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BRIEF COMMUNICATION

Suppression of Juvenile Social Behavior Requires Antagonism of Central Opioid Systems

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JALOWIEC, J. E., D. J. CALCAGNETTI AND M. S. FANSELOW. *Suppression of juvenile social behavior requires antagonism of central opioid systems*. PHARMACOL BIOCHEM BEHAV **33**(3) 697–700, 1989.—Pairs of male and female rats were injected with either tertiary naltrexone (NTX) which readily crosses the blood-brain barrier, or quaternary naltrexone (QNTX) which does not, to determine the importance of central opioid systems in the elaboration of juvenile social behavior. In the first experiment, only intraperitoneal injections of NTX (1.0 mg/kg) suppressed the frequency of wrestling pins. Peripheral injections of QNTX (10.0 mg/kg) were without effect. In a second experiment, QNTX (2.0, 4.0, or 8.0 μg/4.0 μl) was injected directly into the lateral ventricles. Intracerebroventricular injection of the moderate dose reliably reduced frequency of pinning while the higher dose was severely incapacitating and the low dose was without effect. The results of these two experiments confirm an important role for brain opioid systems in the control of juvenile social interaction.

Naltrexone Quaternary naltrexone Endogenous opioids Social behavior Play Intracerebroventricular

SEVERAL recent studies have implicated endogenous opioid systems in the elaboration and maintenance of juvenile social interactions, defined operationally by wrestling-like "pins" in young rats (8,11). Considerable evidence indicates that the relevant systems are localized, at least in part, in opioid-rich regions of the limbic system (7,12). However, the critical demonstrations that opioid receptor activation enhances this social interaction and that opiate antagonism significantly depresses pin frequency (9) have relied upon systemic injections of morphine and naloxone which bind readily to peripheral as well as central receptors. Accordingly morphine and naloxone, respectively, may increase and decrease pins via their direct action on opioid systems lining the gastrointestinal tract and other peripheral sites (3).

The development of a quaternary (methobromide) form of naltrexone, a potent and selective opiate antagonist that does not cross the blood-brain barrier in moderate doses as readily as the tertiary form (1), has made the distinction between the effects of central versus peripheral action fairly straightforward (2).

In order to determine whether the reduction of some forms of social interaction following opiate antagonism is due to blockade of brain opioid receptors, we monitored the social interaction of juvenile rats following peripheral injections of either tertiary naltrexone (NTX) or quaternary naltrexone (QNTX). If antagonism of central opiate systems is necessary for reducing social interaction, then "pinning" behavior should decrease only after injections of tertiary naltrexone.

In addition, pairs of juvenile rats implanted with lateral ventricle cannulae received intracerebroventricular (ICV) injections of QNTX to determine its effectiveness in reducing social interaction. If antagonism of central opioid systems is essential in reducing social interaction then ICV injections of QNTX should be more effective than systemic treatment.

EXPERIMENT 1

METHOD

Subjects

Twelve male and 12 female juvenile Long-Evans hooded rats, born and raised in the Dartmouth Department of Psychology colony, were weaned at 21 days of age into individual suspended wire cages (24 × 24 × 17 cm). They were maintained at an ambient temperature of 22 ± 1°C with ad lib access to Agway

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Prolab 3000 food pellets and water. Fluorescent lighting was on a 14:10 hr light/dark schedule, and all behavioral tests were completed during the first half of the light phase.

Apparatus

For observation of social interaction, pairs of rats were placed in a 32 × 32 × 27 cm clear Plexiglas box with approximately 2 cm of wood chips covering the floor. This test cage was situated within a larger (34 × 50 × 28 cm) sound-attenuated Grason-Stadler chamber equipped with an exhaust fan, a 25 W red light bulb centered on one side and a 15-cm diameter viewing window in the front door.

Procedure

Twenty-four and 48 hr after weaning, pairs of rats (matched for sex and body weight) were observed for five minutes, and the frequencies and durations of pinning behavior were recorded with computerized counters and time accumulators. Pairings were maintained throughout the experiment. Pinning was defined as the event in which one rat overturned its partner and, using its forepaws and upper torso, held it dorsal surface to the ground. This discrete behavior usually occurs briefly during rough-and-tumble play (play-fighting) although some bouts may last several seconds (11). Thirty minutes prior to these initial habituation trials, each rat was given a sham intraperitoneal injection.

