PARTIAL AGONISTIC ACTION OF MORPHINE IN THE RAT VAS DEFERENS

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Abstract—Effects of morphine on the force of contraction of rat vas deferens were investigated. Morphine and β-endorphin decreased the electrically evoked twitch tension, in a dose dependent manner. The inhibitory effect of morphine, however, was much weaker than that of β-endorphin. These effects of both morphine and β-endorphin were completely antagonized by naloxone. In the presence of 30 μM morphine, the dose-response curve of β-endorphin shifted to the right by about 10-fold. Moreover, morphine partly reversed the contraction depressed by 0.3 μM β-endorphin, in a dose dependent manner. These findings suggest that morphine acts as a partial agonist on the rat vas deferens. Marked tolerance to β-endorphin and change in the antagonist potency of morphine were not observed in the vas deferens isolated from morphine-dependent rats.

The rat vas deferens is highly sensitive to β-endorphin, but practically insensitive to morphine (1, 2). On account of these unique properties, this tissue has been given much attention in recent years. A new type of opiate receptor, ε-receptor, has been postulated to be present in this tissue (3). Extensive studies mainly focused on the agonist potency have provided many data concerning the characteristics of ε-receptor (1-5). However, the question of why morphine and many other opiate agonists are ineffective in the rat vas deferens remains unknown. To acquire more detailed information on the action of morphine in the rat vas deferens, the interactions between morphine and β-endorphin were investigated in naive and morphine-dependent rats.

MATERIALS AND METHODS
Male Wistar rats weighing 250–300 g were sacrificed by a blow on the head and exanguinated from the common carotid arteries. A pair of vasa deferentia were dissected out and carefully desheathed. The seminal contents were gently extruded. About 2 cm of the mid-portion was cut out and mounted in a 18 ml organ bath containing Krebs-bicarbonate solution of the following composition (mM); NaCl 119, KCl 4.8, CaCl₂ 2.5, KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 25 and glucose 10. The nutrient solution was maintained at 37±1°C and aerated with a mixture of 95% O₂ and 5% CO₂.

The resting tension was adjusted to 1.0 g. Preparations were driven by platinum ring-hook electrodes at 0.1 Hz with rectangular pulses (0.5 msec, 5 mA) generated from the electronic stimulator (Nihon Kohden, Model MSE-3). Force of contractions was measured isometrically by means of force-displacement transducers (Nihon Kohden, Model TB-
611T) and recorded on an ink-writing oscillograph (Nihon Kohden, Model RM 6,000).

Muscles were allowed to equilibrate for about 15 min, and then, 1 μM β-endorphin was added to the bath to assess the responsiveness of the preparations. When the force of contraction became constant after washing out β-endorphin, the effect of β-endorphin or morphine on the vas deferens was observed. The dose-response curves were obtained by adding each compound cumulatively. β-Endorphin solution was freshly prepared just before the experiment by dissolving the freeze-dry powder in distilled water and kept on ice during the experimental periods.

Rats were made morphine dependent according to the method described by Hosoya (6) with slight modification. These animals were given morphine subcutaneously twice a day for more than 49 days. The first dose of morphine was 20 mg/kg, and this dose was increased by 20 mg/kg each week. Thus, dose of morphine given was 100 mg/kg at 29 days after the start of the repetitive administration, and the dose was not increased further. The experimental procedures were identical to those used in the case of the naive rat, except for the time of equilibration. Here, muscles were exposed to 1 μM β-endorphin immediately after being soaked in the baths and a 5 min incubation period was allowed to obtain full suppression of the contractile force. The thorough washing was repeated and the experiment was started after the contractile force had become constant. This process always required between 12–18 min.

**Drugs used:** Morphine hydrochloride (Sankyo Co. Ltd., Tokyo, Japan), β-endorphin (human) (Peptide Inst., Osaka, Japan) and naloxone hydrochloride (Endo Labs. Inc., New York, U.S.A.).

**RESULTS**

Dose-response curves of β-endorphin obtained in the presence or absence of 30 μM morphine are presented in Fig. 1. β-Endorphin reduced the contractile force of electrically driven rat vas deferens, in a dose dependent manner, and the maximum inhibitory effect was observed at the concentration of 1 μM. As shown in Fig. 1, 30 μM morphine shifted the dose-response curve of β-endorphin to the right by about 10-fold. The mean concentrations of β-endorphin required to decrease the control contractile force by 50% in the absence and presence of 30 μM morphine, were 53.2±10.1 and 484±94 nM, respectively.

