Differential Modulation by $\mu$- and $\delta$-Opioids on Baroreceptor Reflex in Conscious Rabbits

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We examined the role of central $\mu$- and $\delta$-opioids on both neurohormonal responses and baroreceptor reflex in conscious rabbits. Both intracerebroventricular [D-Ala$^2$,N-Me-Phe$^4$,Gly$^5$-ol]-enkephalin, a $\mu$-selective agonist, and [D-Ala$^2$,D-Leu$^5$]-enkephalin, a $\delta$-selective agonist, caused dose-related increases in arterial pressure and renal sympathetic nerve activity, whereas intravenous injection of the same maximum dose of these peptides as that used in the intracerebroventricular experiment did not cause any cardiovascular and neuronal responses. On the other hand, increases in plasma epinephrine, norepinephrine, and glucose levels induced by intracerebroventricular [D-Ala$^2$,N-Me-Phe$^4$,Gly$^5$-ol]-enkephalin were significantly greater than those by [D-Ala$^2$,D-Leu$^5$]-enkephalin. Both enkephalins did not cause any responses in plasma renin activity, plasma vasopressin, and serum sodium and potassium concentrations. The sensitivity of the baroreceptor reflex control of renal sympathetic nerve activity using a logistic model was enhanced by a subpressor dose of intracerebroventricular [D-Ala$^2$,N-Me-Phe$^4$,Gly$^5$-ol]-enkephalin (10 pmol/kg) but not by [D-Ala$^2$,D-Leu$^5$]-enkephalin. Conversely, a $\mu$-selective dose of intravenous naloxone (0.1 mg/kg) attenuated baroreceptor reflex sensitivity. Intravenous naloxone methobromide, which has been shown not to cross the blood-brain barrier, did not change baroreceptor reflex sensitivity, suggesting that naloxone acts at the central nervous system. In conclusion, in conscious rabbits, 1) intracerebroventricular $\mu$- and $\delta$-receptor agonists caused pressor responses and 2) $\mu$-opioid agonist altered baroreceptor reflex control of renal sympathetic nerve activity and produced changes in sympathoadrenal responses. (Hypertension 1992;19:648-652)

KEY WORDS • opioid peptides • naloxone • baroreceptors • central nervous system • sympathetic nervous system • catecholamines

The presence of opioids and opiate receptors in specific brain nuclei known to regulate cardiovascular activity and the potent effects of opioid peptides on blood pressure and heart rate (HR) suggest that opioid peptides may be important endogenous substances in central cardiovascular control. Opioid peptides are suggested to modulate sympathetic nervous system and renin-angiotensin responses through a central action, although these effects are divergent, depending on species, the use of anesthesia, the route of administration, and subtypes of opioid receptors. The opioid peptide system is composed of a family of endogenous peptides (the endorphins) that act at multiple opioid receptor subtypes (i.e., $\mu$, $\delta$, and $\kappa$). To determine the role of the opioid system in central cardiovascular and neuronal regulations, we examined the central effects of $\mu$- and $\delta$-opioids on neurohormonal responses and baroreceptor reflex control of renal sympathetic nerve activity (RSNA) in conscious rabbits.

Methods

Preparation of Animals

Experiments were conducted on 29 male Japanese White rabbits weighing 2.5–3.0 kg. Rabbits were anesthetized with sodium pentobarbital (30 mg/kg i.v.). Three days before experimentation, electrodes were implanted on the left renal sympathetic nerve, and a stainless steel cannula was placed in the right lateral cerebral ventricle. RSNA was recorded as described previously. Briefly, the left kidney was exposed retroperitoneally, and a branch of renal nerve was separated from the renal plexus and the surrounding connective tissues. RSNA was recorded by a pair of electrodes made from Teflon-insulated seven-stranded steel wire. The area of the nerve and wire interface was embedded in silicone cement. A 23-gauge stainless steel cannula was implanted into the right lateral cerebral ventricle. The position of the cannula in the lateral ventricle was confirmed by the staining of all four ventricles after injection of 0.1 ml dye at the end of the experiments. After surgery, disodium sulbenicillin (200 mg i.v.) was given to the rabbits to prevent any postoperative infection.

