

Central Regulation of Urine Production by a Selective μ -Opioid Agonist, [D-Ala², N-Me-Phe⁴, Gly⁵-ol]-Enkephalin, in Rats

Hiromi Tsushima, Mayumi Mori and Tomohiro Matsuda[#]

Department of Pharmacology, Nagoya City University Medical School, Kawasumi, Mizuho-ku, Nagoya 467, Japan

Received November 8, 1996 Accepted February 12, 1997

ABSTRACT—We have investigated opioid mechanisms concerning regulation of urine production in the hypothalamic supraoptic nucleus (SON). In this study, the effect of [D-Ala², N-Me-Phe⁴, Gly⁵-ol]-enkephalin (DAMGO), a potent selective μ -opioid agonist, microinjected into the SON of anesthetized hydrated rats, on the urine outflow rate was examined. DAMGO caused a dose-dependent decrease in the urine outflow rate with no significant changes in blood pressure nor heart rate. The ED₅₀ value for the antidiuresis was calculated to be 0.055 nmol from the dose-response curve. The antidiuresis elicited by DAMGO (0.1 nmol) was partially inhibited by intra-SON pre-injection of naloxone (3 nmol), a relatively μ -selective opioid antagonist, and timolol (100 nmol), a β -adrenoceptor antagonist, but not by intra-SON pre-injection of phenoxybenzamine (20 nmol), an α -adrenoceptor antagonist, nor atropine (300 nmol), a muscarinic antagonist. Intravenous injection of *d*(CH₂)₅-D-Tyr(Et)VAVP (16.7 μ g), a vasopressin receptor antagonist, did not influence the DAMGO-induced antidiuresis. These findings suggest that antidiuresis mediated through μ -opioid receptors in the SON involves β -adrenoceptors in the nuclei, but does not involve an increase in vasopressin release.

Keywords: Vasopressin, Urine production, Supraoptic nucleus, [D-Ala², N-Me-Phe⁴, Gly⁵-ol]-Enkephalin, μ -Opioid receptor

It has been believed that oral and intravenous administrations of morphine elicit antidiuresis, resulting from increased vasopressin release from the neurohypophysis after the opioid affects cell bodies of vasopressin-containing neurons in the hypothalamic supraoptic (SON) and paraventricular nuclei (PVN) and/or their terminals in the neurohypophysis. However, effects of morphine applied in the central nervous system on vasopressin release are controversial. Intracerebroventricular injections of morphine produce antidiuresis, not diuresis (1). On the other hand, electrophysiological studies show morphine-induced inhibition on the activities of the magnocellular neurons (2), suggesting the decrease in vasopressin release from the neurohypophysis into the circulation. Concerning the plasma vasopressin level, both the increase (3, 4) and the decrease (4, 5), and also no effect (1, 6) by morphine injected into the cerebroventricle are reported. We have previously showed antidiuretic effects of morphine, directly microinjected into the SON and PVN (7). The most likely reason for the

discrepancy is differences in action sites for the opioid. A drug injected into the cerebroventricle widely spreads and diffuses over the regions around the ventricle and/or the ventricular wall and then indirectly influences the vasopressin release through neurons. Moreover, it is one of the reasons that morphine has affinity to both μ - and δ -opioid receptors (8). In this study, we used a opioid peptide, [D-Ala², N-Me-Phe⁴, Gly⁵-ol]-enkephalin (DAMGO), which is the most potent and specific for μ -receptors among opioid agonists (8), and investigated the role of μ -receptors in the SON in the regulation of urine production.

MATERIALS AND METHODS

Animals

Male Wistar rats, 9- to 10-week-old (Kitayama Labes Co., Ina) housed in a 12-hr dark/light cycle at 22 \pm 1°C were used. They were deprived of food for 17 hr before the experiment, but allowed free access to tap water.

[#] deceased.

