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Selective D_1 and D_2 Dopamine Antagonists Decrease Response Rates of Food-Maintained Behavior and Reduce the Discriminative Stimulus Produced by Heroin

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CORRIGALL, W. A. AND K. M. COEN. *Selective D_1 and D_2 dopamine antagonists decrease response rates of food-maintained behavior and reduce the discriminative stimulus produced by heroin.* PHARMACOL BIOCHEM BEHAV **35**(2) 351–355, 1990.—Animals were trained to discriminate heroin from saline in a two-lever food-reinforced paradigm. Tests with the heroin metabolites O^6-monoacetylmorphine and morphine suggest that the heroin discriminative stimulus was mediated by monoacetylmorphine. The heroin discriminative stimulus was not blocked by pretreatment with low doses of the D_1 dopamine antagonist SCH23390 or the D_2 antagonist spiperone; higher doses of the antagonists produced decreases both in selection of the drug-appropriate lever after heroin, and in food-maintained responding. The data suggest that dopamine may mediate the heroin discriminative stimulus. When administered in the absence of opioids, the D_2 antagonist spiperone did not have rate-decreasing effects, whereas SCH23390 did. Heroin partially reversed the rate-decreasing effects of SCH23390, possibly as a result of the ability of opioids to release dopamine.

Dopamine antagonist Discriminative stimulus Heroin Monoacetylmorphine Morphine

IT is evident from biochemical studies that opioids can modulate the function of dopaminergic neurons in the brain (16). In addition, there is evidence that dopamine is the neurotransmitter involved in some of the behavioral effects of opioids. For example, increases in locomotor activity following low doses of opioids appear to have both a dopaminergic as well as a dopamine-independent substrate (6). With respect to the rewarding effects of opioids, some studies have shown that conditioned reinforcement, as measured by place preference procedures, is reduced by treatment with dopamine antagonists such as haloperidol and pimozide or by 6-OHDA lesions at the level of the nucleus accumbens (2,12), or is mediated by neuroanatomical sites in the vicinity of the A10 dopamine-containing neurons of the midbrain (1). However, other research has shown that a morphine-conditioned place preference is insensitive to neuroleptic treatment (10), and that heroin self-administration is not altered by dopamine antagonist treatment (4).

Related to the question of a role for dopamine in opioid effects is the possibility that the transmitter underlies in part the discriminative stimulus effect of this class of drugs. The current experiments examined this hypothesis using animals trained to discriminate heroin from saline in a standard two-lever, food-reinforced paradigm, and tested with selective D_1 and D_2 dopamine antagonists.

METHOD

Subjects were 10 male Long-Evans rats, 280–300 g at the time that training procedures were begun. Animals were housed in a reversed light-dark cycle (lights off between 7:00 and 19:00) with ad lib access to food and water. Once habituated to the animal colony, animals were food deprived prior to operant training, and then maintained for the duration of the experiment on approximately 20 g of food per day (total intake, i.e., food pellets received in operant sessions plus a single meal of laboratory rat chow several hours after operant sessions, to yield a daily food allotment of approximately 20 g).

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Operant sessions were carried out in chambers equipped with two levers. Animals were initially trained to press the left-hand lever for food pellets (45 mg size) on a continuous reinforcement schedule. Schedule requirements were increased in steps to a final value of FR10, with a session duration of 15 min. Discrimination training began at this stage of the experiment. During training, animals were given a subcutaneous injection of either saline (1 ml/kg) or heroin hydrochloride (0.3 mg/kg) 30 min before each operant session. Animals were trained to press the left lever after saline injections and the right lever after heroin injections. Choice of saline or heroin injection was made according to a predetermined fixed sequence which repeated every 4 weeks. To control for possible olfactory cues, consecutive subjects running in the same chamber received opposite training injections on some days and the same training injections on other days. That is, two different injection sequences, which alternated every four weeks, were used: DSSDS, SDSDD, SDDSS, DSDSD, and SDDSS, DSDSD, DSDDS, SDSDS, where "S" represents a saline injection and "D" as drug injection. Stimulus lights were not used, except for a house-light which indicated that the session had commenced, and which flashed briefly following completion of the FR10 ratio on the correct lever. Sessions occurred once each weekday. Schedule requirements were such that responding on the incorrect lever reset the response requirement for the correct lever, i.e., the subject was required to complete an uninterrupted FR10 on the correct lever. Training continued until, for a period of 2 weeks, no more than 2 incorrect responses occurred before the delivery of the first food pellet and, in addition, at least 90% of the total responses in the session were made on the correct lever. When these conditions were met, testing procedures were begun.

