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Effects of *d*-Amphetamine and Naloxone on Brain Stimulation Reward

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Abstract. Self-stimulation thresholds were determined in rats by means of a modification of the psychophysical method of limits. Reinforcement values were determined after the administration of *d*-amphetamine alone, naloxone alone, and naloxone administered concurrently with *d*-amphetamine. *d*-Amphetamine yielded dose-related decreases in the threshold (0.25–2.00 mg/kg IP), while naloxone alone (2.0–16 mg/kg IP) caused no consistent changes. For each animal, a dose of *d*-amphetamine that substantially lowered the threshold was then selected to be administered with varying doses of naloxone. The threshold-lowering effect of *d*-amphetamine was blocked by naloxone at doses as low as 2.0 or 4.0 mg/kg. This finding suggests the possible involvement of an opiate receptor in the mediation of the enhancement by *d*-amphetamine of brain stimulation reward.

Key words: ICSS – *d*-Amphetamine – Naloxone – Enkephalin – Reward

Administration of low to moderate doses of amphetamine significantly lowers the reinforcement threshold level necessary for maintaining intracranial self-stimulation (ICSS) in the rat (Cassens and Mills, 1973; Schaefer and Holtzman, 1979; Stein, 1962) and in the squirrel monkey (Spencer and Revzin, 1976). Although a number of stimulus parameters can be varied within the ICSS paradigm (e.g., frequency, pulse width), the threshold determinations in these studies were based on variations in current intensity. Pharmacological analysis of this drug's facilitative effects on ICSS has indicated a critical role for the central catecholamines (Stein, 1964), with recent work highlighting the importance of dopamine in particular (Phillips and Fibiger, 1978). Currently there is interest in discerning the anatomical and possible functional relationships between central catecholamine neurons and the endogenous opioid peptides. This interface has recently been extended to the area of brain stimulation reward, where preliminary evidence has led to the hypothesis that the enkephalins, in conjunction with the catecholamines, may function as natural reward-mediating substrates (Belluzzi and Stein, 1977). To examine a possible catecholamine-endogenous opioid involvement in the mediation of central reward processes, the present study was undertaken to assess the separate and combined effects of amphetamine and the opiate antagonist naloxone on brain stimulation reward thresholds in rats.

Materials and Methods

Subjects. Adult male albino Fischer rats (Charles River Breeding Laboratories), weighing approximately 300 g, were stereotaxically implanted with bipolar stainless steel electrodes (0.0127 cm in diameter and insulated except at the tips). The electrodes were aimed at brain areas that would support ICSS behavior (see below). The animals were singly housed in stainless steel cages and provided continuous access to food and water. The colony room was automatically illuminated on a 12-h light-dark cycle. The weight of each animal was monitored daily during the course of the experiment.

Surgery. Prior to surgery the animals were anesthetized with Equi-Thesin (0.3 ml/100 g body weight). The electrodes were aimed at the medial forebrain bundle (MFB) at the level of the lateral hypothalamus or the ventral tegmentum (VT) at the level of the interpeduncular nucleus. With the skull leveled between bregma and lambda the coordinates were as follows: MFB, 4 mm posterior to bregma, ±1.4 mm from the midline suture, and 8.5 mm ventral from the skull surface; VT, 2 mm anterior to lambda, ±1.4 mm from the midline suture, and 8.0 mm ventral from the skull surface. The electrodes were placed through small burr holes in the skull surface and attached permanently to the surface with an acrylic platform.

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Post-surgery the rats were injected IM with 60,000 units of penicillin (Bicillin), and then given 1 week for post-operative recovery before behavioral testing.