Experimental procedure

The rats were orally administered with tap water (5 ml/100 g body weight) followed by 12% ethanol (5 ml/100 g body weight) for anesthesia 45 min later. After being cannulated in the trachea, urinary bladder and jugular vein, they were mounted in a stereotaxic instrument. The coordinates of the SON and PVN were 7.3 mm from the lambda, 1.3 mm from the midline, 8.8 mm from the dural surface and 5.6 mm, 0.3 mm, 7.8 mm, respectively. When blood pressure was examined simultaneously with the urine outflow rate, another polyethylene cannula connected with a pressure transducer was inserted into the carotid artery. An electrocardiograph was used for measurement of heart rate. During the experiment, Locke's solution containing 3% ethanol was infused at 0.1 ml/min through the cannula in the vein. Ethanol-anesthesia and -infusion brought about a stable anesthetic condition and measurable outflow rate of urine for several hours. Urinary drops flowing from the bladder cannula were continuously counted with a photoelectric drop counter. The urine outflow rate reached a constant level at approximately 30 min after the rats were all prepared, and then a stainless steel cannula (o.d.: 200 μ m) connected to a microsyringe was inserted into the nuclei for administration of DAMGO, phenoxybenzamine, timolol and atropine (1 μ l). Arg-vasopressin and *d*(CH₂)₅-D-Tyr(Et)VAVP (0.1 ml) were intravenously injected through the jugular vein cannula. In the experiments with naloxone (0.3, 3, 30 nmol), phenoxybenzamine (20 nmol), timolol (100 nmol), atropine (300 nmol) and *d*(CH₂)₅-D-Tyr(Et)VAVP (16.7 μ g), DAMGO (0.1 nmol) was microinjected into the nucleus at 30–40 min after the pretreatment with the antagonists. The influence of these antagonists was determined by comparison between the two DAMGO-induced effects on urine production with and without the antagonists. All drugs were dissolved in saline.

Identification of injection sites

After the experiments were finished, methylene blue was injected into the nuclei. The stained site in the coronal 15- μ m sections cut with a freezing microtome were verified under a microscope.

Statistical analyses

Data were reported as values of the mean \pm S.E., and a statistical difference between the means was considered to be significant when the P value was less than 0.05 by Scheffe's test following ANOVA.

Drugs

The following drugs were used: [D-Ala², N-Me-Phe⁴, Gly⁵-ol]-enkephalin and Arg-vasopressin (grade IV) (Sig-

ma Chemical Co., St. Louis, MO, USA); timolol malate (Nippon Merck-Banyu Co., Tokyo); phenoxybenzamine hydrochloride (Nacalai Tasque, Kyoto); atropine sulfate (Iwaki Co., Tokyo); naloxone hydrochloride (Sankyo

