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Reinforcing and Phencyclidine-Like Stimulus Properties of Enantiomers of Metazocine

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SLIFER, B. L., R. L. BALSTER AND E. L. MAY. *Reinforcing and phencyclidine-like stimulus properties of enantiomers of metazocine.* PHARMACOL BIOCHEM BEHAV **25**(4) 785–789, 1986.—The positive reinforcing properties of the racemate and stereoisomers of the benzomorphan, metazocine, were tested in three rhesus monkeys trained to self-administer IV injections of cocaine. The (−)-isomer maintained responding above saline levels at the highest dose tested (30 μg/kg/injection) in two of the three monkeys. Likewise, (+)-metazocine maintained self-administration responding in two monkeys at a dose of 100 μg/kg/injection. Responding for (±)-metazocine was maintained in all three monkeys at doses of 10–100 μg/kg/injection. The discriminative stimulus properties of the three forms of metazocine were tested in rats trained to discriminate phencyclidine (PCP; 3.0 mg/kg) from saline on a two-lever food-reinforced operant task. When metazocine was tested in these animals, only the (+)-isomer produced dose-related increases in responding on the PCP-lever. Both (±)- and (−)-metazocine resulted in only saline-appropriate responding. Thus, the results of these two experiments demonstrate that both enantiomers of metazocine function as positive reinforcers in monkeys and further, the reinforcing properties of (+)-metazocine may be due to the PCP/*sigma* properties of this isomer.

Drug self-administration Drug discrimination Metazocine Benzomorphans Phencyclidine *Sigma* agonists Rhesus monkeys Rats

THE 6,7-benzomorphans represent an interesting series of opioids. Two of the best known members of the group are pentazocine, the first marketed agonist/antagonist analgesic and cyclazocine, a widely studied agonist/antagonist which has had clinical trials for analgesia and for the pharmacotherapy of opioid dependence. Two additional benzomorphans, ketocyclazocine and N-allylnormetazocine (SKF-10,047), have become important research tools because they were proposed by Martin *et al.* [17] as prototypic agonists for *kappa* and *sigma* opioid receptors, respectively. In spite of this intense research interest in these N-substituted benzomorphans, the N-methyl compound in the series, with an N-substitution corresponding to morphine, has been much less systematically studied. This compound has generally been referred to as metazocine or α-metazocine [9].

Early studies of the opiate properties of metazocine were conducted with both enantiomers. May and Eddy [18] found a separation of antinociceptive effects for the isomers of metazocine similar to what is typically found with opioids. That is, the (−)-isomer was potent on the mouse hot-plate test while the (+)-isomer was inactive. The (−)-isomer also produced morphine-like subjective effects in non-dependent human subjects (cited in [19,24]). In spite of these similarities to morphine, (−)-metazocine does not substitute for morphine in morphine-dependent monkeys [24] and has a very low relative potency for suppression of abstinence in dependent humans (cited in [19,24]). This isomer, in fact, may precipitate abstinence in monkeys maintained on chronic morphine [24]. When tested for primary physical-dependence producing properties in monkeys, (−)-metazocine was found to produce negligible dependence [2]. Following chronic administration to humans, however, the compound produced a morphine-like physical dependence (cited in [24]). The morphine-antagonist properties of the (−)-isomer of metazocine in the monkey are unusual, as N-methyl-substituted analgesics in most other opioid classes have not been shown to have such effects. Additionally, metazocine differs from the other N-methyl benzomorphans such as etazocine (the 5,9-diethyl homolog) because the (+)-isomer does not suppress abstinence in the morphine-dependent monkey [2,24].

(+)-Metazocine is devoid of morphine agonist or antagonist effects, but it is not without other behavioral effects. When administered to morphine-dependent monkeys, the compound produced signs of marked CNS depression and prominent muscle weakness which were not antagonized by nalorphine [24]. The racemate, consistent with its being a

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mixture of the optical isomers of metazocine, partially suppresses abstinence in morphine-dependent monkeys only at doses that produced behaviorally toxic effects which include marked ataxia and disorientation [7].

