Intake of and Preference for Sweet Solutions Are Attenuated in Morphine-Withdrawn Rats

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The hypothesis that intake of sweet solutions is partially controlled by endogenous opioid peptides was tested in 2 experiments that examined the effects of repetitive morphine administration and withdrawal on subsequent intake of and preference for saccharin solutions in rats. Experiment 1 established that 17 hr after morphine withdrawal, rats consumed less saccharin, but not less water, than did controls. The groups did not differ 8 days later. In Experiment 2, using a 2-bottle saccharin-preference test, rats exhibited a reduced preference to saccharin solutions (1, 3, 9, 30, or 60 mM) for 6 days after morphine withdrawal. The difference between the groups was most pronounced at 1 day and 4 days after saccharin preference (Yirmiya, Lieblich, & Liebeskind, 1988). In addition, the anatomical distribution of cell bodies containing opioid precursors (Khachatryan, Lewis, Schaefer, & Watson, 1983) and the pharmacological characteristics of taste-activated neurons in the parabrachial nucleus (Herman & Novin, 1980) and lateral hypothalamus (Ono et al., 1980; Oomura, Nishino, Atou, & Lenard, 1986) suggest that opioids are involved in gustatory information processing.

The possibility that the intake of palatable sweet solutions stimulates the release of opioid peptides has also been suggested. For example, animals given highly palatable food or drink demonstrate increased release of β-endorphin in the hypothalamus (Dum, Gramsch, & Herz, 1983) and develop signs of tolerance to low doses of morphine (Bergman, Lieblich, Cohen, & Ganchrow, 1985; Cohen, Lieblich, & Bergman, 1984; Holder, 1988; Lieblich, Cohen, Ganchrow, Blass, & Bergman, 1983).

The findings that endogenous opioid peptides are involved in the initiation of intake of palatable solutions, that drinking of such solutions induces release of opioid peptides, and that tolerance to some effects of exogenous opiates develops after chronic exposure to palatable solutions raise the possibility that tolerance to the rewarding effects of palatable solutions will be developed after chronic exposure to opiates. This possibility was tested in the following two experiments in which the relationship between chronic morphine treatment and the intake of and preference for saccharin solutions was assessed.

Experiment 1

In this first experiment, intake of saccharin was measured during the early period of morphine withdrawal (a 24-hr period, beginning 17 hr after the last morphine injection). Using a morphine administration procedure similar to the one used in this study, rats were shown to be completely tolerant to the analgesic effects of morphine at this period (Kayser & Guilbaut, 1983). In a preliminary experiment, we found that with the same procedure, tolerance to the analgesic
Morphine treatment was a modification of a procedure that was previously found to induce morphine tolerance (Kayer & Guilbaud, 1985). Morphine sulphate in isotonic saline (40 mg/ml sc) was injected daily for 5 days. The initial dose of morphine was 40 mg/kg and was increased daily by 40 mg/kg. Control rats were injected with equal volumes of isotonic saline. The injections were given at 5:00 p.m. Rats were weighed after the last injection and before the start of the drinking tests.

Seventeen hours after the last injection, a single bottle of either 3 mM saccharin solution or water was presented for 24 hr to the Sac and Wat groups, respectively. Eight days later the same procedure was repeated. Fluid consumption was estimated as the difference in weight of the bottles at the beginning and end of the test period.

The results were analyzed by a three-way analysis of variance (ANOVA) with drug and solution as between-subjects factors and test time (17 hr and 8 days after the last injection) as the repeated measure and were followed by post hoc comparisons with the Newman-Keuls procedure (p < .05).

Results and Discussion

The mean consumption of water and saccharin by morphine- and saline-treated rats is presented in Figure 1.

Analysis of the data revealed a significant solution effect, F(1, 55) = 42.88, p < .01, which indicated that more saccharin was consumed than water, and a significant Drug × Solution × Time interaction, F(1, 55) = 8.97, p < .01, which demonstrated a differential recovery of saccharin intake in Mor rats with time. Post hoc tests showed that during the first postdrug testing period Mor rats showed a significantly reduced intake of saccharin to a level resembling the intake of water in Sal rats (Figure 1). By the 8th day after morphine treatment, Mor rats did not differ from Sal rats in amount of saccharin consumed. Water intake was not affected by the morphine regimen.