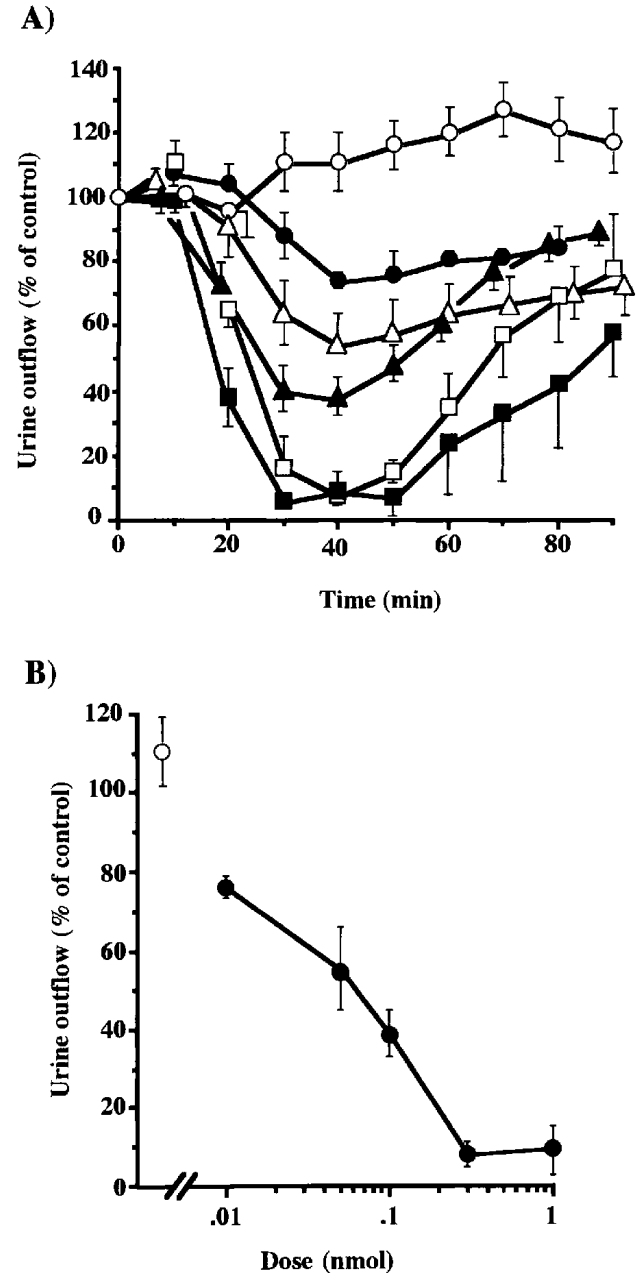


Fig. 1. Antidiuretic effects induced by microinjection of DAMGO into the SON. A) ○: Vehicle, ●: 0.01 nmol, △: 0.05 nmol, ▲: 0.1 nmol, □: 0.3 nmol, ■: 1 nmol of DAMGO. The ordinate and abscissa indicate the urine outflow rate as a percentage of the control level and time in min after administration of DAMGO, respectively. B) ○: Vehicle, ●: DAMGO. The ordinate indicates the urine outflow rate as a percentage of the control level at 40 min after drug administration in panel A. The abscissa shows the dose of DAMGO (nmol). Each value is the mean \pm S.E. of 4–19 experiments.

Co., Tokyo); $d(\text{CH}_2)_5\text{-D-Tyr}(\text{Et})\text{VAVP}$ [1-(mercapto- β,β -cyclopentamethylene propionic acid) 2-(*O*-ethyl) D-tyrosine, 4-valine, arginine vasopressin] (gift from Prof. K.G. Hofbauer, Ciba-Geigy, Ltd., Basel Switzerland). The other chemicals were of the highest analytical grade available.

RESULTS

As shown in Fig. 1 (A and B), microinjection of DAMGO from 0.01 nmol to 1 nmol into the SON produced antidiuretic effects in time- and dose-dependent manners. During antidiuresis, neither blood pressure nor heart rate significantly changed ($n=3$, data not shown). The ED_{50} value was estimated to be 0.055 nmol (Fig. 1B). The antidiuresis induced by intra-PVN injection of DAMGO showed a similar time-course and potency (ED_{50} value: 0.045 nmol) to those in the SON (data not shown).

Pretreatment with naloxone at 0.3 nmol into the SON tended to inhibit the effect of 0.1 nmol DAMGO microinjected into the same nucleus, but not significantly. A tenfold dose of naloxone significantly diminished the DAMGO-induced effect at only 40 min after administration. For a blockade of DAMGO-induced antidiuresis, 30 nmol of naloxone was needed (Fig. 2A). Microinjection of naloxone at 30 nmol itself into the SON elicited weak decreases in the urine outflow rate (urine outflow rate: $100 \pm 5.1\%$ at 10 min, $86 \pm 12\%$ at 20 min, $59 \pm 17\%$ at 30 min, $87 \pm 12\%$ of the control level at 40 min after administration, $n=5$). The smaller doses did not produce any significant change in the urine outflow rate.

Figure 2B shows that timolol at 100 nmol inhibited the DAMGO-induced antidiuresis, but neither phenoxybenzamine at 20 nmol nor atropine at 300 nmol influenced it. Timolol alone increased the urine outflow rate up to $218 \pm 22\%$ of the control level at 40 min after administration. The other antagonists did not alter the rate (phenoxybenzamine: $121 \pm 3.4\%$ at 30 min, atropine: $82 \pm 7.4\%$ of the control level at 40 min after the administration). The vasopressin receptor antagonist did not diminish the DAMGO (0.1 nmol)-induced effect, although it obviously inhibited antidiuresis induced by i.v. injection of 4 mU Arg-vasopressin (urine outflow rate by Arg-vasopressin with versus without the antagonist: $63 \pm 9.3\%$ versus $30 \pm 4.6\%$ at 10 min, $74 \pm 7.5\%$ versus $10 \pm 1.6\%$ at 20 min, $89 \pm 4.4\%$ versus $9.3 \pm 1.4\%$ at 30

