Pharmacology Biochemistry & Behavior, Vol 24, pp 833–836, 1986 © Ankho International Inc Printed in the U S A 0091-3057/86 $3 00 + 00

Opiate Blockade Inhibits Saccharin Intake and Blocks Normal Preference Acquisition

WESLEY C. LYNCH

Department of Psychology, Montana State University, Bozeman, MT 59717

Received 21 June 1985

LYNCH, W C *Opiate blockade inhibits saccharin intake and blocks normal preference acquisition* PHARMACOL BIOCHEM BEHAV 24(4) 833–836, 1986 —Recent evidence indicates a close connection between oral sensory function and opioid effects on feeding Not only is gustatory motivation influenced by opiate drugs [11] but apparently gustatory stimuli can also activate central opiate receptor systems [3] In 3 experiments we studied the effect of opiate receptor blockade on drinking motivated by the sweet taste of saccharin. Experiment 1 established a dose-response function for inhibition of intake by naloxone (NAL) in short (60 min) 2-bottle tests. This experiment demonstrated the extreme sensitivity of nondeprived, nonstressed animals to NAL and estimated the MED_{50} at less than 0 1 mg/kg (SC), well below the threshold for effects due to illness or general motor disturbance Experiment 2 further demonstrated that NAL's effectiveness depends on saccharin concentration. In particular, the lowest NAL dose studied was effective near the threshold for saccharin preference but not at higher concentrations. These data suggest that endogenous opioid systems may be activated by taste stimuli in a graded fashion. Finally, experiment 3 showed that the typical acquisition of preference for a moderate saccharin concentration can be effectively blocked by daily pre-test NAL injection Together these experiments further demonstrate the close functional relationship between opioid systems and gustatory sensory systems

Taste preference Feeding Drinking Appetite Opioids Naloxone

A number of recent studies suggest a connection between oral sensory stimulation and opiate receptor function. Indirect evidence comes from studies of feeding and drinking in which taste provides the primary incentive for intake By far the most numerous of these have employed opiate antagonists which suppress intake and in which intake-suppression is enhanced by flavor [2, 8, 14]. For instance Locke *et al* [10] showed that various opiate antagonists suppress intake of sweetened condensed milk in non-deprived monkeys and rats, and Rockwood and Reid [13] found that sucrose intake was suppressed by the antagonist naloxone even in nondeprived rats sham drinking with open gastric fistulas. They reasoned that naloxone's effect must be due to modification of the "affective reactivity to palatable solutions . and not due to feedback from postabsorptional events" (p 1175). Other studies have shown that intake motivated by sweet taste is highly sensitive to opiate antagonism [11,18]. More direct evidence for a connection between gustatory stimulation and opiate system function comes from a study by Herman and Novin [6] which demonstrated a naloxone-reversible inhibition by morphine of gustatory-evoked unit activity recorded from the parabrachial (gustatory) nucleus. Unit activity evoked by a 1 8% salt solution applied to the tongue was inhibited by morphine but tactile-evoked activity from the tongue, recorded from the trigeminal nucleus, was unaffected

While the above studies suggest that opiate drugs can influence gustatory sensation, the converse influence of gustatory stimulation on endogenous opioid function has also been reported. Lieblich *et al.* [9] compared female rats genetically selected for high versus low intake of saccharin (LC2-Hi vs. LC2-Lo). When LC2-Hi rats were given daily access to 3 mM saccharin they showed a lack of analgesic response (cross-tolerance) to a moderate dose of morphine (2.5 mg/kg), whereas LC2-Lo rats given daily saccharin or LC2-Hi rats given no saccharin showed a typical (nontolerant) analgesic response These data imply that, in this selected group of rats, saccharin intake somehow modifies the functional status of endogenous opioid systems. The same conclusion was more forcefully illustrated by Dum *et al* [3] who reported an increased release of β-endorphin and a corresponding decrease in *in vivo* binding of ^{3}H-etorphine within the hypothalamus of nondeprived, nonstressed animals given a highly palatable food or drink (chocolate candy or chocolate milk) immediately before assays were performed. The decreased etorphine binding, in particular, suggests that palatable foods somehow activate endogenous opioids which in turn compete with etorphine for available binding sites.