Although Sal rats manifested a relatively stable body weight throughout the experiment (251–254 g), Mor rats lost an average of 9 g after 5 days of morphine injections. This difference between Sal and Mor rats was no longer apparent 8 days later. The body-weight data were assessed by a two-way ANOVA (with drug as the between-subjects factor and time as the repeated measure), which revealed a significant drug effect, F(1, 57) = 8.80, p < .01, and a significant Drug × Time interaction, F(3, 55) = 5.00, p < .01.

One possible interpretation of the results of this experiment is that the first injection of morphine, administered to the Mor rats 24 hr after the saccharin familiarization, induced a conditioned aversion to the taste of saccharin. This explanation is unlikely, however, given the long interval between the presentation of saccharin and the injection of morphine (Smith & Roll, 1967). It should also be noted that when the rats were tested for saccharin consumption 8 days after the injections, no differences in drinking between Mor and Sal groups were detected. If the basis of a previously reported reduction in saccharin preference in morphine withdrawn rats had been, as was suggested, an acquired aversion to this solution paired with the onset of withdrawal stress (Parker, Falor, & Weedman, 1973), then some trace of such an aversion should be manifested 8 days later. This conclusion is not supported by the present experiment.

Several studies reported that animals made sick by ad-
ministration of a toxin reduce intake of novel tasting food and fluid (e.g., Domjan, 1977; Kutcher, Wright, & Lisch, 1977). Thus, it is possible that in the present experiment the withdrawal from morphine produced a state of general malaise that contributed to the reduced intake of the novel saccharin solution. Although this explanation cannot be ruled out, it should be noted that during the first test period the mean intake of saccharin was not reduced compared with the mean intake of water in the Mor group, as would be expected from a reaction to the discomfort of acute withdrawal.

A reduction in the intake of palatable solutions could be a characteristic of withdrawal from any addictive drug. This is probably not the case because at least in one case of withdrawal (i.e., from nicotine), rats increased their intake of saccharin-flavored foods (Grunberg, Bowen, Maycock, & Nesper, 1985). Also, morphine withdrawal does not attenuate intake of all flavored solutions. Ho, Chen, and Morrison (1976) reported unchanged intake of ethanol on the 1st day after the interruption of a chronic morphine injection regimen.

In the presence experiment, morphine-treated animals manifested reduced body weight at the time of the first saccharin consumption test and a recovery of body weight by the second test. It is possible that the differences in body weight between Mor and Sal rats, which may have been associated with different feeding patterns, contributed to the observed differences in saccharin consumption. This issue was addressed in Experiment 2.

Experiment 2

This second experiment was designed to extend the results of Experiment 1 by studying preference for, rather than intake of, saccharin. Measuring saccharin preference may be more appropriate because the morphine treatment could affect consumption of all fluids and not just specifically palatable solutions. In addition, the time during which the depressive effects of morphine treatment persisted and the effects of morphine withdrawal on the preference for a range of saccharin concentrations were explored. Others have shown that the suppressive effects of opioid antagonists on the intake of and preference for sweet solutions were most prominent on the most palatable concentrations of saccharin (Cooper, 1983; Lynch & Libby, 1983; Siviy, Calcagnetti, & Reid, 1982) and the opioid agonists seem to widen the range of acceptable concentrations of sweet solutions (Lynch & Libby, 1983; Siviy et al., 1982). Thus, it was expected that the effects of chronic morphine treatment would be reflected differentially across saccharin concentrations.

Finally, the possibility that saccharin preference may be associated with the reduction in body weight during the morphine treatment and withdrawal period and the recovery of body weight after withdrawal was examined.

Method

Subjects. Ninety-one female Sprague-Dawley rats from the same source as those in Experiment 1 were used. They were maintained as before, except as noted.

Procedure. Morphine treatment was the same as described in Experiment 1. Measurement of saccharin preference was initiated only 48 hr after the last drug injection to reduce the possibility of association between saccharin's taste and withdrawal-induced illness. At this time, Mor and Sal rats were assigned to five different saccharin solution subgroups. The rats within each subgroup were presented with two drinking bottles, one that contained water and one that contained 1, 3, 9, 30, or 60 mM sodium saccharin solution. Subgroups were comprised of 8 rats each, except for 9-mM Mor subgroup (n = 10) and the 9-mM Sal subgroup (n = 17).

