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Effects and Interactions of Naloxone and Amphetamine on Self-Stimulation of the Prefrontal Cortex and Dorsal Tegmentum

K B J FRANKLIN AND A ROBERTSON

Department of Psychology, McGill University, Montreal, Quebec, Canada

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FRANKLIN, K B J AND A ROBERTSON *Effects and interactions of naloxone and amphetamine on self-stimulation of the prefrontal cortex and dorsal tegmentum* PHARMAC BIOCHEM BEHAV **16**(3) 433–436, 1982 —Naloxone (0 1 to 10 mg/kg) caused a dose-dependent depression of self-stimulation of the medial prefrontal cortex (PFC), lateral hypothalamus (LH) and a region in the dorsal tegmentum lateral to the central gray (DT) The DT contains many enkephalin fibers and is a site for stimulation-produced analgesia, while the PFC contains little enkephalin and does not support stimulation-produced analgesia However, self-stimulation rates of the PFC, DT and LH were all equally depressed by naloxone In order to study possible opiate-dopamine interactions, we examined the effects of naloxone on the facilitatory effects of D- and L-amphetamine (1 0 mg/kg) on self-stimulation of the DT and PFC If amphetamine mildly facilitated self-stimulation (L-amphetamine on DT self-stimulation and D-amphetamine on PFC self-stimulation), then the addition of naloxone was without effect If amphetamine greatly increased self-stimulation (D-amphetamine on DT self-stimulation), naloxone caused a depression of the amphetamine effect It is argued that naloxone's effects in this and other reports reviewed is related to the level of self-stimulation performance, and not to the level of enkephalin at the self-stimulation site, nor to amphetamine's effects on dopamine activity

Self-stimulation L-Amphetamine Reward Prefrontal cortex Naloxone Lateral hypothalamus D-Amphetamine Central gray

SINCE the discovery that the brain contains endogenous opiate-like peptides [10], there has been interest in the notion that opiate peptide neurons might be involved in the rewarding effects of opiates and natural rewards as well as in analgesia [1,18] If this were so, electrical stimulation of opiate-peptide neurons might be expected to be rewarding and this reward to be blocked by opiate antagonists In this regard, Beluzzi and Stein reported that naloxone depresses self-stimulation (SS) of the enkephalin-rich periaqueductal gray, a site which was chosen because it yields analgesia when stimulated [2] However, other studies have reported no effect of naloxone on SS of the lateral hypothalamus and medial forebrain bundle [6, 9, 12, 20, 21] or caudate nucleus [20], the latter of which contains moderate levels of enkephalin and opiate receptors [3,17] More recently Stapelton *et al* [19] found mild (<30%) depression from naloxone in SS of the periaqueductal gray, lateral hypothalamus, substantia nigra and nucleus accumbens The SS sites that have been tested contain varying amounts of enkephalins and it is not clear whether the effectiveness of naloxone in reducing SS bears any relationship to the presence of enkephalin Moreover, enkephalins might influence SS indirectly via dopamine (DA) neurons which are thought to be presynaptically inhibited by enkephalin [11,15] If this were the case the effects of naloxone might be increased in conditions where DA activity is high such as during amphetamine-induced potentiation of SS [4,9]

The present study compared the effect of naloxone on SS in three sites the dorsal tegmentum, prefrontal cortex and lateral hypothalamus The dorsal tegmental region near the periaqueductal gray contains enkephalin [17] and, like previously tested periaqueductal gray sites [2], is a site for analgesia produced by electrical stimulation (F Abbott and R Melzack, personal communication) The medial prefrontal cortex (PFC) is thought to contain very low levels of enkephalin [3, 16, 17] and does not seem to support stimulation analgesia (Abbott and Melzack, personal communication) SS of the lateral hypothalamus (LH) was tested for comparison with other reports In addition, naloxone was tested on rats self-stimulating in the PFC and DT under the influence of a 1 mg/kg dose of D- or L-amphetamine This dose of D-amphetamine produces a facilitation of SS across all three sites [5] and the difference between the effects of the two isomers is maximal [14]

METHOD

Subjects

Subjects were 25 adult male Wistar rats which self-stimulated through bipolar stainless steel electrodes (Plastic Products, Roanoke, VA), 127 μ in diameter at each tip, implanted under Nembutal anaesthesia (60 mg/kg, IP) in the PFC (10 rats), the LH (7 rats) or the DT (8 rats) Electrode sites are shown in Fig 1