Apparatus. The animals were trained on a threshold procedure in a Plexiglas chamber (20 × 20 cm). Mounted in an opening on one wall of the chamber was a cylindrical wheel manipulandum, which was 16 cm long and 7.5 cm in diameter. Four equally spaced cams were positioned on one of the end plates such that they operated a microswitch when the wheel was rotated. Reinforcement was obtained only after closure of the microswitch (one-quarter of a wheel turn). A constant current stimulator (designed and built in our laboratory by Henry Appleton and Paul Miller and similar to those commercially available by Sunrise Systems, North Scituate, MA, U.S.A.) was used to deliver the stimuli which consisted of half-second trains of biphasic symmetrical pulses. Each train occurred at a frequency of 160 Hz, with a pulse width of 0.2 ms, and a delay of 0.2 ms between the positive and negative pulses. Pulse amplitude was varied according to the procedural requirements for threshold determination. Current was periodically checked with an oscilloscope to insure constancy.

Procedure. Determination of the threshold involved a discrete trial procedure. A trial began with the delivery of a noncontingent 0.5 s pulse train. A response within 7.5 s of this stimulus resulted in immediate delivery of a contingent stimulus, identical in all parameters to the noncontingent stimulus, and terminated the trial. Failure to respond had no scheduled consequences and the trial terminated after 7.5 s. Intervals between trials varied (average 15 s, range 7.5 – 22.5 s). Responses during the intertrial interval resulted in a 15-s postponement of the next trial. The initial noncontingent stimulation thus served both as a discriminative stimulus indicating availability of response contingent stimulation and as a comparative stimulus in the sense that it was a predictor of the parameters of the contingent stimulus. Stimulus intensities were varied according to the classical method of limits with slight modification. Stimuli were presented in alternating descending and ascending series with a step size of 5 or 10 μA (depending upon the individual animal's discriminative capabilities), with a number of trials presented at each step or level. Subjects completed four series (i.e., two ascending and two descending) pre-injection and then four series post-injection, with the entire session lasting 1.5 – 2 h. For further details on this procedure see Esposito and Kornetsky (1977). All experimental events and data collection were collected and stored by an on-line microcomputer.

Animals were run on the above procedure until stable threshold values were obtained, whereupon saline injections were initiated. When the threshold value did not vary more than ± 10 μA each session and where there was no day-to-day trend in either direction, the threshold was considered stable. After the animals had received saline injections for a number of days (at least five in succession), drug injections were initiated. Saline injection days were always interspersed between each day of drug treatments. The sequence of drug treatments was amphetamine, followed by naloxone, and then the two administered concurrently. In the latter phase of the experiment a single dose of amphetamine was tested with a range (2 – 16 mg/kg) of doses of naloxone. The dose of amphetamine selected was one that clearly lowered the threshold in the respective animal. To assess the effects of repeated naloxone injections alone, three separate animals were tested with amphetamine, following which they were tested, receiving daily naloxone (16 mg/kg) injections alone for 5 consecutive days, and then were retested with amphetamine.

After completion of all behavioral testing the animals were killed with an overdose of anesthesia (Equi-Thesin) and perfused intracardially with saline and then formalin. The brains were subsequently removed from the skull, fixed, embedded, and sliced at 40 μ. Mounted sections were stained with cresyl violet plus Luxol Fast blue and subsequently examined under a light microscope to determine electrode placements.

