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Differential Effects of Intracerebral Microinjection of Morphine on Approach and Escape Responses Induced by Lateral Hypothalamic Stimulation in the Mouse

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BENDANI, T. AND P. CAZALA. *Differential effects of intracerebral microinjection of morphine on approach and escape responses induced by lateral hypothalamic stimulation in the mouse.* PHARMACOL BIOCHEM BEHAV **30**(2) 397–401, 1988.—BALB/c mice were implanted with a combined guide-cannula and bipolar stimulation electrode. The tip of the guide-cannula was positioned 1.0 mm above the electrode tip which was located in the lateral hypothalamus (LH). Mice were trained in a shuttle-box to initiate and terminate a continuous electrical stimulation of the LH. Following stabilization of the baseline response latencies two experiments were performed. In the first experiment, isotonic NaCl or morphine sulphate (0.5, 1.0 or 2.0 μg dissolved in NaCl) were injected into the LH (volume of the injection 0.5 μl). The lowest dose (0.5 μg) of morphine rapidly decreased approach latency for LH stimulation over a period of two hours. The same result was observed with both 1.0 and 2.0 μg but with greater magnitude and a longer time course. In some animals, an increase in escape latency appeared but only at the dose of 1.0 μg. In the second experiment, it was observed that intraperitoneal injection of naloxone (2.0 mg/kg) suppressed the shortening of latency of approach responses induced by the microinjection of 2.0 μg of morphine. These results suggest the involvement of opiate mechanisms in the regulation of LH self-stimulation.

Approach response Escape response Electrical stimulation Lateral hypothalamus Microinjection Morphine Mouse

THE effects of morphine on self-stimulation elicited from the lateral hypothalamus (LH) have been investigated in several studies. In the classical lever-press box paradigm, peripheral injections in the range 5.0 to 10.0 mg/kg produce two opposite effects: initially, there is a large reduction in lever-pressing rate, lasting for several hours, followed by an enhancement of self-stimulation also lasting several hours ([11], general review). These biphasic effects, the magnitude and the time course of which were different, appear to be relatively independent. The inhibition of self-stimulation may result from non-specific effects of morphine which particularly produces sedation and/or muscular rigidity. On the other hand, various data suggest that the subsequent facilitatory effect on lever pressing rate may be related to a specific activation of the neuronal mechanisms involved in self-stimulation behavior [12, 19, 20, 23]. The lowering of self-stimulation thresholds observed after injection of morphine [10,22] seems to corroborate this hypothesis. An increase of hypothalamic self-stimulation has also been observed after intracerebral injection of morphine [2,3].

However, in the shuttle-box paradigm, the effects of morphine on self-stimulation in LH appeared more variable. In this experimental situation, the length of time the electrical stimulation is applied or shut-off is determined by the animal. When low current intensities are used, particularly in the case of LH stimulation, the latency to initiate the stimulus (OFF duration) can be considered an index of its rewarding properties, and the latency to terminate it (ON duration) an index of its aversive effects [1, 6, 7]. Depending on the case, peripheral injections of morphine produce a non-selective depressant effect revealed by increases in both ON and OFF durations [26], a selective increase of OFF duration [8] or a selective increase of ON duration [16,17]. None of these results appear to unambiguously identify a morphine-induced increase in the rewarding component of LH stimulation. For this reason, we have studied, in mice, the effects of intra-hypothalamic injection of low doses of morphine on the approach and escape responses induced by LH activation.

