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Modulation of Saccharin Preference by Morphine and Naloxone: Inversion of Drug Effects as a Function of Saccharin Concentration

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TOUZANI, K., K. AKARID AND L. VELLEY. *Modulation of saccharin preference by morphine and naloxone: Inversion of drug effects as a function of saccharin concentration.* PHARMACOL BIOCHEM BEHAV **38**(1) 37–41, 1991.—The aim of the present study was to verify and extend a recent, isolated observation showing that, in rats, a moderate dose of morphine may induce either an increase or a decrease in preference for saccharin, the direction of the response depending apparently on the concentration of the sweetener. Two experiments were performed successively. First, we showed that the preference threshold for saccharin (0.3 mM, two-bottle procedure) of rats placed on a schedule of restricted water access was significantly decreased following injection of 1 mg/kg of morphine. In the second experiment, three groups of naive rats were submitted to the preference test but the concentration of saccharin solution was different for each group, namely 0.3, 1 and 1.7 mM. After stabilization of the baseline responses the effect of morphine (1 mg/kg) was tested in each of the 3 groups. As observed previously morphine decreased the preference of the rats tested with the 0.3 mM solution, but markedly increased the preference of the two other groups tested with the 1 and 1.7 mM solutions respectively. The effects of low doses of naloxone (0.01, 0.1 and 1 mg/kg) were then tested on the same groups of rats with the same saccharin concentrations. The 0.01 mg/kg dose of the antagonist increased the preference for the groups of rats tested with the 0.3 and 1 mM solutions. The other two doses of naloxone decreased saccharin intake whatever the saccharin concentration used. It is suggested that these apparently paradoxical effects of morphine and naloxone could result either from the stimulation of opioid autoreceptors or from the differential stimulation of different opioid receptor subtypes.

Morphine Naloxone Preference test Saccharin concentrations Rats

IN the course of our analysis of reward processes associated with the pontine parabrachial area, the second relay station of gustatory signals, we recently compared the effects of a moderate dose of morphine (2 mg/kg) on the gustatory preference threshold for saccharin solutions in both sham-lesioned rats and in rats whose lateral hypothalamic neurons had been lesioned by ibotenic acid (20). In this study, we observed that the sham-lesioned rats exhibited an unexpected response which was dependent on the concentration of the saccharin solution. In agreement with other data (2), morphine was observed to increase the preference for highly palatable solutions of saccharin. However, the same dose of the drug suppressed the predrug level of preference when the sweetener concentration was near the preference threshold (0.3 mM). In other words, it appeared that at this saccharin concentration morphine exhibited an apparently antagonist action, similar to the well-known effect of naloxone on saccharin preference. This paradoxical result was difficult to interpret, since it was initially observed using only one concentration of saccharin and only one dose of morphine.

The purpose of the present study, therefore, was to verify and to extend our previous observations by conducting an analysis of the relationship between different doses of morphine or naloxone and the preference for saccharin solutions using different concentrations around the threshold value.

METHOD

Subjects

Male rats of the Sprague-Dawley strain (Iffa-Credo, Lyon) were individually housed in wire-mesh cages and maintained on

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a regular 12:12-h light-dark cycle in a temperature-regulated (21–23°C) animal room. The rats were 2 months old at the beginning of experiments.

Saccharin-Water Choice

In each of the following experiments, rats were placed on a schedule of restricted access to water (2,20). Over 5 days, rats were allowed only two periods for drinking, one beginning at 08.30 h lasting one hour and one beginning at 18.30 h lasting 2 h. After habituation to the deprivation schedule, rats were presented with two bottles each equipped with stainless steel drinking spouts. One contained tap water the other contained the saccharin solution. Fresh solutions of the sweetener were mixed daily by dissolving tablets in tap water. Each tablet contained 20 mg of benzoic sulfimide and did not contain glucose. The test session took place each day at 08.30 h and lasted 1 hour. The bottles were weighed at the beginning and at the end of the test to the nearest 0.01 g. The different concentrations of saccharin were tested one per day. The bottle containing saccharin solution was placed on a different side of the home-cage every other day.