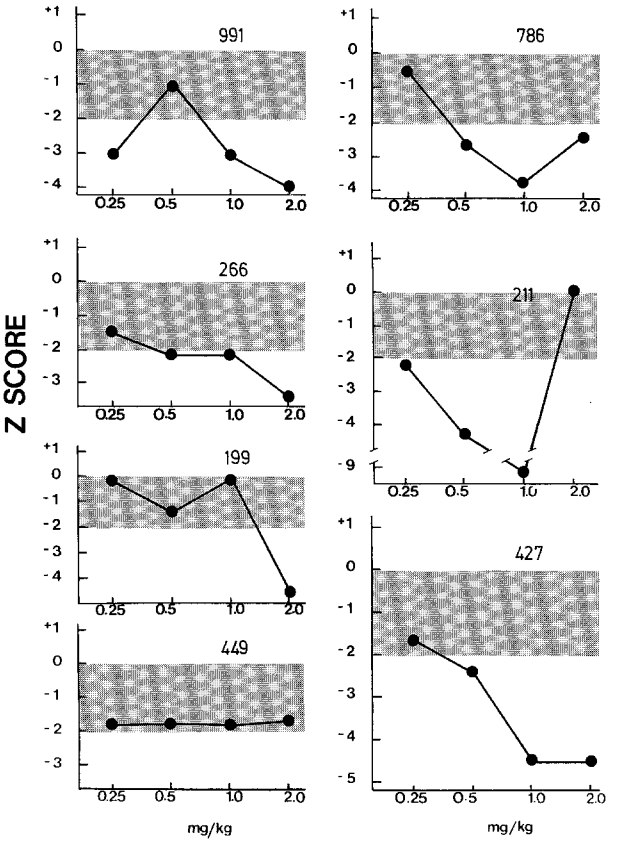

Fig. 1. Standard score (*Z* score) changes in threshold values from pre- to post-drug as a function of dose of *d*-amphetamine for each of seven animals

Drugs. The drugs employed in this study were *d*-amphetamine sulfate and naloxone HCl. These agents were dissolved in a 0.9% saline vehicle, and injected IP in a volume of 1.0 ml/kg. When the two drugs were given together the naloxone was injected first, followed 2 min later by the amphetamine injection. The sequence of doses was randomly arranged for each animal.

Analysis of Data. The subjects were run for four series pre-injection, and four series post-injection. Threshold values were calculated for both the pre-drug and post-drug session, with the difference between these two scores taken as the critical dependent measure. All the change (difference) scores were transformed to standard scores (*Z* scores) to make comparisons between drug-change scores and the distribution of change scores seen following saline injections. A *Z* score of 2.0 ($P < 0.05$) was pre-selected as the level of significance.

Results

The results are summarized in Figs. 1 – 3. Figure 1 displays the effects of 0.25 – 2.0 mg/kg amphetamine. All the subjects showed pronounced reductions in the reinforcing thresholds with maximal effects occurring at 1 – 2 mg/kg.

Figure 2 shows the results obtained after the administration of naloxone. There is little evidence for any

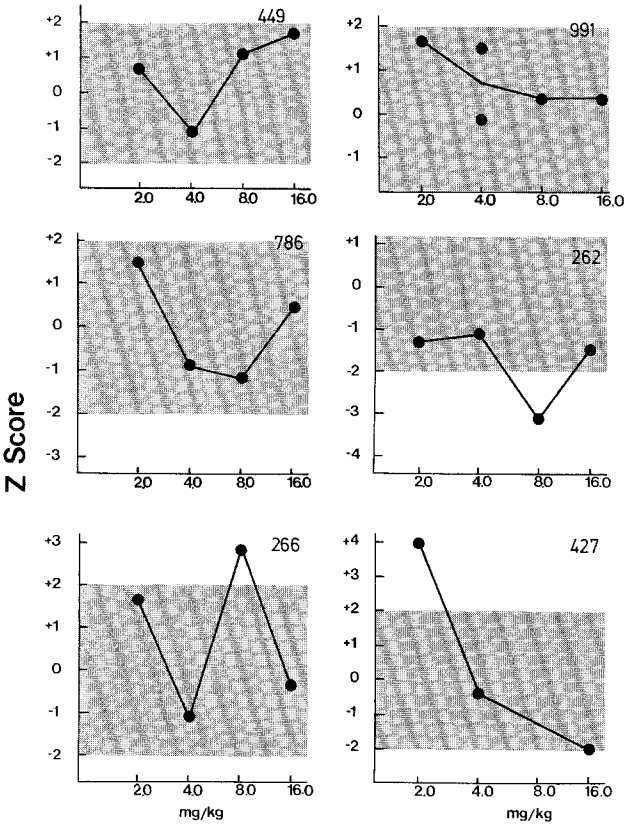

Fig. 2. Standard score (Z score) changes in threshold values from pre- to post-drug as a function of dose of naloxone for each of six animals