The 9-mM Mor and Sal subgroups were each subdivided into two subgroups, one fed as before and one (Food) fed specially to control for the effects that body weight differences might have on saccharin preference. The specially fed morphine (Mor Food) subgroup (n = 7) was given pulverized chow ad lib in a round feeding cup, which was designed for the measurement of food consumption. The mean food intake (grams/animal/24 hr) of this subgroup was supplied in similar dishes to each of 7 rats of the specially fed Sal subgroup (Sal Food). The rats of these two groups were weighed daily, whereas all other rats were weighed as in Experiment 1.

The position of the water and saccharin bottles was alternated daily, and the consumption of the fluids was measured for the next 4 days by weighing the bottles as in Experiment 1. At this time (Day 7 postinjection), saccharin solutions were removed, and rats were given only water. Twenty-four hours later, they were given another 24-hr saccharin preference test.

The effects of the various independent variables on saccharin preference were assessed by a three-way ANOVA (with drug and saccharin concentration as between-subjects factors and time as the repeated measure).

Results and Discussion

Analysis of the results revealed a significant drug effect, F(1, 76) = 54.27, p < .001, which indicated a depressive effect of morphine withdrawal on saccharin preference, a significant concentration effect, F(4, 76) = 12.92, p < .001, a significant Drug × Concentration interaction, F(4, 76) = 2.57, p < .05, and a significant Drug × Time interaction, F(4, 73) = 4.34, p < .01, which indicated an initial drop in saccharin preference in the Mor rats that recovered to about the levels of the Sal group 8 days after the last injection. To explore a possible source for the Drug × Concentration effect, the preference data were collapsed across days, and Mor and Sal groups were compared at each saccharin concentration level using t tests. Given the overall ANOVA significance level of .05 and the fact that five tests were used, the significance level for these t tests was set at p < .01. Compared with Sal rats, Mor animals exhibited significant attenuation of saccharin preference only at the two saccharin concentrations most preferred by the Sal groups in the present experiment, 9 and 30 mM, t(23) = 7.91 and t(13) = 3.97, respectively.

To assess whether recovery of body weight could explain the observed results, we conducted a two-way ANOVA (with drug as the between-subjects factor and time as the repeated measure) on the preference scores of the Mor Food and their pair-fed Sal Food controls. These rats were subgroups of the animals offered the choice of 9 mM saccharin or water. The analysis revealed a significant drug effect, F(1, 12) = 21.83, p < .01, which indicated again that morphine treatment depressed subsequent saccharin preference. Also, a signifi-
A significant Drug × Time interaction was obtained, $F(4, 9) = 5.36$, $p < .01$, which suggested that a recovery of the saccharin preference by the Mor Food rats occurred at Day 8. At 6 days after the last injection, the mean saccharin preference scores of the Mor Food rats was 0.43, whereas this score was 0.83 for the matched Sal Food group. At Day 8 the mean saccharin preference scores were 0.70 and 0.74, respectively. These data are quite similar to the data of Mor and Sal groups as a whole on these same days.

Mor Food rats consumed a mean of 11 g food/day during the period of morphine treatment. After the injections, the mean values were as follows: 13 and 16 g at 24 and 48 hr after the last injections and 16, 16, 19, and 21 g/day during the first 4 days of saccharin preference testing. The pairing feeding procedure was highly successful in affecting a weight drop in the Sal Food rats comparable to that achieved in Mor Food animals. The procedure also permitted a simulation of the recovery in weight observed in Mor Food rats after the drug injections. Although the mean initial weights of the Mor Food and Sal Food were 255 and 257 g, respectively, these mean weights dropped to 246 and 238 g, respectively, 24 hr after the last injection. Body weights subsequently recovered to a mean weight of 267 g in the Mor Food animals and 266 g in the Sal Food rats by Day 8 after the last injection.

A striking finding, seen clearly in Figure 2, is that when given a choice, Mor rats actually reject saccharin at all concentrations on most days. This rejection is not indiscriminate, however, being most pronounced at the concentrations most preferred by Sal rats. The recovery of saccharin preference is abrupt in Mor rats, as is apparent from the comparison of the saccharin preference functions on Days 6 and 8. Mor rats do not show any trace of the previous rejection of saccharin when returned to the saccharin preference test on Day 8. This result argues against the suggestion that the reduction in saccharin intake or preference in morphine-treated rats represents an acquired aversion to the solution paired with the onset of withdrawal stress.

