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The Combined Effects of Morphine and d-Amphetamine on the Threshold for Brain Stimulation Reward

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HUBNER, C. B., G. T. BAIN AND C. KORNETSKY. *The combined effect of morphine and d-amphetamine on the threshold for brain stimulation reward.* PHARMACOL BIOCHEM BEHAV **28**(2) 311–315, 1987.—The effect of morphine and d-amphetamine co-administration on the threshold for rewarding intracranial electrical stimulation was studied in rats with electrodes stereotaxically implanted in the medial forebrain bundle-lateral hypothalamic or ventral tegmental area of the brain. Thresholds were determined by means of a rate-independent psychophysical method. Individually, morphine and d-amphetamine both caused a dose-related lowering of the reward threshold. Low doses of morphine or d-amphetamine which were ineffective or minimally effective in lowering the reward threshold were then tested with various doses of either d-amphetamine or morphine, respectively. In both cases, the combined administration of morphine and d-amphetamine resulted in a lowering of the reward threshold that was greater than for the corresponding doses of morphine and d-amphetamine when given alone. Given that increased sensitivity for rewarding brain stimulation has been suggested to be an animal model of drug-induced euphoria, this effect is congruent with the reported increase in the degree of euphoria produced when amphetamine is used in conjunction with opiate drugs.

Brain stimulation reward Morphine d-Amphetamine Threshold determination

DUE in part to their ability to produce profound euphorigenic effects, the opiates and the central nervous system stimulants are two classes of drugs which are widely abused. While a significant degree of euphoria is associated with the administration of these substances on an individual basis, polydrug abuse between these drug classes has also been reported. Based on clinical reports it has been concluded that addicts self-administer psychomotor stimulants in combination with opiate drugs as a means of maximizing the euphoria caused by the narcotic drug [5,14]. Amphetamines, for example, are used by opiate abusers in an attempt to potentiate the high obtained from methadone or poor quality heroin [4,13]. In a study conducted under controlled experimental conditions, subjects administered various combinations of morphine and d-amphetamine reported that the degree of euphoria they experienced following the co-administration of these substances was greater than for either drug alone [16]. In addition to the enhanced euphoria produced by these drug combinations, animal [23,24] as well as clinical [11] studies have demonstrated that the analgesic efficacy of morphine is also increased by the concomitant administration of d-amphetamine.

The purpose of the present study was to investigate the enhanced euphorigenic effects reported for opiate and psychomotor stimulant combinations by testing the effect of the co-administration of morphine and d-amphetamine on brain stimulation reward. It has been suggested by us [18,19] as well as by others (e.g., [17,28]) that drugs of abuse activate those areas of the brain which are part of the reward system. Increased sensitivity for rewarding brain stimulation, measured in our laboratory as a lowering of the reward threshold, has been used as an animal model of drug-induced euphoria and is thought to be predictive of abuse liability in man. Individually, morphine [19] and d-amphetamine [8] have been shown to significantly lower brain stimulation reward thresholds at appropriate doses. Using a two-lever reset brain stimulation threshold procedure it was reported [26] that the combined administration of d-amphetamine and morphine lowered the reset value.

The specific aim of the research was to determine if ineffective or minimally effective threshold lowering doses of morphine when combined with various doses of d-amphetamine would produce an effect on threshold greater than that seen when the corresponding doses of morphine and d-amphetamine were given alone. The reverse combination was also tested, in that, non-effective threshold lowering doses of d-amphetamine were combined with various doses of morphine and the resulting threshold changes were com-

