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Differential Effects of Naloxone on Approach and Escape Responses Induced by Electrical Stimulation of the Lateral Hypothalamus or the Mesencephalic Central Gray Area in Mice

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CAZALA, P. AND V. DAVID. *Differential effects of naloxone on approach and escape responses induced by electrical stimulation of the lateral hypothalamus or the mesencephalic central gray area in mice.* PHARMACOL BIOCHEM BEHAV **40**(2) 323–327, 1991.—BALB/c mice implanted with a bipolar electrode were trained in a shuttle-box to initiate and to terminate a continuous electrical stimulation applied in the lateral hypothalamus (LH) or in the mesencephalic central gray area (CG). Following stabilization of the baseline response latencies, the subjects were subcutaneously injected with isotonic NaCl or with naloxone HCl (0.5, 2 or 10 mg/kg) 15 min or 45 min before a test session. In LH-stimulated animals no modification of the behavioral responses was observed after injection of 0.5 mg/kg of naloxone. The 2 mg/kg dose increased the value of escape latency (ON time) but had no effect on approach latency (OFF time). The 10 mg/kg dose also increased ON time. At this dose, an increase of OFF time was simultaneously observed but only 15 min after the injection. In CG-stimulated mice an increase of OFF time and a reduction of ON time were recorded 15 min after the injection of 0.5 mg/kg. Only the reduction of ON time was detected for the 45-min delay. The 2 mg and 10 mg/kg doses simultaneously increased OFF time and reduced ON time for the two delays. These results demonstrate 1) that the effects of naloxone on self-stimulation varied as a function of the structure considered 2) that the predominant characteristic of the considered structure (essentially "rewarding" as the LH or "aversive" as the CG) governs the modulations induced by naloxone.

Approach response Escape response Naloxone Electrical stimulation Lateral hypothalamus Mesencephalic central gray area Mouse

SEVERAL experimental data have shown that opioid mechanisms are involved in the regulation of brain reinforcement processes. It has been observed in particular that peripheral (13) (general review) or intracerebral (2–4) injection of morphine highly facilitates intracranial self-stimulation behavior in rat and mouse.

Morphine has high affinity for the μ opiate receptor (27). Consequently, the effects on self-stimulation, of substances blocking activity of μ receptors such as naloxone were studied. The results of these investigations appeared extremely variable. Some authors showed that naloxone reduced the response rate for self-stimulation, whereas others reported that this substance did not significantly change the bar pressing rate (23) (general review). The marked differences between these data may be due to several methodological variables: 1) The doses of naloxone used; 2) the route of administration, intraperitoneal or subcutaneous; the latter appearing to be generally more efficient (20); 3) the delay separating the injection from the beginning of the test (28,31); 4) the brain site where stimulating electrodes are implanted.

Moreover, with a few exceptions, most of the experimental data has been obtained with a bar pressing task in which only one parameter (rate of bar pressing) is recorded. Consequently, it is difficult to determine if the decrease of self-stimulation induced by naloxone is due to the reduction by this substance of the reinforcing value of electrical stimulation, or if it results only from disturbances of locomotor activity (1, 5, 8).

The shuttle-box task has rarely been used to study the effects of opiate antagonists on self-stimulation (15,29). In this task, the behavioral performances are established from the latency to trigger the electrical stimulation (which provides an index of the strength of the approach component of the stimulus) and also by the latency to interrupt it (which provides an index of the strength of the escape component of the stimulus). These two independent parameters allow the study of the behavioral effects of drugs (21).

Naloxone seems not only to reduce the rewarding value of electrical stimulation but, paradoxically, it also increases escape responses induced by activation of various brain regions (22).

