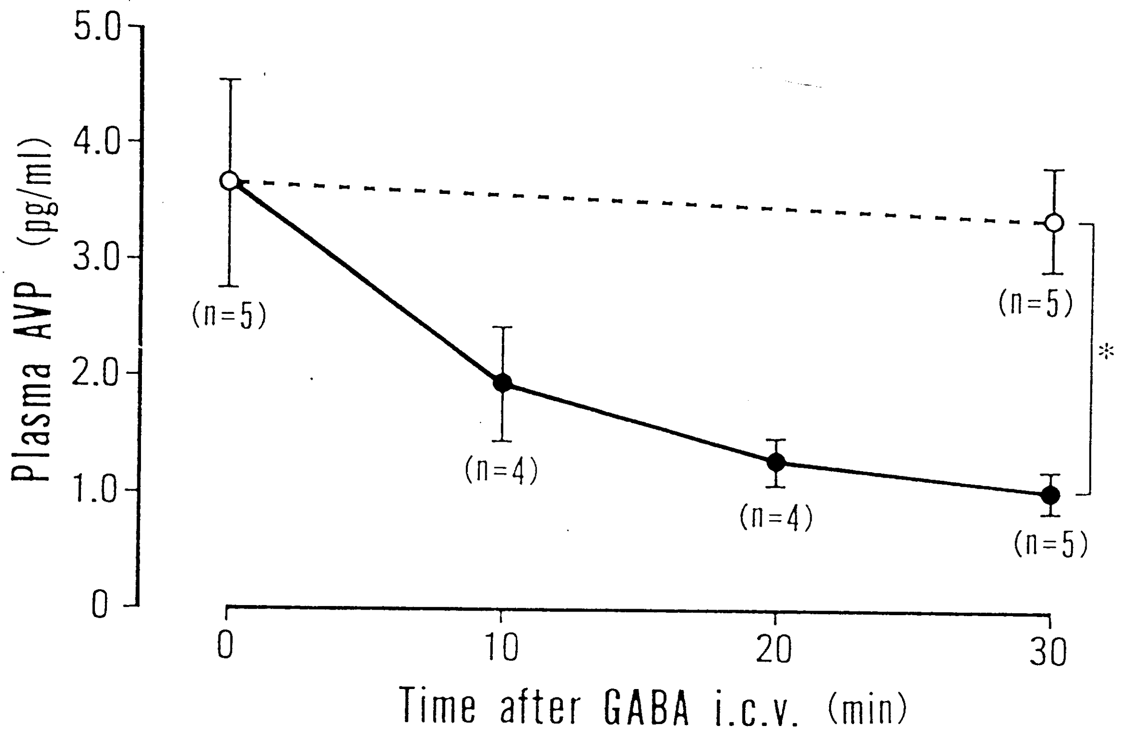

Fig.2

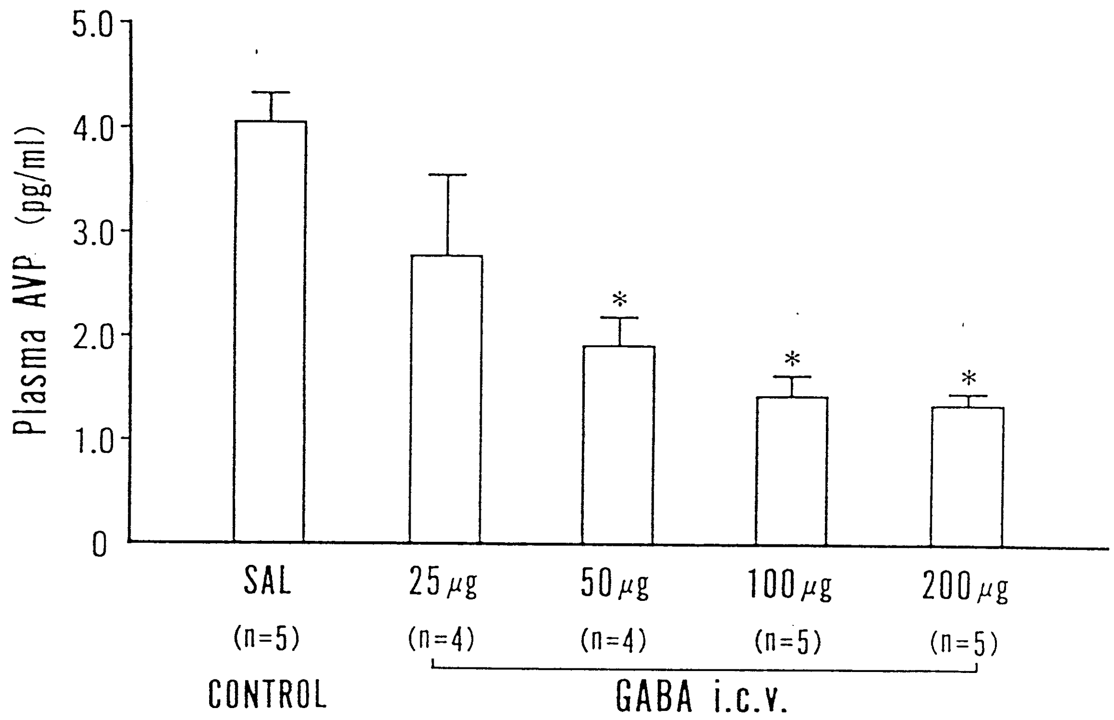

Fig.3

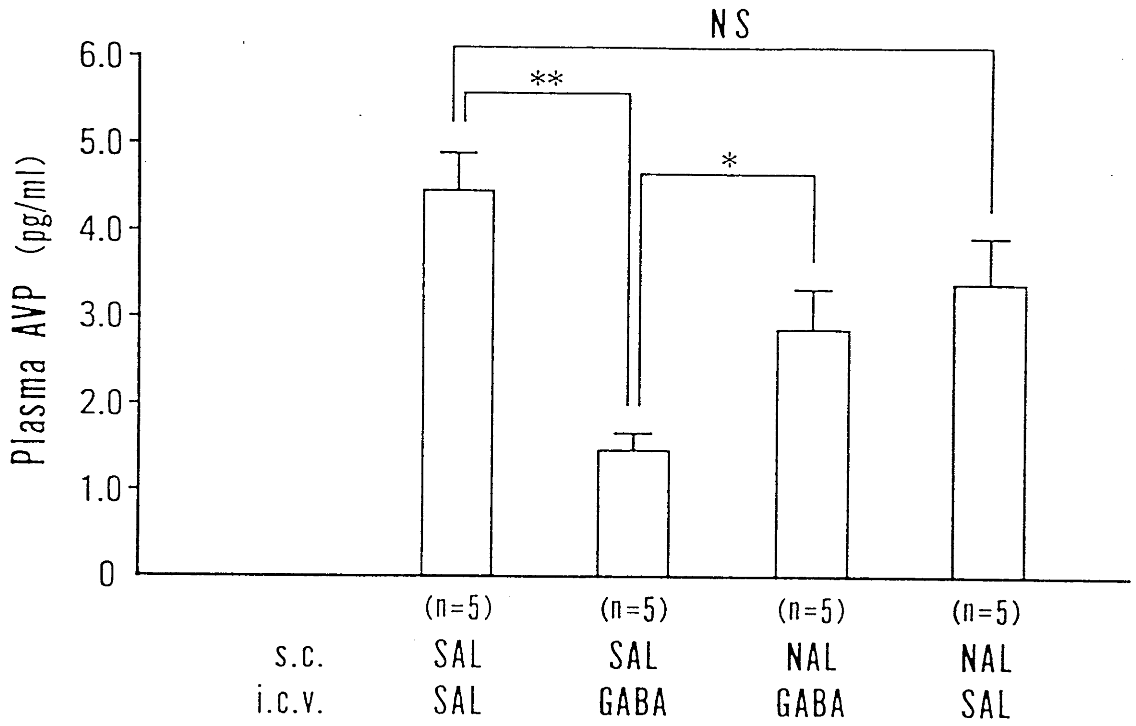

Table 1. Effect of GABA injected intracerebroventricularly (i.c.v.) and/or naloxone injected subcutaneously (s.c.) on plasma AVP and plasma Na in rats given isotonic saline intraperitoneally (mean± SEM).

Pretreatment (s.c.)	Treatment (i.c.v.)	Plasma AVP pg/ml	Plasma Na mEq/L	n
Isotonic saline	Isotonic saline	1.01± 0.16	140.5± 1.2	4
Isotonic saline	GABA 100μg	1.39± 0.29	141.8± 0.3	4
Naloxone 10mg/kg	GABA 100μg	1.13± 0.08	141.2± 1.0	4
Naloxone 10mg/kg	Isotonic saline	1.13± 0.18	141.7± 2.1	4