Differential Effects of Morphine on Operant Escape Behavior and Averse Symptom Induced by Dorsal Central Gray Stimulation in Rats

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ABSTRACT—The involvement of a central opiate mechanism in the operant escape behavior induced by dorsal central gray (DCG) stimulation was investigated in rats. Morphine (2–10 mg/kg, i.p.) produced a rise in the DCG-stimulation threshold, but did not suppress rapid running as an averse symptom. Naloxone alone affected neither the threshold nor the averse symptom. Nevertheless, naloxone counteracted morphine-induced increments in the threshold. These results suggest that the opiate system may be indirectly involved in certain aspects of the operant escape behavior induced by the DCG-stimulation.

It is known that the mesencephalon or the periventricular system respectively plays an important role in the integration of aversion in animals (1–4). Stimulation of the mesencephalic dorsal part of the central gray (DCG) caused strong behavior indicating aversive sensation, such as jumping, running and escape behavior (2–4). The animals learn to stop the DCG stimulation in an operant situation (operant escape response). As to the involvement of endogenous amines of the brain in this behavior, Kiser et al. (5) showed that the averse behavior induced by DCG-stimulation was affected by the manipulation of brain serotonin function. Cazala and Garrigues (4) reported that 5-methoxy-N,N-dimethyl-tryptamine decreased the latency time of the escape response induced by the DCG-stimulation in mice. In addition, our previous study showed that the DCG-stimulation threshold for induction of the escape response was increased by 5-hydroxytryptophan (5-HTP) and chlorimipramine, and it was decreased by p-chlorophenylalanine (PCPA) and cyproheptadine (6). Furthermore, we have observed that cholinergic drugs such as physostigmine and arecoline caused an increase of the stimulation threshold, and anticholinergic drugs such as scopolamine and atropine caused a decrease of the threshold (7). These findings indicate that the operant escape behavior induced by the DCG-stimulation is related not only to the central serotonergic mechanism but also to a cholinergic mechanism.

On the other hand, as to the involvement of the opiate mechanism on mesencephalic stimulation, Kiser et al. (8) and Schmitt et al. (9) observed that latencies of escape behavior induced by DCG-stimulation were increased by electrical stimulation of the dorsal raphe nuclei which contain some serotonergic cells. Kiser and German (10) suggested that serotonin suppresses the susceptibility to foot-shock.
and potentiates the analgesic action of morphine. Furthermore, Jenck et al. (11) and Moreau et al. (12) observed that the microinjection of morphine into the DCG or the ventral tegmentum suppressed the escape responding induced by the DCG-stimulation. However, it is not yet clear whether the opiate mechanism may be directly involved in the development of operant escape behavior induced by the DCG-stimulation or not. So, the purpose of this study was to determine whether an opiate mechanism may be directly involved in the DCG-stimulation, by examining the effect of morphine and the combination of the opiate antagonist naloxone with morphine on the operant escape responding as well as aversive symptom induced by the stimulation.

Seventeen male Wistar rats weighing 250-300 g at the time of electrode implantation were used as subjects. They were housed by one or two in plastic cages (26 × 36 × 25 cm) and were given food and water ad libitum throughout the experiment. All the animals were maintained on a 12-hr light-dark cycle (light on 900 to 2100) at a room temperature of 22-24°C with a relative humidity of 50-60%.

All the animals were anesthetized with Na pentobarbital at 45 mg/kg, i.p., and placed on a stereotaxic instrument (Takahashi). Each animal was chronically implanted with a bipolar stainless steel electrode (250 μm in diameter, insulated except for the tip) aimed at the DCG (coordinates A, 0.6; L, 0.6; H, 0.4 mm, according to the rat brain atlas (13). The electrodes were bilaterally inserted into the target sites at an angle of 15° in order to avoid injuring the sagittal venous sinus as far as possible. All animals were given 150,000 units of penicillin subcutaneously after the surgery.