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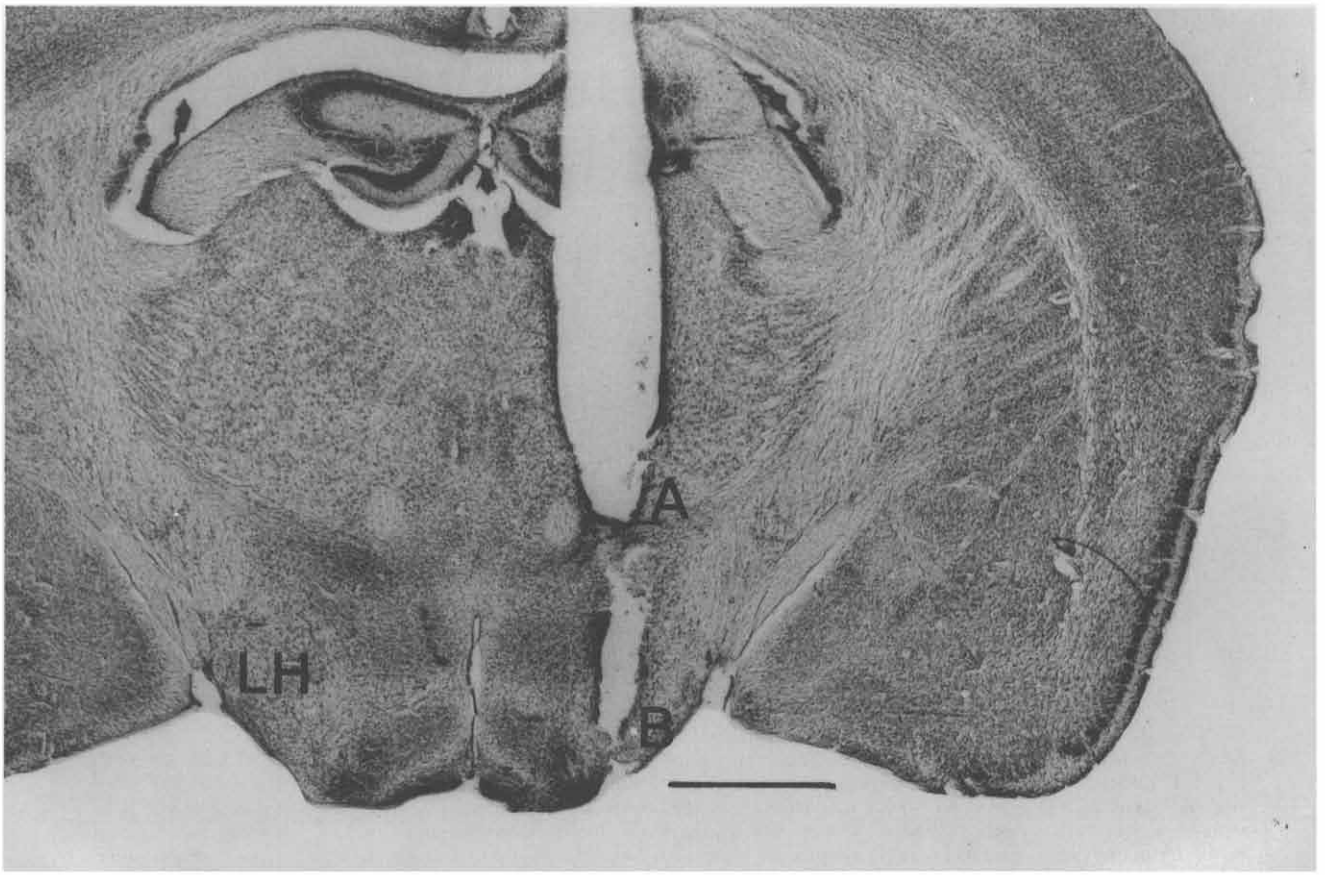

FIG. 1. Photomicrograph of thionin-stained sections (60 μm) through guide-cannula (A) and electrode (B) tracks. LH: lateral hypothalamus (horizontal bar=1.0 mm).

METHOD

Animals and Surgery

The subjects were male mice of the BALB/c strain. At 8 weeks of age, they were housed individually with ad lib access to food and water in a temperature-controlled room (23°C) with a light-dark cycle (12 hr-12 hr). The animals were 10–11 weeks of age (body weight 27–30 g) at the beginning of the experiments.

Under deep sodium thiopental anesthesia (90 mg/kg) the animals were unilaterally implanted with a combined stainless steel guide-cannula (outer diameter 0.460 mm, inner diameter 0.255 mm) and bipolar electrode made of two insulated and tightly twisted strands of 0.09 mm platinum wire. The tip of the guide-cannula was positioned 1.0 mm above the electrode tip which was inserted into the lateral hypothalamus (LH). A slight antero-posterior distance (0.1 to 0.2 mm) separated the guide-cannula from the stimulating electrode. In order to ensure correct electrical insulation the guide cannula and the electrode, except at its tip, were coated with a varnish. The diameter of the guide-cannula-electrode unit at its thickest portion was approximately 0.8 mm. The electrode was located either in front of or behind the guide-cannula in different cases. The following stereotaxic coordinates were used: 2.20 mm anterior to the interaural line; ±1.10 mm lateral to the sagittal line and 5.40 mm below the surface of the skull; the incisor bar was level with the interaural line.

