Effects of D-Ala^2-D-Leu^5-Enkephalin, Microinjected into the Supraoptic and Paraventricular Nuclei, on Urine Outflow Rate

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ABSTRACT—The effects of D-Ala^2-D-Leu^5-enkephalin (DADL, a δ-opioid agonist), microinjected directly into the hypothalamic supraoptic (SON) and paraventricular (PVN) nuclei, on urine outflow rate, urinary osmotic pressure, blood pressure, heart rate, respiratory rate and rectal temperature were investigated in water-loaded and ethanol-anesthetized rats. The microinjection of DADL into both the nuclei decreased urine outflow rate in a dose-dependent manner with an increase in urinary osmotic pressure, but did not change the other recorded parameters. The DADL-induced antidiuretic effect in the SON was inhibited by naloxone, but not by atropine, phenoxybenzamine, timolol nor a vasopressin antagonist, $d(CH_2)_5$-D-Tyr(Et)VAVP. The effect in the PVN was inhibited by naloxone, atropine, timolol and $d(CH_2)_5$-D-Tyr(Et)VAVP, but not by phenoxybenzamine. These results suggest that DADL causes antidiuretic effects mediated through opioid receptors in both the SON and PVN, and the underlying mechanisms are different between them. Involvement of δ-opioid receptors in the DADL-induced antidiureses was discussed.

Keywords: D-Ala^2-D-Leu^5-Enkephalin, Vasopressin, Antidiuretic effect, Supraoptic nucleus, Paraventricular nucleus

The hypothalamic supraoptic (SON) and paraventricular (PVN) nuclei include cell bodies of vasopressin-containing neurons and projecting neurons to the neurohypophysis, the brain stem, the spinal cord and others. Vasopressin release is regulated by various transmitters such as norepinephrine (1 – 3), acetylcholine (1, 4), histamine (1, 3) and angiotensin II (1, 3) in the nuclei, and thus changes the urine outflow rate.

In addition, the SON and PVN contain opioid peptides, derived from proopiomelanocortin, and proenkephalin A and B (5 – 9), some of which coexist with vasopressin (8, 10, 11). Immunohistochemical studies show fibers and terminals of opioid neurons in the nuclei (5 – 7, 11 – 13). These opioid peptides appear to play a role in the vasopressin release and the regulation of urine outflow rate. It was demonstrated that intracerebroventricular (i.c.v.) injection of morphine, a μ-agonist, increases vasopressin release and causes antidiureses (14, 15). On the other hand, i.c.v. injection of dynorphin or bremazocine, κ-agonists, produces the opposite effects (16, 17). Also, we have reported the antidiuretic effect induced by microinjection of morphine or fentanyl into the nuclei (18, 19). However, the roles of δ-agonists in the regulation are not well known. Therefore, the present experiments are designed to study the effects of D-Ala^2-D-Leu^5-enkephalin (DADL), a relatively selective δ-agonist (20), on urine outflow rate in the nuclei.

MATERIALS AND METHODS

Animals

Adult male Wistar rats (300 – 370 g) were used. The animals were housed with a 12-hr light/dark cycle and given food and tap water ad libitum. They were removed from food for approximately 19 hr before the experiments were started.

Experimental protocol

The rats were orally loaded with water (5 ml/100 g body weight); and 45 min later, they were given the same volume of 12% ethanol for anesthesia. The animals were inserted with cannulae into the trachea, the jugular vein and the urinary bladder. Locke solution (3% ethanol) was continuously infused through the jugular vein cannula at 0.1 ml/min. The ethanol-anesthetized and ethanol-infused condition kept the urine outflow rate measurable