The apparent exacerbation with time of the rejection of saccharin, especially of the most preferred concentrations, is consistent with the findings of Parker et al. (1973), who used one concentration. This phenomenon appears once again not to reflect increased general malaise in Mor animals because food intake of the Mor Food rats measured during the withdrawal periods was higher than during the morphine regimen. Moreover, at the time when saccharin preference was decreasing, food intake was actually increasing as seen in the Mor Food groups. This finding is contrary to what would be expected if these results would be explainable by recovery from illness (Green & Garcia, 1971). The exacerbation of the rejection of saccharin with time in Mor rats is consistent with the hypothesis that saccharin drinking itself might add to the tolerance induced by treatment with morphine and in turn further reduce saccharin preference in these animals. Such an hypothesis could account for the abrupt change in preference between Day 6 and 8, which may be associated with the interpolation of a water-drinking period between these test days. Such a saccharin-free period might have reduced
additive effects of habituation to saccharin after the long drinking period and the tolerant state induced by morphine treatment.

General Discussion

The results of these two experiments establish that following a regimen of injections of morphine, which is effective in inducing morphine tolerance and leads to withdrawal (Kayser & Guilbaud, 1985), rats reduce their intake of (Experiments 1) and preference for (Experiment 2) sweet solutions. Experiment 1 demonstrated that Mor rats consumed less saccharin, but not less water, than Sal rats at 17 hr after the last injection. They did not differ from Sal rats in a saccharin-drinking test conducted 8 days later. In Experiment 2, using a saccharin-preference test, Mor rats rejected saccharin up to 6 days after the last injection; this rejection was most pronounced at the most preferred concentrations (9 and 30 mM). In the last test period, Mor and Sal groups were indistinguishable in saccharin preference. These results are consistent with our preliminary finding that shows that by the 8th day after the last morphine injection rats are no longer tolerant to the analgesic effect of morphine (Lieblisch & Yirima, 1986). The disappearance of morphine tolerance at this time may explain the recovery of normal intake of and preference for saccharin.

The present data are not consistent with interpretations of these results relying on acquired aversions, general malaise, or recovery of body weight. Both experiments suggest that previous interpretations of similar phenomena in terms of an acquired aversion to the sweet taste (Parker et al., 1973) are not likely. The results of Experiment 2 are not consistent with the notion that the attenuation of intake and preference of sweet solutions represents a state of general malaise produced by withdrawal from morphine treatment. Finally, the pattern of intake of sweet solutions in morphine-treated rats appears not to represent a simple process of recovery of body weight.

The findings of the present experiments are consistent with the theoretical expectation that repeated injections of morphine would result in cross-tolerance to the effects of opioid peptides associated with the intake of palatable sweet solutions. This conclusion in conjunction with results of previous research suggests that opioid peptides can stimulate an increase in the initial intake of sweet solutions. This intake results, in turn, in increased opioid activity leading to tolerance to the effects of the opioids and consequently to a reduction in saccharin intake. This conceptualization represents a self-limiting mechanism that would protect against excessive incentive-induced consumption, which could result in hyper-hydration. Preliminary studies in our laboratory (Lieblisch & Yirima, 1986), as well as studies by others (Collier & Novell, 1957), are consistent with this hypothesis, showing gradually decreasing consumption of saccharin solutions in naive rats starting 5–6 days after initiation of saccharin exposure. This decrease is particularly apparent in the most preferred concentration of saccharin. The results of Experiment 2 show only a slight reduction in saccharin preference in saline-injected rats consuming the 9-mM solution.

It is likely that more time is needed for tolerance to develop (e.g., chronic saccharin drinking produces tolerance to the analgesic effects of morphine only several weeks after initiation of saccharin exposure; Bergmann et al., 1985; Cohen et al., 1983; Holder, 1988; Lieblisch et al., 1983). In addition, it is possible that less tolerance develops in the preference paradigm, in which rats shuttle between saccharin and water, than in a one-bottle paradigm, in which saccharin is the only solution presented.

The taste of saccharin to humans as well as rats is complex and involves both sweet and bitter qualities (e.g., Bartoshuk, 1979). This fact may explain the actual rejection of saccharin solutions exhibited by morphine-tolerant rats in the present study. It is possible that although in saline-treated rats the operation of an intact endogenous opioid system dominates the aversive effect of the bitter taste, in morphine-treated rats the endogenous opioid system is rendered tolerant and the bitter effects gain importance in determining saccharin preference. Another possibility is that the morphine treatment altered the sensory qualities of saccharin, perhaps amplifying its bitter component. This possibility should be addressed in further studies.

References

Lieberich, I., & Yirmiya, R. (1986) [Time course for tolerance to the analgesic and hedonic effects of morphine]. Unpublished raw data.