Test days occurred on Tuesdays and Fridays, subject to sustained training criteria on intervening days. On test days, both levers were active, and every FR10 on either lever, uninterrupted by responding on the other lever, resulted in the delivery of a food pellet. Animals which failed to meet the training requirements during the test phase were not tested further until their training data returned to criterion levels for one week.

There have been no previous reports in which heroin was used as training drug in discrimination procedures. Therefore, the first tests carried out in these animals were to determine the dose-effect curve for the heroin discriminative stimulus, and to compare it with those for O^6-monoacetylmorphine (the first metabolite of heroin) and for morphine (the second metabolite). The following doses (as the salt) were tested: for heroin hydrochloride, 0.03, 0.10, 0.30, 0.60, and 1.00 mg/kg; for monoacetylmorphine hydrochloride, 0.10, 0.30, 0.60, and 1.00 mg/kg; for morphine sulphate 0.10, 0.30, 1.00, 3.00, and 6.00 mg/kg. For comparison of the data from these opioids, values were plotted against the drug dose expressed in nanomoles/kg.

Dopamine antagonists were tested over a dose range of 0.001 to 0.10 mg/kg (doses expressed as the base) in half-logarithmic steps. The D_1 antagonist used was SCH23390 hydrochloride, the D_2 was spiperone. Due to their different onsets and durations of effect, the antagonists were injected at different times; SCH23390 was injected subcutaneously 30 min before heroin administration (and therefore 1 hour before the drug discrimination session), and spiperone was injected subcutaneously 1 hour before heroin (90 min presession). The effects of the full range of doses of the dopamine antagonists were examined at the heroin training dose of 0.30 mg/kg. In addition, a more limited dose range of antagonists was tested on discrimination of 0.10 mg/kg heroin and saline.

The following drugs were used: heroin hydrochloride (Ward Robertson Chemicals, Toronto, Ontario), O^6-monoacetylmorphine hydrochloride (Bureau of Dangerous Drugs, Ottawa, Ontario), morphine sulphate (B.D.H.), SCH23390 hydrochloride and spiperone (the two latter both from Research Biochemicals Inc.). Heroin and morphine were prepared each week in sterile saline. Monoacetylmorphine was prepared on the day of use; to prevent salting out, the hydrochloride salt was prepared in distilled water, and sufficient sodium chloride added to make an isotonic solution. Both SCH23390 and spiperone were prepared on the day of use; SCH23390 was dissolved in normal saline, and spiperone was dissolved in 0.1 ml of 0.1 N tartaric acid and diluted to the required volume. The vehicle control injections for spiperone consisted of the highest concentration of tartrate used; for SCH-23390, saline was used as the zero-dose test injection. All drugs were injected subcutaneously in a volume of 1 ml/kg.

Data are presented as the group mean of a single determination at each dose. For response rates, data from each subject were expressed as the percentage of its saline-control value before averaging. Data from animals which did not complete at least one FR10 during testing were not included in calculating the % drug-appropriate response score. The number of animals not completing one FR10 is indicated in the figure captions.

RESULTS

Dose-effect curves for heroin, morphine and monoacetylmorphine are shown in Fig. 1. For both drug-appropriate responding and response rates, the curves for heroin and monoacetylmorphine are virtually coincident. In contrast, the curves for morphine are in both cases shifted by approximately 1 logarithmic unit to the right. With respect to discriminative stimulus, the dose-effect curves for each of heroin and monoacetylmorphine were steep; for morphine, however, there was some intermediate responding produced at the 1 mg/kg dose (2990 nanomoles/kg) which resulted in a more graded curve. For heroin, the lowest dose which produced 100% drug-appropriate responding was 0.30 mg/kg; the lowest dose of morphine which produced complete drug lever selection was 3.0 mg/kg.

The effects of SCH23390 and spiperone pretreatments on discrimination of the heroin training dose and on response rate are shown in Fig. 2. Injections of vehicle (saline or tartrate) or of the three lowest doses of SCH23390 or spiperone had negligible effects either on selection of the drug-appropriate lever or response rate; drug-appropriate responding remained between 90% and 100%, and average response rate remained virtually at control values. The two highest doses of the dopamine antagonists did affect selection of the drug-appropriate lever; the highest dose of the antagonists in particular decreased the degree of drug-lever selection. In addition, response rates at the two highest doses of the antagonists were also decreased substantially, particularly at the 0.1 mg/kg dose of either SCH23390 or spiperone in combination with 0.30 mg/kg heroin.

