Pharmacology Biochemistry and Behavior, Vol. 46, pp. 911–915, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Pharmacological Studies of Centrifugation-Induced Analgesia

JON E. SPRAGUE,* MARK A. SUCKOW,† ANNE L. FITZGERALD,† CYNTHIA F. GRIGDESBY,† ROBERT L. UETRECHT* AND ROGER P. MAICKEL*†[1]

Department of Pharmacology and Toxicology, School of Pharmacy and Pharmacal Sciences, and †Laboratory Animal Program, Purdue University, West Lafayette, IN 47907-1334

Received 21 January 1993

SPRAGUE, J. E., M. A. SUCKOW, A. L. FITZGERALD, C. F. GRIGDESBY, R. L. UETRECHT AND R. P. MAICKEL. *Pharmacological studies of centrifugation-induced analgesia.* PHARMACOL BIOCHEM BEHAV **46**(4) 911–915, 1993.—Subjecting rats to a brief period of centrifugal rotation produces a brief analgesia (1–2 min) that is similar to that produced by pretreatment with morphine. The effect of the morphine is blocked by naloxone, while that of the centrifugal rotation is only partially blocked by the same dose of naloxone. Cholinergic blocking agents such as scopolamine are also capable of partially blocking the rotational-induced analgesia. The combination of pretreatment with scopolamine plus naloxone is capable of completely blocking the rotational-induced analgesia, suggesting the involvement of both central cholinergic and endogenous opioid components.

Rotation-induced analgesia Cholinergic factors Endogenous opioids

A BROAD range of stressful and other environmental stimuli have been associated with endogenous analgesic responses (3). For example, stress-induced analgesia has been observed in laboratory rodents exposed to foot shock (14), warm and cold water swim (23), and centrifugational rotation (7,27,28).

Sprague-Dawley rats subjected to 5 min of manual horizontal rotation in a cloth sack demonstrated significant inhibition of the tail-flick response (16). Female F344 rats showed rotation-induced analgesia prevented by pretreatment with the opioid antagonist naloxone when a rotation rate of 110 RPM was used, but not at a higher rate (7). In another study designed to avoid the confounding effect of restraint during rotation, mice were allowed to roam freely in a clear, rotating Plexiglas chamber, then were tested for the latency of a foot-licking response on a hot plate apparatus; the resultant analgesic effect appeared to wear off in less than 30 min (27). In a related study (28), a significant analgesic effect was noted in rotated mice compared with sham-rotated mice. The effect was reversible with naloxone, an indication that rotation-induced analgesia in mice is opioid mediated.

Centrifugation has been found to produce motion sickness in a variety of animals (6,13,17,24–26,29). Indicators of centrifugation-induced motion sickness include the production of a conditioned taste aversion (24) and an increase in defecation levels (26).

The present investigations were designed to further characterize centrifugation-induced analgesia. The results indicate that the analgesia produced is a blend of endogenous opioid effects and vestibular effects of cholinergic nature.

METHOD

All use of animals was in accordance with protocols approved by the Institutional Animal Care and Use Committee. Virus-antibody-free male Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN), weighing 240–300 g, underwent a 7-day quarantine/acclimation period before use. The animals were housed in groups of four in suspended wire cages in a facility accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC). Animal room temperature was maintained at 22.2 ± 1.1°C. The room was ventilated with 100% fresh air at a rate of 10–15 room air changes per hour. The rats were provided with food (Purina Rodent Laboratory Chow 5001) and tap water ad lib, and maintained on a 14 L : 10 D cycle. All testing was performed between the third and sixth hours of the lights-on period. The following drugs were kindly supplied by their respective manufacturers: cyclizine (Burroughs Wellcome), methylscopolamine (Upjohn), metoclopramide (A. H. Robins), naloxone (DuPont), and prochlorperazine (Smith Kline Beecham). Other drugs were purchased from commercial sources. All drugs were dissolved in deionized water except prochlorperazine, which was suspended in distilled water with 5 drops of Tween 80. Solutions were prepared so that the

[1] Requests for reprints should be addressed to Roger P. Maickel, Ph.D., Department of Pharmacology & Toxicology, Purdue University, 1334 R.E. Heine Pharmacy Building, West Lafayette, IN 47907-1334.