At the end of the experiment, the animals were given an overdose of Na pentobarbital, and the head was perfused with 0.9% saline and subsequently with 10% formalin via the heart. Then the brain was immersed into a formalin-saline solution at least for one week. After removing the skull, each brain was cut in 40-μm sections using a cryostat (Chiyoda). The sections were stained with cresylviolet. All electrode tips were located in or on the border of the DCG by the inspection of the stained sections.

The experiment was carried out in a Skinner box (30 × 27 × 25 cm) with a metal lever, which was already described in the previous reports (7). A lever press turned off or decreased the brain stimulation current. A swivel was mounted in the ceiling of the Skinner box holding the electrode lead, allowing the animal to move freely. The stimulation current was derived from a square-wave stimulator (Watanabe) which allowed a decremental stimulation paradigm.

After a recovery period of at least one week from implantation surgery, each animal was placed into the Skinner box, and the stimulation cable was connected to the electrode plug mounted on the animal's head. The DCG was stimulated with negative squarewave pulses (5 trains/sec, train duration = 100 msec, pulse duration = 0.5 msec, stimulation current = 100-600 μA). The stimulation current was gradually increased until the animal began to show aversive behaviors such as rapid running around the box and jumping, defecation and urination. These rats were trained to press a lever to stop the DCG-stimulation (escape response). Only animals that showed the stable escape response were trained with the DLP paradigm in which the rat itself could decrease the DCG-stimulation by pressing a lever.

In the DLP paradigm, one trial consisted of a 120-sec stimulation period alternating with a 60-sec rest period. During the stimulation period, each lever press decreased the DCG-stimulation current by 5% of its initial level. The initial stimulation current was chosen for each animal such that its average lever pressing rate was 4-6 per trial. When the threshold was stable for three successive days, the animals performed 10 trials at 0.5, 1, 2, 4 and 24 hr after the drug injection in the morphine administration experiment or at 0.5 hr after morphine administration in the morphine + naloxone combination experiment. After that, the animals were allowed to rest for at least 10
All drugs were dissolved in saline solution (vehicle) and administered intraperitoneally. Control animals were given vehicle alone (0.1 ml per 100 g body weight).

The experimental data are represented as the mean percent change of the stimulation-threshold. The Mann-Whitney U-test was used for statistical analysis.

Figure 1 shows the effects of morphine at various doses on the DCG-stimulation threshold. Morphine at doses of 2–10 mg/kg caused a dose-dependent increase of the DCG-stimulation threshold at 1–4 hr after the administration. The peak time of this drug effect at the doses used was 1–2 hr after the administration. Significant differences as compared with the vehicle administered control values were found at 1 and 2 hr (U = 2, P < 0.05, respectively) after 2 mg/kg administration; at 1, 2 (U = 0, P < 0.01, respectively) and 4 hr (U = 2, P < 0.05) after 5 mg/kg administration; and at 1, 2 (U = 0, P < 0.01, respectively) and 4 hr (U = 2, P < 0.05) after 10 mg/kg administration. By 24 hr after the administration, the threshold increasing effect of morphine was no longer observed. Rapid running behavior was observed 1 and 2 hr after the morphine administration. On the other hand, naloxone, an opioid antagonist, at doses of 5 and 10 mg/kg did not affect the DCG-stimulation threshold and general behavior such as running (without figure).

The combination effect of morphine and naloxone on the stimulation threshold is shown in Fig. 2. These measurements were carried out 60 min after 10 mg/kg morphine administration, and 10 mg/kg of naloxone was administered 30 min before testing the stimulation threshold. The DCG-stimulation threshold was markedly increased by morphine at 1, 2 and 4 hr after the administration, and the effects of morphine were completely antagonized by naloxone. There were significant differences (U = 0, P < 0.01, respectively) between the morphine group and the morphine + naloxone group at 1 and 2 hr after morphine administration.