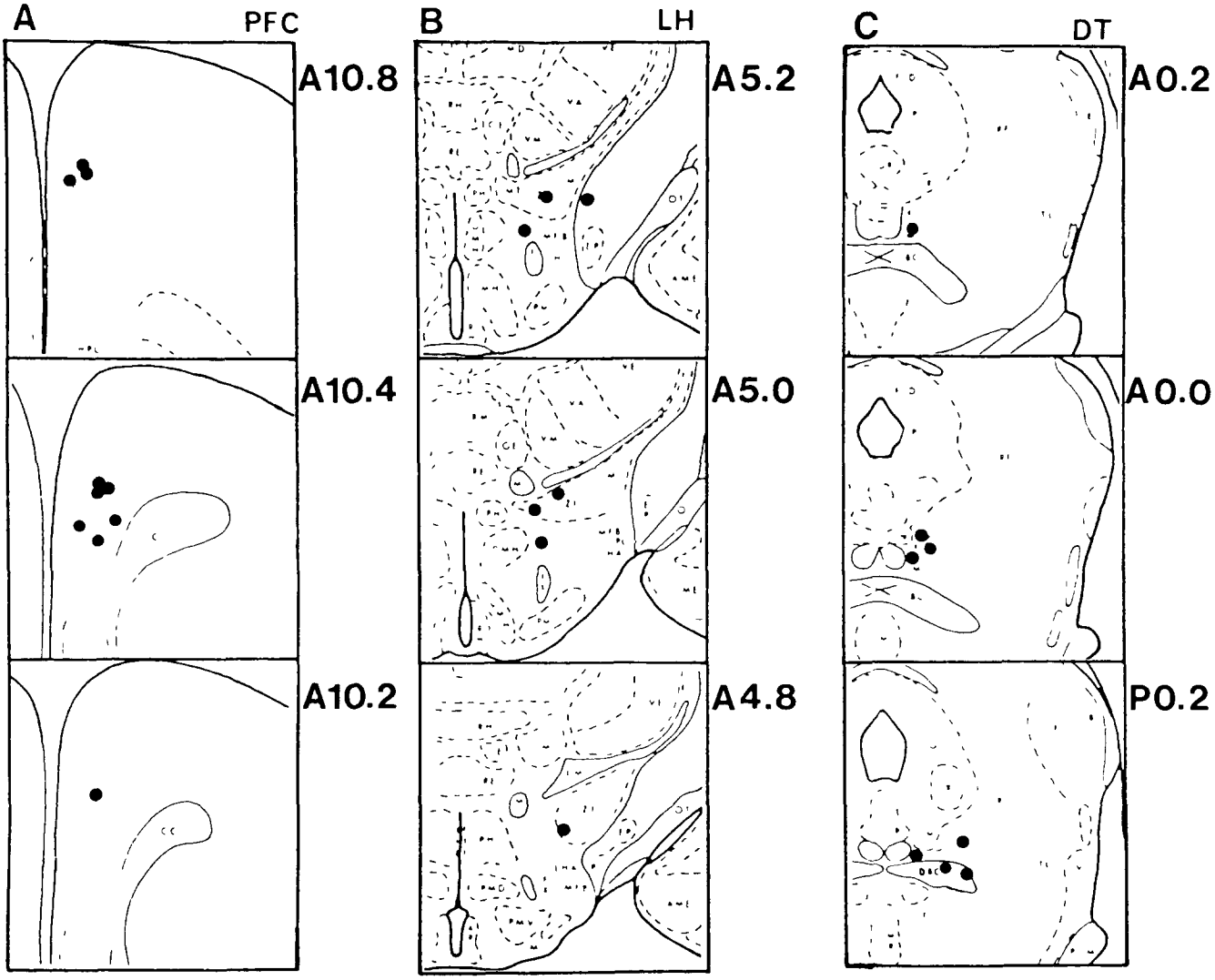

FIG 1 Locations of self-stimulation electrode tips shown on planes from the atlas of Pellegrino and Cushman [14] Numbers alongside each frame show the position in millimeters anterior (A) or posterior (P) to the ear bars