deceased

and constant for 5–6 hr. The rats were implanted with another cannula, attached to a transducer (MPU-0.5-290-0-III; Nihon Kohden Kogyo, Tokyo), into the jugular artery for measurement of blood pressure; and thermister probes were inserted into the tracheal cannula and rectum (SR-115 and MGA III-219, Nihon Kohden Kogyo, Co.) for measurement of respiratory rate and rectal temperature, respectively. Heart rate was monitored by cardiography (FD-14; Fukuda, Tokyo). Then the animal was placed in a stereotaxic instrument (Takahashi Co., Tokyo), and a stainless steel cannula (diameter: 200 μm) was inserted into the SON or PVN according to the atlas of König and Klippel (21). After 30–60 min, the urine outflow from the bladder cannula reached a constant rate of 0.04–0.20 ml/min. All drugs were dissolved in saline, which was used as the vehicle. Solutions of vasopressin (0.1 ml) and $d(CH_2)_5$-D-Tyr(Et)VAVP (0.2 ml) were injected intravenously. The other drug solution (1 μl), followed by an artificial cerebrospinal fluid (2 μl) (22) for washing-out the dead space of the cannula, was microinjected into the nucleus for 10 min through a microsyringe which was connected to the stainless steel cannula in the nucleus. The number of urine drops was counted every 10 min by a photoelectric counter (DCT 102; Unique Medical, Inc., Tokyo) and was expressed as a percentage of the control (the number of urine drops for 10 min before the microinjection). Urinary osmotic pressure was measured by the freezing point depression method (The Fiske Osmometer, Model G-62; Fiske Associates, Inc., Uxbridge, MA, USA).

Injection sites in the SON and PVN were verified from coronal 15-μm sections, which were cut using a freezing microtome (Tissue-Tek II; Miles Inc., Erkhart, IN, USA) and were stained with hematoxylin-eosin.

Statistical analyses

All values are presented as the mean±S.E. Significance of differences was determined by Student's *t*-test at $P<0.05$.

Drugs

D-Ala^2-D-Leu^5-Enkephalin and vasopressin (Sigma Chemical Co., St. Louis, MO, USA), phenoxybenzamine hydrochloride (Nacalai Tesque, Kyoto) and atropine sulfate (Iwai Co., Tokyo) were purchased. Naloxone hydrochloride and timolol malate were generous gifts from Sankyo Co. and Nippon Merck-Banyu Co., Tokyo, respectively. The vasopressin antagonist $d(CH_2)_5$-D-Tyr(Et)VAVP (1-(mercapto-β,β-cyclopentamethylene propionic acid) 2-(*O*-ethyl) D-tyrosine, 4-valine, arginine vasopressin) was kindly provided by Prof. K.G. Hofbauer (Cardiovascular Division, Ciba-Geigy, Ltd., Basel, Switzerland). The other chemicals were of the highest analytical grade available.

RESULTS

Effects of DADL microinjected into the SON and PVN

Urine outflow rate from the bladder cannula was constant, 0.04–0.20 ml/min, for 5–6 hr from 30–60 min after all was set. The microinjection of vehicle (saline) into the SON and PVN did not change the urine outflow rate nor the other recorded indices.

As shown in Fig. 1, 4 nmol of DADL, microinjected into the SON, began to decrease the urine outflow rate within 20 min, reached a maximum response at 30–40 min and returned it to the control level by 80 min after the microinjection. The antidiuretic effects were dose-dependent from a dose of 0.5 nmol to 10 nmol (Fig. 2). The microinjection of DADL into the PVN also elicited antidiuretic effects, which showed similar potency to those in the SON (Fig. 2).

Urinary osmotic pressure was measured to examine the intervention of vasopressin release in the antidiuretic effects. The microinjection of 10 nmol DADL significantly increased the osmotic pressure to 202±26% of the control level at 30 min in the SON (n=4) and 228±17% of the control level at 40 min in the PVN (n=6). At these times, the urine outflow rates showed the maximum decrease, which were 6±4% and 7±3% of the control level in the SON and PVN, respectively. When the urine outflow rate recovered to the control level at 80–120 min after the injection, urinary osmotic pressure also returned to the control level. The control urinary osmotic pressure

