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# Effects of Opioid Antagonists and Their Quaternary Derivatives on Locomotor Activity and Fixed Ratio Responding for Brain Self-Stimulation in Rats

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SCHAEFER, G. J. AND R. P. MICHAEL. *Effects of opioid antagonists and their quaternary derivatives on locomotor activity and fixed ratio responding for brain self-stimulation in rats.* PHARMACOL BIOCHEM BEHAV **23**(5) 797–802, 1985.—Rats were implanted with stimulating electrodes aimed at the midbrain-central gray area (MID-CG) and trained to lever press for brain stimulation (ICSS) on a fixed ratio:30 (FR:30) schedule of reinforcement. When response rates were stable, animals were administered either naloxone hydrochloride, naltrexone hydrochloride, naloxone methobromide or naltrexone methobromide in a dose range of 0.1–30 mg/kg. Fifteen minutes after the subcutaneous administration of either drug or vehicle, animals were tested for 45 min in the ICSS procedure and changes in response rates following drug administration were compared with those following vehicle administration. Both naloxone and naltrexone hydrochloride produced graded decreases in responding over the entire dose range, while naloxone and naltrexone methobromide did not alter response rates at any dose level. In a separate testing procedure, 30 mg/kg naloxone and naltrexone hydrochloride produced modest reductions in motor activity, while the methobromide derivatives did not. These results demonstrated that the fixed ratio procedure was sensitive to changes in responding for ICSS produced by opioid antagonists, and this effect depends upon the entry of these opioid antagonists into the brain.

Brain self-stimulation Midbrain-central gray Naloxone Naltrexone Quaternary opioid antagonists Locomotor activity

WHILE there has been some controversy regarding the effects of opioid antagonists on the rate of responding for brain self-stimulation (ICSS), it now appears that these drugs do alter this behavior under certain conditions [10, 11, 20, 21, 24]. One important consideration is the schedule of reinforcement employed. When a continuous reinforcement schedule is used, generally only small changes in response rates occur. However, with partial reinforcement schedules, response rates decrease conspicuously and the effect is a sensitive one [7, 19, 25]. To characterize further the effects of opioid antagonists on schedule-controlled ICSS behavior, we have examined whether the changes in responding are due primarily to the blockade of central opioid receptors or whether peripheral sites of action are involved. One approach to discriminating between these sites of action is to compare the effects of the tertiary forms of the antagonists, which rapidly diffuse across the blood-brain barrier, with their quaternary derivatives which do not cross membranes readily. This approach has been used previously to study the effects of opioids on fluid consumption [2,5], on brain stimulation-induced feeding [4] and on plasma corticosterone levels [6].

The purpose of the present study was to compare the effects of two opioid antagonists, naloxone and naltrexone, with the effects of their quaternary derivatives on the rate of lever pressing for ICSS on a fixed ratio:30 (FR:30) schedule of reinforcement. Since we had previously found that the midbrain-central gray (MID-CG) area was more sensitive to the effects of the antagonists than the medial forebrain bundle-lateral hypothalamus (MFB-LH) [19], in this study animals were implanted in MID-CG sites. In addition, the effects of these compounds on spontaneous locomotor activity were measured to help assess the role of any generalized changes in behavior on the effects of these compounds on lever pressing activity.

## METHOD

### *Animals*

The subjects were 13 adult male Sprague-Dawley rats (King Animal Labs, Inc., Oregon, WI) that weighed 325–400 g when electrodes were implanted. The animals were housed in group cages in a colony room with free access to food and

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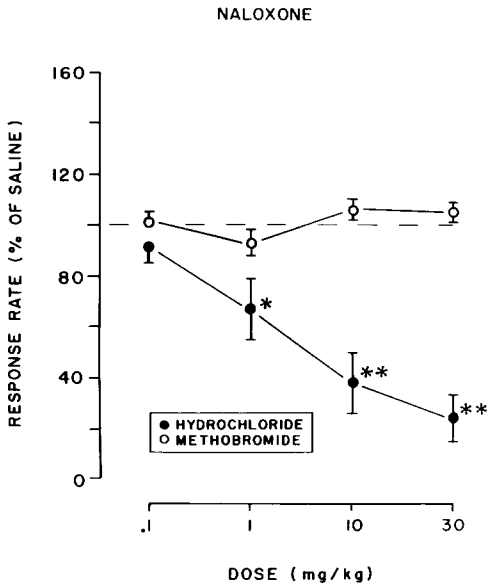