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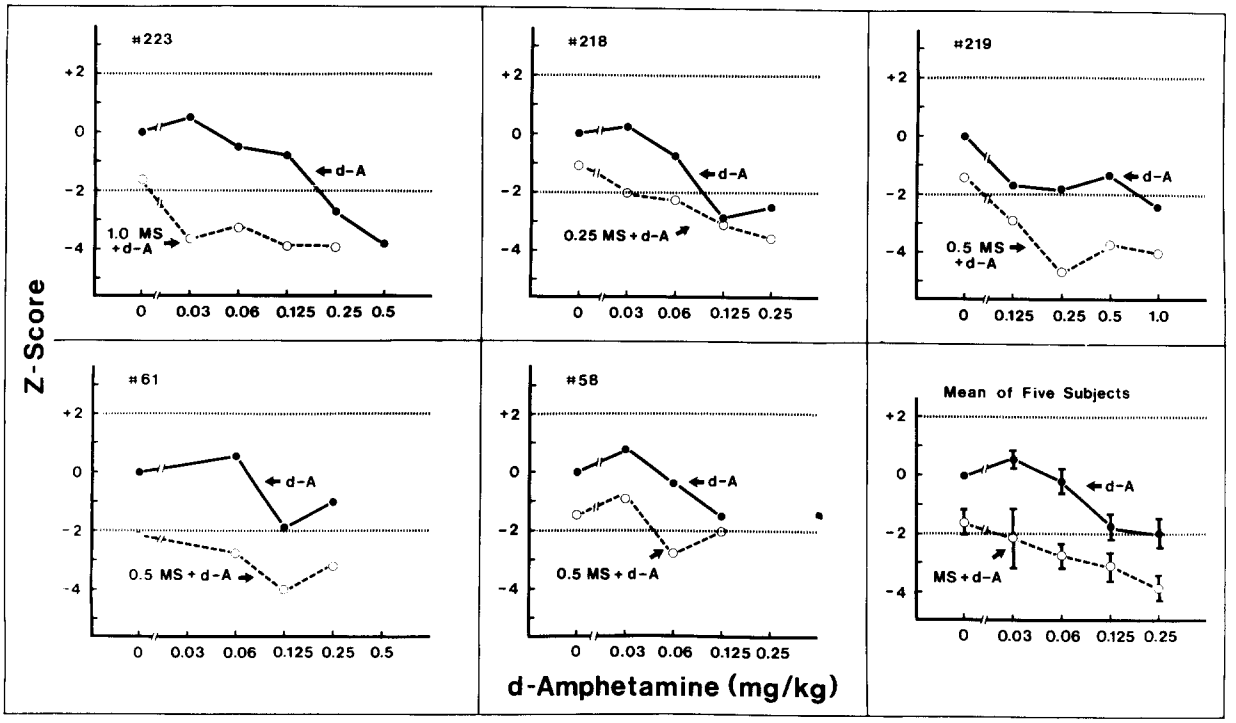

FIG. 1. Standard score (z-score) changes in reward threshold value from pre- to post-drug as a function of dose for each of five animals. The effect of d-amphetamine alone is represented by the solid line. The effect of morphine alone and in combination with various doses of d-amphetamine is represented by the dotted line. A z-score of ±2.0 indicates the 95% confidence limits. Mean z-scores for the five animals are also shown in the lower right corner. Note: the N contributing to the mean at any dose varied since not all animals were tested at each dose. Also, the SEM is only indicated if three or more animals were tested at a particular dose.

pared with those obtained for either drug alone. Given the clinical reports concerning the euphorigenic effects produced by d-amphetamine in combination with opiate drugs, it would be expected that a potentiation should be seen in both drug treatment schedules.

EXPERIMENT 1

Method

Five male albino rats (F-344 Charles River Laboratories), weighing approximately 300 g, were anesthetized with Equi-Thesin® (0.9 ml) and bipolar stainless steel electrodes (0.13 mm in diameter) (Plastic Products, Roanoke, VA) were stereotaxically implanted with the electrode tips aimed for the lateral hypothalamic region of the medial forebrain bundle (MFB-LH coordinates with the dorsal surface of the skull level to the horizontal: 4.0 mm posterior to bregma, 1.4 mm lateral from the midline suture, and 8.5 mm ventral to the skull surface). The electrodes were placed through small burr holes in the skull and attached permanently to the surface with an acrylic platform. After surgery, animals received 60,000 units of penicillin (Bicillin®) IM and were given at least one week for post-operative recovery before behavioral testing was begun. Animals were maintained on a 12 hour light/dark cycle, singly housed in standard stainless steel cages and had ad lib access to food and water.

Animals were trained and tested in an acrylic chamber (20×20 cm). A cylindrical manipulandum (7.5×15 cm) was located within one wall of the test chamber. Four equally spaced cams on one endplate of the manipulandum operated a microswitch which resulted in immediate delivery of a stimulation when the cylinder was rotated one-quarter of a turn. A constant current stimulator (Sunrise Systems, Pembroke, MA) was used to deliver the biphasic symmetrical pulses. Each stimulus consisted of a 500 msec train with a pulse width of 0.2 msec and a delay of 0.2 msec between the positive and negative pulses at a frequency of 160 Hz. Thresholds were determined by a rate independent procedure for determining the threshold for rewarding brain stimulation. This procedure has been previously described [6–8].