Morphine sulfate and naloxone HCl (Sigma) were dissolved in sterile 0.9% NaCl. The drugs as well as the vehicle were given subcutaneously, 30 min before the test at the nape of the neck in 1 ml/kg volumes.

Two successive experiments were performed.

Experiment 1 first established the preference threshold of a group of 20 rats. Two different concentrations of saccharin were used successively, 0.2 and 0.3 mM. Despite repeated testing the 0.2 mM solution did not induce a significant preference for saccharin over water indicating that this concentration was below the threshold. Using the 0.3 mM solution, all rats exhibited a significant preference for saccharin. After stabilization of the response (over 4 days), all rats received 30 min before the test and over 3 consecutive days an injection of the vehicle. Subsequently, on Days 4, 6 and 9, the rats were injected with increasing doses of morphine namely 0.1 mg/kg on Day 4, 0.5 mg/kg on Day 6 and 1 mg/kg on Day 9. On Days 5, 7, 8 and 10 the rats received the vehicle.

In the second experiment, 3 groups of naive rats (10 rats per group) were placed on the usual water deprivation schedule and then submitted to the saccharin-water choice test. One group was tested with the 0.3 mM solution, the second and the third groups were tested with 1 and 1.7 mM saccharin solutions respectively. After stabilization of the preference, all rats initially received vehicle injections before the choice test, and then morphine at a dose derived from the first experiment and known to effectively suppress preference (1 mg/kg). The next day the animals were tested once again following vehicle injection.

At the end of testing with morphine, water was given ad lib for 3 days. Then the rats were placed once more on the water deprivation schedule and given a choice between the previous concentration of saccharin and water. For 2 consecutive days, each test was preceded by vehicle injection. Then on Days 3, 5 and 8, the rats received increasing doses of naloxone, namely 0.01 mg/kg on Day 3, 0.1 mg/kg on Day 5 and 1 mg/kg on Day 8. On Days 4, 6, 7 and 9, they were injected with the vehicle.

RESULTS

Figure 1 shows the results from the first experiment. Mean difference scores for each group were calculated by subtracting liquid volumes consumed following vehicle injection (mean of 3 days) from volumes consumed following morphine injections, for each animal. Positive and negative values indicate increased and decreased intakes respectively. In order to ascertain that the placebo scores remained stable despite repeated injection of increasing doses of morphine, a one factor ANOVA with repeated measures was performed. The following placebo scores for water and saccharin intake were compared. Mean intake recorded during the 3 days before the first injection of morphine, intake recorded the days before the second and third administration of the drug (Days 5 and 8) and lastly the day following the third injection of morphine (Day 10). Neither water consumption nor saccharin consumption were significantly modified: water, $F(3,57) = 0.99$, ns; saccharin, $F(3,57) = 0.54$, ns. Thus given the stability of the placebo scores, the effect of each dose of morphine was tested by comparing for each rat the saccharin and water intake after the administration of the drug to the mean values recorded during the 3 first days (paired *t*-test). Each dose of morphine was observed to significantly decrease saccharin intake and the highest dose (1 mg/kg) was also observed to induce a significant compensatory increase in water intake. Given the significant and opposite effect of the 1 mg/kg dose of morphine on saccharin and water intakes, this dose was used in the second experiment.