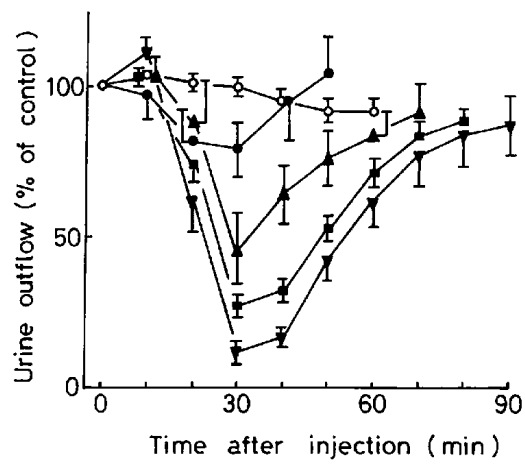

Fig. 1. The effects of DADL microinjected into the SON on urine outflow rate. ○: vehicle, n=10; ●: 0.5 nmol DADL, n=6; ▲: 1 nmol DADL, n=6; ■: 4 nmol DADL, n=32; ▼: 10 nmol DADL, n=10. Ordinate: Urine outflow rate expressed as a % of the control level (0.04–0.20 ml/min). Abscissa: Time in min after the microinjection. Data are presented as the mean, and the bars show the S.E.

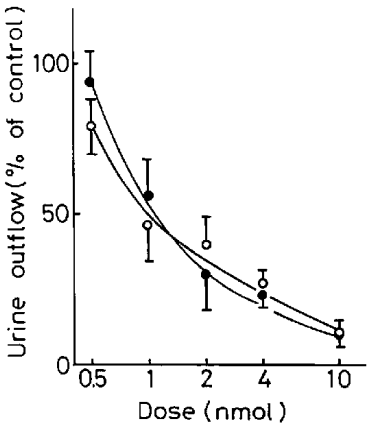

Fig. 2. Dose-response curves for the DADL-induced antidiuretic effects in the SON (○) and PVN (●). Ordinate: Minimum urine outflow rate, at 30 min after the microinjection of DADL into the nuclei, expressed as a % of the control level. Abscissa: Dose in nmol of DADL. Data are presented as the mean, and the bars show the S.E. of 4–32 experiments.

was 264±8 mOsm/kg in the SON and 291±20 mOms/kg in the PVN.

Neither blood pressure, heart rate, respiratory rate nor rectal temperature were changed during the antidiuretic effects induced by 10 nmol DADL in the nuclei.

Effects of the antagonists on the DADL-induced antidiuretic effects

The DADL (4 nmol)-induced effects on urine outflow rate before and after the microinjection of the various antagonists into the nucleus were compared to investigate mechanisms underlying the antidiureses. The antidiureses induced by the first and second injection of DADL into the same nuclei were not significantly different.

The i.v. injection of $d(CH_2)_5$-D-Tyr(Et)VAVP (16.7 μg), a vasopressin V_1V_2-antagonist (23) that alone did not show any effect on the urine outflow rate, inhibited the vasopressin (4 mU)-induced antidiuretic effect, being almost the same as the DADL (4 nmol)-induced effect (Fig. 3a). The vasopressin-induced antidiureses were reproducible in the same rat. Figure 3 (b and c) shows the effects of $d(CH_2)_5$-D-Tyr(Et)VAVP on the DADL-induced effects in the SON and PVN, respectively. The antagonist partially inhibited the antidiuretic effect in the PVN, but did not do so in the SON.

In Figs. 4 and 5, the effects of an opioid antagonist, naloxone (NAL, 600 nmol); a muscarinic antagonist, atropine (ATR, 300 nmol); an α-adrenergic antagonist, phenoxybenzamine (PHE, 80 nmol); and a β-adrenergic antagonist, timolol (TIM, 100 nmol), microinjected into the nuclei, on the DADL-induced antidiureses are shown. NAL at 300 nmol partially inhibited the DADL-induced antidiureses in both the nuclei (SON: 13±7.0% vs. 41±8.4% at 30 min, 22±7.4% vs. 48±9.9% at 40 min, n=4; PVN: 6±2.8% vs. 53±14% at 30 min, 10±3.4% vs. 53±11% at 40 min, n=4; $P<0.05$). A previous study showed that this dose of NAL produced complete inhibition of the antidiureses induced by 10 nmol morphine, a