FIG. 1. Effects of graded doses of naloxone hydrochloride (●) or naloxone methobromide (○) on lever pressing for intracranial self-stimulation on a fixed ratio: 30 schedule of reinforcement. Horizontal interrupted lines in this and the subsequent figures give saline values during control periods. Vertical bars give standard errors of means. N=10 per group. Significantly less than saline control at $p<0.05$* and at $p<0.01$**.

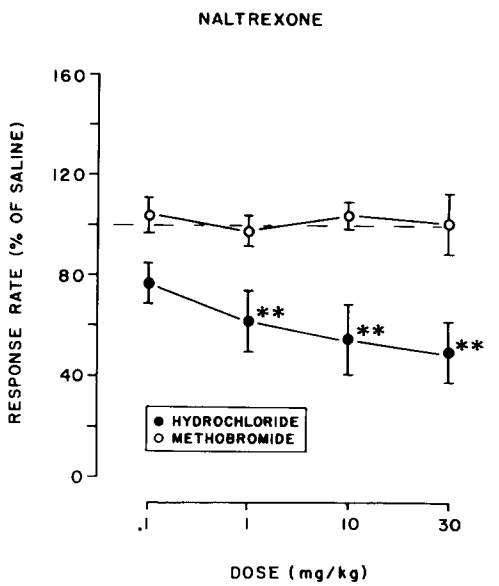


FIG. 2. Effects of graded doses of naltrexone hydrochloride (●) or naltrexone methobromide (○) on lever pressing for intracranial self-stimulation on a fixed ratio: 30 schedule of reinforcement. Other symbols as in Fig. 1.

water. The colony lights were on between 7:00 a.m. and 7:00 p.m.

*Apparatus*

The operant test chamber was similar to that used previously [19]. Electrical pulses were produced by a biphasic, constant-current stimulator [18], 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 160–600 $\mu$A. The stimuli were delivered to the animal's brain through a commutator (Model CAY-675-6, Airflyte Electronics, Bayonne, NJ) connected to the skull by a length of spring-shielded hearing-aid wire. Solid-state modules were used to control the test sessions and provide digital information about the number of lever presses and reinforcements, while a cumulative recorder was used to produce an analogue record of lever pressing activity.

Locomotor activity was measured using an Omnitech Digiscan Model RXY activity monitor (Omnitech Electronics, Inc., Columbus, OH) that was equipped with two counters. Total horizontal activity was measured on one counter as the total number of infrared beam interruptions. Ambulatory activity, defined as animal movement from one location to another, was measured on the second counter and counts were made only when different beams were broken in sequence. During monitoring, the animal was placed inside a clear acrylic cage (39.4×39.4×30.5 cm high, inside dimensions), positioned within the Digiscan monitor, which was itself placed within a sound attenuating chamber.

*Surgery and Histology*

Rats were deeply anesthetized with sodium pentobarbital (50 mg/kg IP) and were also given atropine sulfate (0.25 mg, SC) to reduce any respiratory distress. A bipolar platinum electrode (tip diameter=0.125 mm, Plastic Products, Roanoke, VA) was aimed at the MID-CG as previously described [19] using the atlas of Pellegrino *et al.* [15]. When the experiments were completed, animals were killed with sodium pentobarbital and perfused via the heart with 10% formalin with electrodes in place. After one week in formalin, brains were removed and frozen sections were cut at 50 $\mu$m. Alternate sections were stained with cresyl violet and Weil stain to locate the electrode tips.