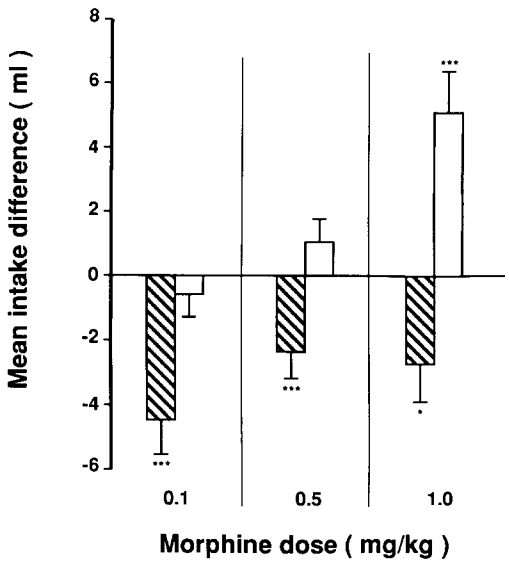

FIG. 1. Dose-effect of morphine on the intake of an 0.3 mM solution of saccharin as compared to water. Abscissa: doses of morphine; ordinate: differences (ml ± SEM) between the intake after vehicle injection and the intake after morphine injection. The predrug intakes (ml) of saccharin and water, respectively were: 12.5 ± 0.7 and 4.7 ± 0.4. Hatched bars: mean differences in saccharin intake; white bars: mean differences in water intake. For this and the following figures the statistical significance is indicated as follows: $*p<0.05$; $**p<0.01$; $***p<0.001$.

Figure 2 shows the influence of 1 mg/kg of morphine on preference for saccharin using increasing concentrations of the sweetener solutions. The statistical significance of the differences observed in each group of rats was computed by comparing for each animal the water and saccharin intake after morphine administration to the corresponding values recorded after vehicle injection the day before. As observed in the first experiment the opiate agonist decreased saccharin intake and increased water consumption with the 0.3 mM solution of the sweetener. However, with the two more concentrated solutions, the same dose of morphine was observed to produce opposite effects: the intake of saccharin was significantly increased whereas water consumption was si-

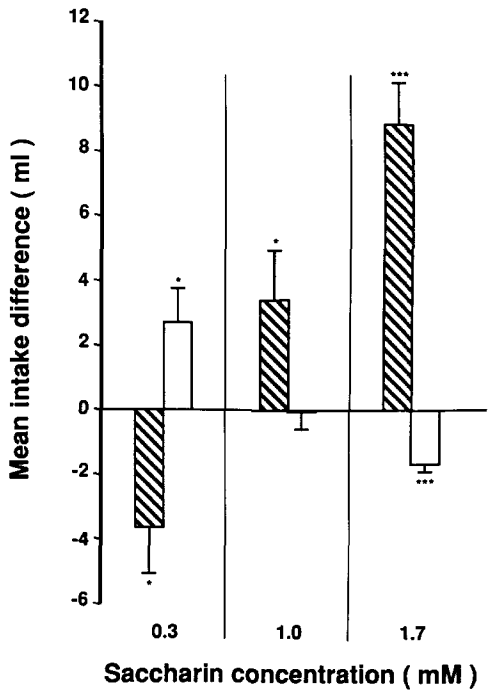

FIG. 2. Effects of the 1 mg/kg dose of morphine on the preference for saccharin over water with increasing concentrations of saccharin solutions. Abscissa: saccharin concentrations; ordinate as in Fig. 1. The predrug intakes (ml) for each saccharin solution were as follows: 0.3 mM: Sacc: 13.3±1; water: 5±0.5; 1 mM: Sacc: 18.4±1; water: 2.9±0.5; 1.7 mM: Sacc: 21.7±2.2; water: 2.4±0.2.

multaneously decreased.

Figure 3 summarizes the interaction between increasing doses of naloxone and increasing concentrations of saccharin solutions. As in the first experiment, a one-factor ANOVA with repeated measures was used to verify the stability of the placebo scores throughout the experiment. Four placebo scores of water and saccharin intake were compared: mean score recorded during the 2 days before the first injection of naloxone, the scores recorded on the days before the second and the third administration of the drug (Days 4 and 7) and lastly the day following the third injection of naloxone (Day 9). No significant difference was observed between the 4 values in each of the 3 groups of rats. Group 0.3 mM: water, $F(3,27)=0.97$, ns; saccharin, $F(3,27)=1.35$, ns. Group 1 mM: water, $F(3,27)=0.90$, ns; saccharin, $F(3,27)=1.51$, ns. Group 1.7 mM: water, $F(3,27)=1.41$, ns; saccharin, $F(3,27)=0.65$, ns. Thus given the stability of the placebo scores, the individual values of saccharin and water intake after each naloxone administration were compared to the corresponding mean intake recorded during the 2 days before the first injection of naloxone. With the lowest dose of the antagonist (0.01 mg/kg), the effect on saccharin intake was dependent on the concentration of the sweetener. Naloxone at this dose produced an increase of saccharin intake and a decrease in water consumption. These effects were significant, however, only with the 1 mM solution. Using the most concentrated solution of the sweetener (1.7 mM) both saccharin and water intakes were slightly decreased.