One day subsequent to the second habituation trial, drug tests began using a completely counterbalanced within-subjects protocol (pairs as subjects) in which each pair received each of three treatments four times over the course of twelve trials. Thirty minutes prior to observations both members of a pair received intraperitoneal injections of either 0.9% sodium chloride (1.0 ml/kg), naltrexone (NTX, 1.0 mg/kg), or quaternary naltrexone (QNTX, 10.0 mg/kg). Drug tests occurred every 48 hr beginning at age 24 days and ending at age 46 days to take advantage of the developmental period during which pinning is most prominent (6). The repeated-measures design was used because pinning first increases and then decreases over this age range (an inverted U-shaped function). The observer of the social interaction periods was blind to the drug treatments.

RESULTS

Only injections of NTX significantly reduced the mean frequency of pinning behavior (Fig. 1) during the five-minute observation periods [repeated measures ANOVA, $F(2,22) = 28.77$, $p<0.0001$]. Similar reductions occurred in both male and female pairs and their results were combined. Injections of QNTX produced no significant changes in mean frequency of pinning.

Mean duration of individual pins (Fig. 1) was increased significantly by NTX but not by QNTX [repeated measures ANOVA, $F(2,22) = 21.44$, $p<0.0001$]. However, the average total amount of time spent in the pinning posture during the observation periods (Fig. 2) was essentially unchanged [repeated measures ANOVA, $F(2,22) = 0.51$, $p>0.60$]. Again, no sex differences were noted.

DISCUSSION

The results of this experiment clearly support the contention that central opioid systems are involved in the reductions in the frequency of rat social interaction noted after peripheral treatment with opiate antagonists. Injections of the tertiary form of naltrexone, which readily passes into the central nervous system, reduced the frequency of pinning behavior. Treatments with the quaternary form, which is excluded from the brain, did not.

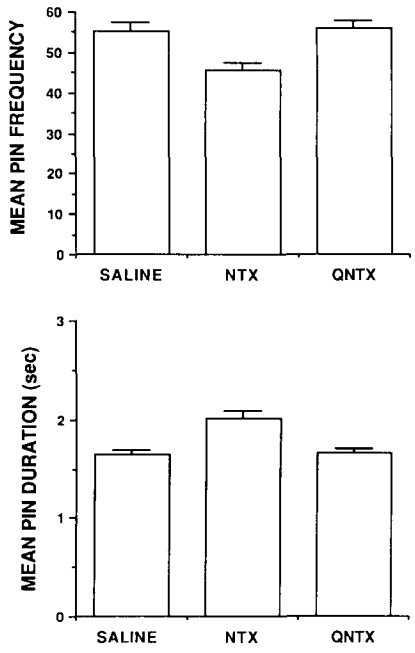

FIG. 1. Mean (+SEM) frequencies of pinning (top) and mean (+SEM) durations of individual pins (bottom) after peripheral injections of saline, tertiary naltrexone (NTX—1.0 mg/kg) and quaternary naltrexone (QNTX—10.0 mg/kg).

The reliable increase in mean duration of pins following NTX injections (Fig. 1) was surprising and may reflect some difficulty in establishing stable dominance relationships within the pairs because of the changing pharmacological context of their social interaction. In previous work, pin frequencies and the average duration of each bout tended to change in concert (9). Others (11) have noted that pin durations are not as reliable as pin frequencies for discriminating between manipulations. The present results suggest that even though NTX reduced the frequency of pinning, total time in the pinning posture was conserved by extension of pin duration (Figs. 1 and 2). Essentially, the rats pinned less, but held onto each other longer after injections of NTX. Accordingly, antagonism in opiate systems may interfere with the initiation of

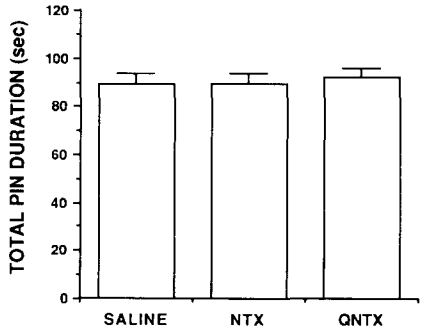

FIG. 2. Mean (+SEM) total pin durations after peripheral injections of saline, tertiary naltrexone (NTX—1.0 mg/kg) and quaternary naltrexone (QNTX—10.0 mg/kg).

pins and also modify the reinforcing value of maintaining the pinning posture.