*Procedure*

Animals were allowed at least one week to recover, and were then placed in the operant chamber and trained to press a lever to deliver electrical stimuli to their brains. These animals were trained to respond for ICSS on a FR:30 schedule using the procedure described earlier [19]. When responding on the FR:30 schedule had stabilized, rats were tested with drugs or vehicle in this paradigm for 45 min per day, four days per week. The starting current remained constant for each animal through each experiment. Animals were injected with saline vehicle (Mon., Thurs.) or drug in saline (Tues., Fri.), and 15 min after injection the animals were placed in the operant chamber for 45 min. At the beginning of each session, two noncontingent "priming" reinforcements were always given. For each drug series, the same doses were used (0.1, 1.0, 10 or 30 mg/kg) and were administered in random sequences. Of the 13 rats implanted, 10 were trained satisfactorily on the FR:30 schedule and these 10 animals were used for drug tests.

Approximately one month after completion of ICSS testing, 9 of the 10 animals were used to measure the effects of

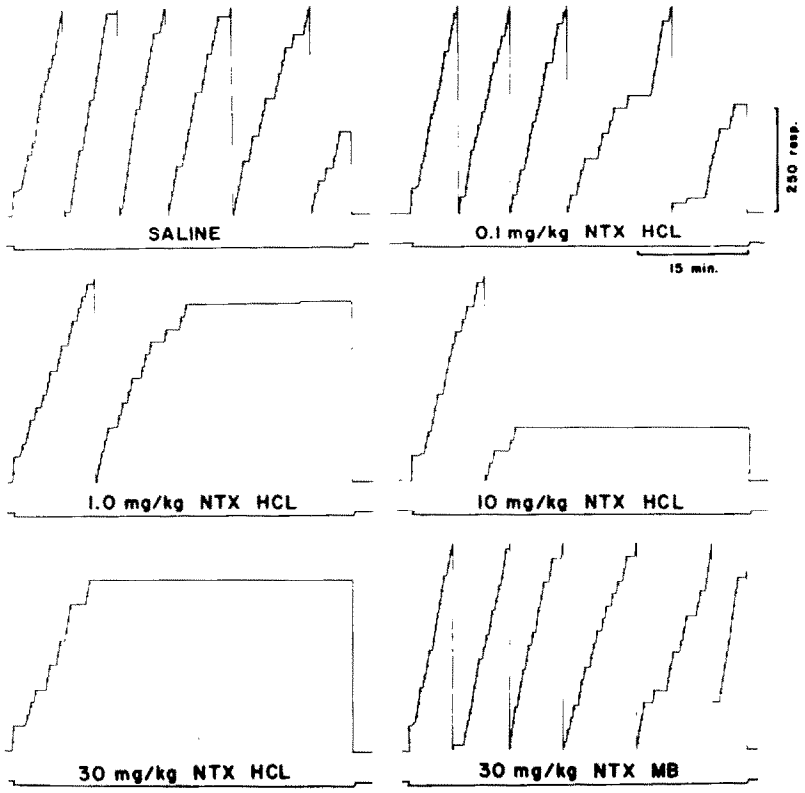


FIG. 3. Cumulative response records for one rat illustrating the effects of saline, naltrexone hydrochloride (NTX HCL) (0.1, 1.0, 10 and 30 mg/kg) and naltrexone methobromide (NTX MB) (30 mg/kg) on the pattern of responding in the fixed ratio: 30 procedure. The records represent 45 min test sessions during which the event pen is offset downward. The upper left record is of a representative control session in which saline was administered. Downward deflections of the response pen indicate the presentation of brain stimulation reward. The response pen reset automatically after a total of 500 responses or the session ended.

the highest dose of each drug on locomotor activity. Animals were allowed three days to habituate to the activity apparatus and to habituate also to receiving saline injections, and then they were administered either saline or 30 mg/kg of one of the four test compounds 15 min prior to the activity session. Infrared beam interruptions during the last 10 min of the 12-min activity session provided the measures of both total horizontal activity and of ambulatory activity.

