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Effects in the Rat of Intranigral Morphine and DAGO on Eating and Gnawing Induced by Stress

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HAWKINS, M. F., R. D. FULLER, A. A. BAUMEISTER AND M. D. McCALLUM. *Effects in the rat of intranigral morphine and DAGO on eating and gnawing induced by stress.* PHARMACOL BIOCHEM BEHAV **49**(3) 737-740, 1994.—Stress produced by pinching the tail is known to increase feeding behavior in rats, and endogenous opioids have been implicated in the mediation of this effect. We have reported previously that a nonspecific opioid antagonist and a mu-selective antagonist decrease this stress-induced eating (SIE) when they are microinjected into the substantia nigra (SN). The present study investigated the possibility that activation of opioid receptors in the SN might also alter SIE. Because oral stereotypy and nociception are affected by opioid mechanisms in the SN, measurements of gnawing and of tail flick and hot plate response latencies were also made. Bilateral injection of morphine (0.1-20 nmol) and the mu-selective agonist D-Ala^2,N-Me-Phe^4,Gly^5-ol-enkephalin (DAGO; 0.03-1 nmol) increased response latency on the hot plate test and decreased gnawing produced by tail pinch. Tail flick latency and SIE were not affected. It is concluded that activation of opioid receptors in the SN does not produce an alteration in SIE as has been seen with opioid antagonists.

Stress Tail pinch Feeding Gnawing Nociception Tail flick Substantia nigra Hot plate Morphine DAGO

ONE OF THE behavioral responses to stress in a variety of species is increased food intake. Stress-induced eating (SIE) has been observed in species ranging from slugs (11) to humans (25), and can be produced by stressors such as forced swimming in cold water (27), sleep deprivation (22), and defeat in aggressive encounters (26) [see (23) for a review]. One of the most common means of producing SIE in the rat is to apply a moderate pinch to the tail. Shortly after the application of tail pinch the animal begins to eat; although oral stereotypies of licking and vacuous chewing are observed also (1,2).

Endogenous opioids have been implicated as mediators of SIE. Opioid antagonists reduce or abolish SIE when they are administered parenterally (6,13-15,18,27) or centrally (10,12). Recently, the pharmacological and anatomical specificities of opioid antagonism of SIE have received some experimental attention. Previous research in our laboratory has shown that the substantia nigra (SN) may be an important anatomical locus of action of opioids in SIE (10). Intranigral microinjection of the nonspecific opioid antagonist naloxone or the selective mu antagonist CTOP reduces eating produced by tail pinch without affecting gnawing. Blockade of delta (naltrindole) and kappa (norbinaltorphimine) receptors in the SN, however, has no effect on eating or gnawing produced by tail pinch stress. The importance of the mu opioid receptor in the regulation of SIE produced by tail pinch has been confirmed by others who have reported that intracerebroventricular injection of an irreversible mu antagonist (β-funaltrexamine) reduces SIE, while antagonists of kappa, delta$_1$, and delta$_2$ receptors have no effect (12).

These data suggest that mu opioids in the SN may play an important role in the regulation of SIE. No research to date has investigated the possibility that microinjection of opioid agonists into the SN might alter the food intake produced by tail pinch. In the present study, morphine and the highly selective mu agonist D-Ala^2,*N*-Me-Phe4,Gly^5-ol-enkephalin (DAGO) were employed. Because oral stereotypy (17) and nociception (4,5) are affected by opioid mechanisms in the SN, measure-

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ments of gnawing and of tail flick and hot plate response latencies were also made.

METHOD

Animals

Male and female Sprague-Dawley rats (250-350 g) were used. The animals were obtained from a breeding colony maintained at Louisiana State University. Animals to be used for this investigation were prescreened by applying a moderate pinch to the tail for 2 min (procedures described below). Only those animals that displayed eating or gnawing for at least 5 s during the 2-min interval were used for further investigation. The animals were housed individually in a temperature-controlled vivarium (22 ± 2°C) with free access to laboratory chow (Purina Rat Chow) and water. Lights were cycled on a 12 : 12 photoperiod (on 0700 h).