In the present study, we have compared, in mice, the effects of 3 doses of naloxone on approach and escape responses induced by activation of two brain structures showing a distinct reactivity to electrical stimulation: the lateral hypothalamus, a "rewarding" structure and the dorso-lateral part of the mesencephalic central gray area, an "aversive" structure in which a

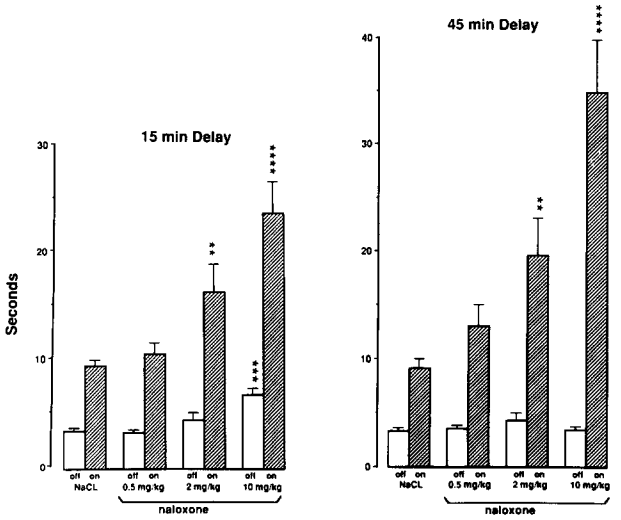

FIG. 1. Variations of approach (OFF duration) and escape (ON duration) latencies either 15 min or 45 min after the injection of isotonic NaCl or naloxone in LH-stimulated animals (**$p<0.02$; ***$p<0.01$; ****$p<0.001$).

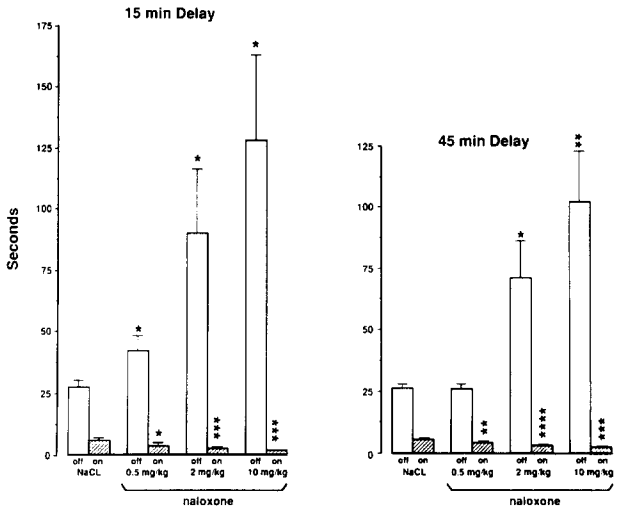

FIG. 2. Variations of approach (OFF duration) and escape (ON duration) latencies either 15 min or 45 min after the injection of isotonic NaCl or naloxone in CG-stimulated animals (*$p<0.05$; **$p<0.02$; ***$p<0.01$; ****$p<0.001$).

paradoxical self-stimulation behavior can be nevertheless observed (6). The effects of naloxone were studied either 15 min or 45 min after the injection.

METHOD

Animals and Surgery

The subjects were male mice of the BALB/c By Jlco strain. At 9 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 h–12 h, light on at 0800 h). The animals were aged 11–12 weeks (body weight 27–30 g) at the beginning of the experiment.

Under deep sodium thiopental anesthesia (90 mg/kg) the animals were unilaterally implanted with a bipolar electrode made of two insulated and tightly twisted strands of 0.09 mm platinum wire. The following stereotaxic coordinates were used: LH: 2.10 mm anterior to the interaural line, ±1.10 mm lateral to the sagittal line and 5.40 mm below the surface of the skull. CG: 0.40 mm posterior to the interaural line, ±0.30 mm lateral to the sagittal line and 2.70 mm below the surface of the skull; the incisor bar was level with the interaural line. Mice were allowed to recover from operation over one week.

Materials and Experimental Protocol

Behavior was studied in a 40×8×12 cm shuttle-box. A photoelectric cell was placed 7.5 cm from each end of the box. Interruption of a first light beam triggered 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 s.

After a first habituation session during which no current was applied, the animals learned to trigger and to interrupt the electrical stimulation of the LH (n=17) or of the CG (n=14). For each animal, the current intensity was adjusted so that clear approach and escape responses could be elicited and that all the mice implanted in a same structure (LH or CG) had very similar baseline responses. The mice were submitted to 15-min daily sessions during ten days, until their baseline responses had entirely stabilized.