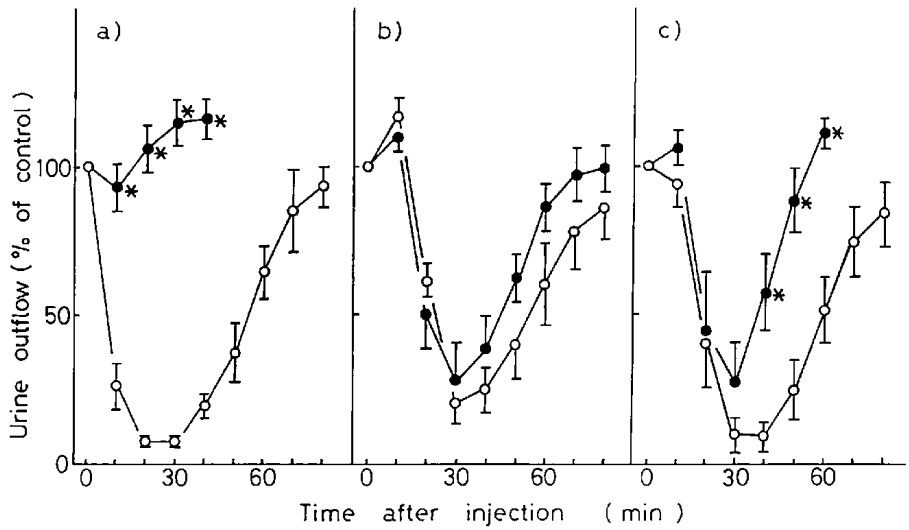

Fig. 3. Effects of $d(CH_2)_5$-D-Tyr(Et)VAVP on the vasopressin- and DADL-induced antidiuretic effects. Ordinate: Urine outflow rate expressed as a % of the control level. Abscissa: Time in min after the administration of a) 4 mU vasopressin (n=4, i.v.), b) 4 nmol DADL (n=8, intra-SON) and c) 4 nmol DADL (n=5, intra-PVN). Open and solid circles indicate the urine outflow rate induced by the drugs before and after the pretreatment with $d(CH_2)_5$-D-Tyr(Et)VAVP (16.7 μg, i.v.), respectively. The period for the pretreatment was 30–40 min. Data are presented as the mean, and the bars show the S.E. * indicates a significant difference from open circles at the same time ($P<0.05$).

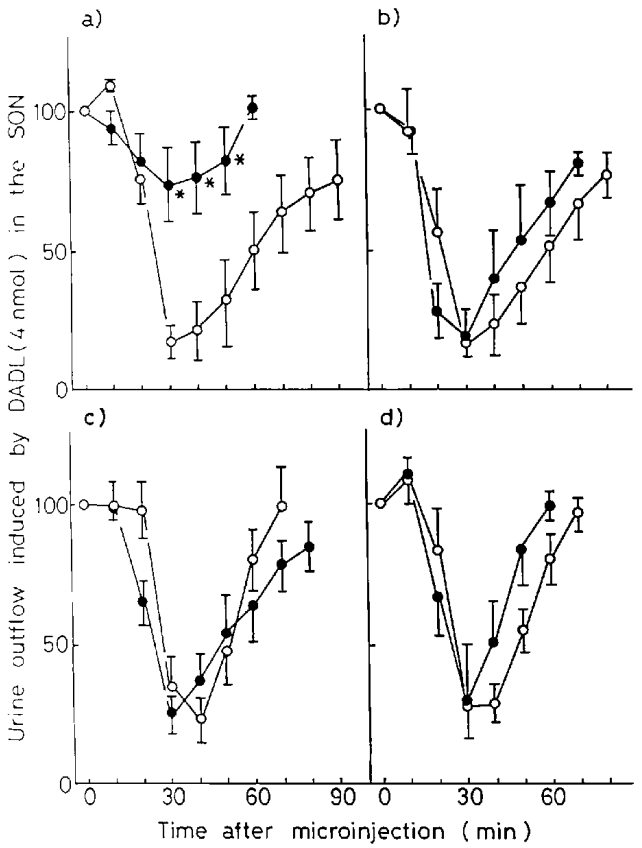

Fig. 4. Effects of the antagonists on the DADL-induced antidiuretic effects in the SON. The ordinate and abscissa show the urine outflow rate expressed as a % of the control level and the time in min after the microinjection of DADL into the SON, respectively. ○: 4 nmol DADL, ●: 4 nmol DADL after pretreatment with a) naloxone (600 nmol, n=5), b) atropine (300 nmol, n=4), c) phenoxybenzamine (80 nmol, n=4) and d) timolol (100 nmol, n=5). Naloxone, atropine, phenoxybenzamine and timolol were microinjected into the SON at 80, 60–70, 30–50 and 30–50 min before the microinjection of DADL into the same nucleus, respectively. Data are presented as the mean, and the bars show the S.E. * indicates a significant difference from open circles at the same time ($P<0.05$).

