ABSTRACT—The mechanisms for the antidiuretic effects of dynorphin (DYN), an endogenous κ-agonist, microinjected into the hypothalamic supraoptic (SON) and paraventricular (PVN) nuclei were investigated. DYN decreased the urine outflow rate dose-dependently from 5 to 20 nmol in the SON and PVN, and it increased vasopressin release. Microinjection of des-Tyr-DYN (a non-opioid peptide) into the SON produced antidiuretic effects with similar potency to that of the DYN-induced effects. However, in the PVN, the effects of des-Tyr-DYN were very markedly weaker than those of DYN. The DYN-induced antidiuresis in the SON were partially inhibited by phenoxybenzamine, timolol and atropine, but not by naloxone. Those in the PVN were partially inhibited by naloxone, timolol and atropine, but not by phenoxybenzamine. Synthetic specific κ-agonists, U50,488H and Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Arg-Leu-Arg-Gly 5-aminopentylamide (DAKLI), microinjected into the PVN also produced antidiuretic effects in a dose-dependent manner. The order of antidiuretic potency was DAKLI > DYN > U50,488H, which was the same as that of κ-receptor binding affinity. The DAKLI-induced antidiuresis in the PVN were not inhibited by naloxone. These results suggested that DYN caused antidiuresis by vasopressin release, through adrenergic and cholinergic mechanisms in the SON and PVN. Only the DYN-induced effects in the PVN were mediated, at least partially, through opioid receptors, perhaps the κ-subtype.

Keywords: Dynorphin, Vasopressin, Antidiuretic effect, Supraoptic nucleus, Paraventricular nucleus

In our preliminary study, however, microinjection of dynorphin(1–13) (DYN), an endogenous κ-agonist (20, 21) which co-exists with vasopressin (22), into the SON and PVN decreased the urine outflow rate (23). Thus, we investigated the mechanisms of the DYN-induced antidiuretic effects in this study.

MATERIALS AND METHODS

Male Wistar rats (280–330 g), starved overnight but allowed free access to water, were used. They were orally loaded with tap water (5 ml/100 g body weight); and 45 min later, they were anesthetized by oral administration of the same volume of 12% ethanol. The trachea, the urinary bladder and the jugular vein were cannulated with polyethylene tubes. The rats were placed in a stereotaxic instrument (Takahashi Co., Tokyo). A stainless steel cannula (outer diameter: 200 μm) was implanted into the SON (coordinates: 6.3 mm anterior to the lambda, 1.3 mm lateral to the midline, 9.0 mm from the dura) or PVN (coordinates: 5.6 mm, 0.3 mm, 8.0 mm), according to at-
Injection sites of the drug into the nuclei were verified by microinjection of methylene blue or by burning with an electric current (3 mA, 60 sec, Lesion Generator Model RFG-4A; Radiomics, Inc., Burlington, MA, USA) after the experiments. After 15-μm coronal sections were cut with a microtome (Tissue-Tek II; Miles, Inc., Erkhard, IN, USA) and then stained with Hematoxylin-Eosin, the sites of the tip of the cannula were confirmed under the microscope.

The data were presented as the mean ± S.E. Statistical analysis for the two means was performed by Student’s t-test. To judge the total inhibition by the antagonists, two-way ANOVA was used. Differences were considered significant when the P value was less than 0.05.

The following drugs were used: dynorphin(1-13), Tyr-Gly-Gly-Phe-Leu-Arg-Ile-Arg-Pro-Met-Leu and Met-enkephalinamide methanesulfonate (U-50,488H), dynorphin(1-13) (des-Tyr-dynorphin), EDTA, bovine serum albumin and Arg⁸-vasopressin (grade VI) (Sigma Chemical Co., St. Louis, MO, USA); atropine sulfate (Iwaki Co., Tokyo); phenoxybenzamine hydrochloride (Nacalai Tesque, Kyoto); [¹²⁵I]-vasopressin (Amersham Japan, Ltd., Tokyo); vasopressin antibody (Calbiochem-Behring, A Division of American Hoechst Co., La Jolla, CA, USA). Naloxone hydrochloride (Sankyo Co., Tokyo) and timolol malate (Nippon Merck-Banyu Co., Tokyo) were generous gifts. The other chemicals were of the highest analytical grade available.

RESULTS

Effects of DYN on urine outflow rate

Vehicle alone, microinjected into the SON or PVN, had no effect on the urine outflow rate (Fig. 1). The control urine outflow rate was constant at approx. 0.1 ml/min.