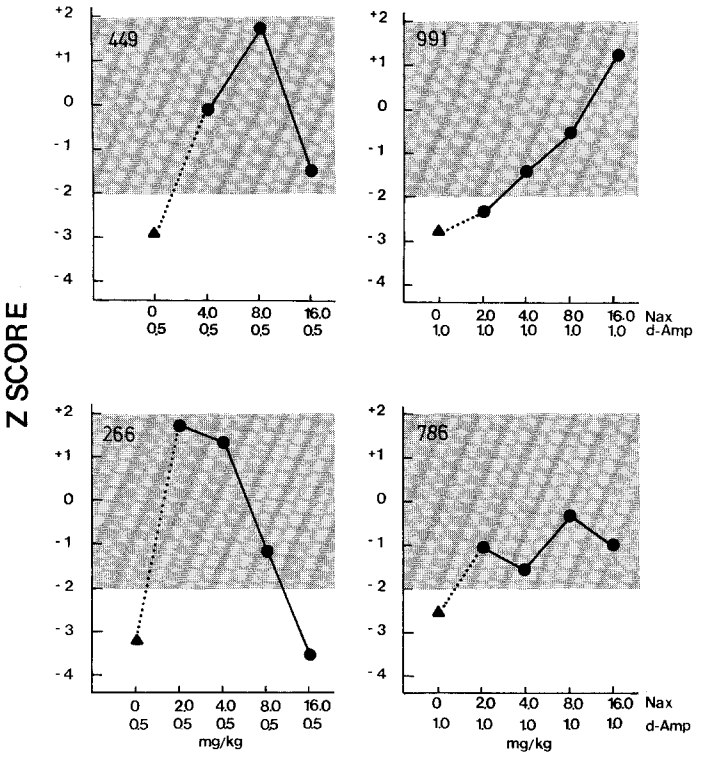

Fig. 3. Standard score (Z score) changes in threshold values from pre- to post-drug as a function of naloxone concurrently administered with a fixed d-amphetamine in each of four animals. The effects of d-amphetamine alone in each animal is indicated (▲) on the left. The smaller N was due to the loss of the electrode platform in three of the original seven animals

consistent or significant effects. However, there were two instances (rat 262 at 8 mg/kg and rat 427 at 16 mg/kg) where there were significant threshold reductions, and two instances (rat 266 at 8 mg/kg and rat 427 at 2 mg/kg) of significant increases.

Figure 3 shows the effects of various doses of naloxone given concurrently with a threshold-lowering dose of amphetamine. Naloxone clearly attenuates the amphetamine effect; however, except for rat 991, this interaction does not seem to be monotonically dose-related. The three control animals who received repeated naloxone injections (16 mg/kg) for 5 days subsequently displayed a typical lowering response to amphetamine when the latter was administered alone. It is important to note that repeated (5 days) injections of naloxone did not alter this agent's effects on the reward level.

Histological analysis showed animals 786, 427, 449, and 199 to have electrode tips within the dorsal aspect of the MFB at the level of the zona incerta. Animals 262, 266, and 991 had their electrode tips approximately 0.5 mm dorsal to the ventral tegmental nucleus, lateral to the substantia nigra, and medial to the medial lemniscus (266).