On the other hand, the inhibitory effect of morphine on the force of contraction of vas deferens was much weaker than that of β-endorphin. Maximum reduction in twitch
tension observed with a concentration of 10–30 µM, was not over 20% (Fig. 2). Since morphine at concentrations higher than 30 µM produced significant increases in the force of contraction, 30 µM or less morphine was used throughout the experiments.

Although the inhibitory effects induced by both β-endorphin (data not shown) and morphine (Fig. 2) were antagonized by 3 µM naloxone, the excitatory effect of morphine observed at concentrations over 30 µM, was not altered by naloxone (data not shown).

When the contraction was suppressed almost maximally by 0.3 µM β-endorphin, a dose dependent increase in the contractile force was observed with the addition of morphine (Figs. 2 and 3). The force of contraction was recovered by 30 µM morphine to the level of 63% of the control contractile force. Thus, contraction of the rat vas deferens depressed by β-endorphin was to some extent recovered by morphine.

**Fig. 2.** Effects of morphine on the force of contraction of electrically driven vas deferens in the absence (■) or presence of 3 µM naloxone (△) or 0.3 µM β-endorphin (□). Morphine was added cumulatively. Preincubation with naloxone and β-endorphin were allowed to equilibrate for 10 and 5 min, respectively. Each point with a vertical bar represents the mean ±S.E. of 5 experiments. Ordinate: Changes in the contractile force. Values are expressed as percent of the initial level. Abscissa: Negative logarithm of the concentration of morphine (M).

**Fig. 3.** Typical tracing of the effect of morphine and naloxone on the force of contraction of the vas deferens depressed by 0.3 µM β-endorphin. Drugs were added at the time indicated by the arrows. At the end of the experiment, 3 µM naloxone was added to check the full recovery in contractile force. Vertical and horizontal calibrations represent 200 mg and 2 min, respectively. Concentrations of drugs are expressed as µM. β-EN=β-endorphin; Mor=morphine; Nal=naloxone.

**Fig. 4.** Effects of β-endorphin on the force of contraction of electrically driven vas deferens in the absence (●) or presence (○) of 30 µM morphine. Vasa deferentia were obtained from morphine-dependent rats. β-Endorphin was added cumulatively. Tissues pretreated with morphine were allowed to equilibrate for 15 min. Each point with a vertical bar represents the mean±S.E. of 5 experiments. Ordinate: Changes in contractile force. Values are expressed as percent of the initial level. Abscissa: Negative logarithm of the concentration of β-endorphin (M).
Complete recovery in the force of contraction was attained with application of 3 \( \mu M \) naloxone (Fig. 3).

The agonistic effect of \( \beta \)-endorphin and antagonistic property of morphine were further investigated using vas deferens isolated from morphine-dependent rats. As shown in Fig. 4, results closely resembled to those obtained in experiments on the vas deferens isolated from naive rats (Fig. 1). The concentrations of \( \beta \)-endorphin which induced a 50% reduction in the control force of contraction in the absence and presence of 30 \( \mu M \) morphine were 64.5 ±17.4 and 704±237 nM, respectively. These values were not significantly different from the corresponding values obtained in the vasa deferentia of the naive rats. A similar inhibitory effect of morphine was observed in the vas deferens of both morphine-dependent and naive rats (data not shown).

**DISCUSSION**

Morphine has been reported to exert no or only a weak inhibitory effect on the rat vas deferens (1–3, 5). In the present study, morphine at a concentration of 30 \( \mu M \) or less produced a slight decrease in the electrically evoked twitch tension, in a concentration dependent manner. Since this effect was completely antagonized by 3 \( \mu M \) naloxone, certain types of opiate receptors are probably involved in the process of the inhibition. Miranda et al. (7) and Jacquet (8) using rather high doses of morphine, demonstrated the direct excitatory effect of this opiate in the rat vas deferens, and also showed the failure of naloxone to block this excitatory effect of morphine. Jacquet (8) proposed that the weakness of the morphine effect was due to the co-existence of inhibitory and excitatory actions. The excitatory effect of morphine at concentrations over 30 \( \mu M \) was also apparent in our experiments, therefore the effect of morphine was investigated using a smaller concentration than that which produced an excitation of the tissue. As shown in Fig. 2, morphine did not increase the contractile force up to 30 \( \mu M \), even in the presence of naloxone. The above result suggests that the weak inhibitory effect of morphine cannot be attributed to the combined effects of the inhibitory and excitatory actions of this agent.

As shown in Figs. 2 and 3, morphine induced a partial recovery of force of contractions which had been already suppressed by \( \beta \)-endorphin. Full recovery to the initial level was observed with the additional 3 \( \mu M \) naloxone. Since the excitatory effect of morphine can be ruled out at the concentrations we used, this partial restoration in contraction by morphine may be due to the substitution of \( \beta \)-endorphin at the so called "\( \tau \)-receptor" sites. Full restoration by naloxone could also be attributed to the same mechanism of substitution of \( \beta \)-endorphin and morphine.