The two other doses of naloxone (0.1 and 1 mg/kg) had the same general effect, namely they both greatly decreased saccharin intake while at the same time moderately decreasing water intake.

DISCUSSION

In agreement with our previous observations (20), the present data show that morphine can induce either a decrease or an increase in preference for saccharin. Naloxone produces a symmetrically opposite pattern of influences on preference.

When the concentration of saccharin was at the preference threshold value (0.3 mM) low doses of morphine (1 mg/kg) significantly decreased saccharin intake. In contrast, the 10 μg/kg dose of naloxone induced an increase in saccharin consumption with both the 0.3 and 1 mM solutions. This effect of naloxone has already been reported for sucrose solutions (17). The first possible explanation of the paradoxical effect of morphine is to suppose that the agonist induced some general malaise which would affect saccharin intake. A number of studies have previously shown that morphine produces taste aversion (3, 8, 23), but in these experiments, the doses injected were greater than the doses presently used and the deficit appeared only after repeated injections. Nevertheless, it is worth noting that, using a place-preference paradigm, Bechara and Van der Koy (1) observed a positive reinforcing effect of a small dose of naltrexone and an aversive effect of a small dose of morphine, after intraperitoneal but not after subcutaneous injections. They demonstrated that these responses were due to an action at peripheral opioid receptors, especially in the gut. The decrease of preference following small doses of morphine could also be explained by inhibition of drinking, since it was demonstrated that morphine, as well as opioid selective agonists, injected subcutaneously (12) intraventricularly (19) or into the hypothalamus [(4), review in (5)], suppress water intake. It seems, however, that neither peripheral aversion, nor inhibition of drinking can explain the present findings. In particular, these possibilities cannot explain the reversal of the effect of morphine when the saccharin concentration was increased while the dose of morphine was kept constant (Fig. 2). In other words, the main result indicates that the concentration of the sweetener is the primary significant factor which is responsible for the reversal of the morphine and naloxone's effects. This observation suggests that the intensity of the gustatory signals modulates the activation level of certain endogenous opioid systems and possibly, that this modulation reverses the action of the opiate agonist and antagonist. Although these observations are presently difficult to explain, some possibilities can be considered. For example, the suppressive effect of morphine and the enhanced preference following low doses of naloxone could be due to a preferential stimulation of opiate autoreceptors, while the reverse effects of these two drugs would result from the stimulation of postsynaptic opioid receptors. The possibility that the release of opioid peptides is modulated by presynaptic feedback inhibition has received some support. It has been shown that low doses of opiate antagonists, like naloxone, produce analgesia in mice and rats (13,21). Furthermore, morphine has been shown to decrease (Met)-enkephalin release from the brain, while naloxone enhanced the release of this peptide (16,21). Thus if we suppose as have others (15) that the state of activation of certain endogenous opioid systems increases as a function of saccharin concentration, it is possible that at the lowest concentration of saccharin presently tested (0.3 mM) the state of activation of the opioid systems was very low, leading to a preferential action of small doses of morphine and naloxone on the putative opioid autoreceptors.

