Effect of Intracerebroventricular Administration of Opioid Peptides on Basal Serum Adrenaline Levels in Conscious Rats

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Abstract—It is considered that adrenomedullary secretion of adrenaline is centrally regulated by various endogenous substances. Recently, opioid peptides have been reported as one of this group of endogenous substances. However, the receptor through which these endogenous opioid peptides regulate adrenomedullary adrenaline secretion has not been elucidated. In the present study, various opioid peptides were administered intracerebroventricularly in conscious, unrestrained rats, and blood catecholamine levels were measured at various periods to determine which cerebral opioid peptide receptor is involved in the mechanism of basal adrenomedullary adrenaline secretion. The results indicate that δ-receptor stimulation has a positive effect on basal adrenomedullary adrenaline secretion, while μ- and κ-receptors are not involved in this mechanism.

Various endogenous cerebral opioid peptides such as β-endorphin (β-ED), methionine-enkephalin (M-EK), leucine-enkephalin (L-EK) and dynorphin (DYN) have been identified, and it has been well-established that these opioid peptides are involved in various physiological functions. Experiments using their ligands have revealed the presence of several types of central opioid peptide receptors (1), although their relation with physiological functions has not been fully understood.

Administration of morphine to rats causes a remarkable increase in adrenaline secretion from the adrenal medulla, and this effect is mediated by the CNS (2). A study of blood pressure and heart rate changes in rats revealed that endogenous central opioid peptides are closely involved in central regulation of adrenomedullary adrenaline secretion (3–7). Intracerebroventricular administration of β-ED is followed by a significant increase in the plasma levels of dopamine, noradrenaline (NA) and adrenaline (AD), which suggests that endogenous opioid peptides centrally mediate the increase in catecholamine secretion from the peripheral sympathetic nerve terminals and adrenal medulla (8). However, many points remain unelucidated; especially, it is not known which of the receptors in the brain, μ-, δ- or κ-subtypes, mediates the action of endogenous opioid peptides.

The aim of the present study was to identify the subtypes of opioid receptors which regulate basal adrenal secretion of AD using conscious, unrestrained rats.

Materials and Methods

Male Donryu rats weighing 300 to 350 g were used as experimental animals. Each rat was fitted with an L-type fine stainless steel tube, inserted into the ventricle using a brain stereotaxis under pentobarbital anesthesia (30 mg/kg). A silicone tube with a 0.3 mm inner diameter was connected to this L-type tube, and the other end of the silicone tube was passed through a spring outside the cage for protection. After 24 hr, a venous catheter for blood collection (0.8 mm in inner diameter) was inserted into the right cervical vein, and the other end of the catheter was
placed outside the cage through the spring, in a fashion similar to the ventricular tube (9). After 48 hr, 25 µl of physiological saline was administered intracerebroventricularly to the conscious, unrestrained rats. At 10, 20 and 60 min after saline administration, serum AD levels were determined and the measurements obtained were used as the basal levels. Opioid peptides were administered intracerebroventricularly in a similar manner after dilution with physiological saline, and 2 ml blood samples were collected for 30-60 sec at 10, 20 and 60 min after administration of opioid peptides in conscious, unrestrained rats.

For the determination of serum AD levels, the blood sample was separated into serum from which the protein was then removed. Subsequently, it was adsorbed on alumina, according to the method described by Anton and Sayre (10), eluted, separated into adrenaline and noradrenaline by high performance liquid chromatography (LC-1, Shimadzu) and assayed fluorometrically (11).

Chemicals used including β-endorphin (β-ED), methionine-enkephalin (M-EK), (D-Ala²) methionine-enkephalin (DAME), (D-Ala², D-Leu⁵) enkephalinamide (DADL), dynorphin (DYN) and morphiceptin (MCP) were purchased from Sigma, and naloxone was supplied through the courtesy of Sankyo Company, Ltd.