Apparatus and Pretraining

Following a one-week recovery period after the surgery, rats were trained to lever press for 0 2 sec trains of 100 Hz, 0 2 msec pulses of electrical stimulation in a test chamber (30×30×30 cm) with a lever at one end, 6 cm above the floor Stimulation current was monitored on an oscilloscope

When a rat had learned to bar press for stimulation, the current was adjusted to twice that of the threshold for responding (to about 160 μA for LH, 185 μA for DT, and 400 μA for PFC) and remained at that level for the duration of the experiment Rats were then trained to respond on a random interval 10 sec schedule of reinforcement in daily 90 min sessions The number of presses was automatically recorded every 5 min After 5 to 10 sessions on this regimen, response rates had stabilized (744 resp/hr for LH, 720 resp/hr for DT and 533 resp/hr for PFC) Drug tests were then begun

Drugs

D- and L-amphetamine sulphate were dissolved in isotonic saline to a concentration of 1 0 mg/ml (expressed as the salt) Naloxone HCl was dissolved in isotonic saline to concentrations of 0 1, 1 0 and 10 mg/ml Both drugs were administered IP in a volume of 1 0 ml/kg

Testing Procedure

Rats were tested during 90 min sessions The first 25 min of the session were used to calculate baseline responding The drugs were injected between the 25th and 30th min of the session If both naloxone and amphetamine were administered, naloxone was always injected 2–3 min before amphetamine Response rates were then measured for the following 60 min A minimum of 3 days was allowed between successive drug sessions to avoid cumulative drug effects

All 3 groups of rats received naloxone in doses of 0 1, 1 0 or 10 0 mg/kg Only rats with DT or PFC electrodes received naloxone (1 0 or 10 0 mg/kg) in combination with D- or L-amphetamine (1 0 mg/kg)

Five rats with PFC electrodes and four rats with DT elec-

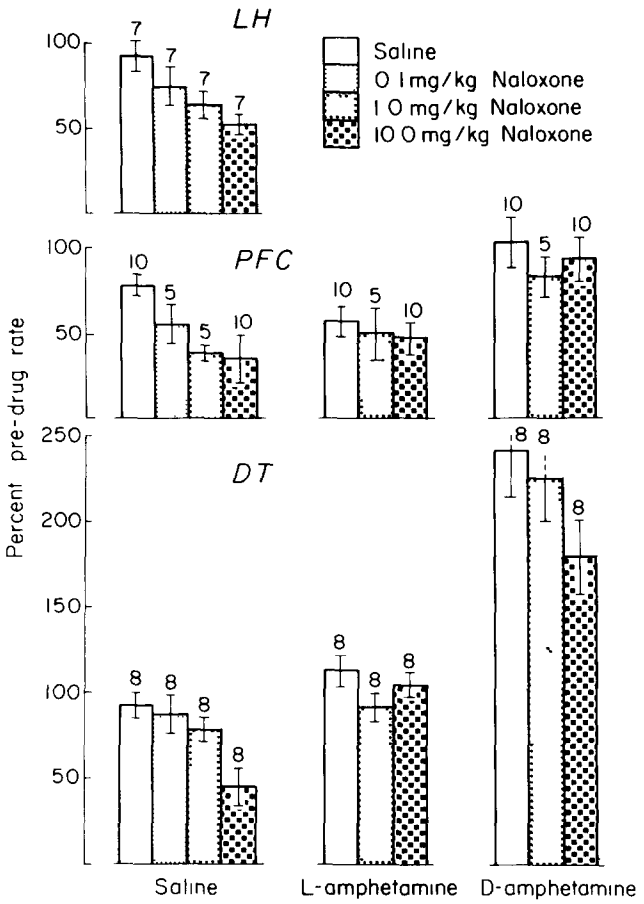

FIG 2 Self-stimulation responding after saline or naloxone (0 1, 1 0 or 10 mg/kg) given alone (left side of figure) or in combination with 1 0 mg/kg L- or D-amphetamine (middle and right side of figure) Electrodes were implanted in lateral hypothalamus (LH-top), medial prefrontal cortex (PFC-middle), or dorsal tegmentum (DT-bottom) Vertical bars represent standard errors, number above bars represent n per group

trodes and all seven rats with LH electrodes were also tested with another combination of drug treatments methysergide, a serotonin antagonist, alone and in combination with D- and L-amphetamine These data have been reported elsewhere [5] The additional drug treatments were delivered in different orders for each rat, and one week separated any tests with different drugs