*Data Analysis*

The total number of lever presses made during the 45-min test sessions provided the data for analysis. The scores for all saline days for a given dose-response curve were averaged and the response rate for a given dose of drug is expressed as a percentage of the mean saline score. By using this method direct comparisons between each dose-response curve can readily be made and comparisons between animals having different baseline response rates are facilitated. To compare dose-response curves for the two naloxone compounds, a split-plot factorial analysis was performed on these data [14]. This analysis allowed us to test for parallelism and assess the significance of any difference in slopes. Subsequently, a randomized block design analysis of variance was performed on each of the two dose-response curves, followed by Dunnett's test (two-tailed) to compare

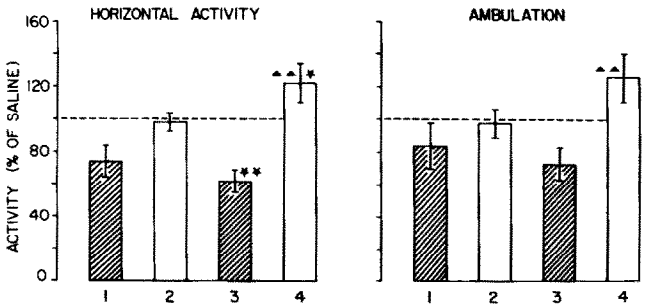


FIG. 4. Effects of 30 mg/kg naloxone hydrochloride (1), naloxone methobromide (2), naltrexone hydrochloride (3), or naltrexone methobromide (4) on horizontal activity and ambulation during 10 min test sessions. The mean number of infrared beam counts (±SEM) for the horizontal activity parameter following saline administration was 786±102 for the naloxone compounds and 537±99 for the naltrexone compounds. The corresponding values for the ambulation parameter were 313±53 for the naloxone compounds and 189±42 for the naltrexone compounds. Significantly different from saline control at $p<0.05$*, and at $p<0.01$**; significantly different from naltrexone hydrochloride at $p<0.01$ ▲▲.

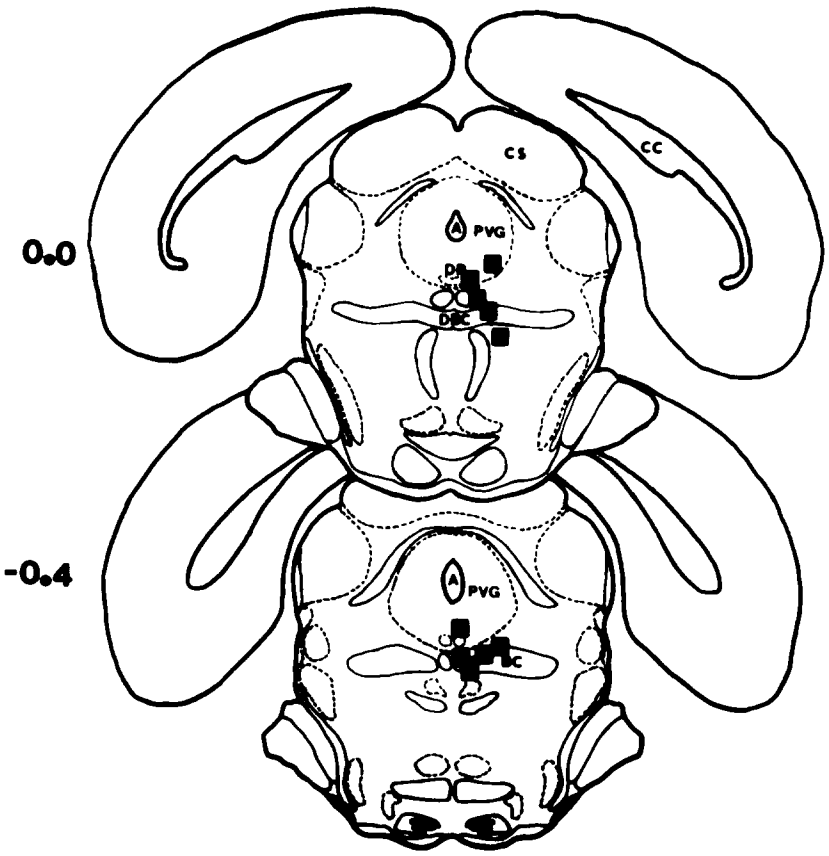


FIG. 5. Maps of the locations of electrode tips for the 10 animals used in the brain stimulation studies. Numbers to the left of the sections give anterior-posterior coordinates. Abbreviations: A, aqueduct of Sylvius; BC, brachium conjunctivum; CC, corpus callosum; CS, superior colliculus; DBC, decussation of the brachium conjunctivum; DR, dorsal raphe nucleus, PVG, central gray substance. Atlas of Pellegrino *et al.* [15].