It is said that the operant escape behavior induced by DCG-stimulation may be related not only to a central serotonergic mechanism but also to a cholinergic mechanism (6, 7). Especially as to the involvement of serotonin in this behavior, a serotonin precursor and agonist, 5-HTP and chlorimipramine, increase the DCG-stimulation threshold while the antagonists, cyproheptadine and PCPA, decrease the threshold (5, 14). On the other hand, serotonnin decreases susceptibility to foot-shock, i.e., the analgesic action of morphine is potentiated by an increase of the brain serotonin level, and a microinjection of morphine near to the dorsal raphe nuclei causes a strong analgesic action that is antagonized by anti-serotonergics (5). These data indicate that the opiate mechanism may be involved in operant escape behavior induced by DCG-stimulation.

In the present experiment, morphine increased dose-dependently the DCG-stimulation threshold, but the symptoms such as rapid running behavior induced by DCG-stimulation were not affected. The opiate antagonist naloxone did not have any effect on the stimulation threshold and behaviors such as rapid running. However, naloxone suppressed completely the morphine-induced increase of the stimulation threshold. The authors already observed that morphine did not suppress the lever pressing for stopping the DCG-stimulation under the intensity of initial stimulation in the DLP paradigm (M. Moriyama et al., unpublished data). These observations indicate that morphine may affect the threshold of DCG-stimulation. On the other hand, Kiser and German (10) observed that escape behavior induced by stimulation of various brain sites was suppressed by 15 mg/kg morphine, and catalepsy was simultaneously induced by that dose. Moreau et al. (12) reported that the depressant effect of morphine when administered into the ventral tegmentum in the operant escape behavior induced by the DCG-stimulation was unlikely to be due to the impairment of gross motor activity (morphine conversely provoked a behavioral activation).
Fig. 1. Effect of morphine on the operant escape behavior induced by DCG-stimulation. Morphine was administered intraperitoneally. Each point represents a mean % change (± S.E.) of the DCG-stimulation threshold compared to the pre-administration test. ••••, vehicle, 1 ml/kg (N = 5); ••••, morphine, 2 mg/kg (N = 4); ••••, morphine, 5 mg/kg (N = 4); morphine, 10 mg/kg (N = 4); *, P < 0.05; ***, P < 0.01.

Fig. 2. Combined effect of morphine with naloxone on operant escape behavior induced by DCG-stimulation. Each column represents a mean % change (± S.E.) of the DCG-stimulation threshold compared to the pre-administration test. Measurements were performed at 1, 2, 4 and 24 hr after intraperitoneal administration of 10 mg/kg morphine. Naloxone, 10 mg/kg, i.p., was administered 1 hr before the DCG-stimulation threshold test. v, vehicle group; m, morphine group; m + n, morphine + naloxone group; *, P < 0.05; ***, P < 0.01.
lization was induced by peripheral averse stimulation such as foot-shock, but not by intracranial averse stimulation. Morphine does not have such a strong action on escape behavior for brain averse stimulation, suggesting that DCG-stimulation may not be considered the same phenomenon as sensory pain. In the present study, the morphine-induced increment in the DCG-stimulation threshold was observed at 2 mg/kg and higher doses, but rapid running as an averse symptom was not suppressed. If DCG stimulation itself would be the same as sensory pain, morphine should suppress simultaneously the lever pressing for escaping the DCG stimulation and the rapid running induced by the stimulation. This indicates that the opiate system may be involved in certain aspects of operant escape behavior induced by the DCG-stimulation, but this involvement is not a direct one.

Recently, Ableitner and Herz (16) demonstrated that small doses of a selective \( \kappa \)-opioid agonist, U-50,488H, dose-dependently attenuated the response to noxious stimulation such as heat and pressure, suggesting the involvement of \( \kappa \)-opioid receptors in the averse response. So, the application of a selective \( \kappa \)-opioid receptor agonist or its antagonist in the experimental series should clarify whether opioid receptors participate in the DCG-stimulation induced behavior.

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