It has been suggested that the dependence-producing properties of benzomorphans are underestimated in rhesus monkeys because, while many of the compounds produce effects in humans similar to those of morphine, they have low potency for producing or maintaining dependence in rhesus monkeys [24]. It has been demonstrated, however, that rhesus monkeys do not underestimate the reinforcing properties of benzomorphans, further suggesting that the abuse liability of these compounds can be separated from their physical dependence liability [10]. Furthermore, it has recently been reported that reinforcing properties of two other benzomorphans, N-allylnormetazocine and cyclazocine, reside in the (+)-isomer of these compounds [22]. Their reinforcing properties appear to be PCP-like or *sigma*-agonist effects [3, 5, 6]. A report by Woods *et al.* [25] suggests that metazocine may also have *sigma*-agonist effects in the (+)-isomer with morphine-like effects in the (−)-isomer.

It is possible, then, that the (+)-isomer of metazocine has reinforcing properties and, in addition, that these properties may be PCP/*sigma*-agonist effects. Moreover, unlike other N-alkylated benzomorphans that have *sigma*-like effects in the (+)-isomer, metazocine may also have reinforcing properties similar to morphine in the (−)-isomer. To test this hypothesis we used IV drug self-administration procedures in rhesus monkeys and drug discrimination procedures in rats to evaluate the reinforcing and PCP/*sigma* agonist-like discriminative stimulus properties of metazocine and its pure stereoisomers.

METHOD

Self-Administration Study

Subjects. Three adult male rhesus monkeys *(Macaca mulatta)* were used as subjects in this experiment. Two of the monkeys (M432, 4173) had previous experience in IV drug self-administration studies while the third animal (M319) was experimentally naive at the start of this study. The animals had free access to water and were maintained on a diet of approximately 200 g of Purina Monkey Chow given in a single daily feeding. Each received a chewable vitamin supplement daily and fresh fruit at least once a week.

Apparatus. The monkeys were housed individually in self-administration cubicles (0.8×0.8×1.0 M) and each wore a tubular stainless steel restraint harness [8] which was attached to a spring arm fastened to the rear wall of the cubicle. This served to restrain the monkeys while allowing free movement within the cubicle.

A response lever with three corresponding stimulus lights was mounted on the front Plexiglas door of each cubicle. During experimental sessions the two red stimulus lights were illuminated. An infusion was signalled by illumination of the center white light and offset of the red lights. Events within the cubicles were controlled and recorded by solid-state programming equipment which was located in an adjoining room. Drug injections were delivered by peristaltic pumps (Masterflex, Cole-Parmer Instrument Co., Chicago, IL) at a rate of 1.0 ml per 10-sec infusion.

Procedure. Prior to behavioral training, the monkeys were surgically prepared with chronic indwelling IV catheters. Under PCP-pentobarbital anesthesia, a silicon catheter (0.79 mm lumen, Ronsil Rubber, Bell Mead, NJ) was implanted into a major vein (i.e., internal or external jugular or femoral). The catheter was passed subcutaneously and exited on the animal's back. An external catheter was attached and this was threaded through the spring arm and out the rear of the cubicle where it was connected to the pump. Following recovery from surgery, each monkey was trained to respond for an infusion of 50 μg/kg cocaine hydrochloride under a fixed-ratio 10 (FR-10) schedule of reinforcement. Sessions were 1 hr in duration and were conducted daily.

Once stable FR responding for cocaine injections occurred, doses of the test drugs or saline were substituted for four consecutive sessions. After each dose of a drug the animals were returned to cocaine for at least three days or until responding was stable (<10% variation in the mean number of cocaine injections over 3 consecutive sessions). Saline was tested between each drug. Doses were tested in an ascending order in some monkeys and a descending order in others; the order of testing the compounds was also varied between animals. The following drugs and doses were tested in all three monkeys: (±)-metazocine (3–100 μg/kg/injection), (−)-metazocine (1–30 μg/kg/injection) and (+)-metazocine (3–100 μg/kg/injection). Limited supplies of these drugs precluded testing higher doses.

Drug Discrimination Study

Subjects. The subjects were nine male albino Sprague-Dawley rats (Flow Laboratories, Dublin, VA) weighing 265–305 grams. They had previous experience in PCP discrimination studies where various benzomorphans were tested for generalization. They were individually housed in wire mesh cages with free access to water in a colony room. They were maintained at a constant weight by post-session feedings of Purina Rodent Chow.

Apparatus. Four standard two-lever operant chambers (Model E10-10, Coulbourn Instruments, Lehigh Valley, PA) housed inside light and sound attenuating cubicles (Model E10-20, Coulbourn Instruments) were used. A food cup, into which 45-mg Noyes pellets could be delivered, was mounted between the two response levers, which were 13 cm apart. A stimulus light was located 20 cm above the food cup. Events in the chamber were controlled and recorded by solid-state programming equipment located in an adjacent room.