Materials and Experimental Protocol

Experiment 1. Nine mice were used in this experiment. Behavior was studied in a 40×8×12 cm shuttle-box [4]. A photoelectric cell was placed 7.5 cm from each end of the box. By interrupting one light beam, the mouse learned to trigger a continuous sinewave (100 Hz) stimulation which terminated only when the animal interrupted the beam at the other end of the cage. The time periods during which the mouse received stimulation (ON duration which corresponds to escape latency) and did not receive stimulation (OFF duration which corresponds to approach latency) were recorded automatically with a precision of 0.01 sec.

After a first habituation session in which no current was applied, the animals learned to trigger and to interrupt the electrical stimulation of the LH. For each animal the current intensity was adjusted so that, on the one hand, clear approach and escape responses could be observed, and on the other hand all the mice would have very similar baseline responses (mean current intensity applied: 24.8 (SEM ±2.3) μA peak to peak). The mice were tested daily, over two weeks, until their baseline response latencies had entirely stabilized (mean values: approach latency 14 sec, escape latency 11 sec).

The experiment was conducted over five sessions, each successive session being separated by a one week interval. Isotonic NaCl was injected at the beginning of the first and the fifth sessions. Increasing doses of morphine sulphate

(0.5, 1.0 and 2.0 μg dissolved in NaCl) were injected at the beginning of the second, third and fourth sessions. The doses of morphine were expressed in terms of the salt. The volume of each injection was 0.5 μl.

The microinjections were carried out by inserting a stainless steel injection cannula (outer diameter 0.229 mm, inner diameter 0.127 mm) into the guide-cannula. The injection cannula was connected by polyethylene tubing to a 5.0 μl Hamilton syringe. The tip of the injection cannula extended 1.0 mm beyond the tip of the guide-cannula. Consequently, the substances were injected into the immediate proximity of the tip of the stimulating electrode. Each injection lasted 3 min. Following injection, animals were first replaced in their home cage for 10 min. Their behavior was then studied in the shuttle-box during a 12 min period every hour for up to 6 hours. For each period the first 2 min served as a warm up; approach and escape latencies were recorded during the other 10 min. The total amount of time that stimulation was ON or OFF during the 10 min was divided by the number of ON or OFF responses. The mean values of ON and OFF durations were thus determined. During the week which separated two successive injections the stabilization of ON and OFF durations was controlled in daily 10 min sessions.

Experiment 2. After a two-week rest period, 6 mice were selected randomly. They were again injected with morphine into the LH (2.0 μg dissolved in isotonic NaCl; volume of the injection: 0.5 μl). The animal's behavior was observed during two 12-min periods in the shuttle-box, 10 min and 30 min respectively after the microinjection. An intraperitoneal injection of 2.0 mg/kg of naloxone HCl dissolved in NaCl 0.9% was then performed. Three other mice underwent exactly the same experimental procedure with the exception that the naloxone injection was replaced by the vehicle. The combined effects of morphine and naloxone, or of morphine and NaCl were studied during four supplementary 12 min periods respectively 10 min, 1 hr, 2 hr and 3 hr after injection of naloxone or of the vehicle.

Histology

At the end of the experiments, animals were anesthetized and perfused with 0.9% saline followed by 10% formalin solution. The brains were removed and frozen sections (60 μm) obtained through the guide cannula and electrode tracks. The sections were stained with 0.1% thionin solution. Figure 1 shows typical guide cannula and electrode tracks.

RESULTS

Experiment 1

No significant differences were observed between the effects of NaCl injected before or after microinjections of morphine. Therefore ON and OFF values respectively recorded during these two control sessions were pooled. Moreover, statistical analysis showed that the values of ON and OFF duration recorded during the 7 successive daily sessions remained stable [analysis of variance of single factor with repeated measures [27] OFF duration, $F(6,48)=1.50$, n.s.; ON duration, $F(6,48)=1.42$, n.s.].

