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The Effects of Naloxone on Body Rotation-Induced Analgesia and Anorexia in Male Mice

KLAUS-PETER OSSENKOPP, MARCIE A. BETTIN* AND MARTIN KAVALIERS

**Department of Psychology and Division of Oral Biology—Faculty of Dentistry*
University of Western Ontario, London, Ontario, Canada N6A 5C2

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OSSENKOPP, K.-P., M. A. BETTIN AND M. KAVALIERS. *The effects of naloxone on body rotation-induced analgesia and anorexia in male mice.* PHARMACOL BIOCHEM BEHAV **34**(2) 317–320, 1989.—The effects of body rotation in a horizontal plane and the opiate antagonist, naloxone, on the nociceptive responses and the feeding behavior of male mice were examined. In the first experiment the mice were rotated (70 rpm, schedule of 15 sec on; 5 sec off) for 60 minutes or exposed to sham rotation for the same duration. Midway through the rotation or sham procedure the mice were either injected with naloxone (1 mg/kg) or isotonic saline. At the end of the 60-minute treatment period the animals were placed on a warm surface (47.5°C) and their latency to show a foot-licking response was measured. The rotation procedure produced a significant ($p<0.01$) increase in response latency in the saline-injected mice and the naloxone injections blocked this analgesic effect. This finding provides evidence for opioid involvement in the rotation-induced analgesia. In Experiment 2 mice on a food restriction schedule were rotated (70 rpm, 15 sec on; 5 sec off) or sham exposed for 60 minutes. Midway through this treatment period the mice were either injected with naloxone (1 mg/kg) or isotonic saline. Following the treatment period the mice were given access to food for 2 hours. The rotation procedure produced a significant ($p<0.01$) reduction in feeding (anorexia) in the first 30 minutes of food access for the saline-injected mice. Injections of naloxone significantly ($p<0.05$) enhanced the rotation-induced anorexia. These experiments demonstrate that rotation-induced analgesia in mice is blocked by the opiate antagonist, naloxone, whereas rotation-induced anorexia is not. Further, naloxone was found to enhance rotation-induced anorexia, possibly by intensifying rotation induced motion sickness in the mice.

Body rotation Naloxone Motion sickness Analgesia Anorexia Stress Mice

A broad range of laboratory and environmental stressors have been shown to alter pain sensitivity and render animals less responsive to aversive stimuli by activating endogenous analgesic mechanisms. Stress-induced analgesia (SIA) has been observed in laboratory rodents exposed to stimuli such as footshock, warm and cold water swim, immobilization, body or tail pinch, social conflict, agonistic encounters, brief exposure to a natural predator, and centrifugal rotation (2, 9, 18, 19, 21, 24, 27, 36). Moreover, depending on the characteristics of the stressful stimuli (e.g., duration, intensity, temporal patterning), the analgesia may be mediated by either endogenous opioid peptide or nonopioid hormonal and neurochemical mechanisms (3–5, 9, 22). Several studies on the effects of centrifugal rotation on analgesic responses in rats have found that this type of stimulation can produce SIA and that at some rotation speeds (110 rpm) the mediating mechanisms seems to be opioid in nature (7), but at other speeds (150 rpm), nonopioid in nature (7, 15, 18). It should be noted that opioid mediation of this effect is probably not a simple function of rotation speed since duration of the stimulation differed among the various studies as well.

Body rotation has been found to produce motion sickness in a variety of animals (6, 16, 20, 25, 28, 29, 32, 35, 37). Evidence for the stressful nature of body rotation includes postrotation reductions in activity (10,30) and in feeding or drinking (12, 13, 17, 31), as well as increases in defecation levels in rats (26). Measurements of hormonal changes produced by motion sickness have indicated elevations in corticosterone, among others (11,34), and suggest that certain types of vestibular stimulation can be stressful. Results of recent studies with laboratory mice have shown that body rotation can also induce significant analgesic responses (27). A variety of different schedules of rotation (duration, temporal patterning) were observed to induce analgesia during the immediate postrotation period.

The present investigations examined two issues. The first experiment examined the effect of the prototypic opiate antagonist, naloxone (33), on the analgesia produced by horizontal rotation in mice. Sensitivity of the analgesia to the systemic administration of naloxone would provide evidence for opioid mediation of the analgesia. The second experiment examined the effects of naloxone on postrotation alterations in feeding in food-deprived mice. If the reductions in feeding typically observed following exposure to rotation are related to activation of the endogenous opioid systems, then one might expect to find an attenuation of this type of anorexia following administration of naloxone. On the other hand, if the rotation-induced anorexia is related to the effects of the motion sickness, then administration of naloxone could, in fact, exacerbate the anorexia since several previous studies have shown that systemic naloxone enhances

motion sickness in cats (8) and humans (1).