Results
Changes in AD levels after intracerebroventricular administration of physiological saline (25 µl) in conscious, unrestrained rats are shown in Fig. 1. The serum AD level was 0.35±0.03 ng/ml (means±S.E.M., n=15) before i.c.v. administration of physiological saline. It increased to 0.51±0.14 ng/ml, 0.8±0.11 ng/ml and 1.09±0.23 ng/ml (n=15) at 10, 20 and 60 min after administration of physiological saline, respectively, although there was no significant difference from the values obtained for those before physiological saline administration (control group, n=15).

The serum AD level following i.c.v. administration of 12.5 µg of β-ED was 1.12±0.03 ng/ml (n=5) at 10 min, showing a slight increase compared to the control group, although the difference was not statistically significant. However, the serum AD level increased to 1.72±0.13 ng/ml (n=5) at 20 min and to 2.1±0.33 ng/ml (n=5) at 60 min. These increases are significant compared to the values recorded for the control group (Fig. 1). When the dose of β-ED was increased to 25 µg, the serum AD levels were elevated to 1.44±0.15 ng/ml (n=5) at 10 min, 1.70±0.3 ng/ml (n=5) at 20 min and 3.02±0.6 ng/ml (n=5) at 60 min, significantly higher levels than the control group (Fig. 1). The serum AD levels at 60 min after i.c.v. administration of β-ED exhibited a linear increase in a dose-dependent manner (Fig. 2). Following i.c.v. administration of M-EK at 25 µg, serum AD levels were 1.5±0.4 ng/ml, 1.2±0.06 ng/ml and 0.97±0.07 ng/ml (n=5) at 10, 20 and

![Fig. 1](image-url)  
*Fig. 1. Time course of serum adrenaline levels after intracerebroventricular administration of saline (n=15) and β-endorphin (12.5 µg, n=5 and 25 µg, n=5) in conscious unrestrained rats. Points are means±S.E.M. *Significantly different from the control (P<0.05).*
60 min, respectively, with a significant increase compared to the control value at 20 min (Fig. 3). The serum AD levels following administration of 25 μg of DAME, a stable analog of M-EK, were 1.4±0.3 ng/ml, 1.0±0.8 ng/ml and 2.3±0.9 ng/ml (n=6) at 10, 20 and 60 min, respectively, showing an increase, although the differences from the control group were not statistically significant due to the great variations of measurements. Since these opioid peptides interact with all of μ-, δ- and κ-receptors, subsequent experiments were performed using individual agonists specific for each receptor. When DADL, a δ-receptor agonist, was administered at 12.5 μg by the i.c.v. route, a remarkable increase in serum AD levels was observed with values reaching to 2.5±0.79 ng/ml, 7.3±1.63 ng/ml and 9.02±2.87 ng/ml (n=5) at 10, 20 and 60 min, respectively (Fig. 3). However, following i.c.v. administration of 12.5 μg of MCP, an agonist specific for μ-receptor, little or no increase in serum AD level was seen (Fig. 4). Moreover, the serum AD levels after i.c.v. administration of 12.5 μg of DYN, a specific κ-receptor agonist, showed little or no difference from control values at any of the assessment periods (Fig. 4).

The effect of naloxone, an opioid peptide receptor antagonist, on increases in serum adrenaline level was studied next. Administration of β-ED (12.5 μg), a δ-, μ- and κ-agonist, and DADL (12.5 μg), a δ-agonist was performed. When β-ED (12.5 μg) was
administered immediately after pretreatment with i.c.v. administration of naloxone (125 μg), serum AD levels of 0.53±0.1 ng/ml, 0.89±0.1 ng/ml and 1.73±0.11 ng/ml (n=5) were recorded at 10, 20 and 60 min, respectively. These levels were lower than those obtained after administration of 8-ED (12.5 μg) alone. Especially, a significant inhibition was seen at 20 min (Fig. 5). Similarly, the increase in serum AD level induced by DADL, a 8-agonist, was remarkably inhibited by pretreatment with naloxone (125 μg), with AD levels of 0.5±0.11 ng/ml, 0.47±0.08 ng/ml and 0.98±0.33 ng/ml (n=4) at 10, 20 and 60 min, respectively (Fig. 6).