Microinjections of morphine rapidly decreased the latency of the approach response induced by activation of the LH in all mice studied since a statistically significant decrease of OFF duration was observed starting 10 min after the injection. This change lasted for 2 hours with 0.5 μg of morphine and for 4 hours after injection of 1.0 or 2.0 μg (Fig. 2). Moreover, the magnitude of this effect was proportional to the dose injected [analysis of variance of two factors (dose and passage) with repetition on the second factor [27]: dose effect, $F(2,24)=3.74$, $p<0.05$; passage effect, $F(6,144)=32.63$, $p<0.001$].

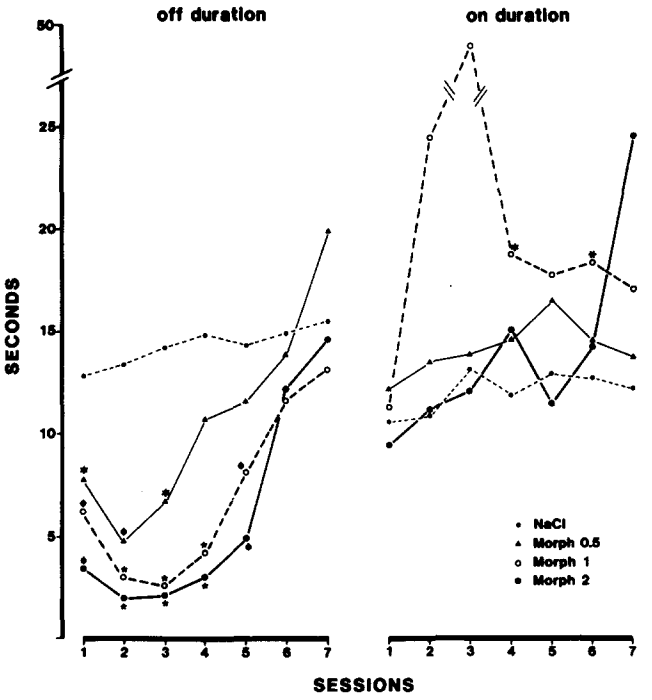

FIG. 2. Changes in the mean value of the latency to initiate (OFF duration) or of the latency to escape (ON duration) lateral hypothalamic stimulation after intra-hypothalamic injections of morphine (0.5, 1.0 or 2.0 μg) or of NaCl 0.9% (comparison between morphine and vehicle sessions). *$p<0.05$; ϕ$p<0.01$; ★$p<0.001$. (Student's t-test, paired test.)

At the dose of 1.0 μg morphine produced an increase in the ON duration which was marked in 3 animals, but much weaker or absent in others. Because of this heterogeneity the increase is only statistically significant for the 4th and 6th sessions. No comparable significant modulation of ON duration was detected at 0.5 and 2.0 μg. Statistical analysis confirmed that the observed effect is independent of the dose injected, $F(2,24)=1.45$, n.s.

Experiment 2

As in Experiment 1, intracerebral injection of morphine (2.0 μg) markedly reduced approach latency for LH stimulation, without concomitant modification of escape latency. This improvement immediately disappeared after the injection of 2.0 mg/kg of naloxone (Fig. 3). The statistical comparison between the values of OFF duration recorded respectively 30 min after injection of morphine alone and 10 min after injection of naloxone indicated: t(paired test) $=5.74$, $p<0.01$. In mice having received the vehicle in place of naloxone (data not shown in Fig. 3) the approach response remains facilitated by morphine throughout the duration of the test.