The present experiments examined further the influence of endogenous opioid systems on fluid intake motivated by taste. All experiments were carried out in nondeprived, nonstressed adult male rats tested in their home cages. In this situation the primary incentive to drink is provided by the gustatory quality of solutions presented during short (60 min) 2-bottle test sessions. Experiments 1 and 2 determined

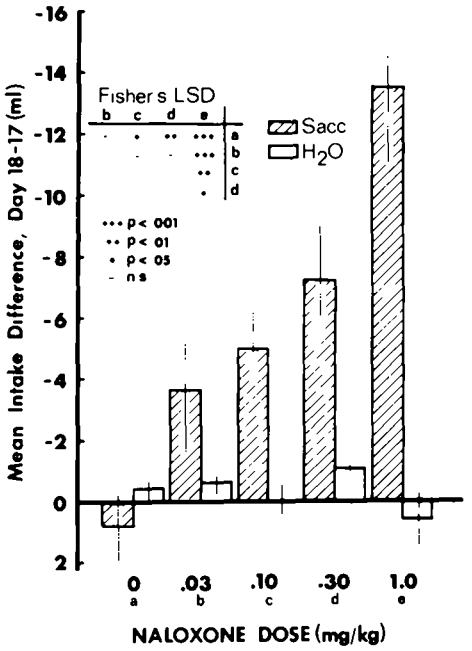

FIG 1 Dose-effect of naloxone-HCl on intake of a highly preferred (0 1%, w/v) solution of Na-saccharin versus water Group mean difference scores (Days 18–17) ±SEM Negative values indicate suppression of drinking Mean (±SEM) pre-drug intake (ml) of saccharin and water, respectively, was 15 3±0 89 and 1 6±0 38 Inset shows statistically significant group differences (Fisher's LSD test)

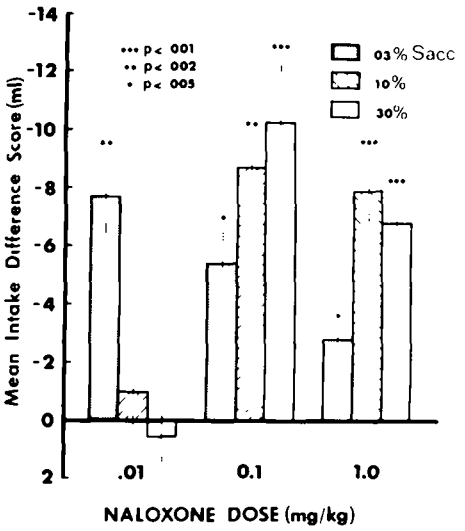

FIG 2 Dose-effect of naloxone-HCl on intake of 3 saccharin solutions spanning the peak preference range (0 03–0 30%, w/v) Mean intake difference scores (±SEM) are from a single group of rats (n=10) tested repeatedly over 10 weeks, calculated by subtracting scores for the week immediately preceding (SAL) and during drug (NAL) treatment Mean (±SEM) pre-drug intake volumes (ml) for the 3 doses (0 01, 0 1 and 1 0 mg/kg) were respectively, 15 1±1 53, 15 1±2 11 and 9 2±2 47 The lower volume associated with the 1 mg/kg dose reflects the fact that this dose was given earlier in training

the lowest effective doses of naloxone (NAL) that acutely suppress intake of highly preferred solutions of saccharin Experiment 3 examined the effects of moderate daily doses of NAL given throughout testing, on saccharin preference acquisition.

METHOD

In each of the following experiments adult male Holtzman albino rats were housed individually with ad lib access to food and water except during test sessions. All tests were carried out in home cages during the light portion of a 14·10 hr light/dark cycle (on at 10 a m.) Room temperature was maintained at approximately 25°C. Naloxone-HCl (Endo Laboratories) was dissolved in 0.9% bacteriostatic NaCl (SAL). All injections were given subcutaneously at the nape of the neck in 1 ml/kg volumes.

Experiment 1 established a dose-response function for NAL's effect on saccharin intake in fifty rats randomly assigned to 1 of 5 dose groups (n=10) For 11 days prior to 2-bottle testing all animals were allowed 1 hr daily access to a highly preferred (0.1% w/v) saccharin solution. Two-bottle presentations (saccharin vs water) began on day 12 with each session preceded (15 min) by a vehicle injection (days 11–17) or by 1 of 5 NAL doses (day 18). Doses for the 5 groups were. 0 0, 0.03, 0.10, 0.30, and 1 0 mg/kg (SC).

