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Effects of Naloxone and Diprenorphine on Spontaneous Activity in Rats and Mice[1]

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DEROSSETT, S. E. AND S. G. HOLTZMAN. *Effects of naloxone and diprenorphine on spontaneous activity in rats and mice.* PHARMAC. BIOCHEM. BEHAV. 17(2) 347–351, 1982.—The narcotic antagonist naloxone has been reported to decrease locomotor activity in the rat, presumably by blocking endogenous opiate systems. Naloxone has a greater affinity for receptors which preferentially bind morphine and other opiate alkaloids as compared to receptors that bind endogenous opioid peptides. Diprenorphine, another pure opiate antagonist, binds with equal affinity to both receptor subtypes. Therefore, the effects of the narcotic antagonists naloxone and diprenorphine on spontaneous activity were compared in rats and mice, tested individually and in pairs. Only naloxone (10 mg/kg) affected spontaneous activity in rats tested individually, decreasing both gross and fine activity. In rats tested in pairs, naloxone (1.0 and 10 mg/kg) decreased both fine and gross activity, while diprenorphine (10 mg/kg) produced significant decreases only in fine activity. In mice tested individually, naloxone produced modest (nonsignificant) decreases in activity while diprenorphine (10 mg/kg) significantly enhanced activity. Neither opiate antagonist produced consistent effects on activity in paired mice. These results illustrate the species and situation dependence of the effects of opiate antagonists and point out the need for testing more than one narcotic antagonist in research designed to provide inferential information concerning possible physiological functions of endogenous opioid peptides.

Naloxone Diprenorphine Rats Mice Spontaneous activity Narcotic antagonist

NALOXONE is a widely used opiate antagonist that has become an important pharmacologic tool in efforts to identify the possible physiological functions of the endogenous opioid peptides. Although naloxone is essentially devoid of intrinsic activity at low doses [3,18], it nonetheless produces behavioral changes when administered to otherwise drug-free subjects, presumably as a consequence of the blockade of opiate receptors and the resultant disruption of the activity of endogenous opiate systems [18,19].

Recent investigations have suggested that naloxone decreases locomotor activity [1, 11, 17] as well as social interaction in the rat [8]. Arnsten and Segal [1] have demonstrated that naloxone produces dose-related decreases in locomotor activity in the rat as reflected by a decrease in compartment entries in a nine-compartment chamber. In this study, a low dose of naloxone (0.5 mg/kg) was associated with an increase in contact with environmental stimuli, suggesting that low doses of naloxone may have effects on exploration which are secondarily reflected as changes in locomotor activity and which may be regulated via endogenous opioid peptides. A high dose, 25 mg/kg, produced a decrease in locomotor activity which was unassociated with an increase in contact with environmental stimuli, and which may reflect non-specific behavioral suppression. Rodgers and Deacon [17] have shown that naloxone produces a decrease in locomotor activity in the rat when assessed in the novel open field for two minutes, but were unable to demonstrate a simple dose response relationship. File [8] has shown that naloxone decreases the amount of time spent in social interaction by pairs of rats as assessed by an observer rating scale and, in addition, reduces the amount of motor activity displayed by the pairs of rats. The same dose (2.0 mg/kg) did not reduce locomotor activity in animals tested individually. Due to the multi-dimensional character of the interacting behaviors which we collectively call spontaneous activity, and due to the wide variety of protocols and paradigms used to assess spontaneous activity, it was of interest to more fully characterize the effects of naloxone on activity in the rat by examining spontaneous activity in another experimental context. Therefore, we examined the effects of naloxone over a 100- to 1000-fold range of doses on both locomotor and fine activity in rats tested individually as well as in pairs.

