Implication of Leumorphin in Inhibitory Control of Vasopressin Secretion in Conscious Rats

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SUMMARY The effects of leumorphin, a k-agonist derived from proenkephalin B (neoendorphin and dynorphin precursor), on vasopressin secretion were studied under basal and stimulated conditions in conscious, unrestrained rats. Intracerebroventricular injection of leumorphin (60 or 600 pmol) significantly inhibited basal vasopressin secretion. The vasopressin response induced by intracerebroventricular injection of angiotensin II (100 pmol) was significantly suppressed, in a dose-dependent fashion, by the simultaneous intracerebroventricular injection of leumorphin (6, 60, or 600 pmol). Intravenous pretreatment with naloxone (0.5 mg/kg body weight) diminished the inhibitory action of leumorphin (60 pmol) on vasopressin secretion. Moreover, naloxone (0.5 mg/kg body weight) prolonged the vasopressin secretion induced by intracerebroventricular injection of angiotensin II (100 pmol). These results indicate that leumorphin possesses a potent inhibitory effect on vasopressin secretion and that, alone or in combination with other endogenous opioid peptides, it plays an important role in the control of vasopressin secretion.

(KEY WORDS • opioid peptides • vasopressin • angiotensin II • naloxone)

**LEUMORPHIN** is a proenkephalin B (neoendorphin and dynorphin precursor)—derived, 29 amino acid polypeptide containing a leucine-enkephalin (leu-enkephalin) sequence at its N-terminus. Using a specific radioimmunoassay (RIA) for leumorphin coupled with high performance liquid chromatography, we demonstrated the presence of leumorphin in the pituitary and brain, and parallel distribution of leumorphin and dynorphin in discrete regions of the brain. We also reported that leumorphin acts as an agonist at the k-type opioid receptor, and that intracerebroventricular (i.c.v.) injection of leumorphin inhibits water drinking induced by water deprivation, angiotensin II (Ang II), or carbachol in rats, which suggests its involvement in the central control of water and electrolyte balance. In addition, dynorphin derived from the same precursor as that of leumorphin coexists with arginine vasopressin (AVP) in the hypothalamic cells, and messenger RNA (mRNA) for both proenkephalin B and provasopressin increases parallel with salt loading in magnocellular neurons in supraoptic and paraventricular nuclei. These observations raise the possible implication of leumorphin in the control of AVP secretion.

In this study, we examined the effects of i.c.v. injection of leumorphin on basal and Ang II–induced AVP secretion in conscious, unrestrained rats.

**Materials and Methods**

**Peptides and Chemicals**

Synthetic Ang II was purchased from the Protein Research Foundation, Osaka, Japan. Human leumorphin was synthesized by a conventional, solid phase method. Naloxone hydrochloride was obtained from Sigma Chemical (St. Louis, MO, USA). These substances were dissolved in physiological saline solution and used for i.c.v. injection at a volume of 5 μl and intravenous (i.v.) injection at a volume of 50 μl.

**Animals**

Male Sprague-Dawley rats (Shizuoka, Japan) weighing 240 to 280 g were housed at constant room temperature (25 ± 1°C) with a 12-hour light-dark cycle (light on at 0700). Standard rat biscuits and water were available ad libitum until the time of the experiments.
The rats were anesthetized with sodium pentobarbital (Nembutal, Abbott Laboratories, Chicago, IL, USA) 50 mg/kg body weight intraperitoneally (i.p.), for implantation of a stainless steel cannula stereotaxically into the left lateral ventricle, as reported elsewhere. They were housed individually, and all experiments were carried out under conscious, unrestrained conditions after a 3-day recovery period. At the end of experiments, 5 μl of 1% Evans blue solution was injected through the cannula to confirm that the placement of the cannula was appropriate.

**Leumorphin Injection and Basal AVP Level**

To elucidate the effect of leumorphin on basal plasma AVP level, each rat received i.c.v. injection of leumorphin (6, 60, or 600 pmol) or the vehicle (saline). Five minutes later, blood samples (1 ml) were drawn through the jugular catheter.

**Leumorphin Injection and Ang II–Induced AVP Secretion**

To study the effect of leumorphin on Ang II–induced AVP secretion, rats received simultaneous i.c.v. injection of Ang II (100 pmol) and leumorphin (0, 6, 60, or 600 pmol). Blood samples (0.25 ml) were taken from the jugular catheter at 0, 1.5, 5, and 10 minutes.

**Naloxone Injection and Inhibitory Action of Leumorphin**

Rats were given an i.v. injection of naloxone (0.5 mg/kg body weight) or the vehicle (saline) 5 minutes prior to simultaneous i.c.v. injection of Ang II (100 pmol) and leumorphin (0 or 60 pmol). The protocol of the blood sampling was the same as that in the Ang II experiment.

**Radioimmunoassay**

Blood samples were collected in chilled polypropylene tubes containing NaEDTA (1 mg/ml). Plasma was separated immediately and stored at −20°C until measurement within a few days.