Procedure. At the start of this experiment the animals had been trained to perform a PCP-saline discrimination as described previously [4]. They received either 3.0 mg/kg PCP or saline IP before each session in a double-alternation sequence (PCP, PCP, saline, saline, PCP, etc.). Thirty-minute sessions were conducted Monday through Friday each week. All injections were given 15 min before the session, and the animals were placed in the test chamber just prior to the start of the session which was signalled by illumination of the stimulus light. Responding on one of the two levers was reinforced according to a fixed-ratio 32 (FR-32) schedule during sessions following PCP administration, and responding on the other lever was reinforced under the same schedule during sessions following saline administration. The position of the correct lever (left or right) following PCP administration was counterbalanced within the group. Incorrect responses during training sessions reset the FR requirement on the correct lever. These experienced subjects nearly always made over 85% correct responses on training sessions throughout the study.

Test sessions were conducted on Tuesdays and Fridays if the first 32 consecutive responses were made on the correct lever on the preceding training day. During test sessions, responding on neither lever was reinforced and the session was terminated after 2 min. Various doses of the isomers and racemate of metazocine were administered to each rat prior to these test sessions. The order of drug testing was counterbalanced. Single determinations were made for each dose except for doses in the middle of the dose-effect curves for each drug (3 and 6 mg/kg of (±)-metazocine, 3 mg/kg of (−)-metazocine, and 10 mg/kg of (+)-metazocine). For these doses, the averages of two tests were used in the data analyses. In addition, the training dose of PCP (3 mg/kg) was tested once at the beginning and once at the end of this portion of the study. There were no systematic differences so the results of these two determinations were averaged for each subject. Also, saline vehicle was tested before and after the testing of each drug. The averages of these two control determinations for each drug were used in the analyses.

Data analyses. For the self-administration study, the mean number of injections from the last three days of substitution for each dose of the test drugs and saline were determined for each animal. Rates of responding for cocaine injections were calculated from all cocaine days throughout the study. A dose of a drug was considered to be reinforcing if the mean number of injections exceeded saline levels and the ranges did not overlap.

For the drug-discrimination study, the percentage of responses on the PCP lever as well as the rate of responding on both levers during the 2-min test sessions were calculated. These values for each subject were averaged for all 8 or 9 subjects and standard errors determined.

Drugs. Phencyclidine hydrochloride (PCP) and cocaine hydrochloride were obtained from the National Institute on Drug Abuse. The isomers of metazocine (2′-hydroxy-2,5,9α-trimethyl-6,7-benzomorphan) were synthesized as described by May and Eddy [18]. The racemate was in the form of the HCl salt while the HBr salts of the (−)- and (+)-isomers were used. Doses were calculated based on the salt weights and solutions were prepared with saline vehicle.

RESULTS

Self-Administration

Figure 1 shows the data for the three monkeys in the self-administration study. Cocaine maintained stable rates of responding with the overall mean injections for the three animals ranging from 38 to 52 injections per session. Saline substitution significantly reduced responding and resulted in mean injection rates between 6 and 12 injections per session.

The results of substitution of the (−)-isomer of metazocine indicates that this isomer maintained responding above saline levels at least at one dose in all three monkeys, however monkey 4173 had highly variable rates and thus the data from this animal did not meet our criteria for (−)-metazocine serving as a reinforcer. In the remaining two monkeys, the highest dose (30 μg/kg/injection) consistently maintained the highest rates. (+)-Metazocine, at the highest dose of 100 μg/kg/injection, maintained self-administration behavior in monkeys M432 and M319, but not in monkey 4173. Racemic metazocine was the only compound that met the criteria for a reinforcer in all three monkeys. Doses of 10–100 μg/kg/injection maintained rates of responding which exceeded the range of saline responding in the individual monkeys.

