Pharmacology Biochemistry & Behavior, Vol. 29, pp. 209–212. © Pergamon Journals Ltd., 1988. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

BRIEF COMMUNICATION

Naloxone and Diprenorphine Reduce Responding for Brain Self-Stimulation in a Fixed-Ratio Schedule in Rats

GERALD J. SCHAEFER[1] AND RICHARD P. MICHAEL

Department of Psychiatry, Emory University School of Medicine
Georgia Mental Health Institute, 1256 Briarcliff Rd., N.E., Atlanta, GA 30306

Received 12 March 1987

SCHAEFER, G. J. AND R. P. MICHAEL. *Naloxone and diprenorphine reduce responding for brain self-stimulation in a fixed-ratio schedule in rats.* PHARMACOL BIOCHEM BEHAV **29**(1) 209–212, 1988.—Rats were implanted with bipolar stimulating electrodes in the midbrain-central gray area (MID-CG) and trained to lever-press for intracranial self-stimulation (ICSS) on a continuous reinforcement schedule (CRF). When behavior was stable, animals were tested in 30 min ICSS sessions following the administration of either naloxone or diprenorphine, both over the dose-range 0.001–10 mg/kg, or with vehicle. Following testing on the CRF schedule, animals were re-trained on a fixed-ratio:30 (FR:30) schedule. When behavior had again stabilized, testing with naloxone, diprenorphine and vehicle was repeated. In the CRF tests, neither naloxone nor diprenorphine had any effects on response rates over the 10,000-fold dose-range used. In the FR:30 tests, however, both drugs significantly reduced response rates at the 10 mg/kg dose, and the reduction produced by naloxone was significantly greater than that produce by diprenorphine. These results suggested that diprenorphine is qualitatively similar to naloxone in altering the rate of responding maintained by ICSS, but is less potent than the prototypical opioid antagonist in this paradigm.

Naloxone Diprenorphine Brain self-stimulation Response rates Continuous reinforcement schedule Fixed-ratio:30 schedule

THERE is now substantial evidence that opioid antagonists can alter behavior reinforced with intracranial self-stimulation (ICSS) under certain experimental conditions (see [18] for review). When lever-pressing rate is the dependent variable, a critical factor is the schedule of reinforcement. While antagonists, such as naloxone, may not reliably change responding for ICSS in a continuous reinforcement schedule (CRF), these drugs produce dose-dependent reductions when partial reinforcement schedules are used [20]. Furthermore, the effect appears to be due to changes in the central nervous system since the quaternary forms of naloxone and naltrexone, which do not readily enter the brain, are inactive [18]. We now report that the antagonist, diprenorphine, produces effects on ICSS behavior that are similar to those of naloxone and naltrexone. The latter two drugs bind with greater relative affinity to the mu-opioid receptor than the delta-opioid receptor, but diprenorphine binds with equally high affinity to both receptor subtypes [1,9]. These three antagonists, however, share some pharmacological properties in common: they precipitate withdrawal in morphine-dependent monkeys and dogs, and they lack analgesic effects [2,10]. To examine whether diprenorphine is similar to the prototype-antagonist, naloxone, both drugs were tested over a 10,000-fold dose-range in two ICSS designs. One experiment used a CRF schedule and the other used a fixed-ratio:30 schedule; a paradigm previously demonstrated to be sensitive to the agonistic actions of opioid antagonists [18].

METHOD

Animals

Nine male Sprague-Dawley rats bred in the laboratory were used. The breeding stock was purchased from Charles-River Laboratories (Wilmington, MA). They weighed 360–460 at the time of surgery. Except while being tested, animals were housed in group cages (2–4 per cage) in a colony room with food and water available ad lib. The colony room was artificially illuminated between 07:00 and 19:00 hr and animals were tested during the lights-on phase of the cycle.

[1]Requests for reprints should be addressed to Gerald J. Schaefer, Ph.D., Biological Psychiatry Research Laboratory, Georgia Mental Health Institute, 1256 Briarcliff Rd., N.E., Atlanta, GA 30306.

Apparatus

The operant test chamber was similar to that used previously [16]. Briefly, it measured 31×30×29 cm high (inside dimensions), and contained a single conventional lever (G6312, Ralph Gerbrands Co., Arlington, MA) mounted 10 cm above a grid floor. Electrical pulses were produced by a biphasic, constant-current stimulator [15], and consisted of 200 msec trains of square-wave pulses at 100 Hz with a pulse duration of 0.5 msec. Current intensity ranged from 80–225 μA during testing in the CRF schedule and 167–370 μA during testing in the FR:30 schedule. The stimuli were delivered to the animal's brain through a commutator (Model 590, Mercotac, San Diego, CA) connected to the skull by a length of spring-shielded hearing-aid wire. Test sessions were controlled by, and data were collected with, the use of solid state modules. An analogue record of the number of lever-presses, and of the number of reinforcements was obtained using a cumulative recorder (Gerbrands, Arlington, MA).