Surgery and Histology

Bilateral guide cannulae were implanted under Rompun (xylazine, 5 mg/kg, IM) and ketamine HCl (90 mg/kg, IM) anesthesia. The cannulae were positioned 1.3 mm dorsal to the substantia nigra at 2.7 mm anterior to interaural zero, 2.2 mm to either side of the midline, and 7.0 mm ventral to the surface of the skull (21). Animals were allowed to recover from surgery for 7 days prior to experimentation.

At the conclusion of testing animals were sacrificed with an overdose of anesthetic and were perfused transcardially with 10% formalin. Frozen sections through the site of implantation were stained with cresyl violet for subsequent analysis of cannula placement. Only the data from animals with bilateral injection sites within the substantia nigra (pars reticulata) are reported below.

Methods

A between-group design was used. Animals were randomly assigned to nine groups as follows: water control (n = 9), 0.1 nmol morphine (n = 9), 1.0 nmol morphine (n = 10), 5.0 nmol morphine (n = 11), 20.0 nmol morphine (n = 10), 0.03 nmol DAGO (n = 10), 0.1 nmol DAGO (n = 6), 0.3 nmol DAGO (n = 8), and 1.0 nmol DAGO (n = 6). Doses refer to the amount injected per side. Morphine sulfate was obtained from United States Pharmacopeial Convention, Inc. (Rockville, MD) and DAGO was obtained from Sigma Chemical Co. (St. Louis, MO).

Behavioral testing began at 1230 h each day. An animal was removed from its home cage and received the appropriate intranigral injection via an injector needle that extended 1.3 mm below the ventral tip of the guide cannula. The injection volume was 0.5 μl per side and was dispensed over a 1-min period with the aid of an infusion pump. The injector was left in place for 1 additional min after infusion was complete. The animal was then returned to its cage and was allowed to remain undisturbed for 5 min prior to tail pinch.

Tail pinch was performed by passing the animal's tail through the wire bottom of the cage. Moderate pressure was applied 4 cm from the distal tip of the tail with a spring-loaded clip cushioned with latex tubing. Immediately after the clip was applied the animal was provided with a portion of laboratory chow that had been preweighed to the nearest 0.01 g. Tail pinch continued for 5 min, after which the remaining laboratory chow and spillage were collected.

Tail flick latency was measured immediately after tail pinch stress. The animal was placed in a Plexiglas restraining cage and was positioned in an analgesia meter (IITC, Inc.) so that an intense beam of light could be focused on a spot approximately 2 cm from the distal tip of the tail. Latency to reflexive flick of the tail was measured to the nearest 0.01 s. Testing was discontinued if no tail flick occurred within 12 s.

Latency to paw lick was measured immediately after tail flick latency by placing the animal in a Plexiglas cylinder (26 cm × 21 cm dia) equipped with a stainless steel floor heated to 55 ± 1°C. The animal was removed from the cylinder when it licked either rear paw and the latency to paw lick was recorded to the nearest 0.01 s. If no paw lick occurred within 30 s the test was halted and the animal was removed.

Statistical Analyses

Differences between groups were assessed by analysis of variance and post hoc analysis. Pearson correlation coefficients were calculated to evaluate dose-response relationships.

RESULTS

Analysis of variance revealed no sex differences in the effects of morphine or DAGO on eating, gnawing, or measures of nociception. Therefore, the data for males (n = 49) and females (n = 30) were combined. The results reported below are based upon analyses of the pooled data.

Eating and Gnawing

The effects of morphine and DAGO on eating and gnawing are depicted in Figs. 1 (morphine) and 2 (DAGO). Although stress-induced eating was not affected by either of the opiates, both morphine, $F(4, 44) = 2.91$, $p = 0.0319$, and DAGO, $F(4, 34) = 5.04$, $p = 0.0027$, significantly decreased stress-induced gnawing relative to control injections. Post hoc analysis revealed that the effect of morphine on gnawing was attributable to the 1, 5, and 20 nmol doses, and that these doses did not differ significantly from one another. There was a significant correlation between the dose of morphine and