As shown in the dose-effect curve of Fig. 1, the 0.10 mg/kg dose of heroin (246 nanomoles/kg) was discriminated as saline. At the time of testing with the dopamine antagonists, several animals had begun to select the drug-appropriate lever after the 0.10 mg/kg dose of heroin, producing scores for drug-appropriate responding between 30% and 40%. Pretreatment with either dopamine antagonist at doses of 0.01 or 0.03 mg/kg did not affect selection of the drug-appropriate lever (data not shown). In addition, pretreatment with these antagonist doses did not alter selection of the saline-trained lever following saline injections, except in the case of SCH23390, which at the 0.03 mg/kg dose caused 30% drug-appropriate responding after saline injections (data not shown).

In spite of the similarity of the effects of the antagonists on heroin or saline discrimination, the rate-decreasing properties of the two compounds were different from one another when administered alone, as well as in their interaction with heroin (Fig. 3).

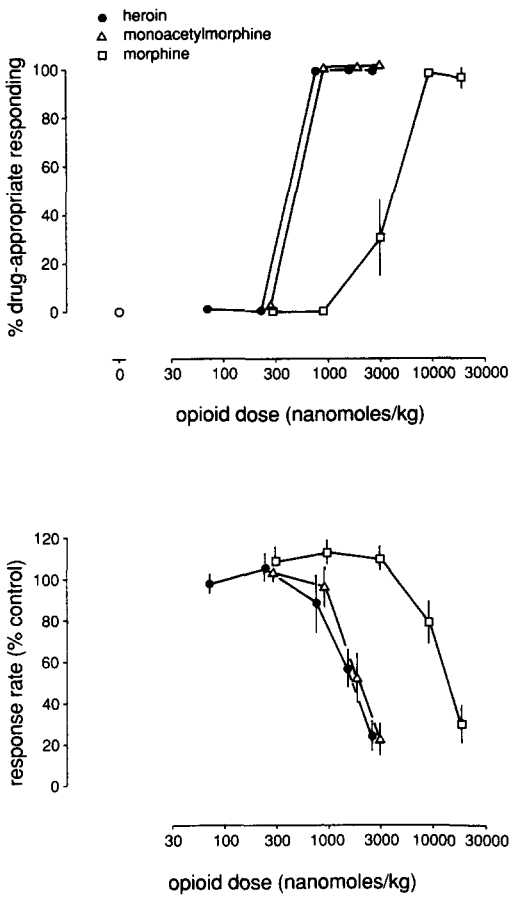

FIG. 1. Molar dose-effect curves. The upper graph shows selection of the drug-appropriate lever by heroin-trained animals after injections of heroin, monoacetylmorphine or morphine over a range of doses. The lower graph illustrates the response rate at the various test doses as a percent of the response rate after saline administration (average response rate after saline was 1.24 per second, with standard error of the mean of 0.07 per second). In both cases, note the similarity in the dose-effect curves for heroin and monoacetylmorphine, and the shift of the morphine curve to the right. The nanomoles/kg dose range used in this figure can be converted to mg/kg with the following values: 1 mg/kg heroin hydrochloride is equivalent to 2460 nanomoles/kg; 1 mg/kg monoacetylmorphine hydrochloride is equivalent to 2880 nanomoles/kg; and 1 mg/kg morphine sulphate is equivalent to 2990 nanomoles/kg. At the highest doses of each opioid, some animals failed to complete even a single FR10, as follows: 3 animals at 2460 nanomoles/kg heroin; 2 animals at 17,944 nanomoles/kg morphine; and 1 animal at 1730 nanomoles/kg and 2 animals at 2880 nanomoles/kg monoacetylmorphine. In this and subsequent figures, error bars show plus and minus one standard error of the mean (SEM).