Surgery and Histology

Following the administration of sodium pentobarbital (50 mg/kg, IP) and atropine sulfate (0.25 mg, SC), standard stereotaxic procedures were used to implant a bipolar platinum electrode (tip diameter=0.125 mm, Plastic Products Co., Roanoke, VA) in the midbrain-central gray area at coordinates AP 0.0, L 0.5, H −2.5 [12]. The electrode was angled 10° toward the midsagittal plane, and the top of the electrode assembly was covered with cranio-plastic cement forming a durable anchor for the electrode. When the experiments were completed the animals were killed with an overdose of sodium pentobarbital and perfused via the heart with 10% formalin. Blocks of brain were frozen on a microtome stage and cut at 50 μm. Alternate sections were stained with cresyl violet and Weil's stain, and these sections were viewed under a microprojector to determine exactly the sites of the electrode tips.

Procedure

After allowing a week to recover from surgery, animals were introduced to the operant chamber and trained to press the lever on a CRF schedule. Training continued for at least four weeks during which time stimulus currents were adjusted for each animal to produce consistent responding that was well below maximum rates. This was followed by five days during which saline was administered 15 min before the 30 min sessions and during these five days the individual response rates were constant for each animal. However, response rates for the group varied between 1400–3600 presses per 30 min. The tests with drugs or vehicle were conducted for 30 min per day, four days per week. The starting current remained constant for each animal throughout the CRF experiment. For the tests with naloxone, which were conducted first, animals were injected with saline (vehicle) on Mondays and Thursdays and with naloxone on Tuesdays and Fridays. Fifteen min after injection, animals were placed in the test apparatus for 30 min. Doses of naloxone (0.001, 0.01, 0.1, 1.0 or 10 mg/kg) were administered in a random sequence. Tests with diprenorphine followed using the same dose-range. The procedure was similar to that for naloxone except that the vehicle used was distilled water and the interval between injection and testing was 30 min to allow for the slower onset of action of diprenorphine.

When animals had completed testing on the CRF schedule, they were trained to lever-press for ICSS on a FR:30 schedule using procedures described previously [16]. One animal failed to respond satisfactorily on the FR:30 schedule and was replaced by another animal. When response rates had stabililized on the FR:30 schedule, animals were again tested first with naloxone and then with diprenorphine using the same procedures as those followed for the CRF experiments.

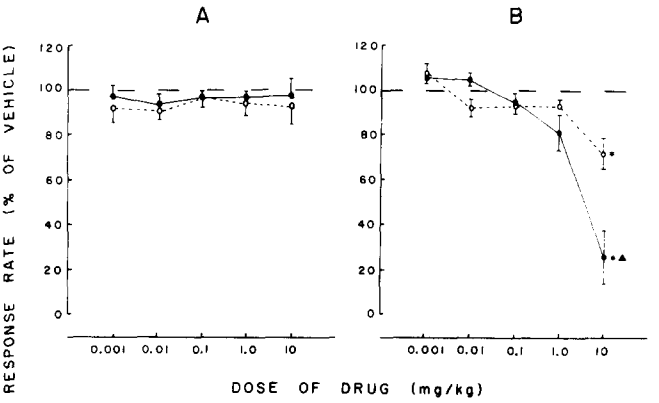

FIG. 1. (A) Effects of graded doses of naloxone (●) or diprenorphine (○) on lever pressing for brain self-stimulation on a continuous reinforcement schedule. Horizontal interrupted lines give control values when vehicle was administered. Vertical bars give standard errors of means. N=8 per group. (B) Effects of graded doses of naloxone (●) or diprenorphine (○) on lever pressing for brain self-stimulation on a fixed-ratio:30 schedule of reinforcement. N=8 per group. *Significantly less than vehicle at $p<0.01$; ▲ = significantly less than 10 mg/kg diprenorphine at $p<0.01$.

Data Analysis

The total number of lever presses made during the 30 min test sessions provided data for analysis. The scores for all control (vehicle) days for a given dose-response curve were averaged and the response rate for each dose of drug was expressed as a percentage of the mean control scores. To test the overall effects for both CRF and the FR:30 schedules, a three-way analysis of variance (drug×dose×animal) was used (SPSS/PC+, SPSS Inc. Information Analysis Systems, Chicago, IL). When results showed significant differences, randomized block design analyses of variance [7] were performed separately. This was followed by Dunnett's test (two-tailed) to compare differences between response rates after vehicle administration and after different doses of either drug. Finally, to compare changes at the highest dose of naloxone (10.0 mg/kg) with those of diprenorphine, a t-test for related measures was used.