At least 3 days after the surgical procedures, the following experiments were carried out on a conscious rabbit placed in a plastic box. On each experimental day, polyethylene catheters (PE-50) were inserted into the central ear artery and marginal ear vein with rabbits under 1% lidocaine local anesthesia. The arterial catheter was connected to a pressure transducer (P50, Gould Statham Instruments Inc., Hato Rey, Puerto Rico) for measurement of arterial pressure. HR was
monitored by a cardiotachometer (model 1332, NEC San-ei, Tokyo).

**Experiment 1**

Three and 6 days after the surgical procedures, the following opioids were injected intracerebroventricularly (n=5) to determine the central effects of μ- and δ-opioids on cardiovascular (arterial pressure and HR) and neurohormonal (RSNA and circulating hormones) responses: 10, 50, 100, or 1,000 pmol/kg of [d-Ala₂,N-Me-Phe₁,Gly₅-oic]-enkephalin (DAGO, μ-agonist¹¹,¹²) (Sigma Chemical Co., St. Louis, Mo.) and [d-Ala₂,δ-Leu⁶]-enkephalin (DADLE, δ-agonist¹¹,¹²) (Sigma). DAGO was injected on one day and DADLE injected on another day in randomized order in the same rabbit. These doses of DAGO and DADLE were dissolved in 80 μl of 0.9% saline. The administration of each dose of DAGO or DADLE was separated by a period of 30 minutes. Both at the control period and at 30 minutes after 1,000 pmol/kg DAGO or DADLE injection, blood samples (2.4 ml) were drawn from the arterial catheter for measurement of plasma catecholamines (epinephrine and norepinephrine), plasma renin activity, plasma vasopressin, plasma glucose, and serum sodium and potassium and were replaced by the same volume of 0.9% saline.

**Experiment 2**

Three and 6 days after the surgical procedures, the maximum dose of DAGO or DADLE (1,000 pmol/kg) as that used in the intracerebroventricular experiment was injected intravenously to examine cardiovascular and neuronal responses (n=6). DAGO was injected on one day and DADLE injected on another day in randomized order in the same rabbit. Fifteen minutes after intracerebroventricular vehicle or agonist (DAGO or DADLE, 10 pmol/kg in 80 μl isotonic saline) was considered as the sensitivity of the baroreceptor reflex of intravenous naloxone methobromide (Boehringer Ingelheim KG, Ingelheim, FRG) (0.116 mg/kg in 0.2 ml/kg isotonic saline; the same molar dose of naloxone as that used in Experiment 4), which does not cross the blood–brain barrier,¹³ was determined (n=6). Fifteen minutes after intravenous vehicle or naloxone methobromide, the sensitivity of the baroreceptor reflex control of RSNA was determined the same as in Experiment 3.

**Baroreceptor Function Curve**

For data analysis, RSNA was plotted at 5 mm Hg intervals of MAP. Data for MAP–RSNA relations during increases and decreases in MAP were collected and fitted to a sigmoid logistic function curve. The equation used for the data analysis was based on the following mathematical model¹⁶:

\[
\text{RSNA} = \frac{P_1}{1 + \exp\left[\frac{P_2(MAP-P_3)}{P_4}\right]} + P_5
\]

where \(P_1\) is the range between the upper and lower plateau, \(P_2\) is a range-independent measure of slope or normalized gain, \(P_3\) is median blood pressure, and \(P_4\) is the lower plateau. Data were fitted to the logistic function with the nonlinear regression program in the Statistical Analysis System (SAS Institute Inc., Cary, N.C.). The maximum slope (\(S_{\text{max}}\), [-P₃/P₂]) calculated from the parameters of the logistic function curve was considered as the sensitivity of the baroreceptor reflex.¹⁶

**Recording Procedures of Renal Sympathetic Nerve Activity**

RSNA was amplified (model DPA-100E, Dia Medical System Co., Tokyo) and filtered (100–3,000 Hz), and the waveforms were integrated after a full wave rectification using an integrator amplifier (model 1322, NEC San-ei) with the sample-hold function reset to baseline by an internal timer set at 5 seconds. Absolute values for integrated RSNA were corrected before data analysis by subtracting the residual electrical output (noise level) recorded from the integrator induced by intravenous phenylephrine (16 μg/kg).