DISCUSSION

Intrahypothalamic injection of morphine at doses of 0.5,

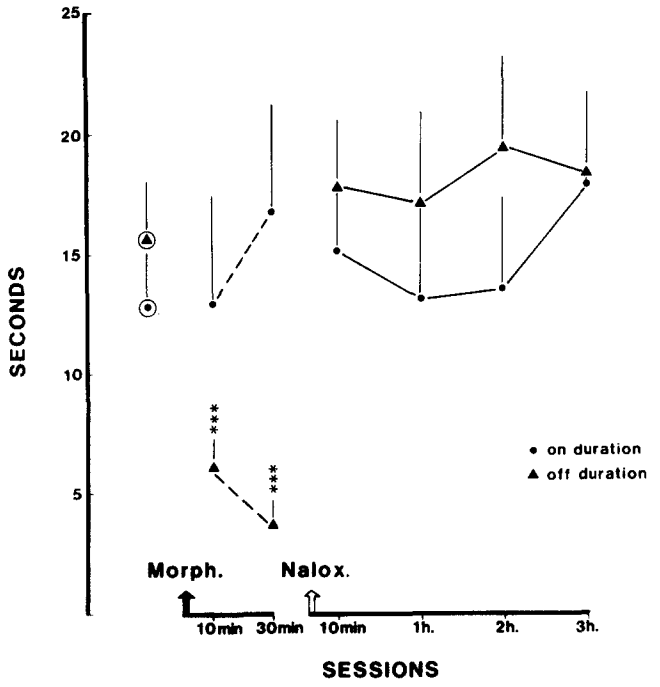

FIG. 3. Mean value (±SEM) of the latency to initiate (OFF duration) or of the latency to escape (ON duration) lateral hypothalamic stimulation recorded before any injection (black triangle in circle, black dot in circle) after microinjection of morphine (2.0 μg) alone (dotted line) and after intraperitoneal injection of 2.0 mg/kg of naloxone (solid line) (comparison between OFF duration values recorded before and after injection of morphine: ***$p<0.01$. (Student's t-test, paired test.)

1.0 and 2.0 μg induces a rapid and large decrease in the latency of the approach response induced by LH stimulation. The amplitude and time course of these effects were dose-dependent. Simultaneously the mean value of the escape latency either remained unchanged in comparison to NaCl injection or in some cases increased. These results suggest: (1) that the increased approach responding is not due to an increase of motor activity induced by intracerebral morphine injection [14]; (2) that the modulation of the ON and OFF duration are the result of an independent action on distinct neuronal mechanisms. Effectively, on the one hand, and in contrast to what is observed for the OFF duration, the amplitude of the effect on the ON duration does not appear to be dose-dependent. On the other hand, whenever a decrease in OFF duration and an increase in ON duration are simultaneously observed at the same dose (1.0 μg), they do not have the same time course; the modulation of the OFF duration preceded that of the ON duration. Furthermore, if all mice without exception show a marked increase in their approach responses only a few subjects increase notably their stimulation duration. Taken together these observations suggest a homogeneous substrate is implicated in approach responses whereas that of escape responses appears to be heterogeneous.

If it can be accepted that approach latencies constitute a valid index of the rewarding component of central stimulation [1, 6, 7] the decrease in OFF duration could therefore be interpreted as resulting from a sensitising effect of morphine on the positive reinforcement system activated by LH stimulation. Morphine has been observed to decrease self-stimulation thresholds in the LH [10,22] and to decrease the amplitude of the EEG waves recorded in this same region [15]. The facilitation of self-stimulation by morphine could be due to a potentiation of the activity of the reward system. Inherent in this possibility, in agreement with the suggestion of Maroli *et al.* [23], is that morphine and rewarding brain stimulation could act upon a common neural substrate. The self-administration of morphine in naive mice, at the same LH sites [5] where morphine facilitation on self-stimulation was observed, lends further support for this hypothesis. The antagonism by naloxone of the facilitating effect of morphine on approach responses shows that the facilitation is the result of the specific activation of certain opiate receptors. The increase in the ON duration observed in certain subjects is in agreement with the observations of Levitt *et al.* [16,17]. This effect could be due to an attenuation by morphine of the aversive component of central stimulation [13,22].

According to Broekkamp *et al.* [2] the facilitation of hypothalamic self-stimulation by intracerebral injection of morphine results from the activation of opiate receptors located in the ventral tegmental area. The activation of neuronal elements at long distances from the injection site would necessarily imply a delayed behavioral effect in order to account for diffusion time. This hypothesis is not supported by our results since: (1) our injections in the immediate proximity of the electrode rapidly increase approach responding; (2) histological controls revealed that all injections were made between 1.6 and 2.3 mm anterior to the ventral tegmental area. According to Lomax [18] an injection of 1.0 μl of ^{14}C-morphine into the anterior hypothalamus occupies a sphere of 0.6 mm radius from the injection cannula; the volume of each of our injections was limited to 0.5 μl. Both morphine and naloxone have high affinity for μ type opiate receptors. These μ opiate receptors have been observed in the hypothalamus by various authors although with varying density [9, 21, 24, 25]. It is therefore probable that these hypothalamic receptors are implicated in the facilitatory effect of morphine on the approach component of LH stimulation.

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