Naloxone has also been reported to decrease activity in several strains of mice [2, 11, 12, 16] under a variety of conditions. For example, naloxone decreases the activity of Swiss Webster mice tested individually in circular activity cages [2] and of Swiss OF_1 and CD-1 mice tested in motility actimeters [11,16]. In addition, Gorris and van Abeelen [10] have shown that grid crosses are decreased in response to naloxone in the SRH mouse strain, but are unaffected by naloxone in strains SRL, C57, and DBA. Because of the well-documented species dependency of many of the effects of opiates, the effects of naloxone on activity in the CF-1

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mouse were determined in the same apparatus and under similar conditions used to assess activity in the rats.

Recent studies have suggested the existence of multiple opiate receptors which preferentially bind either morphine or the enkephalins [6, 7, 14]. The putative opiate receptor μ is postulated to selectively bind morphine and mediate the effects of opiate alkaloids while the δ receptor is proposed to bind enkephalins and mediate the effects of the endogenous peptides [6, 7, 14]. In support of this concept, recent studies have indicated that naloxone and related antagonists bind with greater affinity to the μ opiate receptor relative to the δ receptor [6,7]. Diprenorphine, on the other hand, is a pure antagonist of the oripavine series, which binds to both receptors with equal affinity [6,7]. Since the behavioral effects of narcotic antagonists could be mediated principally through the δ receptor as a result of endogenous peptide blockade, diprenorphine may be more useful than naloxone in elucidating the roles of endogenous peptides in behavior. Therefore, another purpose of the present study was to systematically compare the effects of diprenorphine and naloxone on spontaneous activity in the rat and mouse.

METHOD

Subjects

The rats used in the study were 48 males (Sprague-Dawley derived) obtained from Holtzman (Madison, WI) weighing 190–265 grams at the beginning of testing. Rats were housed in groups of 6 in standard plastic cages. The subjects for the experiments utilizing mice were 64 male CF-1 mice (Charles River Breeding Laboratories, Wilmington, MA) weighing 24–38 grams. Mice were housed in groups of 25–30 in metal cages. All animals were housed in a ventilated room maintained on a 12:12 on:off illumination schedule commencing at 7 a.m. with food (Rodent Laboratory Chow #5001, Ralston Purina Company) and water available ad lib.

Apparatus

The four activity chambers used for the testing of rats were standard polycarbonate cages (51×41×22 cm). Each cage was covered with a wire top and centered on a sensor platform that was connected to a two-channel Electronic Activity Monitor (#31464, Stoelting Co., Chicago, IL) coupled by shielded cable to electromagnetic sensors which measured spontaneous activity. Each cage and platform was housed in a well-ventilated sound-attenuating chamber illuminated by a 6 W fluorescent light bulb located eight cm above the cage top.

For rats, the sensitivity of each sensor was individually adjusted to correspond closely (±5%) to the sensitivity of one sensor (Threshold Reset-S) which had been calibrated with a swinging pendulum to measure gross motor movements corresponding to locomotion. The other channel (Threshold Reset-N) was calibrated to record total activity, with the difference between total and gross activity being equal to fine activity. Counts for each sensor were cumulatively recorded every three minutes by a North Star Horizon microcomputer.

The same two-channel activity monitors were used to measure the activity of mice. The four activity chambers were polycarbonate cages measuring 27.5×17×13 cm covered with a ventilated Plexiglas top. Each cage and platform was housed in the same sound attenuating chambers used in the previous experiment. All other aspects of the apparatus were the same except that the sensitivity for each sensor was individually adjusted to measure total activity, because the small body size of the mouse made it impractical to attempt to reliably differentiate gross from fine movements. Counts were cumulatively recorded every five minutes for a one hour session.

Procedure

Rats were randomly assigned to either the naloxone or diprenorphine drug groups and given either saline, 0.1, 1.0, or 10 mg/kg of drug SC 30 minutes prior to the start of the session which was conducted between 8:30 a.m. and 12:30 p.m. Animals were tested individually in activity monitors for 30 minutes for 1 session each with n=6 for each dose level. Approximately one week later, the same animals were tested in pairs for three 30 minute sessions conducted every other day beginning at 8:30 a.m. The results of a pilot experiment showed that the activity of undrugged rats paired in this manner remained constant over three sessions of testing. An additional dose, 0.01 mg/kg of diprenorphine or naloxone, was added to the drug series. Pairs were formed each session by taking animals from different cages, and both members of the pair were injected with the same drug dose. Assignment of subjects to activity monitors was systematically varied so that all doses were tested on each monitor. In addition, assignment of pairs to doses was varied so that 9 pairs were tested for each dose level.