Schulz et al. (2) demonstrated that the in vitro effect of \( \beta \)-endorphin on the rat vas deferens was only transient while Britton et al. (9) stated that decay of the \( \beta \)-endorphin effect was very slow in the mouse vas deferens, even with a low concentration. During our experimental period, however, a time dependent weakening in \( \beta \)-endorphin effects was rarely observable.

In the experiment using vas deferens isolated from morphine dependent rats, tolerance to \( \beta \)-endorphin was not observed. According to Hosoya (6), the dose of morphine used here is sufficient to make the rat dependent on morphine. Although the possibility of the quick disappearance of the tolerance (10) cannot be ruled out completely, if the strong tolerance to \( \beta \)-endorphin actually did occur in the vas deferens, it can hardly be expected that the tolerance would disappear almost completely in 10–20 min.
Therefore, a marked tolerance to β-endorphin does not seem to occur in the vas deferens of the morphine-dependent rats. Moreover, significant changes were not observed either in the agonistic or antagonistic effects of morphine in the vas deferens from the morphine-dependent rats. These findings strongly suggest that the vas deferens of morphine-dependent rats possesses almost the same responsiveness to opiate compounds as that of the naive rats and support the concept of selective tolerance proposed by Schulz et al. (11) and Wüster et al. (5). During preparation of this manuscript, Huidobro et al. (12) reported that morphine acted as an antagonist on the inhibitory effect of β-endorphin in the isolated vas deferens from naive rats.

In conclusion, morphine acts as a partial agonist in the rat vas deferens, and chronic treatment with large doses of morphine failed to induce tolerance to β-endorphin in this tissue. Therefore, the opiate receptor in the rat vas deferens may differ from that involved in the establishment of morphine-dependence or -tolerance in other tissues.

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REFERENCES
1) Lemaire, S., Magnan, J. and Regoli, D.: Rat vas deferens: A specific bioassay for endogenous opioid peptides. Brit. J. Pharmacol. 64, 327-329 (1978)
2) Schulz, R., Faase, E., Wüster, M. and Herz, A.: Selective receptors for β-endorphin on the rat vas deferens. Life Sci. 24, 843-850 (1979)
3) Wüster, M., Schulz, R. and Herz, A.: Specificity of opioids towards the μ-, δ- and ε-opiate receptors. Neurosci. Lett. 15, 193-198 (1979)
4) Lemaire, S., Berube, A., Derome, G., Lemaire, I., Magnan, J., Regoli, D. and St-Pierre, S.: Synthesis and biological activity of β-endorphin and analogues. Additional evidence for multiple opiate receptors. J. Med. Chem. 21, 1232-1235 (1978)
5) Wüster, M., Schulz, R. and Herz, A.: The direction of opioid agonists towards μ-, δ- and ε-receptors in the vas deferens of the mouse and the rat. Life Sci. 27, 163-170 (1980)
6) Hosoya, E.: Screening of dependence liability of drugs using rats. Modern Pharmacology-Toxicology Vol. 5: Methods in Narcotic Research, Edited by Ehrenpreis, S. and Neidle, A., pp. 261-291, Marcel Dekker, Inc., New York (1975)
7) Miranda, H., Huidobro, F. and Huidobro-Toro, J.P.: Evidence for morphine and morphine-like alkaloid responses resistant to naloxone blockade in the rat vas deferens. Life Sci. 24, 1511-1518 (1979)
8) Jacquet, Y.F.: Excitatory and inhibitory effects of opiates in the rat vas deferens: A dual mechanism of opiate action. Science 210, 95-97 (1980)
9) Britton, D.R., Fertel, R., Coy, D.H. and Kastin, A.J.: Effect of enkephalin and endorphin analogs on receptors in the mouse vas deferens. Biochem. Pharmacol. 27, 2275-2277 (1978)
10) Opmeer, F.A. and van Ree, J.M.: Quantification of the in vitro induced tolerance to morphine of the isolated guinea pig ileum. Neuropharmacology 17, 887-890 (1978)
11) Schulz, R., Wüster, M., Krenss, H. and Herz, A.: Selective development of tolerance without dependence in multiple opiate receptors of mouse vas deferens. Nature 285, 242-243 (1980)
12) Huidobro, F., Huidobro-Toro, J.P. and Miranda, H.: Interactions between morphine and the opiate-like peptides in the rat vas deferens. Brit. J. Pharmacol. 70, 519-525 (1980)