Experiment 2 determined whether or not the intake-suppressant effect of NAL varied as a function of saccharin concentration Ten rats selected from a larger group of 30 on the basis of their consistent (3 day) consumption of 0 1% saccharin, were given repeated 2-bottle preference tests over 10 weeks All animals were tested 3 days/week once with each of 3 saccharin concentrations selected to cover the peak preference range (0 03, 0.10, and 0 30% w/v) Solutions were each paired with water and the positions and solution concentrations randomized. Injections began on week 3 with each week of drug injections preceded by one or two weeks of SAL injections Over the following 7 weeks 3 NAL doses were given in descending dose order: 1 0, 0.1, 0 01 mg/kg (SC).

In experiment 3, three groups of naive rats (n=8) were each given 15 days of 1 hr daily access to 0 1% saccharin vs water (5 days/week) Group 1 (SAL) received an injection of SAL (1 ml/kg) 15 min before each daily session Group 2 (NAL-pre) received an injection of NAL (1 mg/kg) also 15 min before daily testing Group 3 (NAL-post) received the same NAL dose (1 mg/kg) immediately after each daily session. Group 3 was included to control for the possibility that the intake suppression by NAL might be due to conditioned taste aversion (CTA). By associating the taste of saccharin (CS) with the presentation of NAL (US), an aversion to the taste of saccharin might gradually develop thus reducing overall intake However, if the primary effect of NAL was to act as a US for aversion conditioning, the intake suppressant effect should be greater in group 3 than in group 2 since group 3 represents the optimal condition for CTA learning, namely CS (taste) preceding US (drug)

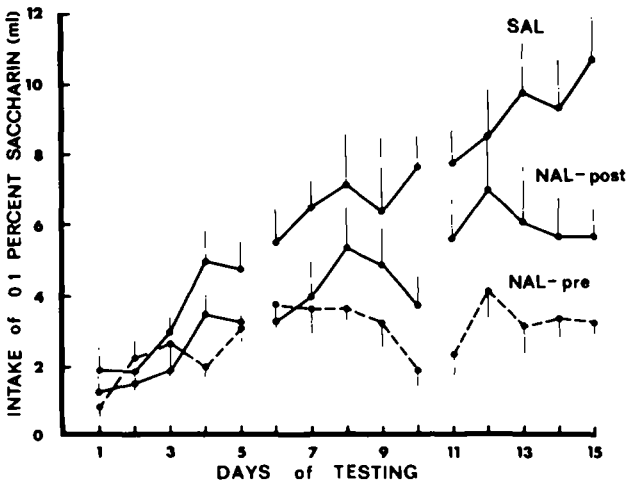

FIG 3 Daily group mean intake of 0 1% saccharin (w/v) for 3 groups tested 5 days per week for 3 weeks. SAL· daily pretreatment with 0 9% NaCl, NAL-pre daily pretreatment with naloxone-HCl (1 0 mg/kg, SC), NAL-post daily post-test treatment with the same dose of naloxone-HCl See text for details and statistics

RESULTS

Figure 1 illustrates the dose-effect of NAL on intake of 0 1% saccharin vs. water Group mean difference scores were calculated by subtracting volumes on day 17 (pre-drug) from volumes on day 18 (drug) for each animal. Negative difference scores (upward) indicate suppression. It is clear that saccharin consumption varies with NAL in a dose-dependent manner. Statistical analysis (one-way ANOVA) confirmed a highly significant main effect of dose, $F(4,45)=8.945$, $p<0$ 001. Multiple comparisons among the 5 groups showed the 3 highest NAL doses were significantly more effective than the zero dose and that the highest dose (1 0 mg/kg) was significantly more effective than any of the 4 lower doses (Fisher's LSD test) The MED_{50} for NAL was estimated to be 0 065 mg/kg by calculating the NAL dose at which half the animals showed a 20% or greater reduction in intake (pre/post).