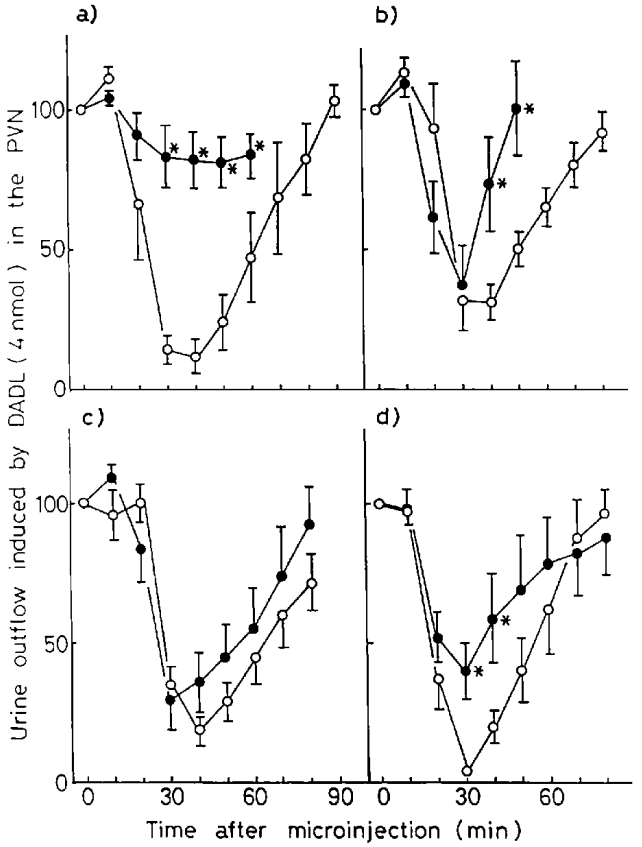

Fig. 5. Effects of the antagonists on the DADL-induced antidiuretic effects in the PVN. The ordinate and abscissa show the urine outflow rate expressed as a % of the control level and the time in min after the microinjection of DADL into the PVN, respectively, ○: 4 nmol DADL. ●: 4 nmol DADL after pretreatment with a) naloxone (600 nmol, n=5), b) atropine (300 nmol, n=5), c) phenoxybenzamine (80 nmol, n=6) and d) timolol (100 nmol, n=5). Naloxone, atropine, phenoxybenzamine and timolol were microinjected into the PVN at 80, 70–80, 40–80 and 30–50 min before the microinjection of DADL into the same nucleus, respectively. Data are presented as the mean, and the bars show the S.E. * indicates a significant difference from open circles at the same time ($P<0.05$).

μ-agonist (18). The higher dose, 600 nmol, of NAL inhibited the DADL-induced antidiureses more strongly than 300 nmol of NAL. However, the weak and significant antidiuretic effect remained (Figs. 4a and 5a). Microinjection of 300 or 600 nmol NAL alone into the nuclei decreased the urine outflow rate. The other antagonists did not influence the DADL-induced antidiureses in the SON (Fig. 4, b–d). On the other hand, in the PVN, ATR and TIM inhibited the effect, but PHE did not (Fig. 5, b–d). All of these inhibitory effects were partial. In the PVN, ATR at the smaller dose, 2 nmol, showed a tendency to inhibit the effects, but not significantly (n=7). The doses of the cholinergic and adrenergic antagonists were sufficient to inhibit the effects of each agonist in the same method (24–26).