TABLE 1

PRODUCTION OF ANALGESIA IN RATS BY BODY ROTATION OR MORPHINE SULFATE (n = 24)

Treatment	Analgesia*	
Vehicle	6.6 ± 0.4	(1)
Vehicle + rotation	10.2 ± 0.5†	(9)
Morphine sulfate (5.0 mg/kg)	10.0 ± 0.4†	(6)
Morphine sulfate (10.0 mg/kg)	10.7 ± 0.4†	(14)

*Duration (mean ± SEM) of tail-flick latency measured in seconds. Numbers in parenthesis are numbers of animals reaching the 12 s cutoff.

†Significantly greater than vehicle ($p < 0.0001$); post hoc analysis.

desired doses could be administered in a volume of 0.1 ml/100 g body weight; all injections were by the intraperitoneal (IP) route. Control animals were given a similar volume of the appropriate vehicle.

In Experiment 1, rats were randomly assigned to four test groups with six animals per group. In a 4 × 4 Latin square design, rats were pretreated with one of the following: morphine sulfate, 5 mg/kg; morphine sulfate, 10 mg/kg; vehicle, 30 min prior to testing for analgesia. Rotated rats were placed in a Plexiglas restraint device and swung in a head out orientation at arms length in a vertical plane (radius of the circle = 65 cm) by the same person each time for 20 revolutions over 15–20 s (60–80 rpm) and tested for analgesia within less than 1 min, using the standard tail-flick apparatus, which automatically measures the latency of a rat withdrawing its tail from a radiant heat source. The time limit for exposure of the animal's tail to the heat source was established at 12 s to prevent tissue damage. Confinement in the restraint device was necessary to immobilize rats for the tail-flick test. Rotated rats usually became temporarily immobile. However, for consistency, all animals were placed in the restraint device. Rats were retested at intervals of not less than 3 days.

In Experiment 2, four groups of six rats each pretreated with vehicle were subjected to manual rotation in the manner described above. The resultant degree of analgesia was measured immediately after rotation and at 1, 2, and 5 min after rotation.

In Experiment 3, rats were randomly assigned to three groups of six each. In a crossover testing design, treatments prior to measuring analgesia consisted of morphine (10 mg/kg), naloxone (1 mg/kg) followed by morphine (10 mg/kg), or vehicle, given 30 min before testing. Rotation was performed as described previously, immediately before testing for analgesia. Rats were tested at intervals of no less than 3 days.

In Experiment 4, rats were randomly assigned to five groups of six each. In a crossover testing design, treatment prior to measuring analgesia consisted of metoclopramide (0.5 mg/kg), prochlorperazine (8.0 mg/kg), scopolamine (2 mg/kg), methylscopolamine (1.0 mg/kg), or vehicle. Injections, rotations, and testing intervals were as described previously. All treatments were given with and without rotation.

In Experiment 5, rats were randomly assigned to four groups of six each. In a crossover testing design, treatments prior to measuring analgesia consisted of cyclizine (20 mg/kg), atropine (5 mg/kg), naloxone (1 mg/kg) plus scopolamine (20 mg/kg), or vehicle. All treatments were given with and without rotation.

All measurements were made in seconds and are reported as mean ± SEM. Data was processed using a two-way ANOVA with Scheffe post hoc analysis (30). Post hoc comparisons were made only when a significant overall drug effect was observed in the initial ANOVA.

RESULTS

In Experiment 1 (Table 1), the mean tail-flick latency in control (vehicle-injected, nonrotated) animals was 6.6 ± 0.4 s. The tail-flick latencies of vehicle-injected, rotated rats, and rats treated with either a low dose or a high dose of morphine did not differ significantly from one another (10.2 ± 0.5, 10.0 ± 0.4, and 10.7 ± 0.4 s, respectively) but were significantly higher than controls ($p < 0.0001$). While the degree of analgesia provided by the high dose (10 mg/kg) of morphine may have been greater than that provided by the low dose (5 mg/kg), this was not detected due to the temporal limit (12.0 s) set to prevent damage to the rats' tails.