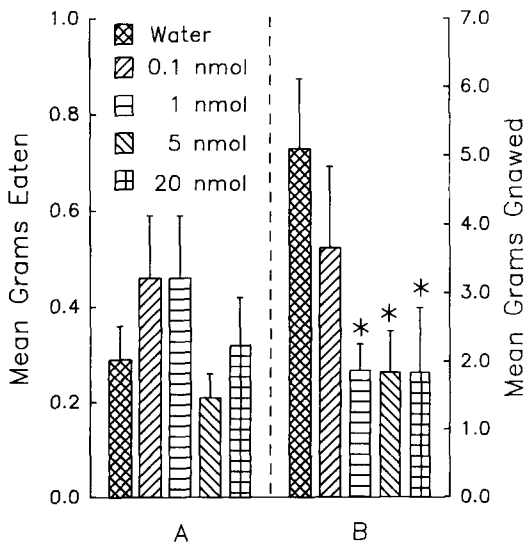

FIG. 1. Mean (±1 SE) grams of food eaten (panel A) or gnawed (panel B) during tail pinch following intranigral injection of vehicle control (water) or morphine. Significant differences ($p < 0.05$) relative to vehicle control are indicated with an asterisk.

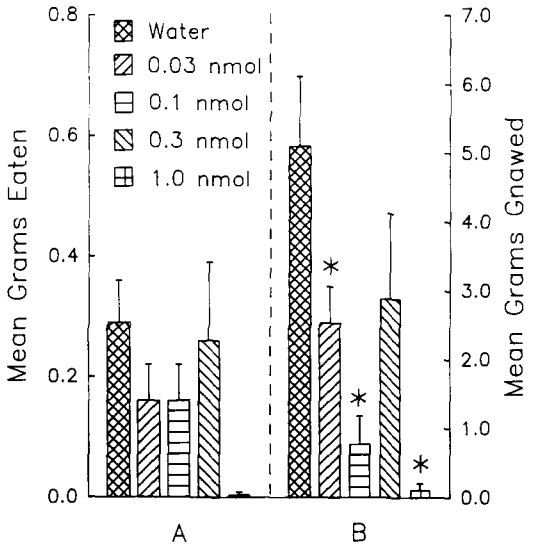

FIG. 2. Mean (±1 SE) grams of food eaten (panel A) or gnawed (panel B) during tail pinch following intranigral injection of vehicle control (water) or DAGO. Significant differences ($p < 0.05$) relative to vehicle control are indicated with an asterisk.

gnawing however, $r(47) = -0.41$; $p = 0.0038$, two-tailed. DAGO suppressed gnawing significantly following microinjection of the 0.03, 0.1, and 1.0 nmol doses. The amount of gnawing after these doses also did not differ significantly but was significantly correlated with dose of DAGO, $r(37) = -0.53$, $p = 0.0006$, two-tailed.

On an equimolar basis DAGO was more potent in its effects on gnawing than morphine. Although 0.1 nmol of DAGO suppressed gnawing, the same dose of morphine had no effect. Additionally, the lowest effective dose of morphine was 1 nmol, while the lowest effective dose of DAGO was 0.03 nmol.

Measures of Nociception

Tail flick latency was not altered by opiate injection (data not shown). Latency to paw lick was increased significantly by both morphine, $F(4, 44) = 2.83$, $p = 0.0354$, and DAGO, $F(4, 34) = 9.27$, $p < 0.0001$. Response latencies for the hot plate test are shown in Fig. 3. The effect of morphine on paw lick latency was due to the 5 and 20 nmol doses, and these two doses did not differ significantly from one another. Paw lick latency was significantly correlated with morphine dose, $r(47) = 0.44$, $p = 0.0015$, two-tailed. Paw lick latency was increased significantly by all four doses of DAGO. The 0.3 and 1.0 nmol doses of DAGO increased response latency significantly more than the 0.03 and 0.1 nmol doses. Following the 1.0 nmol dose, 100% of the animals reached the 30-s cutoff latency without displaying a paw lick response. The correlation between dose of DAGO and paw lick latency was significant, $r(37) = 0.67$, $p < 0.0001$, two-tailed.

As with gnawing, DAGO was more potent in its effect on paw lick latency than morphine. The 0.1 or 1.0 nmol doses of morphine did not alter paw lick latency, while latency was increased following equimolar doses of DAGO. Additionally, the 0.3 nmol dose of DAGO increased response latency significantly more than the 1.0 nmol dose of morphine, and 1.0 nmol of DAGO increased paw lick latency more than the 5 and 20 nmol doses of morphine.