**Blood Collection and Analysis**

Blood samples for measurement of plasma catecholamines, plasma renin activity, and plasma vasopressin were centrifuged at 4°C. Plasma for catecholamines was stored at −80°C, and other samples were stored at −20°C until assay. Plasma catecholamine concentration was measured by radioenzymatic assay,¹³ and plasma renin activity and plasma vasopressin levels were measured by radioimmunoassay.¹³ Plasma glucose level was measured by a Glucose Analyzer 2 (Beckman Instruments, Inc., Fullerton, Calif.). Serum sodium and potassium concentrations were measured by flame photometry (model 265D, Hitachi, Tokyo).

**Statistics**

All values are expressed as mean±SEM. A paired t test was performed to determine the effects of intracerebroventricular DAGO and DADLE on hormonal responses and the effects of DAGO, DADLE, nalox-
one, and naloxone methobromide on baroreceptor reflex control of RSNA. A one-way analysis of variance for repeated measures was performed to determine the effects of intracerebroventricular DAGO and DADLE on cardiovascular and neuronal responses, followed by Duncan's multiple range test. A value of \( p < 0.05 \) was considered significant.

**Results**

**Experiment 1**

Baseline values for MAP and HR before DAGO and DADLE intracerebroventricular injections were 81.8±3.4 mm Hg and 206.0±13.6 beats per minute (DAGO) and 82.4±2.0 mm Hg and 208.0±16.6 beats per minute (DADLE). Intracerebroventricular DAGO and DADLE elicited dose-related increases in MAP and RSNA and a decrease in HR (Figure 1). However, DADLE but not DAGO showed a significant increase in MAP at doses of 50 and 100 pmol/kg. Both DAGO and DADLE showed similar increases in RSNA. Conversely, only intracerebroventricular DAGO (1,000 pmol/kg) induced significant increases in plasma epinephrine, norepinephrine, and glucose levels. Neither DAGO nor DADLE showed significant changes in plasma renin activity, plasma vasopressin, or serum sodium and potassium concentrations (Table 1).

**Experiment 2**

The maximum dose of intracerebroventricular DAGO and DADLE (1,000 pmol/kg) as that used in Experiment 1 was injected intravenously. MAP, HR, and RSNA did not show any responses (data not shown).

**Experiment 3**

Intracerebroventricular DAGO (10 pmol/kg) enhanced the sensitivity of the baroreceptor reflex control of RSNA (\( S^\text{max} = -7.1±1.6 \) versus \(-15.7±4.6; p<0.05\)), whereas intracerebroventricular DADLE did not show any changes in control of RSNA (Figures 2A and 2B).

**Experiment 4**

Intravenous \( \mu \)-selective dose of naloxone attenuated the sensitivity of the baroreceptor reflex control of RSNA (\( S^\text{max} = -9.8±1.0 \) versus \(-6.4±0.6; p<0.05\)) (Figure 2C).

**Experiment 5**

Intravenous naloxone methobromide did not show any changes in the baroreceptor reflex control of RSNA (Figure 2D).

**Discussion**

The present study provides evidence for brain \( \mu \)-opioid receptors in the modulation of sympathoadrenal function and baroreceptor reflex control of RSNA in

| Table 1: Effects of Intracerebroventricular [\( \alpha \)-Ala\(^2\),N-Me-Phe\(^4\),Gly\(^5\)-ol]-Enkephalin and [\( \alpha \)-Ala\(^2\),D-Leu\(^5\)]-Enkephalin on Blood Variables |
|-----------------------------|-----------------------------|-----------------------------|
| Variable                  | Control (1,000 pmol/kg)     | Control (1,000 pmol/kg)     |
| Epinephrine (pg/ml)        | 125.2±23.2                  | 3,113.6±582.9*              |
| Norepinephrine (pg/ml)     | 377.4±59.2                  | 929.0±37.7*                 |
| PRA (ng/ml/hr)             | 1.8±1.0                     | 2.4±1.5                     |
| AVP (pg/ml)                | 3.3±2.1                     | 2.9±2.5                     |
| Sodium (meq/l)             | 142.8±1.8                   | 143.8±4.4                   |
| Potassium (meq/l)          | 3.9±0.3                     | 3.8±0.4                     |
| Glucose (mg/dl)            | 105.0±8.3                   | 232.8±24.8*                 |
| Hematocrit (%)             | 31.8±1.2                    | 30.9±1.0                    |