The results of experiment 2 are shown in Fig. 2. Intake difference scores were calculated by subtracting the volumes consumed of each solution during the week of SAL injections from those during the immediately following NAL week, yielding 9 difference scores for each animal (3 solutions × 3 NAL doses) The results illustrated in Fig. 2 suggest that NAL dose interacts with saccharin concentration such that the lowest NAL dose (0.01 mg/kg, SC) is a less effective intake-suppressant at the 2 highest saccharin concentrations than at the lowest concentration. When t-tests were used to evaluate the effect of each treatment separately (α-levels adjusted for multiple tests, rejecting the null-hypothesis only if $p \leq 0.005$) it was found that NAL was uniformly effective except when the lowest NAL dose was given in the presence of the 2 higher saccharin concentrations This suggests that near the threshold of palatability, a very low NAL dose (0.01 mg/kg) effectively suppresses intake, whereas at higher (super-threshold) saccharin concentrations, intake is unaffected by this low dose

The results of experiment 3 (Fig 3) show that acquisition of preference for an otherwise highly preferred 0.1% saccharin solution is effectively blocked by daily pre-treatment with NAL. A trend analysis based on the data in Fig. 3 [5] confirmed a highly significant main effect of treatment on group mean intake, $F(2,21)=12.91$, $p<0.001$, as well as a highly significant overall difference in linear trends, $F(2,21)=13.70$, $p<0.001$ In addition, individual between-group trends were also significant for NAL-pre vs. NAL-post, $F(1,14)=8.84$, $p<0.025$, and for NAL-pre vs. SAL, $F(1,14)=37$ 98, $p<0.001$. Thus pre-test NAL injection more strongly inhibited the acquisition of saccharin preference than either post-test NAL or than pre-test SAL While the group mean difference between NAL-post and SAL was significant, $F(1,14)=7$ 29, $p<0$ 025, the linear trend difference between these groups fell just short of significance, $F(1,14)=4$ 40, $p<0$ 10

DISCUSSION

Taste is a strong incentive to eat and drink in most species including man. The above results suggest that in the absence of other motives to drink (deprivation, stress, etc.) the incentive motivation provided by sweet taste depends upon an adequately functioning endogenous opioid system. These results cannot easily be accounted for in terms of nonspecific motor disturbance since effective suppressant doses of NAL demonstrated in experiments 1 and 2 are nearly an order of magnitude below most estimates for effects on general activity or motor coordination in rodents [1, 7, 12, 15] These effects also are not likely to be due to malaise or to conditioned taste aversion, since the effective doses reported in experiments 1 and 2 are well below those typically required to produce illness or CTA [4,17] and since a direct comparison between pre- and post-test NAL treatments (experiment 3) showed that post-test NAL (an arrangement more likely to produce CTA) was actually less effective than pre-test NAL which virtually eliminated preference acquisition.

The present results, then, suggest the following conclusions First, that fluid-intake motivated exclusively by sweet taste is extremely sensitive to the disruptive effect(s) of opiate receptor blockade and that NAL inhibits fluid intake in a dose-dependent fashion over nearly a 100-fold range with an approximate MED_{50} of 0.065 mg/kg (SC) Second, that the effectiveness of NAL varies with saccharin concentration such that near the preference threshold low NAL doses are effective but these become less so (or entirely ineffective) above threshold. This is a tentative conclusion but one that may shed light on the interaction between opiate system and sensory system functions. It suggests that when external incentives are weak, a fully functioning opioid system is essential to the maintenance of goal-directed behavior Dum *et al* [3] have convincingly demonstrated that highly palatable foods (primarily sweets) activate endogenous opioid pools leading to opiate receptor occupancy Assuming this is a graded response to sweetness (or palatability or perhaps some general motivational quality of food), weaker activation by weaker or less palatable fluids would perhaps be more easily blocked by low doses of opiate antagonists It remains to be determined exactly which qualities of oral stimulation are responsible for opioid system activation and exactly which elements of the central and/or peripheral opioid systems are involved.

Finally, experiment 3 suggests that daily opiate receptor blockade prior to preference acquisition effectively eliminates the normal preference for saccharin. Whether or not preference would recover once antagonist treatment was

ended is unknown An interesting question in light of the results of Dum *et al* [3] is whether the NAL-post effects are due to CTA or to a blockade of rewarding after effects of opioid activation. If palatable foods activate opioid pools and the effects outlast the stimulus, such after effects may contribute to the positive incentive to drink Thus there are 2 apparently contradictory interpretations of the NAL-post effects· Either NAL is itself an aversive stimulus that when paired with a novel sweet taste leads to CTA and suppression of subsequent drinking or, alternatively, NAL antagonizes an otherwise emotionally positive after effect of sweet taste, thereby reducing the incentive to drink The difference between these interpretations lies in the relationship between sensory events and opioid activation In the first case, no special role is given to the gustatory event CTA is assumed to be a direct response to aversive classical conditioning In the second case, gustatory stimulation is seen as activating endogenous opioid pools leading to positive after effects which are then blocked by naloxone