Sixty-four mice were randomly selected from a colony of 92 and were assigned to either the naloxone or diprenorphine drug group. Each group consisted of eight animals and was given either saline, 0.1, 1.0 or 10 mg/kg of either naloxone or diprenorphine IP 15 minutes prior to the beginning of the session. Animals were individually tested once each for one hour between 8:30 a.m. and 12:30 p.m. in the activity chambers. Assignment of subjects to each sensor platform and monitor was systematically varied so that all doses were tested on each monitor.

Approximately one month subsequent to individual testing, the same pool of mice used in the previous experiment were subjected to activity testing in pairs. The same doses used in the individual activity experiment were used in the paired activity experiment (i.e. 0, 0.1, 1.0, or 10 mg/kg of naloxone or diprenorphine). Both members of the pair were injected with 8 pairs/dose being tested. Hence, some animals were tested more than once. Animals which had been tested previously were paired with different animals upon subsequent testing. Pairs were always formed by taking two animals from different cages. All other procedures were identical to those of the previous experiment.

Drugs

Naloxone hydrochloride and diprenorphine hydrochloride (supplied by the National Institute on Drug Abuse) were dissolved in 0.9% saline and were administered to rats SC in a volume of 1.0 ml/kg of body weight. Mice were injected IP with a volume of 1.0 ml per 100 g of body weight. Drug doses are expressed in terms of the free base. Within each drug series, administration of doses (including saline), was either random or varied in a systematic manner such that all doses were tested on each monitor.

Data Analysis

Data were statistically evaluated by means of two-way

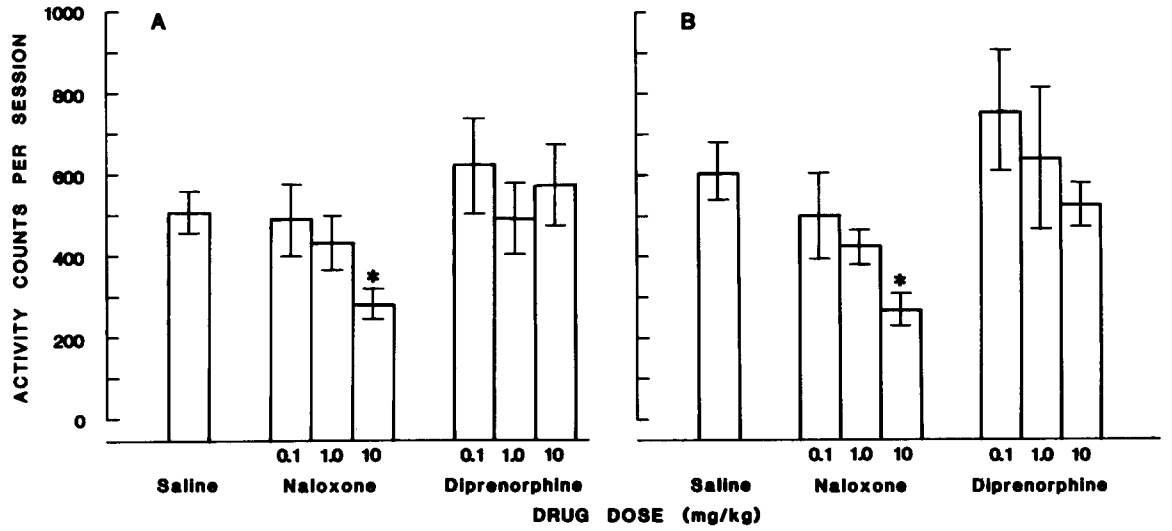

FIG. 1. Effects of naloxone or diprenorphine on gross (A) and fine (B) activity in rats tested individually in activity monitors for 30 minutes. Each point represents the mean±S.E. of 6 observations for each dose level, except for the saline control, where n=12. Significant differences betwwen control and treatment means are indicated as $*p<0.05$.