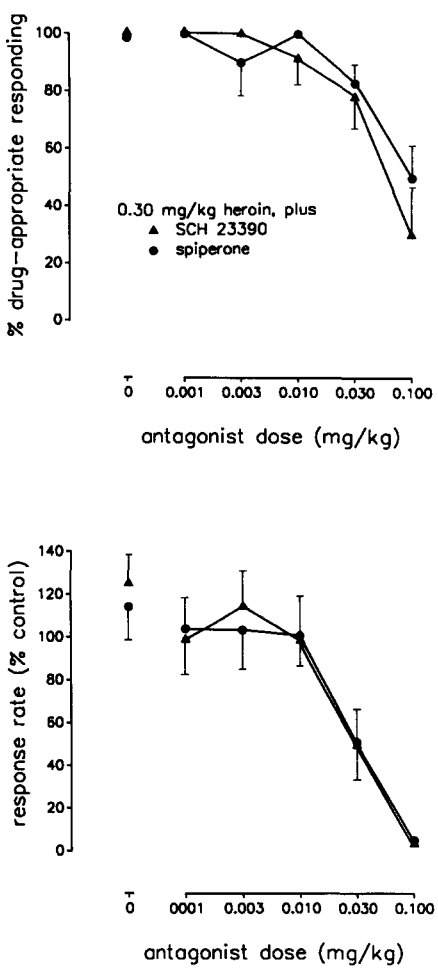

FIG. 2. Effect of a range of doses of the dopamine D1 antagonist SCH23390 and D2 antagonist spiperone on the heroin-trained discriminative stimulus (upper graph) and on response rate (lower graph) after 0.3 mg/kg heroin. At the highest dose of SCH23390, 6 animals failed to complete at least one FR10; 1 animal failed to do so after spiperone. Average response rate after saline for this and the next figure was 1.01 per second, with a SEM of 0.10 per second.

The D_1 antagonist SCH23390 was the more rate-decreasing of the two compounds as measured in operant behavior. In the absence of heroin, SCH23390 produced an almost complete abolition of food-maintained behavior at the 0.03 mg/kg dose. Spiperone, on the other hand, did not suppress response rates, even at the 0.03 mg/kg dose, when administered in the absence of heroin. However, in the presence of heroin, at least at the 0.30 mg/kg training dose, spiperone produced greater response decrement whereas the rate-decreasing effect of SCH23390 was partly attenuated.

DISCUSSION

These experiments demonstrate that heroin can be used as a training drug in discrimination procedures. Previous studies of the biotransformation of heroin to monoacetylmorphine and morphine (14), of opioid receptor binding of heroin and its metabolites (5), and of dose-response relations for heroin, monoacetylmorphine and morphine in tests of nociceptive, diarrheal and diuretic activity (13) have suggested that monoacetylmorphine is the active metabolite of heroin. The present study shows that equi-molar amounts of monoacetylmorphine or heroin produce the same discriminative stimulus and effects on response rate, whereas an approximately 10-fold greater amount of morphine is required to produce the same drug cue. These data are consistent with the idea that the active metabolite of heroin is monoacetylmorphine.

The compounds SCH23390 and spiperone are believed to be specific dopamine antagonists at the D_1 and D_2 receptor subtypes respectively, and have been employed in the dose range used here in a variety of behavioral pharmacological studies (8, 9, 17). The interest in whether these antagonists alter the discriminative stimulus properties of heroin derives from observations that some opioid effects appear to have a dopaminergic substrate.

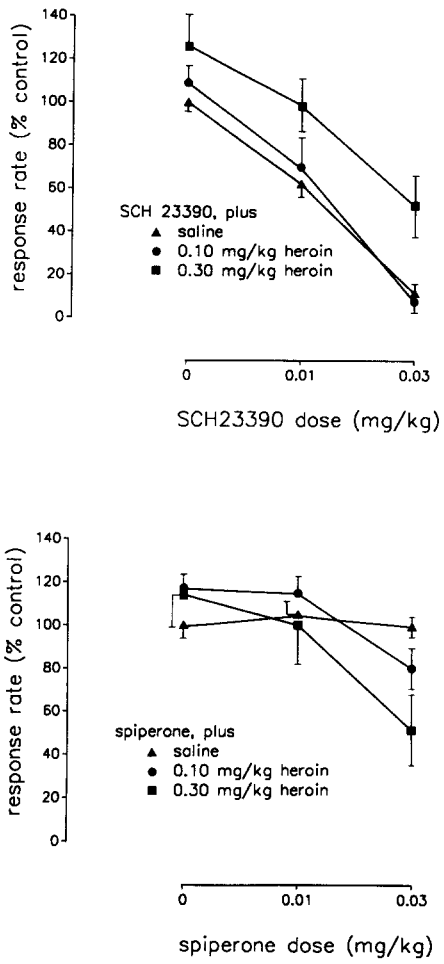

FIG. 3. Effects of the D1 and D2 antagonists on response rate as a function of antagonist and heroin dose. Note that for spiperone, higher doses of heroin in combination with the antagonist produce rate-decreasing effects (e.g., at 0.03 mg/kg spiperone). In contrast, there is a smaller response rate decrease after the highest dose of heroin in combination with SCH23390 than after lower doses.