Data Analysis

Response rates for the 60 min under a drug condition were expressed as a percentage of the baseline rates in the 25 min preceding drug administration The data were then analyzed by repeated measures design ANOVAS followed by Newman-Keuls tests for the comparisons of interest

RESULTS

Effects of Naloxone on SS

It can be seen in Fig 2 that naloxone administered alone depressed self-stimulation in a dose-dependent manner (naloxone main effect, $F(3,51)=10\ 57$, $p<0\ 0001$), and that the amount of depression was very similar in the three sites In both control and drug sessions the response rate of PFC self-stimulators declined more than the rates of DT or LH self-stimulators so that the mean PFC rate over the last 60 min of the session was below the pre-injection baseline rate Because of this there was a significant main effect of site, $F(2,17)=7\ 17$, $p<0\ 006$ However, there was no interaction between naloxone and site, $F(6,51)=0\ 72$, NS

Effects of D- and L-Amphetamine Alone and in Combination with Naloxone on SS

The effects of amphetamine alone depended on both isomer and the site of stimulation, $F(1,16)=8\ 1$, $p<0\ 01$ That is, D-amphetamine facilitated PFC self-stimulation ($p<0\ 05$) but L-amphetamine did not D-amphetamine caused a 149% increase in self-stimulation of the DT ($p<0\ 01$) whereas L-amphetamine only produced a 20% increase ($p<0\ 05$)

There was a significant interaction of naloxone, amphetamine and site of stimulation, $F(1,16)=5\ 7$, $p<0\ 03$) Naloxone combined with D- or L-amphetamine did not significantly alter PFC response rates compared to the effects of D- or L-amphetamine alone Similarly, L-amphetamine combined with naloxone did not alter responding in DT rats compared with L-amphetamine alone However, naloxone (10 mg/kg) added to D-amphetamine in DT rats caused a suppression of the D-amphetamine effect ($p<0\ 05$)

DISCUSSION

Naloxone produced a dose dependent reduction in SS at all three sites tested This result is in agreement with some other reports that naloxone depresses self-stimulation [2,19] However naloxone was as effective in depressing SS of the PFC which contains little enkephalin as it was in the DT where there are many enkephalin fibers [17] A similar lack of reliable association between the presence of enkephalin in the SS site and the effects of naloxone on self-stimulation can be seen in comparing the results of previous studies Belluzzi and Stein [2] found that naloxone (1 and 10 mg/kg) severely (60%) depressed SS of the periaqueductal gray but Stapleton *et al* [19] found periaqueductal gray SS to be only mildly depressed (9%) by 10 mg/kg naloxone, less so than other sites such as the substantia nigra There is also no relationship between the effect of naloxone and sites for stimulation analgesia Self-stimulation of the dorsal tegmentum and periaqueductal gray, sites which support analgesia ([2,19], Abbott and Melzack, personal communication) is no more affected by naloxone than SS of the PFC or SN, which do not show analgesia ([19], Abbott and Melzack, personal communication)

Naloxone did not modify the effects of amphetamine in any simple way No effect of naloxone was seen when SS was mildly facilitated by L-amphetamine in DT self-stimulators or by D-amphetamine in PFC self-stimulators In these cases, the depressant effect of naloxone was antagonized by administration of amphetamine However, when SS of the DT was greatly increased by D-amphetamine, naloxone did reduce responding Thus the effect of naloxone on amphetamine facilitated SS seems to be related to the effectiveness of amphetamine in facilitating SS rather than to the DA releasing effect of the amphetamine isomers, in which D- is 2–5 times as potent as L-amphetamine [7,8]

Similarly, Holtzman [9] found that naloxone was most effective against the dose of D-amphetamine which produced the maximum facilitation

In summary, naloxone seems to depress SS when sessions are long [19] or rewards are intermittent (this paper) but to have unreliable effects when brain stimulation rewards are frequent and strong enough to produce vigorous responding [9,20] The depression of SS can be overcome by amphetamine when amphetamine produces mild facilitation of responding but curiously re-appears when amphetamine ([9], this paper) or other facilitatory drugs [12] would increase SS to very high rates This pattern of results suggests that naloxone influences SS indirectly via opiate receptors in some system which primarily modulates performance of SS rather than the SS reward itself

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