As shown in Fig. la, microinjection of DYN(5-20 nmol) into the SON decreased the urine outflow rate in time- and dose-dependent manners, and DYN(5-20 nmol) microinjected into the PVN also elicited antidiuretic effects with almost the same potency (Fig. 1b). When DYN was microinjected into the nucleus of the same rat twice, the antidiureses induced by the first and second injections were not significantly different at each time point (SON: n = 5, PVN: n = 3; data not shown).

Effects of DYN on urinary osmotic pressure

Urinary osmotic pressure was simultaneously measured with the urine outflow rate after microinjection of 20 nmol DYN into the nuclei and after intravenous injection of 4 mU vasopressin. The vehicle had no effect on the osmotic pressure and the outflow rate of urine.
Fig. 1. Antidiuretic effects induced by microinjection of DYN into
the SON (a) and PVN (b). O: vehicle (SON: n=10, PVN: n=8),
•: 5 nmol (n=8, n=8), △: 10 nmol (n=66, n=60), ■: 20 nmol
(n=13, n=6) of DYN. Ordinate: Urine outflow rate, expressed as a
percentage of the control level (a: 0.099±0.004, b: 0.096±0.004
ml/min). Abscissa: Time in min after microinjection of the vehicle
or DYN. Symbols are the mean±S.E. Significance of difference com-
pared with the open circles (vehicle) at corresponding time-points: *P<0.05.

DYN increased urinary osmotic pressure up to
248±43% and 480±103% of the control level in the SON
and PVN, respectively (SON: n=5, PVN: n=5; P<0.05)
when the antidiuretic effects were maximum: 15±4% of
the control level (0.094±0.016 ml/min) in the SON and
17±5% of the control level (0.085±0.008 ml/min) in the
PVN. The control osmotic pressure was 279±19
mOsm/kg in the SON and 270±23 mOsm/kg in the
PVN. These effects returned to the control level at ap-
prox. 90 min after the injection.

Intravenous injection of 4 mU vasopressin induced anti-
diuretic effects with a time-course similar to that of the
DYN-induced ones. At 20 min after administration of
vasopressin, the urine outflow rate and urinary osmotic
pressure were 10±2% and 338±56% of the control level
(0.067±0.016 ml/min and 315±41 mOsm/kg) (n=4),
respectively. They were approximately equal to those of
20 nmol DYN microinjected into the nuclei.

Effects of DYN on plasma vasopressin level
Effects of DYN (20 nmol), microinjected into the SON
and PVN, on the plasma vasopressin level were studied.
The control vasopressin level was lower than the sensitiv-
ity of the radioimmunoassay, <2.4 pg/ml. The vasopres-
sin level at 30 min after the microinjection of the vehicle
into the nuclei was also less than the sensitivity (SON: n=3, PVN: n=5).

Microinjection of DYN into the SON significantly
elevated the plasma vasopressin level in six out of eight
experiments, with a mean value of 15±5.3 pg/ml. In the
other two experiments, the plasma vasopressin level was
less than the sensitivity of the assay. In the PVN, six
experiments showed an increase up to 23±7.1 pg/ml from
the control level, <2.4 pg/ml by DYN, and only one ex-
periment showed an undetectable level.

Effects of DYN on visceral functions
Mean blood pressure, heart rate, respiratory rate and
rectal temperature were monitored during the antidiuretic
effects induced by DYN (20 nmol). None of the indices
changed significantly (SON: n=3, PVN: n=3; data not
shown).

Effects of naloxone on the DYN-induced antidiuretic
effects
Effects of naloxone (NAL) at 300 or 600 nmol on the
antidiuretic effect of 10 nmol DYN were studied. The
microinjection of 300 nmol NAL alone into both the
nuclei, which was enough to inhibit the morphine-in-
duced antidiuretic effects in the same method (7), did not
show any significant effects on the urine outflow rate.
However, 600 nmol of NAL produced antidiuretic effects
in the nuclei.

NAL at 300 nmol did not influence the DYN-induced
antidiureses in the SON and PVN. NAL at 600 nmol tend-
ed to attenuate the effect in the SON, but not significantly
(Fig. 2a). In the PVN, the high dose of NAL significantly
inhibited the effect at 40–60 min after the microinjection,
but the antidiureses still remained (Fig. 2b). Also, the
total inhibition was valid by ANOVA.