In Experiment 2 (Table 2), vehicle-injected rats tested immediately and at 1 min postrotation had similar tail-flick latencies (10.5 ± 1.0 and 10.4 ± 1.0 s, respectively). These latencies were significantly greater than those of control (vehicle-injected, nonrotated) rats ($p < 0.01$). Rats tested 2 and 5 min postrotation had tail-flick latencies that did not differ significantly from controls.

In Experiment 3 (Table 3), morphine-injected rats and vehicle-injected, rotated rats had significantly greater ($p < 0.001$) tail-flick latencies (10.3 ± 0.7 and 10.0 ± 0.6 s, respectively) than vehicle-injected nonrotated rats (5.8 ± 0.5 s), confirming the findings of Experiment 1. Rats injected with naloxone plus morphine had latencies less than rats injected with morphine alone (6.8 ± 0.7 vs. 10.3 ± 0.7 s respectively,

TABLE 2

DURATION OF POSTROTATIONAL ANALGESIA IN VEHICLE-INJECTED RATS (n = 6)

	Analgesia* Time of Test (Minutes Postrotation)			
Vehicle/No Rotation	0	1	2	5
6.8 ± 1.2	10.5 ± 1.0†	–	–	–
7.3 ± 0.7	9.8 ± 0.9†	10.4 ± 1.0†	–	–
7.2 ± 1.2	10.4 ± 1.0†	–	7.9 ± 1.4	–
6.1 ± 0.5	10.5 ± 1.1†	–	–	7.4 ± 1.4

*Duration (mean ± SEM) of tail-flick latency measured in seconds.

†Significantly greater than vehicle ($p < 0.01$); post hoc analysis.

TABLE 3

EFFECTS OF NALOXONE HYDROCHLORIDE ON ANALGESIA PRODUCED BY BODY ROTATION OR MORPHINE SULFATE

Treatment	*n*	Analgesia*	
Vehicle	18	5.8 ± 0.5	(0)
Vehicle + rotation	18	10.0 ± 0.6†	(11)
Naloxone (1 mg/kg)	12	4.9 ± 0.7	(0)
Naloxone + rotation	12	7.4 ± 0.8‡	(0)
Morphine (10 mg/kg)	12	10.3 ± 0.7†	(9)
Naloxone + morphine	18	6.8 ± 0.7§	(4)

*Duration (mean ± SEM) of tail-flick latency measured in seconds. Numbers in parenthesis are numbers of animals reaching the 12 s cutoff.

†Significantly greater than vehicle ($p < 0.0001$); post hoc analysis.

‡Significantly less than vehicle + rotation ($p < 0.005$), but significantly greater than naloxone without rotation ($p < 0.005$); post hoc analysis.

§Significantly less than morphine ($p < 0.0001$); post hoc analysis.

$p < 0.0001$), demonstrating that the naloxone treatment partially reversed the effects of an exogenous opioid. Naloxone-injected rotated rats had significantly shorter latencies than vehicle-injected rotated rats (7.4 ± 0.8 vs. 10.0 ± 0.6 s, respectively, $p < 0.005$), indicating that pretreatment with naloxone partially reversed postrotational analgesia.

In Experiment 4 (Table 4), the antiemetic agents metoclopramide (dopamine antagonist) and prochlorperazine (dopamine/norepinephrine antagonist) were ineffective in attenuating rotational-induced analgesia, while the antimotion-sickness agent, scopolamine, significantly decreased ($p < 0.01$) the analgesia from 9.3 ± 0.3 s (vehicle-swung) to 7.0 ± 0.9 s. The quaternary amine, methylscopolamine, was ineffective, suggesting that a central anticholinergic agent can partially antagonize centrifugation-induced analgesia.