Discussion

The results obtained with amphetamine alone confirm previous reports that this drug reduces the reward threshold for ICSS (Cassens and Mills, 1973; Schaefer and Holtzman, 1979; Stein, 1962). The literature is ambiguous concerning the effects of naloxone on measures of response rate for ICSS. Belluzzi and Stein (1977) have reported that naloxone will produce dose-related decreases in rat's rate of responding for ICSS to the mesencephalic central gray, and a number of other enkephalin-rich sites in the brain (Stein and Beluzzi, 1978). Other investigators, however, have failed to detect significant effects of naloxone on this behavior (Holtzman, 1976; van der Kooy et al., 1977; Lorens and Sainati, 1978; Wauquier et al., 1974). Our earlier work (Kornetsky et al., 1979) and the present findings support these latter studies, arguing against the nec-

essity for postulating a tonically active endorphinergic system for maintaining ICSS. Our failure to find significant or consistent effects with naloxone cannot be attributed to the absence of enkephalin cell bodies or fibers at the site of stimulation, since the ventral tegmental area near the interpeduncular nucleus shows moderate to high enkephalin immunofluorescence (Uhl et al., 1979), and the dorsal aspect of the medial forebrain bundle has been shown to contain axons with enkephalin-like immunoreactivity (Jacobowitz et al., 1979). However, we did note some instances where a single dose of naloxone in a particular subject produced large threshold increases or decreases. Further investigation is needed to determine if naloxone can, under certain conditions, influence ICSS thresholds.

Naloxone's attenuation of amphetamine's reward-enhancing action cannot be unequivocally interpreted. The lack of effect of naloxone, when given alone, would tend to indicate a specific antagonism of amphetamine's effect. However, the absence of clear dose-response data for their interaction makes it difficult to assume that the antagonism is mediated by competitive mechanisms at a common receptor. Holtzman (1976) has reported that naloxone will attenuate the rate-increasing action of amphetamine on ICSS behavior in rats. However, comparisons of these results with the present findings should be viewed with caution, particularly in view of the recent study by Schaefer and Holtzman (1979), which demonstrated that amphetamine's effects on ICSS reinforcement threshold are not consistently related to its effects on response rates for ICSS in rats. Threshold measurements reflect specific changes in the reinforcing value of the stimulation while response rate measures tend to reflect any drug-induced changes (specific or non-specific) on performance. However, Holtzman (1976) also reported the interaction between amphetamine and naloxone to be qualitatively different than that observed between morphine and narcotic antagonists and, therefore, not consistent with a competitive type of antagonism.

A number of researchers have noted significant effects of naloxone on other amphetamine-induced behaviors. Holtzman (1974) reported that otherwise inactive doses of naloxone consistently and significantly reduced the stimulant effects of amphetamine on avoidance responding and locomotor activity in the rat. Dettmar et al. (1978) reported that naloxone antagonized both amphetamine-induced locomotor activity, and amphetamine-induced ipsilateral turning in unilateral 6-hydroxydopamine lesioned mice. In contrast Haber et al. (1978) reported that naloxone did not alter amphetamine-induced hyperactivity or stereotypy, but did selectively block rearing behavior in rats. Adam-Carrière et al. (1978) reported that naloxone, at otherwise inactive doses (1–10 mg/kg), antagonized the rate-increasing effects of amphetamine on rat's responding for food, while the same doses of naloxone reversed the rate-decreasing effects of morphine.

Viewing the results of our work together with the findings of others it is clear that naloxone has significant effects on a number of amphetamine-induced behaviors, although the mechanism underlying these interactions is not yet clear. However, in light of the probable critical role of dopamine (Phillips and Fibiger, 1978) and the mesolimbic projection system (Koob et al., 1977) in mediating the reward-enhancing action of amphetamine on brain stimulation reward, it would be intriguing to test the hypothesis that naloxone's effects on the reward enhancement may be related to its occupation of opiate receptors located presynaptically on dopamine neurons in the mesolimbic system (Pollard et al., 1977b). It has been suggested that these presynaptic receptors may function to modulate dopamine synthesis and release (Pollard et al., 1977a; Schwartz et al., 1978), and further, it has been demonstrated that intraventricular injections of methionine enkephalin stimulate striatal dopamine synthesis in a manner reversible by naloxone (Biggio et al., 1978). Such modulation by enkephalin neurons could underlie the behavioral effects we have described.

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