The experiment was conducted over eight sessions, each successive session being separated by a 24-h interval. Isotonic NaCl was injected during the first, the fourth and the seventh session. Naloxone hydrochloride dissolved in NaCl was injected at a dose of 0.5, 2 or 10 mg/kg during the second, the fifth and the eighth session. During the third and the sixth session, the animals were not injected but their behavior in the shuttle-box was measured. NaCl or naloxone were subcutaneously administered 15 min (LH n=8; CG n=7) or 45 min (LH n=9; CG n=7) before a test session (mean volume of each injection 0.15 ml). All the animals received the 3 doses of naloxone which were administered in a random order. In each animal two successive injections of NaCl or of naloxone were separated by a 72-h interval. The effects of injections were studied during 15 min.

Histology

At the end of the experiment, the animals were sacrificed under deep chloroform anesthesia; their brains were removed and fixed in a 10% formalin and 30% sucrose solution. Sixty-μm thick frozen sections were made and stained with 0.1% thionin solution.

RESULTS

The results obtained are summarized in Figs. 1 and 2.

Effects of Injections of Isotonic NaCl

Analyses of variance (32) revealed that, in each group studied, no significant difference was observed between the performances recorded during the three sessions with solvent injection [LH: 15-min delay: OFF duration, $F(2,14)=0.32$, n.s.; ON duration, $F(2,14)=2.39$, n.s.; 45-min delay: OFF, $F(2,16)=2.69$, n.s.; ON, $F(2,16)=2.28$, n.s. CG 15-min delay: OFF, $F(2,12)=$

0.16, n.s.; ON, $F(2,12)=1.30$ n.s. Forty-five-min delay: OFF, $F(2,12)=0.28$, n.s.; ON, $F(2,12)=0.39$, n.s.]. Therefore, these three series of values were pooled for each group.

Statistical analysis revealed that for each brain structure, the behavior of animals injected with NaCl either 15 min or 45 min before the session was similar (comparison of the mean values of ON or OFF duration recorded 15 min or 45 min after the injection: LH: OFF, Student's t-test$=0.26$, n.s.; ON, $t=0.42$, n.s. CG: OFF, $t=0.17$, n.s.; ON, $t=0.13$, n.s.).

The latency to trigger the stimulation (OFF duration) was always greater for CG than for LH-stimulated animals (15-min delay groups: $t=7.80$, $p<0.001$; 45-min delay groups, $t=13.39$, $p<0.001$). Conversely, the latency to interrupt the stimulation (ON duration) was shorter in CG than in LH animals (15-min delay groups, $t=3.68$, $p<0.01$; 45-min delay groups, $t=2.93$, $p<0.02$).

The current intensities applied in order to obtain clear approach and escape responses were the following: LH: 15-min delay: 17.4 (SEM $\pm$ 2.4) μA, 45-min delay: 18.5 ± 1.4 μA, $t=0.45$, n.s. CG: 15-min delay: 19 ± 2.6 μA, 45-min delay 18 ± 2.5 μA, $t=0.28$, n.s. The data concerning the two subgroups with 15- or 45-min delays were pooled. Consequently, it appeared that the mean value of the current intensity applied in LH and CG was not statistically different LH: 18 ± 1.3 μA; CG: 18.5 ± 1.7 μA ($t=0.24$, n.s.).

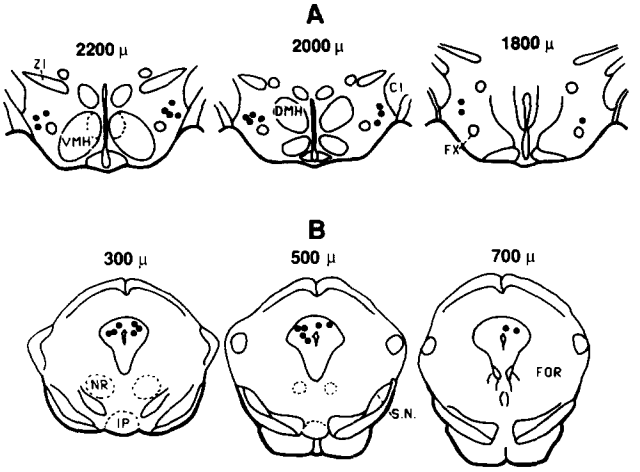

FIG. 3. Histological verification. The placements of the electrode tips in the LH (A) or in the CG (B) are plotted on frontal section diagrams. The values in microns (μ) indicate the distance of the section from the interaural line. Abbreviations: CI, capsula interna; DMH, nucleus dorsomedialis hypothalami; Fx, fornix; FOR, formatio reticularis; IP, nucleus interpeduncularis; NR, nucleus ruber; SN, substantia nigra; VMH, nucleus ventromedialis hypothalami; ZI, zona incerta.