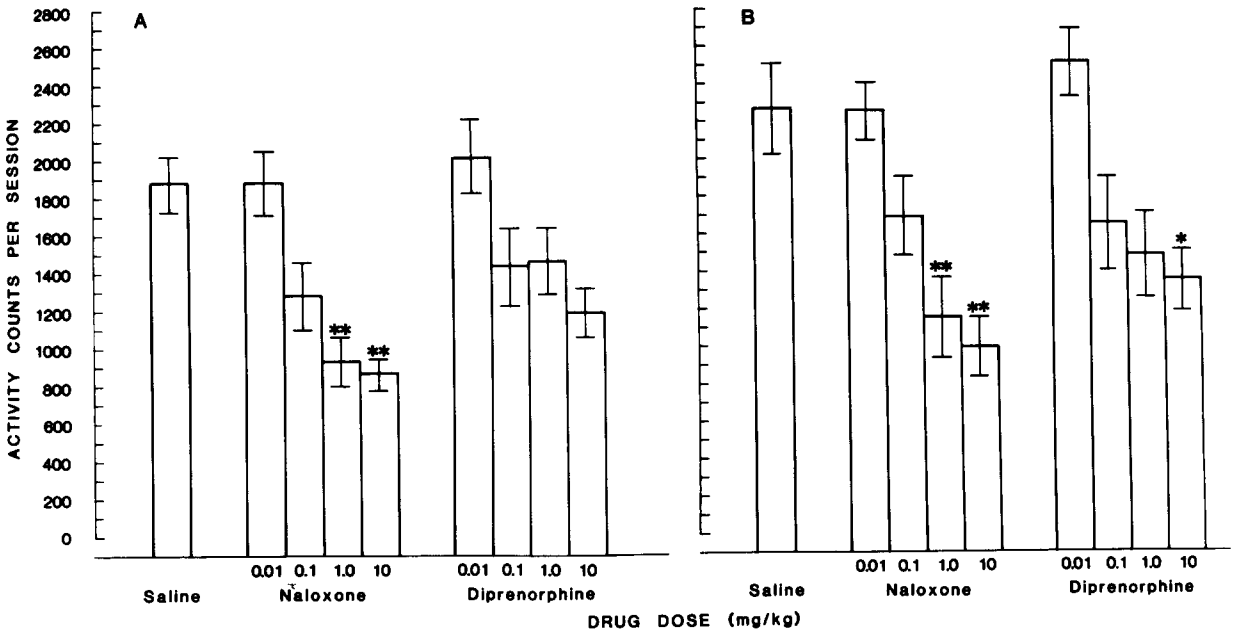

FIG. 2. Effects of naloxone or diprenorphine on gross (A) and fine (B) activity in paired rats tested in activity monitors for 30 minutes. Each point represents the mean±S.E. of 9 observations for each dose level, except for the saline control, where n=18. Significant differences are indicated as $*p<0.05$ and $**p<0.01$.

analyses of variance. Comparison of treatment and control means were accomplished by a two-sided Dunnett's test. Data are presented as treatment means±1 S.E.M.

RESULTS

The effects of naloxone and diprenorphine on spontaneous activity in individual rats during the 30-minute experimental session are shown in Fig. 1. Naloxone treated rats exhibited a dose-dependent decrease in gross activity as compared to saline controls (Fig. 1A). Naloxone decreased gross activity by as much as 44% at the highest dose tested, and produced essentially the same effect on fine activity in these rats; fine activity was decreased by 56% at the 10 mg/kg dose (Fig. 1B). Diprenorphine had no consistent effect on either gross or fine activity in rats tested individually (Fig. 1).