differences between response rates after saline administration and after different doses of the naloxone compounds. These same procedures were used to analyze the effects of the naltrexone compounds.

For the locomotor activity analysis, scores for saline days were also averaged for the two parameters and the data for each drug were expressed as a percentage of the mean saline score. Two analyses of variance were performed, one for the horizontal activity scores and the other for the ambulation scores. Newman-Keuls' procedure was then used to determine if any of the four compounds differed from vehicle or if there were any differences between the hydrochloride and methobromide forms of the drugs.

### *Drugs*

Naloxone hydrochloride and naltrexone hydrochloride (courtesy of Endo Laboratories, Garden City, NY), naloxone methobromide and naltrexone methobromide (courtesy of Boehringer-Ingelheim, West Germany) were used. All drugs were dissolved in 0.9% saline and administered subcutaneously in a volume of 1.0 ml/kg body weight. Doses are expressed as the free base.

## RESULTS

### *Intracranial Self-Stimulation*

Over the dose-range 0.1–30 mg/kg naloxone hydrochloride, a graded decrease in the rate of lever pressing for ICSS on a FR:30 schedule occurred (Fig. 1). A dose of 1.0 mg/kg produced a significant reduction in responding. In these same animals, 0.1–30 mg/kg naloxone methobromide did not alter response rates which remained at control levels. The split-plot factorial analysis of variance showed a highly significant difference between naloxone hydrochloride and naloxone methobromide, $F(1,18)=44.2$, $p<0.001$. There were no differences in baseline lever pressing rates in the two studies. The mean baseline rate with saline in the naloxone hydrochloride study was $2872\pm347$ presses per 45 min compared with $2858\pm359$ presses per 45 min in the naloxone methobromide study.

Over the dose-range 0.1–30 mg/kg naltrexone hydrochloride, a graded decrease in the rate of lever pressing on the FR:30 schedule occurred (Fig. 2). As with naloxone, a dose of 1.0 mg/kg significantly decreased response rates. Inspection revealed that the dose-response curve for naltrexone was flatter than that for naloxone. At the two lower doses,

naltrexone produced somewhat greater decreases in responding, but at the two higher doses effects were less marked. At the 30 mg/kg dose the decrease produced by naltrexone was significantly less than that produced by naloxone, $F(1,9)=7.3$, $p<0.05$. The factorial analysis of variance comparing the two naltrexone compounds revealed that these two drugs had significantly different effects on response rates, $F(1,18)=17.9$, $p<0.001$. There were no differences in baseline response rates in these two studies. With naltrexone hydrochloride, the mean rate when saline was administered was $3135\pm346$ presses for 45 min and with naltrexone methobromide it was $3094\pm391$ presses. Figure 3 shows representative cumulative response records illustrating the effects of saline, naltrexone hydrochloride (0.1, 1.0, 10 and 30 mg/kg) and naltrexone methobromide (30 mg/kg) for one rat. With naltrexone hydrochloride, animals paused longer after each reinforcement as the session progressed.

*Locomotor Activity*

The effects of the highest dose (30 mg/kg) on both total horizontal activity and ambulation for each of the four compounds are given in Fig. 4. The analysis of variance for horizontal activity, $F(4,32)=9.8$, $p<0.001$, and for ambulation, $F(4,32)=3.7$, $p<0.025$, were both significant. Newman-Keuls' procedure indicated that when animals were administered naltrexone hydrochloride, a decrease in total horizontal activity occurred. In contrast, when administered naltrexone methobromide a significant increase in horizontal activity occurred relative to both saline values and to activity scores following naltrexone hydrochloride administration. The only significant difference in the ambulation scores as determined by the Newman-Keuls' procedure was that between naltrexone hydrochloride and naltrexone methobromide.