Effects of des-Tyr-DYN on urine outflow rate
Effects of des-Tyr-DYN, a non-opioid peptide, on the
urine outflow rate were examined to determine if opioid
receptors intervene in the DYN-induced effects.

Des-Tyr-DYN at 20 nmol, microinjected into the SON,
showed similar antidiuretic effects to those of the same
dose of DYN (Fig. 3a). However, in the PVN, it
produced significantly weaker antidiureses as compared
with the DYN-induced ones (Fig. 3b).
Effects of adrenergic and cholinergic antagonists on the DYN-induced antidiuretic effects

Phenoxybenzamine (PHE, 80 nmol), timolol (TIM, 100 nmol) and atropine (ATR, 300 nmol) were used to investigate whether the DYN-induced effects involve adrenergic and cholinergic mechanisms (Fig. 4). These doses of the antagonists were enough to block anti-diureses induced by norepinephrine, isoproterenol or ACh, respectively (26–28).

PHE did not have any effects on the DYN (10 nmol)-induced effects in the PVN. PHE and ATR in the SON and TIM in both the nuclei partially inhibited the effects. In the PVN, ATR significantly inhibited the effects of DYN only at 30 min after the injection, although the total inhibition was not effective according to ANOVA. PHE or ATR alone did not change the urine outflow rate significantly. TIM increased the urine outflow rate to 177±31% of the control level (0.092±0.013 ml/min) in the SON (n=6) and 158±29% of the control level (0.087±0.010 ml/min) in the PVN (n=6).

Effects of U50,488H and DAKLI on urine outflow rate

Synthetic specific α-agonists, U50,488H and DAKLI, microinjected into the PVN decreased the urine outflow rate in a dose-dependent manner (Fig. 5). DAKLI produced the most potent effects among the three α-agonists. The DAKLI (5 nmol)-induced anti-diureses were not inhibited by pre-microinjection of NAL at 600 nmol into the PVN. The following percentages were for the urine outflow rate induced by DAKLI with the pretreatment vs. without the pretreatment: 102±7% vs. 97±6% at 10 min, 24±8% vs. 26±10% at 20 min, 22±6% vs.
13±2% at 30 min, 35±9% vs. 25±4% at 40 min, 73±6% vs. 69±11% at 50 min, and 96±5% vs. 92±19% of the control level (0.086±0.012 ml/min) at 60 min after the injection (n=5).

Fig. 4. Effects of ATR (A), PHE (B) and TIM (C) on the DYN-induced antidiuretic effects in the SON (a) and PVN (b). Ordinate and abscissa are shown in Fig. 1. Control outflow rate: A) a: 0.082±0.012, b: 0.083±0.008; B) a: 0.085±0.009, b: 0.101±0.012; C) a: 0.129±0.014, b: 0.116±0.020 ml/min; ○: 10 nmol DYN without the antagonists. ●: 10 nmol DYN with 300 nmol ATR (A), 80 nmol PHE (B) or 100 nmol TIM (C). Symbols are the mean±S.E. of 5–8 experiments. Significance of difference compared with the effects of the open circles at corresponding time-points: *P<0.05.

Fig. 5. Antidiuretic effects induced by microinjection of U50,488H (a) and DAKLI (b) into the PVN. a) U50,488H ●: 20 nmol (n=3), ▲: 40 nmol (n=6), ■: 70 nmol (n=4); b) DAKLI ●: 0.5 nmol (n=7), ▲: 1 nmol (n=6), ■: 2 nmol (n=6), ▼: 5 nmol (n=5). Ordinate: Urine outflow rate, expressed as a percentage of the control level (a: 0.065±0.006, b: 0.072±0.006 ml/min). Abscissa: Time in min after microinjection. Symbols are the mean±S.E. Significance of difference compared with the open circles in Fig. 1 at corresponding time-points: *P<0.05.
DISCUSSION

The SON and PVN include cell bodies of vasopressin-containing neurons, terminals of DYN-containing neurons (2, 3) and $\kappa$-opioid receptors (7, 29). In this study, we observed that microinjection of the three $\kappa$-agonists, DYN, U50,488H and DAKLI, into the PVN produced antidiuretic effects with the potency order of DAKLI > DYN > U50,488H. This order is consistent with the one for $\kappa$-receptor binding affinity (30). In the SON, the DYN-induced antidiuresis was partially inhibited by a high dose of NAL. In addition, the effects of des-Tyr-DYN, which does not bind to opioid receptors (31, 32), in the PVN were markedly weaker, compared with the effects of the same dose of DYN. These findings suggest that DYN elicits antidiuresis mediated through opioid receptors, perhaps the $\kappa$-subtype, in the PVN. On the other hand, the DAKLI-induced antidiureses were not inhibited by NAL. This is probably explained by the low selectivity and affinity for $\kappa$-receptors of NAL. DAKLI is one of the highest affinity ligands for $\kappa$-receptors (30).