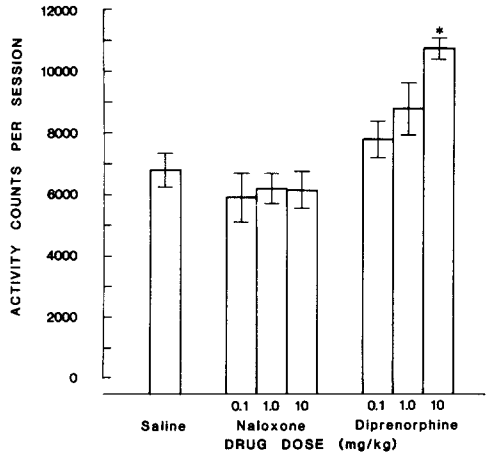

FIG. 3. Effects of naloxone or diprenorphine on total activity in mice tested individually in activity monitors for 1 hour. Each point represents the mean±S.E. of 8 observations for each dose level, except for the saline control, where n=16. Significant differences are indicated as $*p<0.05$.

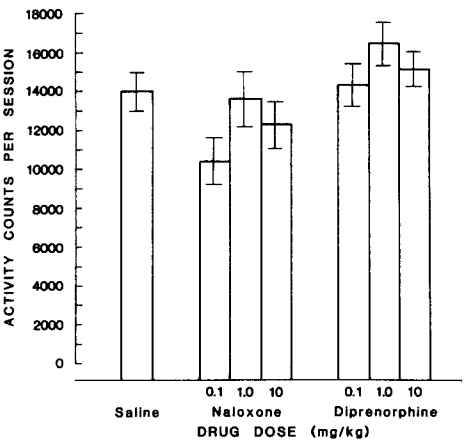

FIG. 4. Effects of naloxone or diprenorphine on total activity in paired mice tested in activity monitors for 1 hour. Each point represents the mean±S.E. of 8 observations for each dose level, except for the saline control, where n=16.

Figure 2 (A and B) shows the effects of naloxone and diprenorphine on rats tested in pairs. Under these conditions, as little as 0.1 mg/kg of naloxone reduced gross activity by 32%. Higher doses of 1.0 and 10 mg/kg produced statistically reliable ($p<0.01$) decreases of 50 and 54%, respectively. Diprenorphine decreased gross activity under these conditions, though the effect was more modest than that produced by naloxone and failed to reach statistical significance. From Figure 2B, it may be seen that naloxone decreased activity by 56%. Diprenorphine decreased fine activity under these conditions by 40% at the 10 mg/kg dose.

Figures 3 and 4 show the dose-response relationships for the total activity of mice given naloxone or diprenorphine and tested under individual and paired conditions. Naloxone produced a negligible decrease in activity in individual mice, whereas diprenorphine produced well-defined, dose-dependent increase in activity that reached 156% of control at the 10 mg/kg doses (Fig. 3). Both naloxone and diprenorphine had inconsistent and non-significant effects on activity when tested in paired mice (Fig. 4).

DISCUSSION

Naloxone has previously been shown to reduce both locomotor activity [1, 8, 17] and social interaction [8] in separate paradigms. Furthermore, naltrexone, an antagonist structurally related to naloxone, has recently been demonstrated to produce dose-related declines in locomotor activity [12]. The present experiment confirmed the results obtained in those previous experiments, i.e., naloxone decreased locomotor activity in rats tested alone. Although the dose necessary to decrease activity was larger as compared to earlier reports [1,17], the decreases in response to low doses of naloxone reported by others may have been a reflection of increased interaction with environmental stimuli [1] or an altered response to a novel environment [17] rather than an effect on activity per se. In addition to its effects on locomotor activity, naloxone also decreased fine activity suggesting, perhaps, that the behaviors involved in social interaction, i.e., behaviors such as grooming, sniffing, etc., are indeed affected by naloxone. These findings cannot be extended, however, to the antagonist diprenorphine. This oripavine-derived antagonist decreased neither gross nor fine activity in animals tested individually.