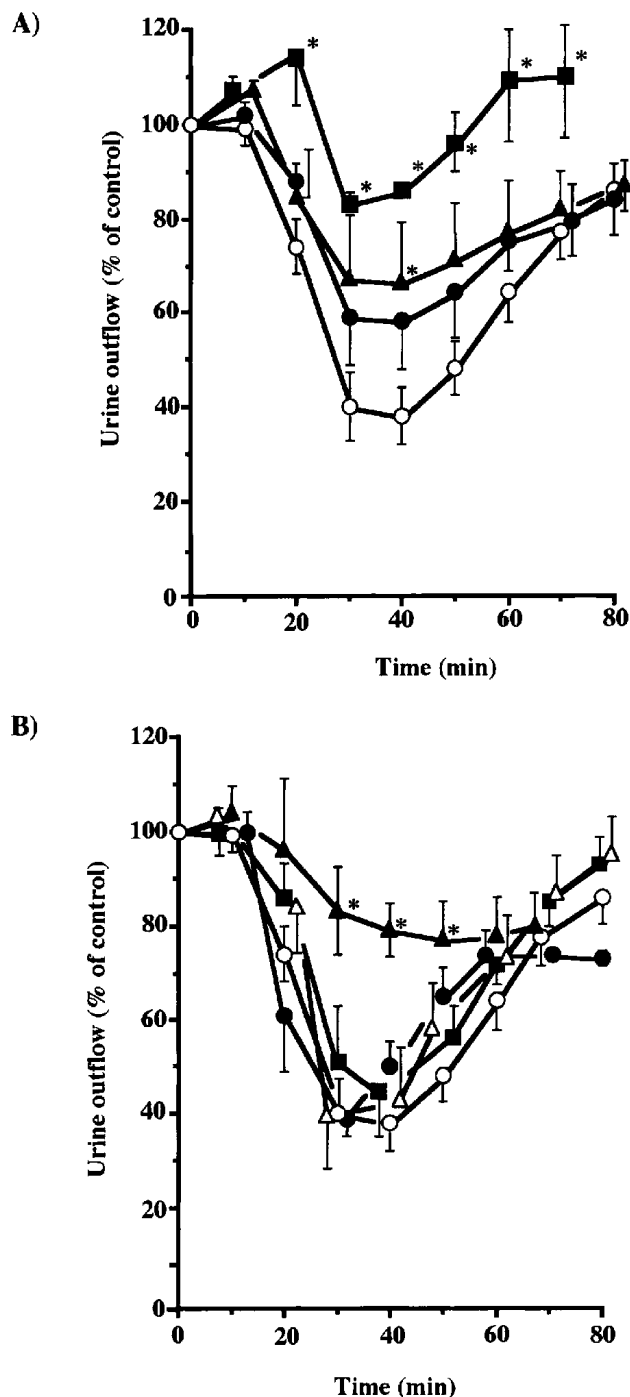


Fig. 2. Influence of various antagonists on the DAMGO-induced antidiuretic effect. Naloxone, phenoxybenzamine, timolol, atropine (intra-SON) and the vasopressin receptor antagonist (i.v.) were pretreated at 30–40 min before administration of DAMGO into the SON. The ordinate and abscissa indicate the urine outflow rate as a percentage of the control level and time in min after administration of DAMGO, respectively. A) ○: 0.1 nmol DAMGO, ●: 0.3 nmol naloxone + DAMGO, ▲: 3 nmol naloxone + DAMGO, ■: 30 nmol naloxone + DAMGO. B) ○: 0.1 nmol DAMGO, ●: 20 nmol phenoxybenzamine + DAMGO, ▲: 100 nmol timolol + DAMGO, ■: 300 nmol atropine + DAMGO, △: $16.7 \mu\text{g } d(\text{CH}_2)_5\text{-D-Tyr}(\text{Et})\text{VAVP} + \text{DAMGO}$. Each value is the mean \pm S.E. of 3–19 experiments. * $P < 0.05$ versus ○ at the corresponding time-points by Scheffe's test following ANOVA.

min, $102 \pm 7.8\%$ versus $15 \pm 3.1\%$ of the control level at 40 min after administration, $n=7$ versus $n=6$).