Animals required approximately 6 one hour training sessions to learn the task and approximately 4 additional sessions for the establishment of a stable threshold level whereupon intraperitoneal (IP) and subcutaneous (SC) vehicle injections were begun. Animals were tested with vehicle injections for 5 days before drug administration was initiated. Also, vehicle days were always interspersed between each day of drug treatment so that animals received drug only twice weekly. On control days, immediately after completion of the pre-injection test session, animals were given a SC injection of saline, followed 10 min later by an IP injection of saline and then the post-injection test session was begun. Morphine sulfate and d-amphetamine sulfate were dissolved in isotonic saline and administered SC and IP, respectively. When morphine was tested alone, a SC injection of morphine was followed 10 min later by an IP injection of saline and then testing began. When d-amphetamine was tested alone, a SC injection of saline was followed by an IP injection of d-amphetamine 10 min later. When the two drugs were given together, morphine was injected SC and followed

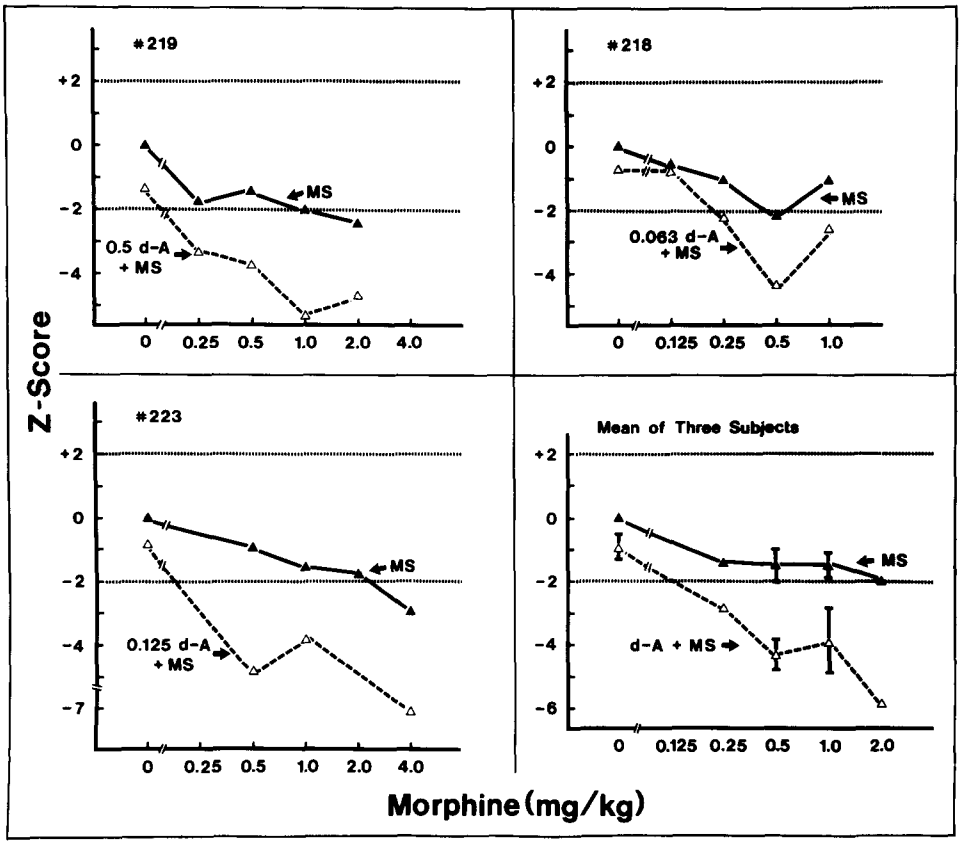

FIG. 2. Standard score (z-score) changes in reward threshold value from pre- to post-drug as a function of dose for each of three animals. The effect of morphine alone is represented by the solid line. The effect of d-amphetamine alone and in combination with various doses of morphine is represented by the dotted line. A z-score of ±2.0 indicates the 95% confidence limits. Mean z-scores for the three animals are also shown in the lower right corner. Note: the N contributing to the mean at any dose varied since not all animals were tested at each dose. Also, the SEM is only indicated if three or more animals were tested at a particular dose.

10 min later by an IP injection of d-amphetamine. All injections were in volumes of 1 ml/kg body weight and the sequence of doses was balanced between animals.

Threshold values were calculated for both the pre-injection and the post-injection sessions, with the difference between the two scores taken as the dependent measure. These difference scores were transformed to standard scores (z-scores) based on the mean and standard deviation of the difference scores for all vehicle control days. A z-score of ±2.0 (95% confidence limits) was preselected as the level of significance.

Dose-effect curves were generated for both morphine and d-amphetamine alone. A dose of morphine which was non-significant or just significant in lowering the threshold for brain stimulation reward was then co-administered with various doses of d-amphetamine. This dose of morphine varied from animal to animal depending on an individual animal's sensitivity to the drug. Once again, difference scores from pre- to post-injection of the combination were converted to z-scores and were then compared to the z-scores obtained from d-amphetamine alone.

After completion of the behavioral testing the animals were killed with an overdose of Equi-Thesin® and perfused intracardially with saline. The brains were then removed from the skull, fixed, embedded and sectioned at 40 μ. Sections were stained with cresyl violet and Luxol fast blue and examined under a light microscope to determine the site of electrode placement.