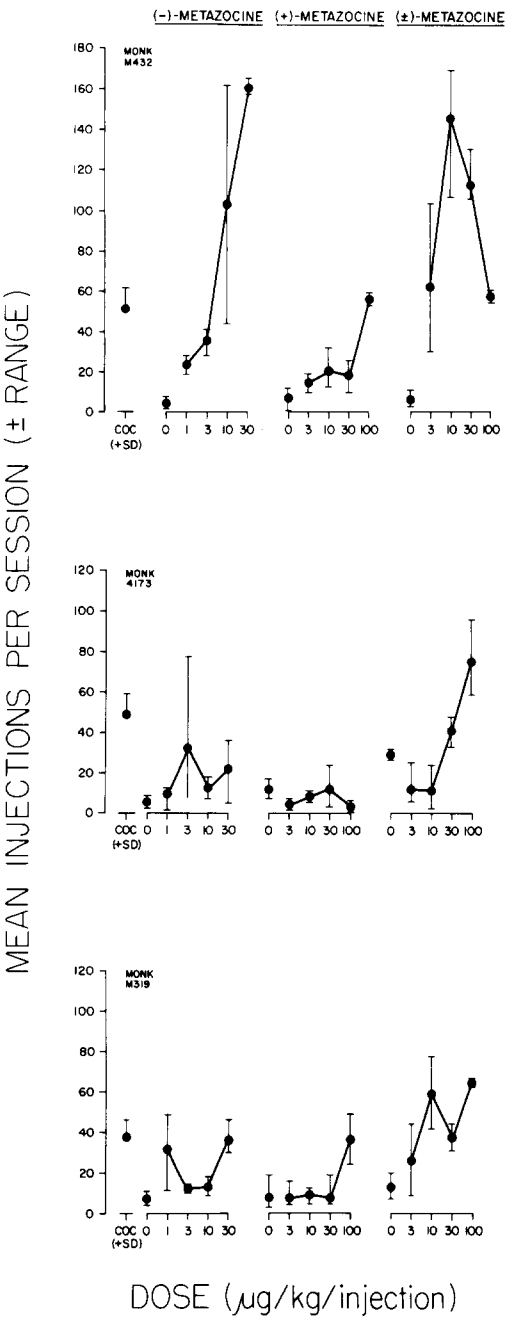

FIG. 1. Intravenous self-administration of (−), (+) and (±)-metazocine by three rhesus monkeys. Values are the mean number of injections (±range) for the last three days of each four-day substitution for vehicle (0 dose) and the doses of the metazocine isomers. Values above COC are the mean number of injections of cocaine (±S.D.) for all cocaine sessions throughout the study.

Drug Discrimination

Phencyclidine (3.0 mg/kg) functioned as a discriminative stimulus to control FR-32 responding in the nine rats in this

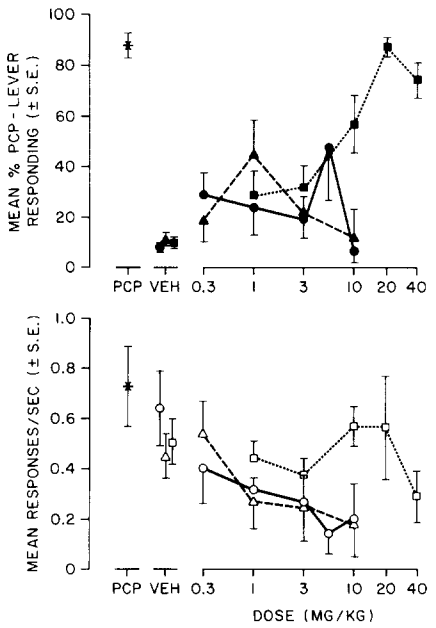

FIG. 2. Phencyclidine (PCP)-like discriminative stimulus properties of (−)-metazocine (triangles), (+)-metazocine (squares) and (±)-metazocine (circles) in rats. Top panel shows mean percent PCP-lever responding and lower panel shows overall response rates on both levers after PCP and vehicle (VEH) control injections and following various doses of the metazocine isomers.

study. When the training dose of PCP was tested, an average of 89% of the total responses occurred on the PCP lever (Fig. 2). Conversely, saline vehicle tests resulted in less than 10% of the total responses occurring on the PCP lever.

When generalization tests were conducted with the three forms of metazocine (Fig. 2), neither the (−)-isomer (0.3–10 mg/kg) nor the racemic mixture of metazocine (0.3–10 mg/kg) produced over 50% PCP-lever responding. (+)-Metazocine, on the other hand, produced dose-related increases in PCP-lever responding with maximum generalization to a dose of 20 mg/kg which resulted in approximately 90% PCP-lever responding.

Response rates (Fig. 2) were decreased below vehicle control rates by increasing doses of both (±)- and (−)-metazocine across a similar dose range (1–10 mg/kg). In contrast, only the highest dose of the (+)-isomer (40 mg/kg) disrupted responding.