In Experiment 5 (Table 5), atropine (cholinergic antagonist) produced a nonsignificant decrease in tail flick latency, while cyclizine (histaminergic/cholinergic antagonist) significantly reduced ($p < 0.005$) the tail flick latency to 7.1 ± 0.7 s. The combination of naloxone + scopolamine significantly decreased ($p < 0.005$) the centrifugation-induced analgesia to 6.5 ± 1.0 s. A comparison of nonswung to swung (naloxone + scopolamine) indicates virtually no change in tail-flick latency, demonstrating that the combination of an opiate antagonist with a cholinergic antagonist completely blocks centrifugation-induced analgesia.

DISCUSSION

The results provide evidence that centrifugation of rats for a brief period of time does, indeed, induce analgesia, comparable in magnitude but not duration, to that produced by 5–10 mg/kg of morphine sulfate. This analgesic effect is short lived, lasting 1 min or less; this duration was less than that (30 min) previously reported in mice after a longer period of centrifugation (27).

Rats pretreated with drug vehicle and rotated had significantly longer tail-flick latencies than rats pretreated with naloxone and rotated, evidence that naloxone (an opioid antagonist) decreased postrotational analgesia. Thus, the analgesia appears to be mediated, at least in part, by endogenous opioids in rats. In meadow voles, postrotational analgesia was blocked by naloxone and also with the mu opioid antagonist beta-funaltrexamine (21), suggesting that rotation-induced analgesia is mediated at mu opioid receptors.

Some manipulations that produce analgesia also produce visceral upset. One example is the administration of morphine, the prototypical opioid, which is associated with the undesirable side effect of nausea in humans and which will support conditioned flavor aversion in rats (4). Centrifugal rotation is also well known to cause nausea in humans and has been shown to produce conditioned flavor aversion in animals (10,12,19,24). The similarities in the effects of the two manipulations are intriguing, leading investigators to question whether analgesia and visceral upset are attributable to the same or separate mechanisms. To investigate the possibility of an interaction between the two systems, we tested agents used in the therapeutic treatment of visceral upset, namely metoclopramide (dopamine antagonist) and prochlorperazine (dopamine/norepinephrine antagonist). Metoclopramide was ineffective at blocking centrifugation-induced analgesia. Prochlorperazine produced a significant analgesia in both the nonswung test group and in the swung test group; this may be an artifact attributed to the sedative effects of the drug. Thus, the dopaminergic and noradrenergic systems do not appear to contribute to the rotation-induced analgesia.

Naloxone has been reported to block the analgesic effect of rotation-induced analgesia but to enhance postrotational

TABLE 4

PRODUCTION OF ANALGESIA BY BODY ROTATION IN RATS: PHARMACOLOGICAL MODIFICATION I

Treatment	*n*	Nonswung	Analgesia*	Swung	
Vehicle	30	7.0 ± 0.5	(1)	9.3 ± 0.3†	(6)
Metoclopramide (0.5 mg/kg)	12	6.6 ± 0.7	(0)	9.8 ± 0.5†	(2)
Prochlorperazine (8.0 mg/kg)	12	9.5 ± 0.6‡	(4)	11.8 ± 0.2†,§	(9)
Scopolamine (2.0 mg/kg)	12	5.5 ± 0.8	(1)	7.0 ± 0.9#	(1)
Methylscopolamine (1.0 mg/kg)	12	6.6 ± 0.6	(0)	9.9 ± 0.7†	(3)

*Duration (mean ± SEM) of tail-flick latency measured in seconds. Numbers in parenthesis are numbers of animals reaching the 12 s cutoff.

†Significantly greater than vehicle ($p < 0.01$); post hoc analysis.

‡Significantly greater than vehicle/non-swung ($p < 0.01$); post hoc analysis.

§Significantly greater than vehicle/swung ($p < 0.05$); post hoc analysis.

#Significantly less than vehicle/swung ($p < 0.01$); post hoc analysis.