Results

The results obtained with each of the five subjects as well as the mean effects are shown in Fig. 1, which shows the dose response curves for d-amphetamine alone and in combination with an ineffective or minimally effective threshold lowering dose of morphine. All subjects showed a lowering of the reward threshold for d-amphetamine with the effective doses varying from animal to animal. Pronounced decreases in threshold were seen when an ineffective or minimally effective dose of morphine was administered in combination with various doses of d-amphetamine.

Histological analysis revealed that the electrode tips in two of the subjects (Nos. 218 and 219) were within the MFB-LH area while in the other three animals (Nos. 58, 61 and 223) the electrodes were within the ventral tegmental area. There were no significant differences in results as a function of these two sites.

EXPERIMENT 2

Method

Three of the five animals (Nos. 218, 219 and 223) used in Experiment 1 were used as subjects in a second experiment.

The testing procedure and analyses were identical to that previously described except that in this experiment a dose of d-amphetamine which was ineffective in lowering the threshold for brain-stimulation reward was co-administered with various doses of morphine. The dose of d-amphetamine varied from animal to animal depending on the animal's sensitivity to the drug.

Results

The results obtained with each of the three subjects as well as the mean effects are shown in Fig. 2, which shows dose response curves for morphine alone and in combination with an ineffective or minimally effective dose of d-amphetamine that had no significant effect on the threshold. The results obtained from this experiment are similar to those from the first experiment. All subjects showed a lowering of the reward threshold for morphine with the effective doses varying from animal to animal. Combining various doses of morphine with a fixed dose of d-amphetamine resulted in a greater lowering of threshold.

GENERAL DISCUSSION

The results from this study are in accordance with clinical reports which find that a potentiation of the euphoria produced by opiates and psychomotor stimulants can be achieved by their co-administration [4,13]. Furthermore, these results suggest that the enhanced euphoria seen in man after the combined administration of morphine and amphetamine is due to their combined action on the neural substrate that defines the reward system.

It is currently believed that the reward system is mediated by the catecholamines, with particular emphasis on dopamine [10], and the endogenous opioid system [2]. Our findings that morphine [19] as well as the psychomotor stimulants d-amphetamine [8] and cocaine [7] will, at appropriate doses, lower the threshold for rewarding brain stimulation supports an opiate and catecholaminergic mechanism mediating reward. There is still considerable debate, however, concerning the relationship between these neural systems in mediating drug reinforcement. While the present study did not address the mechanisms of action responsible for the greater than additive lowering of the reward threshold resulting from the co-administration of morphine and d-amphetamine, this effect suggests that the reinforcing properties of the opiates and the psychomotor stimulants are, at some point, mediated by an interaction between the endogenous opioid and catecholamine systems. Evidence to support a complementary role between these neural systems in mediating reinforcement is the finding that naloxone [8], at doses which are ineffective in altering the reward threshold, will reverse the threshold lowering effects of d-amphetamine [8] and cocaine [1]. Furthermore, several studies have produced results implicating a role for dopamine in mediating the rewarding properties of the opiates. For example, various reports have determined that changes in morphine self-administration rates result following the administration of haloperidol, a neuroleptic which blocks dopamine receptors [12,15]. In the place preference paradigm, pretreatment with the neuroleptics, haloperidol [25,27] or pimozide [3], or the destruction of dopaminergic nerve terminals with 6-hydroxydopamine [25,27] can produce an attenuation of morphine- and heroin-induced place preference. Further evidence for an opiate interaction with a dopamine substrate in central reward is given by the finding that morphine administered systemically or microiontophoretically applied to the ventral tegmental area (VTA) caused excitation of the VTA dopamine neurons [20]. These findings, coupled with the localization of opiate receptors on presynaptic dopamine neurons in the mesolimbic system [22] are evidence for a complementary role between these two neural systems in mediating reinforcement.

There is also evidence, however, indicating that independent neural substrates mediate the reinforcing properties of opiate and central nervous system stimulant drugs. Koob and coworkers [9,21] have suggested that while central dopamine neurons are critical for mediating cocaine reinforcement, it is the activation of the endogenous opioid, but not the dopaminergic system that is responsible for the reinforcing properties of heroin. This conclusion is based on their findings that selective increases in cocaine and heroin self-administration are produced by the administration of the dopamine antagonist, alpha-flupenthixol, and the opiate antagonist, naltrexone, respectively [9] and that destruction of the dopamine terminals in the nucleus accumbens with 6-hydroxydopamine produces an attenuation in cocaine but not heroin self-administration [21].

The question of whether independent neurochemical substrates mediate opiate and psychomotor stimulant reinforcement or whether an interaction between the opiate and dopamine systems exists is still subject to further research. However, the present finding that greater than additive decreases in threshold were obtained with the co-administration of morphine and d-amphetamine suggests that some type of mechanistic interaction between the dopamine and endogenous opioid systems which defines reward is operating to mediate this effect.

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