DISCUSSION

Intranigral injections of morphine and DAGO were found to increase the latency to nociceptive response on the hot plate test and to decrease the gnawing produced by tail pinch. These findings suggest that activation of a nigral opioid system mediating analgesia may have decreased the aversiveness of tail pinch and, therefore, reduced the gnawing response. Although it is possible that the reduction in gnawing represents an action of nigral opioids that is independent of nociception, this seems unlikely, based on other reports that have shown that opioid agonists in the SN activate oral stereotypies. Stimulation of mu receptors in the SN, for example, has been reported to produce stereotyped gnawing that is blocked by mu-opioid or dopamine antagonists (17).

Previous research in our laboratory has shown that intranigral injection of a nonspecific opioid antagonist or a selective mu antagonist decreases the eating produced by tail pinch without affecting gnawing (10). The importance of central mu opioid receptors in SIE has been confirmed by others (12). The data reported here demonstrate that activation of opioid receptors in the SN with morphine and DAGO does not increase SIE, as one might predict from the previous results with antagonists.

In general, it appears that opioid agonists increase food intake less reliably than antagonists decrease it [see (13) for a review]. Repeated injections of opioid agonists frequently are necessary to demonstrate an increase in feeding (8). Nevertheless, other investigators have observed that, in unstressed animals, central injections of morphine and DAGO increase food intake after a considerable time delay. Morphine (1–30 nmol) injected into the ventral tegmental area, hypothalamus, septum, amygdala, or nucleus accumbens, stimulates feeding at 1 to 5 h after injection (9,16,19,20,24). Similarly, DAGO (1–3 nmol) has been reported to increase food intake at 2 to 4 h

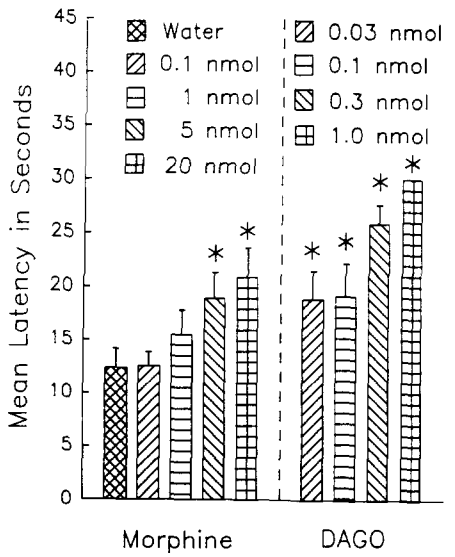

FIG. 3. Mean (±1 SE) latency to paw lick response on the hot plate test following intranigral injection of vehicle control (water), morphine, or DAGO. Significant differences ($p < 0.05$) relative to vehicle control are indicated with an asterisk.

(but not 1 h) after injection into the amygdala and hypothalamus (7).

The long latency to the prophagic effect of opiates contrasts with the rapid effect of opiates on nociceptive responding and of opiate antagonists on SIE. Changes in nociceptive threshold are observable within 5 min following intranigral injection of morphine and DAGO (3,5). SIE is reduced within 5 min of intranigral injection of naloxone or the mu antagonist CTOP (10). It is clear from the present findings, however, that activation of nigral opioid receptors does not produce a rapid alteration in SIE as is seen following blockade of those receptors (10).