ACKNOWLEDGEMENTS

Thanks to Drs Rick Block and Barbara Fernandez for statistical assistance and to Ms Leslie Libby and Mr Bob Jones for laboratory assistance This work was supported in part by MONTS-NSF project ISP-801149, MSU Research Creativity Award and a grant from Procter and Gamble Naloxone-HCl was a gift from Endo Laboratories

REFERENCES

1 Amir, S , H Galina, R Blair, Z W Brown and Z Amit Opiate receptors may mediate the suppressant but not the excitatory action of ACTH on motor activity of rats *Eur J Pharmacol* **66**: 307–313, 1980
2 Apfelbaum, M and A Mandenoff Naltrexone suppresses hyperphagia induced in the rat by a highly palatable diet *Pharmacol Biochem Behav* **15**: 89–91, 1981
3 Dum, J , C H Gramsch and A Herz Activation of hypothalamic β-endorphin pools by reward induced by highly palatable food *Pharmacol Biochem Behav* **18**: 443–447, 1983
4 Frenk, H and G H Rogers The suppressant effects of naloxone on food and water intake in the rat *Behav Neural Biol* **26**: 23–40, 1979
5 Grant, D A Analysis-of-Variance tests in the analysis and comparison of curves *Psychol Bull* **53**: 141–154, 1956
6 Herman, G and D Novin Morphine inhibition of parabrachial taste units reversed by naloxone *Brain Res Bull* **5**: Suppl 4, 169–173, 1980
7 Katz, R J Naltrexone antagonism of exploration in the rat *Int J Neurosci* **9**: 49–51, 1979
8 Levine, A S , S S Murray, J Kneip, M Grace and J E Morley Flavor enhances the antidipsogenic effect of naloxone *Physiol Behav* **28**: 23–25, 1982
9 Lieblich, I , E Cohen, J R Ganchrow, E M Blass and F Bergmann Morphine tolerance in genetically selected rats induced by chronically elevated saccharine intake *Science* **221**: 871–873, 1983
10 Locke, K W , D R Brown and S G Holtzman Effects of opiate antagonists and putative mu- and kappa-agonists on milk intake in rat and squirrel monkey *Pharmacol Biochem Behav* **17**: 1275–1279, 1982
11 Lynch, W C and L Libby Naloxone suppresses intake of highly preferred saccharin solutions in food deprived and sated rats *Life Sci* **33**: 1909–1914, 1983
12 Pert, A., L A DeWald, H Liao and C Sivit Effects of opioid peptides on motor behaviors sites and mechanisms of action In *Endorphins in Mental Health Research*. edited by E Usdin, W E Bunney, Jr and N S Kline New York Oxford U Press, 1979, pp 45–61
13 Rockwood, G A and L D Reid Naloxone modifies sugar-water intake in rats drinking with open gastric fistulas *Physiol Behav* **29**: 1175–1178, 1982
14 Siviy, S M , D J Calcagnetti and L D Reid Opioids and palatability In *The Neural Basis of Feeding and Reward*. edited by B G Hoebel and D Novin Brunswick, ME Haer Institute, 1982, pp 517–524
15 Ukai, M and T Kameyama Naloxone specifically blocks the linear locomotion in mice *Brain Res* **328**: 378–380, 1985
16 Walker, J M , G G Bernston, T S Paulucci and T C Champney Blockade of endogenous opiates reduces activity in the rat *Pharmacol Biochem Behav* **14**: 113–116, 1981
17 Wu, M -F , S E Cruz-Morales, J R Quinan, J M Stapleton and L D Reid Naloxone reduces fluid consumption Relationship of this effect to conditioned taste aversion and morphine dependence *Bull Psychonom Soc* **14**: 323–325, 1979
18 Wu, M -F , M D Lind, J M Stapleton and L D Reid Dose-relationship between naloxone injections and intake of sucrose solution *Bull Psychonom Soc* **17**: 101–103, 1981