DISCUSSION

The results of the drug self-administration experiment demonstrated that the N-methyl substituted benzomorphan, metazocine, has reinforcing properties in two of three monkeys in both of its optical isomers. Because of the similarity of other pharmacological and behavioral effects of (−)-metazocine and morphine it is not surprising that the (−)-isomer of metazocine, like the (−)-isomers of other N-methyl compounds such as morphine and levorphenol, maintains self-administration responding in rhesus monkeys. This result is also consistent with data from Woods *et al.* [25] showing that the discriminative stimulus properties of (−)-metazocine were morphine-like.

Evidence for reinforcing properties for (−)-metazocine is also consistent with reports that this drug produces morphine-like subjective effects in former opioid abusers (cited in [19,24]). Griffiths and Balster [10] found a good correlation between opioids which are self-administered by rhesus monkeys and those which produce morphine-like acute subjective effects in humans. This is particularly interesting in the case of (−)-metazocine because, in physical dependence studies in monkeys, (−)-metazocine is not morphine like. Surprisingly for an N-methyl opioid, it precipitated withdrawal in morphine-dependent monkeys [24], and when tested for primary physical dependence by giving it chronically to non-dependent monkeys, it produced little evidence of dependence [2]. Primary dependence studies in humans with (−)-metazocine (cited in [24]) found clear evidence for morphine-like physical dependence. Thus, as is the case with some other benzomorphans [10], the monkey underpredicts the physical dependence potential of (−)-metazocine. However, self-administration studies with (−)-metazocine and other benzomorphans in monkeys do predict the morphine-like effects in humans. This further establishes the predictive value of IV self-administration studies in monkeys for pre-clinical abuse potential assessment of opioids.

Self-administration of (+)-metazocine, on the other hand, was not predicted. Historically, the (+)-isomers of most N-methyl substituted opioids are thought to be devoid of opioid-like activity including reinforcing or dependence properties. For example, dextrorphan and *d*-methadone have been found to lack morphine-like subjective effects and to be without dependence liability [13,14]. The demonstration of reinforcing properties in the (+)-isomer of metazocine is not without precedence, however. The (+)-isomers of certain other benzomorphans have recently been shown to have reinforcing properties. Operant responding in rhesus monkeys was maintained by injections of (+)-cyclazocine and (+)-N-allylnormetazocine [22]. These compounds, like (+)-metazocine, have been shown to lack classical morphine-like opiate agonist effects in laboratory animals [1, 16, 23]. On the other hand, (+)-cyclazocine and (+)-N-allylnormetazocine have been found to have PCP-like effects [5,6]. Because of considerable evidence showing an overlap in the discriminative stimulus properties of PCP and the prototypic *sigma* agonist N-allylnormetazocine [3, 6, 12, 20, 21], these PCP-like effects are likely due to *sigma*-agonist actions. The results of the present drug-discrimination experiment support this conclusion for metazocine as well. The (+)-isomer of metazocine mimicked the PCP stimulus while (−)-metazocine did not. Two drug discrimination studies by Shannon [20,21] also indicate PCP-like effects for metazocine. In these studies (±)-metazocine produced some PCP or N-allylnormetazocine responding in rats which was increased by the concurrent administration of naloxone, most likely due to antagonism of the (−)-isomer effects, unmasking the PCP/*sigma*-agonist effects of the (+)-isomer. Similar results were reported in pigeons by Grippo *et al.* [11]. Although the discriminative stimulus effects of opioids with mixed agonist-antagonist properties are, in some cases, species dependent [13], it appears that the PCP/*sigma* properties of metazocine are consistent across at least three species. The present results and those of Shannon were found in rats [20,21], Woods *et al.* [25] reported generalization from ketamine to (+)-metazocine in rhesus monkeys, and the results of Grippo *et al.* [11] were in pigeons.

In summary, the results of the present drug self-

administration study demonstrated that metazocine has reinforcing stimulus properties in both enantiomers. Further, it appears that the reinforcing and discriminative stimulus properties of the enantiomers of metazocine may be through different mechanisms. Previous data suggests that the (−)-isomer effects may be mediated by the *mu* receptors [25], while the results of the present drug-discrimination study indicate that the reinforcing properties of the (+)-isomer may be PCP/*sigma*-agonist effects.

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