DISCUSSION

This study showed that the microinjection of DADL into the SON and PVN elicited the antidiuretic effects that were antagonized by NAL, suggesting opioid-receptor-mediated effects. The opioid receptors seem to be δ-subtypes, at least in part, for the following reasons: 1) A higher dose of NAL was required to inhibit the DADL-induced antidiuretic effects than the morphine-induced antidiuretic effects. In receptor binding studies, the affinity of NAL for δ-receptors is 15-times less than that for μ-receptors (27); 2) The effects of the adrenergic and cholinergic antagonists on the DADL-induced effects were different from those on the morphine-induced one (18, mentioned below), and, at least, the DADL-binding opioid receptors

are not μ-subtypes; 3) DADL is thought to be relatively selective for δ-receptors (20), and 4) autoradiography shows the presence of δ-receptors in the nuclei (28).

Microinjection of NAL alone into the nuclei produced the antidiureses. Because the doses used were relatively high, the antidiureses may be an agonistic effect. We have already investigated the effects of the drug solution osmotic pressure on urine outflow rate using NaCl. The ED_{50} values for NaCl-induced antidiureses were >3.2 μmol in the SON and 1.1 μmol in the PVN (25, 26). This suggests that the osmotic pressure of the drug solution influences urine outflow rate, but it is weak.

ATR and TIM inhibited the DADL-induced effect in the PVN, but not in the SON. From these results, it is suggested that the antidiuretic effect of DADL in the PVN involves adrenergic and cholinergic mechanisms, but that in the SON does not involve them. The DADL-binding opioid receptors may have different properties from μ-receptors in the nuclei. That is, μ-receptors induced the antidiuretic effects mediated only through a cholinergic mechanism in both the nuclei, because the morphine-induced effects were inhibited by ATR, but not by PHE nor TIM (18).

Microinjection of DADL into the PVN is suggested to promote vasopressin release, because $d(CH_2)_5$-D-Tyr(Et)VAVP inhibited the DADL-induced antidiuretic effect. However, this inhibitory effect was partial, although the dose of the antagonist used in this study was sufficient to block the antidiuretic effect induced by 4 mU vasopressin, which was almost the same as the DADL (4 nmol)-induced one. In addition, the increase in urinary osmotic pressure induced by DADL was not as much as that by vasopressin. DADL increased the urinary osmotic pressure to approximately 2 times the control level in the PVN. Our previous result shows that the increase in osmotic pressure by vasopressin (4 mU, i.v.), which elicited antidiureses with a similar potency to DADL, was 3.5 times the control level (29). Therefore, it is possible that other mechanisms may intervene in the effect. On the other hand, because in the SON, the DADL-induced effects before and after the pretreatment with $d(CH_2)_5$-D-Tyr(Et)VAVP were not different significantly, the antidiureses did not seem to be mediated through an increase in vasopressin release. This finding is consistent with the electrophysiological result that DADL does not influence the firing rate of vasopressin-containing neurons in the SON (30). DADL did not change the blood pressure nor heart rate when it induced the antidiuretic effects. Electrophysiological studies show that there is a neuronal connection between the hypothalamic nuclei and the kidney (31–33). In addition, some drugs in the hypothalamus produced antidiuretic effects without increasing vasopressin release (32, 34, 35). Taken together, DADL may affect the neurons from the hypothalamic nuclei to the kidney directly or indirectly and may induce antidiuretic effects.

In summary, DADL produced the antidiuretic effects mediated through opioid receptors, probably the δ-subtype in the SON and PVN, and the underlying mechanisms were different between them. The mechanism in the PVN, at least in part, involves an increase in vasopressin release through adrenergic and cholinergic neurons.