TABLE 5

PRODUCTION OF ANALGESIA BY BODY ROTATION IN RATS: PHARAMACOLOGICAL MODIFICATION II

Treatment	*n*	Nonswung	Analgesia*	Swung	
Vehicle	21	5.0 ± 0.5	(1)	9.7 ± 0.5†	(3)
Atropine (5.0 mg/kg)	12	4.1 ± 0.4	(0)	7.8 ± 0.9†	(1)
Cyclizine (20.0 mg/kg)	12	4.8 ± 0.6	(0)	7.1 ± 0.7†,‡	(0)
Naloxone (1.0 mg/kg) + Scopolamine (2.0 mg/kg)	12	6.1 ± 0.9	(0)	6.5 ± 1.0‡	(2)

*Duration (mean ± SEM) of tail-flick latency measured in seconds. Numbers in parenthesis are numbers of animals reaching the 12 s cutoff.

†Significantly greater than vehicle ($p < 0.01$); post hoc analysis.

‡Significantly less than vehicle/swung ($p < 0.005$); post hoc analysis.

anorexia (a sign of visceral upset), leading to the conclusion that the mechanisms for the two phenomena are not the same (28). In addition, visceral upset and some postrotational analgesia have been attributed to activity at histamine receptors, because rotation-induced analgesia, but not conditioned flavor aversion, was blocked by cimetidine, an H2 receptor blocker (19). This is consistent with the finding that cimetidine attenuated nonopioid foot shock-induced analgesia (11). Thus, postrotational analgesia may be attributed to both opioid (mu-receptor) and nonopioid (H2 receptor) mechanisms.

To test the possible role of the vestibular system, agents common to the treatment of motion sickness were also tested. Scopolamine (cholinergic antagonist) significantly attenuated the rotational-induced analgesia, while methylscopolamine, which does not cross the blood-brain barrier, was ineffective, indicating a central mechanism for the effects of scopolamine. Cyclizine (histaminergic/cholinergic antagonist) also significantly reduced the rotational-induced analgesia, suggesting that the analgesia is partially mediated by cholinergic vestibular effects.

While it may be argued that restraint alone is capable of inducing analgesia (stress-induced), confinement stress has been reported to play little or no part in rotation-induced analgesia (12). Further, animal models of restraint-induced analgesia require very close confinement (e.g., restraint by rolling the animal up in wire mesh) for relatively long periods, e.g., 30 to 60 min (1-3,14,18). In the present experiments, animals were loosely confined for a maximum of 3-5 min. Subjecting rats to IP injection may also cause a degree of stress-induced analgesia; however, the stress of an IP injection given 30 min (as in the present experiments) or longer, before manipulation, does not potentiate the resultant analgesia (11).

The results presented herein suggest that centrifugation-induced analgesia is produced by a blend of endogenous opioid effects and vestibular effects of a cholinergic nature; this is supported by the virtually complete blockade of rotation-induced analgesia produced by pretreatment with the combination of scopolamine and naloxone.