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REFERENCES

1. Antelman, S. M.; Szechtman, H. Tail pinch induces eating in sated rats which appears to depend on nigrostriatal dopamine. Science 189:731–733; 1975.
2. Antelman, S. M.; Szechtman, H.; Chin, P.; Fisher, A. E. Tail pinch-induced eating, gnawing and licking behavior in rats: Dependence on the nigrostriatal dopamine system. Brain Res. 99: 319–337; 1975.
3. Baumeister, A. A. The effects of bilateral intranigral microinjection of selective opioid agonists on behavioral responses to noxious thermal stimuli. Brain Res. 557:136–145; 1991.
4. Baumeister, A. A.; Hawkins, M. F.; Anticich, T. G.; Moore, L. L.; Higgins, T. D. Bilateral intranigral microinjection of morphine and opioid peptides produces antinociception in rats. Brain Res. 411:183–186; 1987.
5. Baumeister, A. A.; Nagy, M.; Hebert, G.; Hawkins, M. F.; Vaughn, A.; Chatellier, M. O. Further studies of the effects of intranigral morphine on behavioral responses to noxious stimuli. Brain Res. 525:115–125; 1990.
6. Bertiere, M. C.; Sy, T. M.; Baigts, F.; Mandenoff, A.; Apfelbaum, M. Stress and sucrose hyperphagia: Role of endogenous opiates. Pharmacol. Biochem. Behav. 20(5):675–679; 1984.
7. Gosnell, B. A. Involvement of μ opioid receptors in the amygdala in the control of feeding. Neuropharmacology 27:319–326; 1988.
8. Gosnell, B. A.; Grace, M.; Billington, C. J.; Levine, A. S. Effects of streptozotocin-induced diabetes on feeding stimulated by centrally administered opioid agonists. Life Sci. 45:31–40; 1989.
9. Hamilton, M. E.; Bozarth, M. A. Feeding elicited by dynorphin (1–13) microinjections into the ventral tegmental area in rats. Life Sci. 43:941–946; 1988.
10. Hawkins, M. F.; Cubic, B.; Baumeister, A. A.; Barton, C. Microinjection of opioid antagonists into the substantia nigra reduces stress-induced eating in rats. Brain Res. 584:261–265; 1992.
11. Kavaliers, M.; Hirst, M.; Teskey, G. C. Opioid systems and feeding in the slug, Limax maximus: Similarities to and implications for mammalian feeding. Brain Res. Bull. 14:681–685; 1985.
12. Koch, J. E.; Bodnar, R. J. Involvement of mu_1 and mu_2 opioid receptor subtypes in tail-pinch feeding in rats. Physiol. Behav. 53: 603–605; 1993.
13. Levine, A. S.; Billington, C. J. Opioids: Are they regulators of feeding? Ann. NY Acad. Sci. 575:209–220; 1989.
14. Lowy, M. T.; Maickel, R. P.; Yim, G. K. W. Naloxone reduction of stress-related feeding. Life Sci. 26:2113–2118; 1980.
15. Lowy, M. T.; Yim, G. K. W. Selective reduction by dexamethasone of stress-related hyperphagias. Life Sci. 27:2553–2558; 1980.
16. Majeed, N. H.; Przewlocka, B.; Wedzony, K.; Przewlocki, R. Stimulation of food intake following opioid microinjection into the nucleus accumbens septi in rats. Peptides 7 (5):711–716; 1986.
17. Morelli, M.; Fenu, S.; Di Chiara, G. Substantia nigra as a site of origin of dopamine-dependent motor syndromes induced by stimulation of μ and β opioid receptors. Brain Res. 487:120–130; 1989.
18. Morley, J. E.; Levine, A. S. Stress-induced eating is mediated through endogenous opiates. Science 209:1259–1261; 1980.
19. Mucha, R. F.; Iversen, S. D. Increased food intake after opioid microinjections into nucleus accumbens and ventral tegmental area of rat. Brain Res. 397:214–224; 1986.
20. Nencini, P.; Stewart, J. Chronic systemic administration of amphetamine increases food intake to morphine, but not to U50-488H, microinjected into the ventral tegmental area in rats. Brain Res. 527:254–258; 1990.
21. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1986.
22. Rechtschaffen, A.; Gilliland, M. A.; Bergmann, B. M.; Winter, J. B. Physiological correlates of prolonged sleep deprivation in rats. Science 221:182–184; 1983.
23. Robbins, T. W.; Fray, P. J. Stress-induced eating: Fact, fiction or misunderstanding?. Appetite 1:103–133; 1980.
24. Stanley, B. G.; Lanthier, D.; Leibowitz, S. F. Multiple brain sites sensitive to feeding stimulation by opioid agonists: A cannula-mapping study. Pharmacol. Biochem. Behav. 31:825–832; 1988.
25. Strober, M. Stressful life events associated with bulimia in anorexia nervosa: Empirical findings and theoretical speculations. In: Miller, T. W., ed. Stressful life events. Madison, CT: International Universities Press; Inc.; 1984:237–256.
26. Teskey, G. C.; Kavaliers, M. Aggression, defeat and opioid activation in mice: Influences of social factors, size and territory. Behav. Brain Res. 23:77–84; 1987.
27. Vaswani, K.; Tejwani, G. A.; Mousa, S. Stress induced differential intake of various diets and water by rat: The role of the opiate system. Life Sci. 32:1983–1996; 1983.