GENERAL METHOD

Subjects

Sexually mature male CF-1 mice (Charles River, Quebec), 25–40 g, were housed in groups of four in standard polyethylene cages and maintained in a colony room at 22 ± 1°C and a 12-hr light:12-hr dark cycle. The lights were on from 0630 to 1830 hr and food (Purina lab pellets) and tap water were available ad lib throughout the duration of the experiments unless noted otherwise.

Rotation Apparatus

Body rotation (about a vertical axis) was produced in a ventilated Plexiglas chamber (32 × 32 × 32 cm) mounted on a motor-driven horizontal turntable [see (27)]. The turntable was programmed to rotate at 70 rpm for 15-sec periods separated by 5-sec periods of no rotation. The Plexiglas chamber was partitioned into four compartments of equal size enabling rotation of four mice at the same time. A sham-rotation condition consisted of placing an identical Plexiglas chamber on a surface of the apparatus adjacent to the turntable such that the mice in this condition experienced the noise and vibration of the motor-driven apparatus, but did not experience any rotary stimulation.

EXPERIMENT 1

Since previous research had found an analgesic effect following body rotation in mice (27), the present experiment was designed to replicate this rotation-induced analgesia effect and, more importantly, to examine the role of endogenous opioids in mediating this phenomenon in mice. The prototypic opiate antagonist, naloxone (33), was used to test for opioid involvement.

METHOD

Subjects

Sixty-four CF-1 male mice were used as subjects and these animals were housed as described in the General Method section.

Procedure

All of the mice were exposed to the experimental treatments and tested during the mid-portion of the light phase of the light-dark cycle. Four groups of animals were used in the experiment. Two groups (Naloxone-R, n = 16 and Saline-R, n = 16) were exposed to the rotary stimulation for 60 min (see the General Method section). After 30 minutes of rotation exposure the mice in the Naloxone-R group received an intraperitoneal (IP) injection (1.0 mg/kg in 1.0 ml of sterile isotonic saline) of naloxone hydrochloride (Sigma) and were then rotated for another 30 min. Mice in group Saline-R received an injection of isotonic saline (1.0 ml/kg, IP) midway through the rotation session. Two other groups of mice (Naloxone-S, n = 16 and Saline-S, n = 16) were exposed to the sham-rotation condition for 60 minutes and midway through this procedure received an injection of naloxone (1.0 mg/kg, IP) or isotonic saline (1.0 ml/kg, IP), respectively.

Immediately following the 60-min of rotation or sham rotation, the mice were individually tested for latency of a foot-licking response when placed on a warm surface (47.5 ± 1°C, "hot-plate" apparatus, Omnitech Electronics). Previous research in our laboratory (27) had shown that a temperature of 47.5°C produced a baseline response latency in mice that was not compromised by a "floor effect." One week after the first exposure and test sequence the mice were exposed and tested a second time. In this second session they were subjected to the opposite exposure condition used initially, i.e., those mice initially rotated were now exposed to sham rotation and vice versa. Drug treatment conditions remained the same.

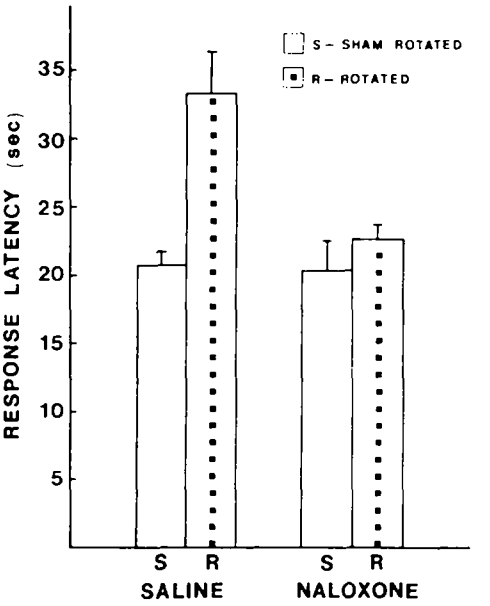

FIG. 1. Mean response latencies to the thermal stimulus for rotated (R) and sham-rotated (S) mice after injections of saline or naloxone. The error bars are standard errors of the mean.