Discussion

In order to observe changes in basal serum levels in rats without allowing the testing method to increase stress, we devised a method to administer drugs intracerebroventricularly and to collect blood samples from outside the cage with animals kept in a conscious, unrestrained condition. After physiological saline was administered to rats in this fashion, a slight increase was seen in serum AD levels at 10, 20 and 60 min, although the increases observed were not statistically significant as compared to the preadministration levels. This slight increase
may be explained by an elevation in intracranial pressure caused by administration of 12.5 μl of physiological saline, resulting in a slight stress (9). We considered that this small increase does not remarkably influence our experiments and subsequent experiments were performed on the basis of this concept.

The serum AD level was elevated in a dose-dependent manner after i.c.v. administration of β-ED, a δ-, μ- and κ-receptor agonist, and this elevation was inhibited by naloxone, a δ-, μ- and κ-receptor antagonist. These findings indicate that central endogenous opioid peptides may contribute to the control of adrenal AD secretion. Following i.c.v. administration of M-EK, an endogenous opioid peptide, serum AD level showed an increase at 10 and 20 min after administration, but was restored to the basal level at 60 min. This short duration of action of M-EK may be explained by its rapid decomposition by enkephalinase which is present in the brain (12, 13). When DAME, a stable M-EK, was administered by the i.c.v. route, serum AD levels were increased at all of the assessment periods. Since M-EK is a δ- and κ-agonist, 12.5 μg of DYN, a κ-receptor agonist, was administered to determine if κ-receptor was involved. Little or no changes from the basal level were seen, indicating that the increase in serum AD level is not mediated by a central κ-receptor. To confirm the subtype of opioid receptor which mediates the increase in serum AD level, 12.5 μg of DADL, which has a high specificity for the δ-receptor, was administered by the i.c.v. route; this caused a remarkable increase in the serum AD level. Moreover, when DADL was administered in doses as small as 1.25 μg, a significant increase was seen in the serum AD level. This increase in serum AD level induced by i.c.v. administration of 12.5 μg of DADL was inhibited by pretreatment with 125 μg of naloxone. Intracerebroventricular administration of 12.5 μg of MCP, a μ-receptor agonist, did not induce an elevation in serum AD levels. This result indicates that the central μ-receptor is not involved in adrenal AD secretory control. When peripheral noradrenaline levels were investigated, a tendency similar to that observed for peripheral AD levels was seen (M. Nakamura et al., unpublished data). Only one paper by Van Loon et al. (7) concerns the increases in serum AD levels induced by central opioid peptides in conscious unrestrained rats. However, they did not address the question of receptor-specific central opioid peptide effects (14, 15). Yukimura et al. (16) and Feuerstein and Faden (17) described various receptor-specific subtypes in studies using blood pressure and heart rate as indices. Feuerstein and Faden (17) reported that μ- and δ-agonists administered directly to the
hypothalamus increased the blood pressure and heart rate significantly, while a \( \kappa \)-agonist exhibited an inhibitory effect. In addition, Pfeiffer et al. (18, 19) also described the administration of a \( \mu \)-agonist and a \( \delta \)-agonist into the anterior portion of the hypothalamus and the resultant elevation in blood pressure and heart rate as well as \( \kappa \)-agonist administration which resulted in no change in blood pressure or heart rate. Petty et al. (20) administered \( \beta \)-ED into the nucleus tractus solitarius in rats under urethane anesthesia and observed significant increases in blood pressure and heart rate. Considering changes in blood pressure and heart rate after administration of \( \delta \)-, \( \mu \)- and \( \kappa \)-agonists into the nucleus tractus solitarius in cats under chloralose-urethane anesthesia, Hassen et al. (21) proposed that DADL, a \( \delta \)-agonist, when administered into the nucleus tractus solitarius, is the most active one of the central opioid peptides for this effect. These results, coupled with the findings obtained in our study indicate that central opioid peptides acting on central \( \delta \)-opioid receptors elevate peripheral adrenal AD levels with influences on blood pressure and heart rate.

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References
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