DISCUSSION

From pharmacological and binding studies, DAMGO was demonstrated to be a selective and potent μ -opioid receptor agonist (8). Its affinity to μ -receptors is more than 100-fold higher than its affinities to δ - and κ -opioid receptors. Autoradiography shows the presence of μ -receptors in the SON (9, 10). Therefore, most of the DAMGO-induced antidiuretic effects in this study involved an activation of μ -opioid receptors. This was supported by the experiments with naloxone: the small doses of naloxone (3–30 nmol) diminished the DAMGO-induced effect. In our previous studies, higher doses of naloxone, 600 nmol or more, were required for inhibition of the antidiuresis induced by δ - or κ -agonists using the same methods (11, 12). Binding studies show that naloxone has slightly higher affinity to μ -receptors than the other opioid receptors (8, 13). Indeed, central injection of naloxone at 2–30 nmol are reported to inhibit μ -receptor-mediated effects induced by administration of DAMGO into the same regions as injection of naloxone (14–16).

The ED_{50} value, 55 pmol, for the DAMGO-induced antidiuresis in the SON is the lowest among those for the other opioid agonists that we have tested under the same experimental condition: 19 nmol for morphine (a μ - and δ -agonist, Ref. 7), 13 nmol for fentanyl (a μ -agonist, Ref. 17), 111 nmol for Met-enkephalin (a δ -agonist, Ref. 18), 1.3 nmol for D-Ala²-Met⁵-enkephalinamide (a μ - and δ -agonist, Ref. 18), 1 nmol for D-Ala²-D-Leu⁵-enkephalin (a δ -agonist, Ref. 11) and 10 nmol for dynorphin (a κ -agonist, Ref. 12). This shows that μ -receptors in the SON play a major role in the regulation of urine production, although the potent effect may be partially due to the long metabolic half life of DAMGO since it is a D-form amino acid.

μ -Receptors are shown to exist on adrenergic and cholinergic nerve terminals and to change the release of neurotransmitters. Therefore, involvement of adrenoceptors and/or cholinceptors on the DAMGO-induced effect was investigated. In the adrenoceptor and cholinceptor antagonist-pretreated experiments, only the β -antagonist blocked the DAMGO-induced effect. This did not result from an increase in the urine outflow rate after administration of the β -antagonist into the nucleus, because morphine (7) and D-Ala²-Met⁵-enkephalinamide (18) after the pretreatment with timolol caused antidiuretic effects to similar extents as those induced without the pretreatment. Moreover, timolol inhibited the antidiuresis of D-Ala²-D-Leu⁵-enkephalin

microinjected into only the PVN, but not the SON, in spite of the diuretic condition after administration of timolol into both the nuclei (11). Therefore, it is supposed that β -adrenoceptors are involved in the DAMGO-induced antidiuresis and DAMGO promotes the release of norepinephrine/epinephrine in the SON. In general, opioid receptors are known to inhibit neurotransmitter release. However, some studies demonstrated that opioids promote neurotransmitter release, and the mechanisms for the release are suggested to be an increase in intracellular calcium concentration and prolongation of action potential duration following an increase in calcium conductance and a decrease in potassium conductance (19–21).

The vasopressin receptor antagonist did not diminish the DAMGO-induced effect, suggesting that it did not result from increased vasopressin release. There are some reports that intracerebroventricular injection of morphine produced antidiuresis without any change in the plasma vasopressin level (1, 6). Our finding indicated that μ -receptors in the SON, as well as in the tissues surrounding the ventricle, did not participate in the regulation of vasopressin release. During the DAMGO-induced antidiuresis, no changes in blood pressure nor heart rate were observed. Koepke et al. demonstrated that intrahypothalamic injection of a β -agonist elicited a decrease in urine production that resulted from modification of renal functions through neurons (22). It is consistent with our previous finding that microinjection of β -agonists into the PVN did not increase vasopressin release, but decreased the urine outflow rate (23). Moreover, electrophysiological studies show neural connections between the hypothalamus and the kidney (24). Taken together, there is a possibility that the neurons in the hypothalamus contribute to renal function.

In conclusion, microinjection of DAMGO into the SON elicited a potent antidiuretic effect that involves μ -opioid and β -adrenoceptors in the SON. However, DAMGO did not promote vasopressin release. The underlying mechanism might be an increase in water reabsorption in the urinary tubules through neurons to the kidney from the hypothalamus.

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