The data were analyzed with a mixed design repeated measures analysis of variance and post hoc comparisons used Tukey's HSD procedure. An alpha level of 0.05 was used in interpretation of statistical significance.

RESULTS AND DISCUSSION

Figure 1 displays the group mean response latencies. The analysis of variance revealed no significant effect of order of testing ($F<1$), i.e., whether the mice were initially rotated or sham rotated. A significant main effect of the treatment, $F(1,62) = 13.42$, $p<0.001$, as well as a significant main effect of the drug condition, $F(1,62) = 8.47$, $p = 0.005$, were obtained. There was also a significant treatment by drug condition interaction, $F(1,62) = 6.61$, $p = 0.012$. Multiple comparisons of the group means confirmed that a significant analgesia effect was obtained in the mice rotated and injected with saline ($p<0.01$), but not in the mice rotated and injected with naloxone ($p>0.50$). The two sham-rotated groups and the Naloxone-R group did not differ significantly among themselves.

The present findings are in agreement with previous demonstrations of rotation-induced analgesia in mice (27) and meadow voles, *Microtus pennsylvanicus* (23). They are also consistent with previous demonstrations of centrifugal rotation-induced analgesia in rats (7, 15, 18). The finding that naloxone blocked this rotation-induced analgesia in mice is a new finding and is consistent with similar observations for meadow voles (23) and for rats rotated at 110 rpm (7), and suggests that the rotation-induced analgesia in mice is also opioid mediated, at least at a rotation speed of 70 rpm.

EXPERIMENT 2

A variety of procedures have been used to index the aversive

consequences of body rotation in rodents. Rotation results in decreases in spontaneous locomotor activity, both during and following the rotary stimulation (10). Both rats and mice deprived of water will exhibit suppression of drinking following the rotation procedure (13,17) and if food deprived will show postrotation reductions in feedings (12,31). Since the results of Experiment 1 suggested that body rotation in mice activates the endogenous opioid systems, it was of interest to determine the possible role of this activation in postrotation anorexia. Opioid involvement was assessed by injecting the animals with the opiate antagonist, naloxone.

METHOD

Subjects

Fifty-two CF-1 mice were used as subjects and these animals were housed as described in the General Method section.

Procedure

All of the animals were individually housed and, over a four-day period, adjusted to eating their food in powder form (ground-up Purina pellets) during a restricted time period (from 1300 to 1500 hr). On Day 5, starting at 1200 hr, the mice were either rotated (Groups Saline-R, n = 12 or Naloxone-R, n = 16) or sham rotated (Groups Saline-S, n = 12 or Naloxone-S, n = 12) for 60 min. Drug injections (naloxone hydrochloride, 1.0 mg/kg, IP) or injections of the vehicle (1.0 mg/kg, sterile isotonic saline, IP) were given midway through the rotation or sham-rotation sessions. Immediately following these exposure periods (i.e., during the normal feeding period), the mice were returned to their home cages and given access to powdered food for a two-hour period. Food intake was measured to the nearest 0.01 g at the end of each 30-min segment during the two-hour access period. Water was available ad lib throughout the experiment.

RESULTS AND DISCUSSION

Group mean food intake values for each 30-min segment of the two-hour postrotation food access period are shown in Fig. 2. Inspection of this figure reveals a substantial reduction in food intake (anorexia) immediately following the rotation treatment relative to the sham-rotation condition (compare the two saline groups). When treated with naloxone the mice exhibited additional reductions in feeding. The sham-rotated mice given naloxone ate less in the first 30-min segment than the sham-rotated saline-injected mice, indicating that this type of feeding regimen leads to opioid activation and a stress-induced feeding effect. More importantly, the rotated mice given naloxone ate less in the first 30-min segment than the rotated mice given saline. The analysis of variance and post hoc comparisons confirmed these impressions. A significant treatment main effect, $F(1,48) = 6.50$, $p = 0.013$, and a significant drug main effect, $F(1,48) = 11.90$, $p = 0.001$, were obtained. Post hoc comparisons indicated that naloxone reduced food consumption in both the sham-rotation ($p<0.05$) and in the rotation condition ($p<0.05$) during the first 30-min segment of the food access period.