Some studies demonstrated diuresis or a decrease in vasopressin release by $\kappa$-agonists injected into the ventricle (14–16). This is not consistent with our present results. The discrepancy is probably due to differences in: 1) action sites of drugs administered and 2) condition of animals used. 1) The drugs, injected into the ventricle, seem to diffuse in it and act in the periventricular tissues. Thus the stimulant would be transferred to several nuclei containing projections from the periventricular tissues. Therefore, the $\kappa$-agonists injected into the ventricle show the diuresis or decrease in vasopressin release as final actions. It is likely that the mechanisms underlying will be more than one. The diuresis are elicited not only by decreased vasopressin release, but also by an increased glomerular filtration rate of the kidney through the central nervous system (16). As compared with intracerebroventricular injection, the action sites of drugs microinjected into the nuclei appear to be limited, because methylene blue microinjected into the SON and PVN using the same method does not spread over more than a volume equivalent to a 1-mm cube, and norepinephrine or oxotremorine microinjected into sites at 1 mm from the nuclei does not show any effects on urine outflow rate (26–28). Taken together, the action sites of the drugs administered by the two methods are different from each other. Met-enkephalin is reported to show diuresis by intracerebroventricular injection and antidiuresis by microinjection into nuclei (6, 33). 2) In the studies for the diureses or decrease in vasopressin release by the $\kappa$-agonists, conscious and non-hydrated (14–16), water-deprived (14), hydrated (16) or angiotensin II-stimulated (15) rats are used. Basal vasopressin level in the plasma under these conditions will be higher than that under the ethanol-anesthetized, ethanol-infused and hydrated condition in the present study, although under the present condition, it is possible to produce diureses by some drugs (this study, ref. 33). This may be one of reasons for the discrepancy. It is reported that intracerebroventricular injection of morphine shows antidiuretic effects, increase in, decrease in or no effects on vasopressin release under various conditions (4).

On the other hand, microinjection of DYN into the SON produced antidiureses, like those in the PVN. However, the DYN-induced antidiureses were not diminished by the high dose of NAL, and des-Tyr-DYN produced diuretic effects equivalent to the DYN-induced effects in the SON. From these results, the DYN-induced effects in the SON were not mediated through opioid receptors. DYN is reported to have another effect which is not connected with opioid receptors, although the mechanisms have not been clarified (31, 32, 34). Also, this mechanism may be partially involved in the effects of the SON, because des-Tyr-DYN produced weak effects in the PVN.

In the SON, the DYN-induced antidiuretic effect was slightly reduced by pretreatment with PHE, TIM and ATR and in the PVN, by pretreatment with TIM and ATR. These findings suggest the possibility that DYN promotes release of epinephrine/norepinephrine and Ach. However, the intervention of adrenergic and cholinergic neurons in the DYN-induced effects does not seem to be very important, because all the inhibitions were not remarkable. Urine outflow rate is regulated by many mechanisms. Therefore, these inhibitions may reflect the complicated regulation of various neurons. There are a few papers suggesting that DYN and morphine increase transmitter release in the central nervous system in vivo studies (35, 36). Many terminals of adrenergic and cholinergic neurons exist in these nuclei (37, 38). In addition, $\alpha$- and $\beta$-adrenergic, and cholinergic agonists increase the firing rate of vasopressin-containing neurons or plasma vasopressin level and cause antidiuretic effects in the central nervous system (26–28, 39, 40).

In both the nuclei, DYN increased vasopressin release. Furthermore, the increase in urinary osmotic pressure, when DYN showed the maximum antidiureses, was almost the same as that of vasopressin injected intravenously. These results suggest that DYN causes antidiureses by increased vasopressin release.

In summary, in the SON and PVN, DYN increased vasopressin release and resulted in the antidiuretic effects involving adrenergic and cholinergic mechanisms. In addition, only the DYN-induced effects in the PVN were mediated, at least partially, through opioid receptors, perhaps the $\kappa$-subtype.
REFERENCES