Effects of Injections of Naloxone

In LH-stimulated mice (Fig. 1) no modification of behavioral responses was observed after injection of 0.5 mg/kg of naloxone, whatever the delay of the injection.

The 2 mg/kg dose induced a significant increase of the mean value of ON time (15-min delay, $t=3.20$, $p<0.02$; 45-min delay, $t=3.27$, $p<0.02$). No concomitant modification of OFF time was observed.

The 10 mg/kg dose also produced an increase of ON time (15-min delay, $t=4.65$, $p<0.01$; 45-min delay: $t=5.52$, $p<0.001$). We observed an increase of OFF time at this dose, but only 15 min after the injection ($t=4.51$, $p<0.01$). An analysis of variance confirmed the dose $\times$ delay interaction for OFF duration, $F(2,30)=11.1$, $p<0.001$.

In CG-stimulated animals (Fig. 2) an increase of OFF time ($t=2.56$, $p<0.05$) and a reduction of ON time ($t=2.84$, $p<0.05$) were recorded 15 min after the injection of 0.5 mg/kg of naloxone. On the other hand, only the reduction of ON time ($t=3.48$, $p<0.02$) was detected 45 min after the injection.

The 2 mg/kg naloxone dose increased OFF duration and reduced ON duration for the two delays (OFF duration: 15-min delay, $t=2.66$, $p<0.05$; 45-min delay, $t=3.0$, $p<0.05$. ON duration: 15-min delay, $t=4.17$, $p<0.01$; 45-min delay, $t=7.71$, $p<0.001$). The modifications of ON and OFF values were similar for the two delays [OFF duration: $F(1,12)=0.40$ n.s.; ON duration, $F(1,12)=0.42$, n.s.].

Similar results were obtained with the 10 mg/kg dose: increases of OFF duration (15-min delay, $t=2.97$, $p<0.05$; 45-min delay, $t=3.18$, $p<0.02$); reductions of ON duration (15-min delay, $t=4.91$, $p<0.01$; 45-min delay, $t=4.93$, $p<0.01$). The mean values recorded were similar for the two delays [OFF duration, $F(1,12)=0.36$, n.s.; ON duration, $F(1,12)=3.47$, n.s.].

These results demonstrate that the effects of the treatment by naloxone are very different in LH- and CG-stimulated mice. Indeed, for each of the considered parameters (OFF and ON duration) and for the two delays (15 and 45 min) the statistical analysis revealed a strong structure $\times$ treatment interaction [15-min delay: OFF duration, $F(2,26)=5.98$, $p<0.01$; ON duration, $F(2,26)=17.32$, $p<0.001$. Forty-five-min delay: OFF duration, $F(2,28)=12.5$, $p<0.001$; ON duration, $F(2,28)=16.90$, $p<0.001$].

Histology

Histological controls have confirmed the correct location of the electrode tips (Fig. 3). In the LH, they were implanted in a frontal plane corresponding to the medial or posterior part of the ventromedialis nucleus of the hypothalamus. In the CG, electrode tips were located in the dorso-lateral part of the structure. In the two brain regions the scattering was identical for animals injected either 15 min or 45 min before a test session.

DISCUSSION

Our results clearly show that before any injection of naloxone, electrical stimulation of the LH induced strong approach responses (short turn-on latencies) and weak escape responses (long switch-off latencies) in the shuttle-box. The opposite was recorded in CG-stimulated mice showing, at the same current intensities, weak approach responses followed by strong escape responses, which confirms our previous data (6).

In CG-stimulated mice, injection of naloxone had two effects. First, it markedly reduced the escape latency, which confirms the results of Sasson and Kornetsky (22) who reported that naloxone increases the aversive effects of stimulation applied in the mesencephalic reticular formation. We observed a reduction of ON time starting at 0.5 mg/kg, 15 min as well as 45 min after the injection. This effect increased at the doses of 2 and 10 mg/kg.