*Histology*

Figure 5 shows locations of the electrode tips for the 10 animals used in these experiments. As in our previous studies [19,25], the tips were located in the ventral part of the midbrain-central gray area, including the dorsal raphe, brachium conjunctivum and medial longitudinal bundle.

## DISCUSSION

The fixed ratio ICSS paradigm provides a sensitive means by which to evaluate the influence of opioid antagonists on positively reinforced behavior. We reported previously that, when ICSS was available on a continuous reinforcement schedule, the paradigm was relatively insensitive to the administration of naloxone in doses as high as 30 mg/kg. As the ratio of lever presses to rewards was increased, small-to-moderate doses of naloxone produced graded decreases in response rates [25]. We have clear evidence from the present study that the changes in fixed ratio responding produced by opioid antagonists are due to the effects of these drugs at receptor sites within the central nervous system rather than at receptor sites located peripherally. These data complement the work of others suggesting that naloxone-induced reductions in ICSS responding result from the blockade of central reward mechanisms [12]. In fact, the increase in the post reinforcement pause produced by naloxone and naltrexone suggest that the rewarding effect of ICSS is attenuated following drug administration in a process analogous to that of extinction. Further, we have observed a similar increase in post reinforcement pauses when the current intensity is reduced (Schaefer, unpublished).

The use of the tertiary and quaternary forms of these opioid antagonists to localize their sites of action is now well established (for review see [1]). From the considerable literature available, it is apparent the quaternary derivatives do bind and act at opioid receptor sites in both the central and peripheral nervous systems. When administered directly into the brain, both forms suppress water intake [2], produce antinociception [16], block morphine-induced catalepsy [3], and increase plasma levels of corticosterone [6]. The quaternary antagonists are active in *in vitro* tests on opiate receptor binding and on the activity of the isolated guinea pig ileum [13,23]. Similar activity is shown in such test systems in the dog as morphine-induced gut stimulation [17], and leucine-enkephalin-induced increases in blood pressure and heart rate [9]. The tertiary forms are more potent than the corresponding quaternary congeners, but potency ratios differ with the assay system and animal species used. Had we tested doses above 30 mg/kg, we might have observed changes in responding with the methobromide forms. However, such high doses might also have proved toxic.

In addition to the importance of the schedule of reinforcement, other variables influence results. With longer test sessions the rate decreasing effects of antagonists become more evident, and it has been suggested that animals undergo extinction [22]; a view with which we agree. The precise location of the stimulating electrode also appears to determine the sensitivity of the drug effect; areas containing higher concentrations of opioid receptors and peptides, such as the MID-CG, are more sensitive to the effects of the antagonists than are areas with lower concentrations such as the MFB-LH [8, 19, 21].

Changes in motor activity did not parallel those in lever pressing ([25], and this report). With naloxone hydrochloride, there was a significant 75% reduction in lever pressing but only a non-significant 25% reduction in horizontal activity and a 15% reduction in ambulation. With 30 mg/kg naltrexone hydrochloride there was a significant 50% reduction in lever pressing and also a significant 40% reduction in horizontal activity and a non-significant 25% reduction in ambulation. It seems unlikely, therefore, that a generalized reduction in motor activity would be of sufficient magnitude to account for the reduction in lever pressing. When the highest dose of the quaternary compounds was administered, the naloxone derivative produced no effect, whereas the naltrexone derivative increased both horizontal and ambulatory activity; this stimulation has not previously been reported (see [1]), and we are uncertain about its significance.

These experiments demonstrated that the effects of opioid antagonists on lever pressing for ICSS on a fixed ratio schedule depend upon the entry of the drugs into the central nervous system and, presumably, upon their entry into the neural substrate of the reward system. This interpretation will be supported or refuted by the results of on-going experiments involving micro-injections of antagonists locally in neural sites while animals are studied in the ICSS paradigm.

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