Values are mean±SEM. DAGO, [\( \alpha \)-Ala\(^2\),N-Me-Phe\(^4\),Gly\(^5\)-ol]-enkephalin; DADLE, [\( \alpha \)-Ala\(^2\),D-Leu\(^5\)]-enkephalin; PRA, plasma renin activity; AVP, arginine vasopressin.

\* \( p<0.01 \) compared with control period by paired \( t \) test.
conscious rabbits. Both the intracerebroventricular \( \mu \)-selective opioid receptor agonist DAGO and the \( \delta \)-selective opioid receptor agonist DADLE produced dose-related increases in arterial pressure and RSNA, whereas only intracerebroventricular DAGO caused increases in plasma epinephrine, norepinephrine, and glucose levels, suggesting that intracerebroventricular \( \mu \)- and \( \delta \)-opioids have a differential effect on adrenal gland. Although it has been reported that \( \mu \)- and \( \delta \)-opioid agonists have a differential modulation on plasma catecholamine responses, the present study showed that increases in RSNA induced by intracerebroventricular DAGO and DADLE were almost at the same level. In addition, because the intravenous injection of DAGO and DADLE at 1,000 pmol/kg did not cause any cardiovascular or neuronal responses, it is unlikely that these effects were caused by a leakage of intracerebroventricular DAGO or DADLE into the systemic circulation.

The effects of opioids on cardiovascular responses and baroreceptor reflex are divergent, depending on the use of anesthesia and the differences between opioid receptor subtypes. Gordon reported that intracisternal DAGO, but not DADLE, impaired baroreceptor reflex function of sympathetic and cardiovascular function in anesthetized rats. Conversely, Petty and Reid reported that intracisternal DADLE caused an increase in baroreceptor reflex sensitivity in anesthetized rabbits. In the present study in conscious rabbits, baroreceptor reflex sensitivity assessed by RSNA using a logistic model was enhanced by a subpressor dose of intracerebroventricular DAGO (10 pmol/kg) but was not influenced by DADLE. In addition, a \( \mu \)-selective dose of intravenous naloxone (0.1 mg/kg) attenuated baroreceptor reflex sensitivity. Because intravenous naloxone methobromide (0.116 mg/kg), which has been shown not to cross the blood–brain barrier, did not change baroreceptor reflex sensitivity, \( \mu \)-opioid receptor centrally modulated the baroreceptor reflex control of RSNA in conscious rabbits. This \( \mu \)-receptor-mediated change in baroreceptor reflex sensitivity might be one of the reasons that a smaller dose of intracerebroventricular DADLE caused a greater pressor response compared with DAGO, despite a similar increase in RSNA. Montastruc et al also reported that the pressor responses induced by stimulation of either the vagus or laryngeal nerve were potentiated by intravenous naloxone. Our results agree with this previous study.

Although the present study did not clarify what exact mechanisms were at work in pressor responses and baroreceptor reflex control of RSNA in response to intracerebroventricular opioid agonists and antagonists, a receptor-mediated action of opioids in the central nervous system might be a candidate for the mechanisms. Autoradiographic studies showed differential distribution of \( \mu \)- and \( \delta \)-opioids in the central nervous system, such as a high density of \( \mu \)- and \( \delta \)-opioid receptors in hypothalamic sites, \( \mu \)-receptors in the...
nucleus tractus solitarius, and δ-receptors in the dorsal motor nucleus of the vagus,\textsuperscript{17} so that μ- and δ-opioids might cause the differential modulation on neurohormonal and baroreceptor reflex regulations in the present study.

In conclusion, μ- and δ-opioid receptor agonists exert a central pressor action, and central μ-receptors modulate sympathoadrenal responses and baroreceptor reflex control of RSNA. Furthermore, μ- and δ-opioids seem to have a differential modulation on central cardiovascular regulations in conscious animals.

References
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