REFERENCES

1. Amir, S.; Amit, Z. Endogenous opioid ligands may mediate stress-induced changes in the affective properties of pain related behavior in rats. Life Sci. 23:1143-1152; 1978.
2. Amir, S.; Amit, Z. The pituitary gland mediates acute and chronic pain responsiveness in stressed and nonstressed rats. Life Sci. 24:439-448; 1978.
3. Amit, Z.; Galina, Z. Stress-induced analgesia: adaptive pain suppression. Physiol. Rev. 66:1091-1120; 1986.
4. Blair, R.; Amit, Z. Morphine conditioned taste aversion reversed by periaqueductal gray lesions. Pharmacol. Biochem. Behav. 15: 651-653; 1981.
5. Bodnar, R. Neuropharmacological and neuroendocrine substrates of stress-induced analgesia. Ann. NY Acad. Sci. 467:345-360; 1986.
6. Braun, J. J.; McIntosh, H. Learned taste aversion induced by rotational stimulation. Physiol. Psychol. 1:301-304; 1973.
7. Cannon, J.; Shavit, Y.; Liebeskind, J. Rotation speed can determine whether the resulting stress-induced analgesia is blocked by naloxone. Ann. NY Acad. Sci. 467:425-427; 1986.
8. Eskin, A.; Riccio, D. The effects of vestibular stimulation on spontaneous activity in the rat. Psychol. Rec. 16:523-527; 1966.
9. Faupel, R.; Seitz, H.; Tarnowski, W.; Thiemann, V.; Weiss, C. The problem of tissue sampling from experimental animals with respect to freezing technique, anoxia, stress, and narcosis. Arch. Biochem. Biophys. 148:509-522; 1972.
10. Fox, R.; Daunton, N. Conditioned feeding suppression in rats produced by cross-coupled and simple motions. Aviat. Space Environ. Med. 53:218-220; 1982.
11. Gogas, K.;, Hough, L.; Glick, S.; Su, K. Opposing actions of cimetidine on naloxone-sensitive and naloxone-insensitive forms of foot shock-induced analgesia. Brain Res. 370:370-374;1987
12. Green, K.; Lee, D. Effects of centrifugal rotation on analgesia and conditioned flavor aversions. Physiol. Behav. 40:201-205; 1987.
13. Green, L.; Rachlin, H. The effects of rotation on the learning of taste aversion. Bull. Psychon. Soc. 1:137-138; 1973.
14. Greenberg, R.; O'Keefe, E. Thiorphan potentiation of stress-induced analgesia in the mouse. Life Sci. 31:1185-1188; 1982.
15. Hayes, R.; Katayama, Y. Range of environmental stimuli producing nociceptive suppression: Implication for neural mechanisms. Ann. NY Acad. Sci. 467:1-13; 1986.
16. Hayes, R.; Bennett, G.; Newlon, P.; Mayer, D. Behavioral and physiological studies of nonnarcotic analgesia in the rat elicited by certain environmental stimuli. Brain Res. 115:69-90; 1978.
17. Hutchinson, S. L. Taste aversion in albino rats using centrifugal spin as an unconditioned stimulus. Psychol. Rep. 33:467-470; 1973.
18. Kulkarni, S. Heat and other physiological stress-induced analgesia: Catecholamine mediated and naloxone reversible response. Life Sci. 27:185-188;1980.
19. Lee, D.; Green, K. Effects of antihistamines on centrifugal rotation-induced analgesia and conditioned flavor aversions. Physiol. Behav. 45:459-464; 1989.

20. Lewis, J. Multiple neurochemical and hormonal mechanisms of stress-induced analgesia. Ann. NY Acad. Sci. 467:194–204; 1986.
21. Lipa, S.; Kavaliers, M.; Ossenkopp, K. Differential inhibitory effects of MIF-1, Tyr-MIF-1, naloxone and beta-funaltrexamine on body rotation-induced analgesia in the meadow vole, *Microtus pennsylvanicus*. Peptides 10:493–497; 1989.
22. Mikeska, J.; Klemm, W. EEG evaluation of humaneness of asphyxia and decapitation euthanasia of the laboratory rat. Lab. Anim. Sci. 25:175–179; 1975.
23. O'Connor, P.; Chipkon, R. E. Comparison between warm and cold water stress in mice. Life Sci. 35:631–639; 1984.
24. Ossenkopp, K. Area postrema lesions in rats enhance the magnitude of body rotation-induced conditioned taste aversion. Behav. Neural Biol. 38:82–86; 1983.
25. Ossenkopp, K.; Tu, G. S. Motion sickness in quail: Body-rotation induced conditioned fluid aversion in *C. coturnix japonica*. J. Comp. Psychol. 98:189–193; 1984.
26. Ossenkopp, K.; Ossenkopp, M. D. Animal models of motion sickness: Are nonemetic species an appropriate choice? Physiologist 28:s61–s62; 1985.
27. Ossenkopp, K.; Macrae, L.; Bettin, M.; Kavaliers, M. Body-rotation induced analgesia in male mice: Effects of duration and type of rotation procedure. Brain Res. Bull. 21:967–972; 1988.
28. Ossenkopp, K.; Bettin, M.; Kavaliers, M. The effects of naloxone on body rotation-induced analgesia and anorexia in male mice. Pharmacol. Biochem. Behav. 34:317–320; 1989.
29. Roy, A.; Brizzee, K. R. Motion sickness-induced food aversion in squirrel monkeys. Physiol. Behav. 23:39–41; 1979.
30. Sokal, R.; Rohlt, F. Biometry, 2nd ed. New York: W. H. Freeman and Co.; 1981.