EXPERIMENT 2

In the previous experiment, reductions in the frequency of pinning occurred only after treatment with NTX. QNTX had no effect on pinning behavior, presumably because it does not readily penetrate the blood-brain barrier (1). However, if central opioid systems modulate pinning, then intracerebroventricular (ICV) injections of QNTX should reliably reduce this aspect of rat social interaction. In this experiment QNTX was injected directly into the lateral ventricle of juvenile rats fitted with permanent cannulae and subsequent pinning behavior was observed.

METHOD

Subjects

Eight male and 14 female juvenile Long-Evans hooded rats, born and raised in the Dartmouth Department of Psychology colony, were weaned at 25 days of age and maintained as in Experiment 1.

Procedure

The apparatus and general procedure for observing and recording social interactions were the same as in Experiment 1.

Five days after weaning all rats were anesthetized with Ketamine hydrochloride (100 mg/kg) and stainless steel guide cannulae (22-gauge; Plastic Products, Roanoke, VA) were stereotaxically-guided and permanently implanted in their right lateral ventricles (0.5 mm posterior and 1.5 mm lateral from bregma, and 3.2 mm ventral from the dura).

One and two days after surgery, the rats were paired by sex and similarity of body weight and observed for 5 minutes as described earlier to habituate the animals to the observation chamber and to verify that vigorous pinning behavior was unimpaired by the cannula implantation. Pairings were maintained throughout the experiment.

Four days after surgery (at 34 days of age), both members of 5 pairs received a 4.0 μl ICV injection of the saline vehicle and 6 pairs were given an injection of QNTX (8.0 μg/4.0 μl). Subsequently, using a counterbalanced design and at 48-hr intervals, pairs were injected with the saline vehicle or QNTX (4.0 μg/4.0 μl) at 36 and 38 days of age and with the vehicle or QNTX (2.0 μg/4.0 μl) at 40 and 42 days of age. Ten minutes after ICV injections, pairs of rats were placed in the observation chamber for 5 minutes and scored for social interaction by an observer blind to drug treatment. In addition to recording the frequency and duration of pinning, dorsal contacts defined as those instances when the forepaws of one rat rested on the dorsal or lateral surface of its partner were counted. This latter measure provided information on play solicitation (11) and general activity levels.

The rats were held gently during removal of the cannulae obturators and the ICV injection procedure. Injections were delivered at a rate of 1.0 μl/10 seconds through a 28-gauge stainless steel internal cannula backloaded with injectant and connected via PE-50 tubing (Intramedic 7411) to a 5 μl Hamilton (7002) syringe. Patency of the injector tubing was established prior to insertion.

Following completion of the experiment, the rats were deeply anesthetized with sodium pentobarbital (70 mg/kg) and given ICV injections of 2.0 μl of India ink. Ten minutes later, the rats were perfused transcardially with saline and buffered formalin. Accurate placement of guide cannulae was verified by visual inspection of the ventricles for traces of ink at the frontal level of the cannula track. Data from one male and one female pair were excluded from further analysis because of misdirected cannula trajectories.

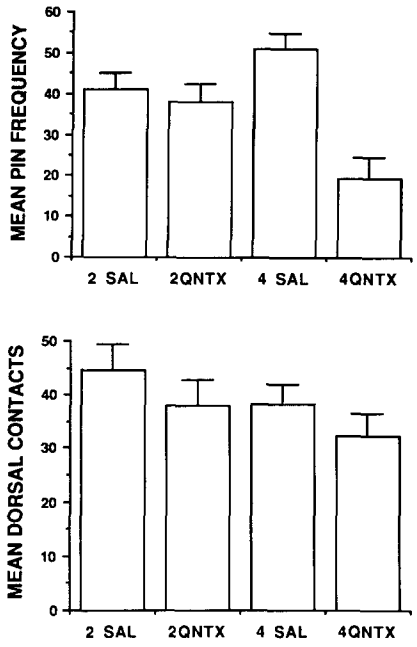

FIG. 3. Mean (+SEM) frequencies of pinning (top) and dorsal contacts (bottom) following intracerebroventricular injections of quaternary naltrexone (QNTX—2.0 or 4.0 μg/4.0 μl) and associated saline controls (4.0 μl).

RESULTS

The initial observations of social interaction following cannula implantation indicated no disruption of pinning behavior. Members of interacting pairs showed little or no interest in each other's exposed cannula guides and caps.

Injections of QNTX at the highest dose (8.0 μg/4.0 μl) completely abolished active social interaction and markedly reduced general activity. During the 5-minute observation periods the rats lay quietly and were unresponsive to their partners or the environment. Accordingly, we did not continue with a counterbalanced treatment at this dose. Subsequent counterbalanced treatments with the moderate (4.0 μg) and low (2.0 μg) doses of QNTX coincided with developmental periods of higher and lower pinning frequencies (6).