REFERENCES

1 Sladek, C.D.: Regulation of vasopressin release by neurotransmitters, neuropeptides and osmotic stimuli. Prog. Brain Res. **60**, 71–89 (1983)

2 Brooks, D.P., Crofton, J.T. and Share, L.: Role of catecholamines in the regulation of vasopressin release. *In* Vasopressin: Cellular and Integrative Functions, Edited by Cowley, A.W., Jr., Liard, J.-F. and Ausiello, D.A., pp. 341–348, Raven Press, New York (1988)

3 Leibowitz, S.F.: Impact of brain monoamines and neuropeptides on vasopressin release. *In* Vasopressin: Cellular and Integrative Functions, Edited by Cowley, A.W., Jr., Liard, J.-F. and Ausiello, D.A., pp. 379–388, Raven Press, New York (1988)

4 Sladek, C.D. and Clough R.W.: Comparison of vasopressin and oxytocin release from hypothalamo-neurohypophyseal explants: Cholinergic and osmotic control. *In* Vasopressin: Cellular and Integrative Functions, Edited by Cowley, A.W., Jr., Liard, J.-F. and Ausiello, D.A., pp. 321–328, Raven Press, New York (1988)

5 Sar, M., Srumpf, W.E., Miller, R.J., Chang, K.-J. and Cuatrecasas, R.: Immunohistochemical localization of enkephalin in rat brain and spinal cord. J. Comp. Neurol. **182**, 17–38 (1978)

6 Wamsley, J.K., Young, W.S., III, and Kuhae, M.J.: Immunohistochemical localization of enkephalin in rat brain. Brain Res. **190**, 153–174 (1980)

7 Watson, S.J., Khachaturian, H., Akil, H., Coy, D.H. and Goldstein, A.: Comparison of the distribution of dynorphin systems and enkephalin systems in brain. Science **218**, 1134–1136 (1982)

8 Weber, E., Evans, C.J. and Barchas, J.D.: Mapping of hypothalamic opioid peptide neurons by a novel immunohistochemical technique: Relation to α-neo-endorphin and vasopressin systems. Adv. Biochem. Psychopharmacol. **33**, 519–526 (1982)

9 Zamir, N., Palkovits, M. and Brownstein, M.: Distribution of immunoreactive Met-enkephalin-Arg^6-Gly^7-Leu^8 and Leu-enkephalin in discrete regions of the rat brain. Brain Res. **326**, 1–8 (1985)

10 Sakanaka, M., Magari, S., Inoue, N. and Lederis, K.: Co-localization of arginine vasopressin-like immunoreactivities in nerve cells of the rat hypothalamus. Cell Tissue Res. **260**, 549–554 (1990)

11 Watson, S.J., Akil, H., Fischli, W., Goldstein, A., Zimmerman, E., Nilaver, G. and van Wimersma Greidanus, T.B.: Dynorphin and vasopressin: Common localization in magnocellular neurons. Science **216**, 85–87 (1982)

12 Difiglia, M. and Aronin, N.: Immunoreactive Leu-enkephalin in the monkey hypothalamus including observations on its ultrastructural localization in the paraventricular nucleus. J. Comp. Neurol. **225**, 313–326 (1984)

13 Kiss, J.Z., Csaaell, M.D. and Palkovits, M.: Analysis of the ACTH/β-end/α-MSH-immunoreactive afferent input to the hypothalamic paraventricular nucleus of rat. Brain Res. **324**, 91–99 (1984)

14 Aziz, L.A., Forsling, M.L. and Woolf, C.J.: The effect of intracerebroventricular injections of morphine on vasopressin release in the rat. J. Physiol. (Lond.) **311**, 401–409 (1981)

15 Huidobro-Toro, J.P. and Huidobro, F.: Central effects of morphine, levorphanol, (−)-methadone and the opioid-like peptides β-endorphin and D-alanine 2-methionine enekapalinamide on urine outflow and electrolytes. J. Pharmacol. Exp. Ther. **217**, 579–585 (1981)

16 Salas, S.P., Roblero, J., Ureta, H. and Huidobro-Toro, J.P.: Diuretic effect of bremazocine, a kappa-opioid with central and peripheral sites of action. J. Pharmacol. Exp. Ther. **250**, 992–999 (1989)

17 Haaf, J.A.T., Maigret, C., Andringa-Bakker, E.A.D. and van Wimersma Greidanus, T.B.: Dynorphin-(1–13) is a potent in vivo supressor of vasopressin levels in the rat. Acta Endocrinol. **114**, 96–101 (1987)

18 Tsushima, H., Mori, M. and Matsuda, T.: Antidiuretic effects of morphine microinjected into the hypothalamic supraoptic and paraventricular nuclei in a water-loaded and ethanol-anesthetized rat. Japan. J. Pharmacol. **45**, 449–457 (1987)