Secondly, naloxone increased approach latency in CG-stimulated mice, a result consistent with the data of Ichitani and Iwasaki (15). We observed an increased approach latency for the 3 doses administered when the test began 15 min after the injection; but this result was also observed for the 45-min delay only

for the two higher doses. In this latter case, it is possible that for the 2 and 10 mg/kg doses the animals hesitate to trigger CG stimulation the aversive effects of which increase after naloxone injection. However, this hypothesis is incompatible with the results obtained at 0.5 mg/kg, because at this dose approach latency was not modified by the drug for the 45-min delay.

These data suggest: 1) that the negative reinforcement mechanisms located in CG which are involved in the escape response induced by electrical stimulation, are the most sensitive to naloxone and 2) that the increase of approach latency observed 15 min after the injection of the 0.5 mg/kg dose probably corresponds to a specific disturbance of positive reinforcement processes by naloxone.

The results obtained in LH-stimulated mice are, at first sight, more difficult to explain. The major effect observed after naloxone injection was a marked lengthening of ON duration for the two delays. Concerning OFF duration, an increase was also observed but only 15 min after the injection of the highest dose of the drug (10 mg/kg).

Various hypotheses have been formulated to explain the switch-off response induced by the "rewarding" LH stimulation. Certain authors believe that the nervous processes responsible for positive reinforcement progressively adapt to the stimulation (9, 10, 16). Others have suggested that the switch-off response is motivated by a change of the effect of LH stimulation: the rewarding effect becomes aversive. In connection with this latter hypothesis, it may be noted that studies analysing the consequences of the variation of stimulation parameters (19, 24, 26), pharmacological (2, 12, 14) or genetical studies (7) tend to demonstrate that the nervous processes respectively involved in the elaboration of approach and escape responses induced by LH stimulation are independent. Our data are consistent with this hypothesis.

In the present study, it is unlikely that the lengthening of ON duration observed after the injection of naloxone is due to a reduction of the secondary aversive effects of LH stimulation, since, at the opposite, aversive consequences of CG stimulation were markedly enhanced by the drug. It also seems unlikely that the increase of ON time is due to a reduction of locomotor activity because at the highest dose of naloxone (10 mg/kg) approach and escape latencies simultaneously increased 15 min after the injection. Conversely, CG-stimulated mice showed at the same dose the shortest escape latencies. Trujillo et al. (30) have recently reported that opiate antagonists reduce the reinforcing value of the brain stimulation but are without effect on the ability of the subject to respond.

Consequently, one can explain the increase of escape latency from LH stimulation by a specific interference with self-stimulation processes. Naloxone, which blocks the activity of μ opiate receptors involved in reinforcement mechanisms (25), could decrease the reactivity of the central nervous system to electrical stimulation. The reduction by naloxone of the stimulation effectiveness would lead the animal to remain under stimulation during a longer time in order to compensate for the reduction of reward. Although the rewarding effect was attenuated, the animal would remain adequately motivated to turn-on stimulation which could explain that approach latency was little modified. The fact that the animal terminates the stimulation suggests that the aversive component remained present.

Therefore, it seems reasonable to assume that approach latency, or OFF duration, represents the degree of motivation of the subject, whereas escape latency accounts for the quality of the reinforcement. The more this latter is rewarding, the more the animal is motivated and the approach latency short. Conversely, the more the reinforcement is negative (aversive) the more the escape latency decreases while approach latency increases, suggesting a loss of motivation. As other authors have previously reported, the effects of naloxone on self-stimulation varied from one brain structure to another. Our study seems to demonstrate that it is the predominant characteristic of the considered structure (in our case essentially "rewarding" or "aversive") which governs the modulations induced by naloxone.

Finally, it may be noted that in the case of CG the changes induced by naloxone were observed starting at 0.5 mg/kg, whereas in LH-implanted mice, no significant behavioral effects occurred before the dose of 2 mg/kg. The stronger reactivity of CG to naloxone may be related to the fact that this structure contains a higher density of μ opiate receptors than LH (11, 17, 18).

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