These results clearly show rotation-induced anorexia in food-deprived mice. These findings are in general agreement with previous research (12,31). In addition, the present results show that systemic naloxone can reduce food consumption in food-deprived sham-rotated mice, a finding also in accord with previous studies [e.g., (14)]. This finding also suggests that this feeding regimen is stressful in nature and may result in the concomitant activation of endogenous opioid systems. Finally, the present

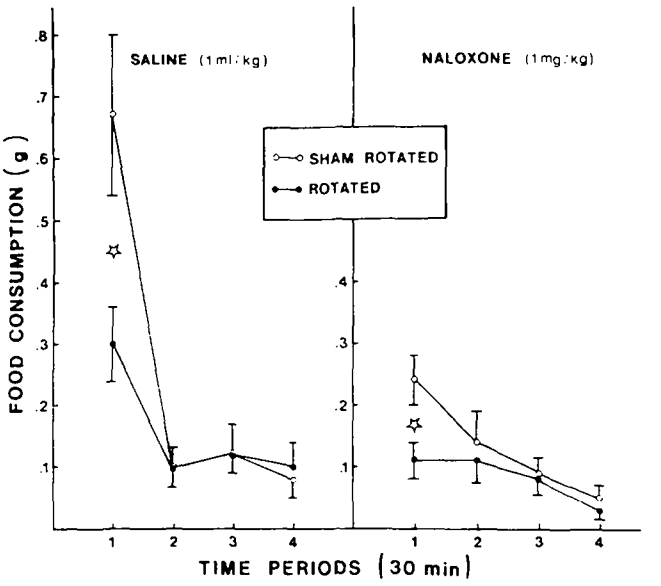

FIG. 2. Mean food intake values for rotated and sham-rotated mice after injections of saline (left panel) or naloxone (right panel) during consecutive 30-minute intervals following the experimental treatment. The error bars are standard errors of the mean and the star indicates significant differences ($p<0.05$) between the two treatment groups.

experiment demonstrated that naloxone enhances, rather than reduces, the rotation-induced anorexia found in male food-deprived mice. This later result suggests that the rotation-induced anorexia is not directly mediated by activation of the endogenous opioid systems following rotary stimulation, since the opiate antagonist failed to block the anorexia effect. It does, however, suggest that in a food-deprivation feeding schedule activation of endogenous opioid systems may, in part, counteract the anorexic effects of body rotation.

GENERAL DISCUSSION

The findings of the present experiments show that horizontal body rotation can induce both analgesia and anorexia in mice. In addition, the prototypic opiate antagonist, naloxone, was found to block the rotation-induced analgesia and to enhance the anorexia. These results suggest that the rotation-induced analgesia is opioid mediated, a finding in agreement with other experiments in our laboratory showing opioid involvement in the mediation of rotation-induced analgesia in meadow voles (23). The experiments with the meadow voles showed that the analgesia could be blocked not only with naloxone, but also with the irreversible mu opiate receptor antagonist, β-funaltrexamine, suggesting mu opioid involvement in the mediation of the rotation-induced analgesia. Further experiments are needed to determine if the same is true in mice. Future experiments should also examine possible interactions between rotation speed and duration with respect to opioid mediation of any resulting analgesia. The findings of the present experiments may be specific to long duration (60 min) vestibular stimulation.

The observation that naloxone injections exacerbated, rather than reduced, the rotation-induced anorexia suggests that the processes mediating the rotation-induced analgesia and anorexia are not the same. Although both effects are probably produced by the rotation-induced vestibular stimulation, the underlying physi-

ological mechanisms responsible for the analgesia and the anorexia are differentially sensitive to naloxone. It should be noted that the feeding schedule used in Experiment 2 induced a naloxone-sensitive, opioid-mediated feeding response. In addition, a variety of other stressful procedures have been shown to enhance food intake in rodents (36). Thus, it is possible that naloxone enhancement of rotation-induced anorexia may actually represent an attenuation of a residual opioid induction of feeding. Investigations with naloxone-insensitive feeding schedules are required to address this possibility. It is also possible that the analgesia arises from the "stress" properties of the rotation-produced motion sickness, whereas the anorexia is related to the putative "nausea" associated with motion sickness. However, we are unable at present to experimentally index the presence of these hypothetical internal processes in rodents. Previous studies found that naloxone increased the frequency of motion sickness symptoms and shortened the latency to retching and vomiting in cats subjected to vestibular stimulation associated with a swing stimulus (8), and in humans exposed to coriolis stimulation in a rotating chair, reduced the time to reach a designated level of reported nausea (1). If the anorexia observed in the present study was a result of motion sickness, then these previous findings would suggest that the enhanced anorexia observed following naloxone injections was likely due to intensification of the motion sickness by administration of the naloxone. It may still be the case that the enkephalins may modulate the production or reception of a mediator important to motion sickness, but this issue needs to be examined in future experiments.

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