The RIA for AVP was performed as previously described. The \(^{125}\)I-VP was obtained from Amersham, Buckinghamshire, England, and AVP was purchased from the Protein Research Foundation, Osaka, Japan. The sensitivity of the assay was 0.05 pg/tube.

**Statistical Analysis**

The values were compared using Duncan’s multiple-range test after one-way analysis of variance.

**Results**

**Effect of Leumorphin on Basal AVP Level**

In euhydrated, freely moving rats, the plasma AVP concentration was 0.7 ± 0.1 pg/ml. Administration of leumorphin (60 or 600 pmol i.c.v.) significantly inhibited basal AVP secretion (Figure 1). However, i.c.v. injection of a smaller dose (6 pmol) had no apparent effect on plasma AVP level as compared with controls.

**Effect of Leumorphin on Ang II–Induced AVP Secretion**

The AVP level was 16.6 ± 1.2 pg/ml 1.5 minutes after i.c.v. injection of Ang II. This AVP secretion was significantly inhibited by the simultaneous i.c.v. injection of leumorphin (6, 60, or 600 pmol) in a dose-dependent manner (Figure 2). The i.c.v. injection of leumorphin also decreased plasma AVP levels 5 and 10 minutes after the injection of Ang II. However, i.v.
Effects of naloxone (NAL) on inhibitory action of leumorphin (LM). Ang II (All) and leumorphin were injected i.c.v. simultaneously in doses of 100 and 60 pmol, respectively. Naloxone in a dose of 0.5 mg/kg body weight or saline was injected i.v. 5 minutes prior to i.c.v. injection. Values are expressed as means ± SEM of five rats in each group. Symbols indicate significant difference from corresponding control values: *p < 0.05, **p < 0.01 compared with Ang II; †p < 0.05, ††p < 0.01 compared with Ang II plus LM.

Effect of Naloxone on Inhibitory Action of Leumorphin on AVP Secretion

As shown in Figure 3, pretreatment with naloxone (0.5 mg/kg) diminished the inhibitory effect of leumorphin on Ang II-induced AVP secretion. Moreover, plasma AVP levels 5 and 10 minutes after i.c.v. injection of Ang II in naloxone-treated rats were significantly higher than those in controls. Thus, naloxone significantly prolonged Ang II-induced AVP secretion (see Figure 3). The i.v. injection of naloxone at a dose of 0.05 mg/kg neither reversed the inhibitory action of leumorphin on AVP secretion nor potentiated Ang II-induced AVP secretion (data not shown).

Discussion

The present study demonstrates that leumorphin possesses a potent inhibitory effect on AVP secretion in conscious, unrestrained rats. The i.c.v. injection of leumorphin inhibited not only basal AVP secretion but also Ang II–induced AVP secretion. Our previous study demonstrated that i.c.v. injection of leumorphin (60 or 600 pmol) reduces both basal blood pressure and Ang II–induced pressor response in conscious rats. The doses used in the present study were the same as those in our study on blood pressure. Thus, the inhibitory action of leumorphin on AVP secretion is not due to a reflex mechanism of an increase in blood pressure. Pretreatment with naloxone abolished the inhibitory effect of leumorphin on AVP secretion, indicating that suppressive action of leumorphin is mediated by opioid receptors. However, i.v. administration of leumorphin had no effect on AVP secretion. It is likely, therefore, that leumorphin inhibits AVP secretion in the brain.

The inhibitory effect on leumorphin on AVP secretion shown in this study was similar to that of other endogenous opioid peptides such as β-endorphin, leu-enkephalin, and dynorphin. However, the inhibitory effect of leumorphin was much greater than that of β-endorphin or leu-enkephalin, and was comparable to that of dynorphin. In addition, the inhibitory action of leumorphin was observed within 5 minutes after i.c.v. injection, whereas β-endorphin was reported to inhibit AVP secretion 30 minutes later. Thus, the inhibitory effect of leumorphin on AVP secretion is rapid as well as potent. Furthermore, leumorphin, dynorphin, and neoendorphin sharing the same precursor (proenkephalin B) coexist with AVP in magnocellular neurons in the supraoptic and paraventricular nuclei, and relatively dense κ-opioid receptors are present in the rat hypothalamus and pituitary. These results suggest the physiological role of leumorphin either alone or in combination with other κ-agonists derived from proenkephalin B.

In the present study, naloxone not only reversed the inhibitory action of leumorphin on AVP secretion but also prolonged the AVP secretion induced by i.c.v. injection of Ang II. Our observation is consistent with previous reports that naloxone promotes the AVP secretion induced by i.v. injection of isoprenaline or Ang II, or i.p. administration of polyethylene glycol. This finding, together with the potent inhibitory effect of leumorphin on AVP secretion, suggests that endogenous opioid peptides, including leumorphin, play inhibitory roles in AVP secretion.

The potent inhibitory effect of leumorphin on AVP secretion, together with its antidipsogenic and depressor action, suggests that leumorphin, alone or in combination with other endogenous opioid peptides, is involved in the central control of fluid homeostasis and blood pressure.

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