Drugs

Naloxone hydrochloride (courtesy of Endo Laboratories, Garden City, NY) and diprenorphine hydrochloride (supplied by the National Institute on Drug Abuse) were used. Naloxone was dissolved in 0.9% saline and diprenorphine was dissolved in distilled water. Both drugs were administered subcutaneously in a volume of 1.0 ml/kg body weight. Doses were expressed as the free base.

RESULTS

Over the 10,000-fold dose-range of 0.001–10.0 mg/kg neither naloxone nor diprenorphine changed the rates of

lever pressing on a CRF schedule compared with vehicle (Fig. 1A). The mean baseline rate with saline in the naloxone study was 2361±187 presses per 30 min compared with a mean baseline rate with distilled water in the diprenorphine study of 2225±176 per 30 min. Results were different when animals were tested in the FR:30 experiment. In this study, the main effects of drug, $F(1,35)=6.9$, $p<0.02$, and dose, $F(5,35)=34.4$, $p<0.001$, as well as the interaction between drug and dose, $F(5,35)=7.9$, $p<0.001$, were all significant (Fig. 1B). Subsequent analyses revealed a highly significant effect of dose for both naloxone, $F(5,35)=24.2$, $p<0.001$, and diprenorphine, $F(5,35)=7.5$, $p<0.001$. This was due to the changes produced by both drugs at the 10.0 mg/kg dose. Finally, a t-test showed that the reduction in responding produced by naloxone was significantly greater than that produced by diprenorphine, $t(7)=3.9$, $p<0.01$. The mean baseline rates were 3813±75 presses for 30 min in the naloxone study and 3797±245 in the diprenorphine study.

Histological findings indicated that the electrode tips in all animals terminated in the ventral part of the periventricular gray area as well as in the medial longitudinal fasciculus near the brachium conjunctivum. These areas, which we refer to as the midbrain-central gray area (MID-CG), are the same as those previously shown to be sensitive to the effects of naloxone and naltrexone [16].

DISCUSSION

Results confirmed previous reports that the fixed-ratio lever-pressing paradigm for ICSS is sensitive to the effects of the opioid antagonists naloxone [16–18, 20], and extended these findings to the antagonist, diprenorphine.

We did not find any changes in response rates in the CRF schedule with naloxone administration but, when tested in the FR:30 paradigm, animals showed significant decreases in response rates at the moderate dose level of 10 mg/kg. Although baseline rates were higher (126 presses per min) on the FR:30 schedule than in the CRF schedule (76 presses per min), a previous study has shown that even when baseline rates were similar, naloxone produced a greater decrease in an FR than in a CRF schedule [20], suggesting that this was not a rate-dependent effect. In addition, current work in our laboratory using a fixed-interval 60 sec schedule has demonstrated that, with baseline rates as low as 10 presses per min, naloxone will produce a dose-dependent decrease in lever-pressing for ICSS.

Diprenorphine failed to alter response rates at any dose level in the CRF experiment. This contrasted with results from a recent study [13] in which increases in the rate of lever-pressing for ICSS occurred over a dose-range of 0.001–0.25 mg/kg. Although a procedure similar to the present one was used, the animals were implanted with electrodes in the lateral hypothalamus and ventral tegmental area. The results of Washburn and Stein [19] are, on the other hand, consistent with the present data. They injected diprenorphine directly into the ventral tegmental area of rats implanted with stimulating electrodes in the medial forebrain bundle, and found no changes in the rates of responding over the dose range 1.5–5.0 μg. This lack of effect of diprenorphine is consistent with the lack of changes in locomotor activity after diprenorphine administration when rats were tested individually in activity monitors [3].

The greater potency of naloxone compared wih diprenorphine in the FR:30 experiment confirms our earlier study and is consistent with the results of other behavioral studies in rats [17]. Naloxone and diprenorphine were tested at FR:15, and while naloxone decreased response rates, diprenorphine did not. It appears that decreases in responding produced by diprenorphine occur only when a critical FR value is reached. In addition, diprenorphine was slightly less potent than naloxone in suppressing the intake of sweetened condensed milk [8] and less potent in altering locomotor activity [3]. While naloxone produced biphasic effects on fighting and escape behavior in rats, diprenorphine was largely without such effects [14]. Perhaps, in the rat some of the behavioral effects of the antagonists represent preferential blockade of the mu rather than the delta receptors. These data stand in marked contrast to those in primates where diprenorphine is highly effective in reducing operant behavior [4,5], and this might also be related to the observation that the populations of receptor subtypes in brain differ between species [6,11]. It would be of considerable value and interest to compare results in rats with a brain stimulation paradigm in primates.

ACKNOWLEDGEMENT

This work was supported by the Georgia Department of Human Resources, which is gratefully acknowledged.

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