Pin frequency (Fig. 3) was reduced significantly by the 4.0 μg/4.0 μl dose of QNTX (correlated $t=9.14$, $p<0.001$), but not by the 2.0 μg/4.0 μl dose (correlated $t=0.83$, $p=0.28$). However, neither dose significantly altered the frequency of dorsal contacts relative to the counterbalanced control tests following ICV vehicle injection (Fig. 3). Average durations of individual pins were not changed significantly by either the 4.0 μg/4.0 μl or the 2.0 μg/4.0 μl doses of QNTX (Fig. 4). Thus, the total amount of time spent pinning was significantly decreased (Fig. 4) following 4.0 μg/4.0 μl QNTX (correlated $t=3.52$, $p<0.01$) because of the reduced frequency of pins.

DISCUSSION

The moderate dose of QNTX (4.0 μg/4.0 μl) injected directly into the lateral ventricle was capable of modestly reducing pinning

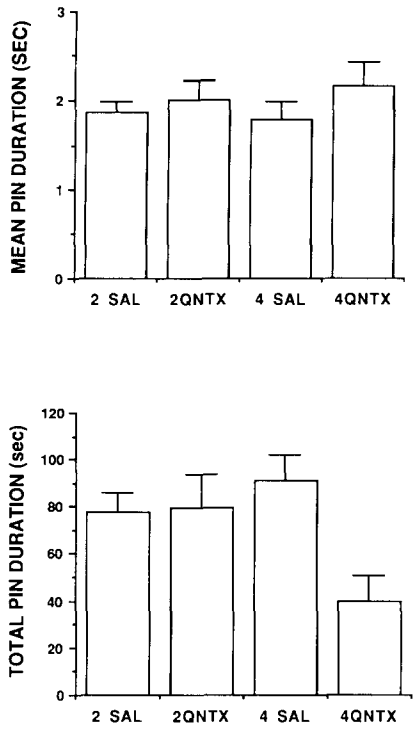

FIG. 4. Mean (+SEM) durations of individual pins (top) and mean (+SEM) total pin durations (bottom) following intracerebroventricular injections of quaternary naltrexone (QNTX—2.0 or 4.0 μg/4.0 μl) and associated saline controls (4.0 μl).

behavior but not dorsal contacts or mean pin duration. The higher dose (8.0 μg/4.0 μl) used initially produced severe general behavioral depression and the lowest dose (2.0 μg/4.0 μl) did not produce statistically significant changes in any measures. These results indicate that ICV treatment with QNTX can suppress social interaction in juvenile rats. The fact that the moderate dose of QNTX did not depress frequency of dorsal contacts indicates that the effect on pin frequencies was not due simply to a general reduction in behavior.

However, these results should be interpreted with caution because complete counterbalancing of doses was not possible and tolerance to the effects of ICV QNTX may have occurred. In other words, the lower dose might have been more effective if it had been given first. Note that counterbalancing of doses would be problematic for a repeated-measures design when baseline levels of pinning fluctuate, albeit predictably, during development.

GENERAL DISCUSSION

The capacity of peripheral or central QNTX for antagonizing morphine analgesia in the rat is uncertain (1). Nevertheless, recent studies have successfully dissociated central and peripheral opioid systems involvement in the enhancement of Pavlovian fear conditioning often seen after opiate antagonist treatment (4) and in conditioned opioid analgesia (2). The current research extended the use of this pharmacological dissociation procedure to juvenile social behavior.

Prior to these studies, a gastrointestinal site of action for the play-enhancing effects of low doses of morphine and the contrasting reductions in play seen after treatment with opiate antagonists was compelling. Low doses of morphine could enhance social interaction by mimicking the releasing effects of a single meal on play in young food-deprived rats (13). Antagonist treatments could contribute to general malaise by direct action on gastrointestinal receptors (3). From this perspective, the dominance-promoting effects of low doses of morphine and the submissiveness seen in naloxone-treated rats (9) could be explained simply as epiphenomena of gastrointestinal upset. Similarly, opioid modulation of ingestive behavior could be primarily a peripheral effect (5).

Taken together, the results of the present experiments provide convincing evidence that antagonism of activity in peripheral opioid systems is not essential for modulation of juvenile rat social behavior. Instead, endogenous brain opioid receptors within the central nervous system are involved in the elaboration of normal social interaction in juvenile rats.

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