Data from these experiments show that blocking either D_1 or D_2 dopamine receptor subtypes affects heroin discrimination, but at doses which reduce response rates markedly. Two previous studies which have used SCH23390 as antagonist in animals trained to discriminate a D_1 agonist can be used as comparisons for our data. In these studies, SCH23390, over dose ranges of 0.015 to 0.06 mg/kg and 0.031 to 0.125 mg/kg, reduced responding on the drug-appropriate lever in rats trained to discriminate the D_1 agonist SKF38393 from saline (3,7). One of these studies (7) used response rate measures comparable to those of the present report, and in that study SCH23390 decreased response rate along with selection of the drug-appropriate lever. Therefore, in comparing these studies with our own, we find consistency in the dose range of SCH23390 necessary to decrease drug-lever selection and in the rate-suppressant effects of these doses. Consequently, while it is somewhat unsatisfying to find that selection of the heroin-appropriate lever is decreased only when response rates are also decreased, the similarity of the doses of SCH23390 necessary to alter either discrimination of a D_1 agonist or discrimination of heroin leads to the conclusion that there is a D_1 receptor component to the heroin discriminative stimulus.

This interpretation is qualified by two other factors, however. One is the high doses of SKF38393 that were necessary to train the D_1 agonist discrimination in the above studies (8 and 10 mg/kg), leading necessarily to the need for similarly high (and response rate-decreasing) doses of antagonist to attenuate the cue. The other factor is our observation that the discriminative stimulus after a lower dose of heroin (0.1 mg/kg) was not decreased by pretreatment with 0.03 mg/kg SCH23390, suggesting that there is not a parallel shift in the dose-effect curve for the heroin discriminative stimulus after treatment with the D_1 antagonist.

Spiperone reduced the heroin cue in a way similar to SCH23390. However, although animals have been trained to discriminate selective D_2 receptor agonists such as quinpirole [e.g., (15)], we are not aware of reports in which the efficacy of spiperone in altering the discriminative stimulus properties of D_2 agonists has been described. Therefore, it is not known what doses of spiperone are required to reduce the discrimination of a D_2 agonist. Consequently, the possible role of D_2 receptors in mediating the heroin discriminative stimulus is not clear. Further studies of the ability of these receptor antagonists to alter the discriminative cue of specific dopamine agonists should facilitate use of the former in research.

The combination of SCH23390 and saline resulted in 30% selection of the drug-appropriate lever after 0.03 mg/kg of the former. This is not the first time that unusual behavioral effects of SCH23390 have been reported. In drug discrimination studies, Schechter and Greer (11) have reported that high doses of SCH23390 (0.1 mg/kg) generalized partially to the apomorphine cue, and Weathersby and Appel (15) have shown that SCH23390 at a dose of 0.25 mg/kg generalized to the quinpirole-trained cue.

The rate-decreasing property of the D_1 antagonist SCH23390 observed in this study has also been reported in other studies with rats [e.g., (7)]. Similarly, Kleven *et al.* (8) have shown that SCH23390, given alone in doses as low as 0.05 mg/kg, stopped responding by monkeys for food in a cocaine discrimination paradigm. This response impairment was overcome when cocaine was administered. A similar phenomenon may have occurred in these experiments, since the depression produced by SCH23390 itself was partially reversed by administration of heroin. This could be due to the ability of heroin to increase synaptic dopamine, which in turn would compete with the D_1 antagonist, although apparently not with the D_2 antagonist.

In summary, these data show that the heroin can be used as a training drug in a discrimination paradigm. The discriminative and rate effects of heroin, which does not bind to opioid receptors, appear to be due to the first metabolite, O^6-monoacetylmorphine. SCH23390 decreases response rates at the doses required to attenuate the heroin discriminative stimulus. Nonetheless, these doses are in the same range as those necessary to reduce the discriminative stimulus of a D_1 agonist, and are rate-decreasing in this test also. These findings, therefore, suggest that the heroin discriminative stimulus is mediated in part by D_1 receptors. The role of D_2 receptors in this discrimination remains uncertain.

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