In subsequent experiments examining paired rats, it was again found that naloxone decreased both fine and gross activity. These findings suggest that the effects of naloxone on motor activity in the rat are of a general nature rather than being situation-specific. However, the effect of naloxone on activity was greater for both fine and gross activity when assessed in paired rather than individual rats. The effects of diprenorphine on activity in paired rats were dissimilar to the effects produced in the same rats tested individually. Diprenorphine decreased gross activity (although the effect was not statistically significant) and caused a significant decrease in fine activity, although not as great as the decrease caused by naloxone. Whether or not the elevated baseline activity seen in the paired condition contributed to the enhanced efficacy of the antagonists remains to be determined. The effect of diprenorphine on paired activity is not a consequence of generalized depression of behavior—since the activity of individual rats was not affected by those same doses. These findings suggest that naloxone affects the behaviors involved in both locomotor activity and social interaction, whereas diprenorphine more selectively affects the behaviors involved in social interaction. More conclusive evidence for this position could be gained by examining the effects of these drugs in paired drug-naive animals. No conclusions concerning the nature of the effect of these drugs on social interaction can be drawn in the absence of constant visual monitoring of the animals.

In contrast to the results obtained in rats, naloxone did not have any consistent effect on either gross or fine activity in the mouse. A few authors have previously demonstrated that naloxone decreases activity in mice in a testing apparatus similar or comparable to ours. In one report, activity was

matched between control and experimental groups (8.0 mg/kg naloxone), hence reducing the between group variability substantially [12]. In another report [11], naloxone at doses in the same range as in the present study was found to decrease activity in the mouse, although the effect was not large, while much higher doses (30 and 90 mg/kg) were required to produce substantial decreases in locomotor activity. In addition, the experimental protocol of that study differed from the design of the present study in that the mice were tested in groups (4/group) and the sample size was large (21 groups/dose). Therefore, it seems plausible that mice are much less sensitive than rats to the effects of naloxone on activity. Alternatively, the discrepancies in results seen between earlier studies and our own may stem from strain differences, in view of the recent demonstration that the effect of naloxone on activity in the mouse is strain-dependent [10]. Diprenorphine, on the other hand, produced a dose-related increase in activity in mice tested individually, contrary to the effects it produced in rats. With paired mice, both diprenorphine and naloxone had variable and inconsistent effects on spontaneous activity. Hence, the effects of both these drugs on activity are species dependent. This conclusion is warranted even though the behaviors recorded for the two species may not be entirely comparable. For example, naloxone produced similar effects on both fine and gross activity in the rat, necessarily resulting in a decrease in total activity, an effect unlike that which is seen in the mouse. In addition, the effects of diprenorphine are more selective; this drug modified fine activity in paired rats while leaving fine activity in individual rats unaffected. The fact that diprenorphine affects fine activity in rats only when they are paired lends credence to the idea that diprenorphine affects social interaction, and suggests that the delta receptor subtypes may be involved in the modulation of social behavior by opioids. In this regard, diprenorphine may be more useful than naloxone in elucidating opiate influences on social behavior, particularly in view of the inconsistent results obtained in tests with naloxone on social behaviors such as proximity maintenance [15]. Detailed studies, including direct observation, of the behavior of diprenorphine treated rats seem warranted by these observations.

These studies emphasize that caution must be exercised when drawing conclusions about the participation of the endogenous opiates in behavior when those conclusions are based on the use of only one antagonist (usually naloxone) and a limited number of behavioral contexts. While diprenorphine has been shown to be more potent and longer acting than naloxone in some situations [20], this observation cannot be extended to the present experimental context. The fact that the two drugs produce different effects in mice as compared to rats may indicate that the species differ in their magnitude of response to the same dose level of the drugs, or alternatively that the behavioral substrate acted upon by the drugs differed for the two species.

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