19 Tsushima, H., Mori, M. and Matsuda, T.: Antidiuretic effects of fentanyl, injected into the hypothalamic supraoptic and paraventricular nuclei, in a water-loaded and ethanol-anesthetized rat. Neuropharmacology **29**, 757–763 (1990)

20 Wüster, M., Schulz, R. and Herz, A.: Multiple opiate receptors in peripheral tissue preparations. Biochem. Pharmacol. **30**, 1883–1887 (1981)

21 König, J.F.R. and Klippel, R.A.: The Rat Brain, a Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem. Williams and Wilkins, Baltimore (1963)

22 Cserr, H.: Potassium exchange between cerebrospinal fluid, plasma and brain. Am. J. Physiol. **209**, 1219–1226 (1965)

23 Hofbauer, K.G., Mah, S.C. and Opperman, J.R.: Chronic blockade of vasopressin receptors in rats. J. Cardiovasc. Pharmacol. **8**, Supp. **7**, S56–S60 (1986)

24 Mori, M., Tsushima, H. and Matsuda, T.: Antidiuretic effects of oxotremorine microinjected into the hypothalamic supraoptic and paraventricular nuclei in a water-loaded and ethanol-anesthetized rat. Japan. J. Pharmacol. **35**, 27–36 (1984)

25 Tsushima, H., Mori, M. and Matsuda, T.: Antidiuretic effects of alpha- and beta-adrenoceptor agonists microinjected into the hypothalamic supraoptic nucleus in a water-loaded and ethanol-anesthetized rat. Japan. J. Pharmacol. **39**, 365–374 (1985)

26 Tsushima, H., Mori, M. and Matsuda, T.: Antidiuretic effects of alpha- and beta-adrenoceptor agonists microinjected into the hypothalamic paraventricular nucleus in a water-loaded and ethanol-anesthetized rat. Japan. J. Pharmacol. **40**, 319–328 (1986)

27 Chang, K.-J.: Opioid receptors: Multiplicity and sequelae of ligand-receptor interactions. *In* The Receptors, Edited by Conn, P.M., Vol. **1**, pp. 1–81, Academic Press, Orlando (1984)

28 Desjardins, G.C., Brawer, J.R. and Beaudet, A.: Distribution of μ, δ and κ opioid receptors in the hypothalamus of the rat. Brain Res. **536**, 114–123 (1990)

29 Tsushima, H., Mori, M. and Matsuda, T.: Antidiuretic effects of methionine-enkephalin and 2-D-alanine-5-methionine-enkephalinamide microinjected into the hypothalamic supraoptic and paraventricular nuclei in a water-loaded and ethanol-anesthetized rat. Japan. J. Pharmacol. **42**, 507–515 (1986)

30 Wakerley, J.B., Noble, R. and Clarke, G.: Effects of morphine and D-Ala, D-Leu enkephalin on the electrical activity of supraoptic neurosecretory cells in vitro. Neuroscience **10**, 73–81 (1983)

31 Ciriello, J. and Calaresu, F.R.: Hypothalamic projections of renal afferent nerves in the cat. Can. J. Physiol. Pharmacol. **58**, 574–576 (1980)

32 Koepke, J.P., Jones, S. and Dibona, G.F.: Hypothalamic β_2-adrenoceptor control of renal sympathetic nerve activity and urinary sodium excretion in conscious, spontaneously hypertensive rats. Circ. Res. **58**, 241–248 (1986)

33 Simon, J.K. and Ciriello, J.: Contribution of afferent renal nerves to the metabolic activity of central structures involved in the control of the circulation. Can. J. Physiol. Pharmacol. **67**, 1130–1139 (1989)

34 Huidobro-Toro, J.P.: Antidiuretic effect of β-endorphin and morphine in brattleboro rats: Development of tolerance and physical dependence after chronic morphine treatment. Br. J. Pharmacol. **71**, 51–56 (1980)

35 Danesh, S. and Walker, L.A.: Effects of central administration of morphine on renal function in conscious rats. J. Pharmacol. Exp. Ther. **244**, 640–645 (1988)