Another possibility would be that the differential responses are due to the differential stimulation of different opioid receptor subtypes. For example, it was shown recently that the intraventricular injection of the selective mu agonist DAGO and the selective delta agonist DTLET increased intake of a palatable saccharin

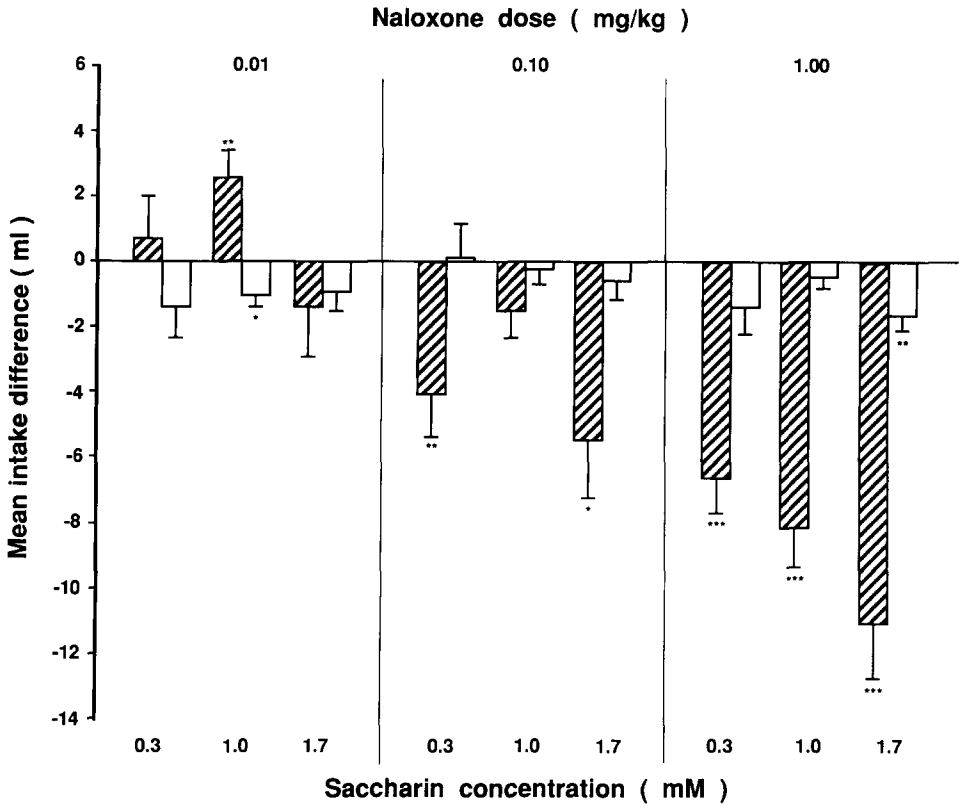

FIG. 3. Dose-effect of naloxone on the preference for saccharin over water with increasing concentrations of saccharin solutions. Ordinate as in Fig. 1. Abscissa: top: doses of naloxone; bottom: saccharin concentrations. The predrug intakes for each saccharin solution were as follows: 0.3 mM: Sacc: 13.6±1; Water: 4.9±0.9; 1 mM: Sacc: 19.7±1.2; Water: 2.1±0.3; 1.7 mM: Sacc: 24.8±2.2; Water: 2.4±0.5.

solution while the injection of the selective kappa agonist U-50,488H decreased the consumption of the same solution (10). However, the failure of the kappa agonist to increase saccharin intake does not agree with other findings showing that the subcutaneous injection of the same agonist stimulates intake of palatable food (6,11). Further data are needed in order to better describe the role of each opioid receptor type on saccharin preference.

The second result of the present study concerns the more classical effects of morphine and naloxone on saccharin preference. When moderately palatable solutions of the sweetener were used (1.7 mM) small doses of morphine (1 mg/kg) increased preference and reciprocally small doses of naloxone (0.1 and 1 mg/kg) decreased the preference. With regard to the morphine effect the present result agrees with our previous observations (20) as well as with other data (2, 10, 18). Likewise, the reduction of saccharin intake by naloxone has also been observed by others [(14, 15, 18), review in (6)]. This significant reduction cannot be explained by motor disturbance or depression of locomotor activity, since the doses of naloxone presently used are at least an order of magnitude below the doses affecting locomotor activity (7,22). Likewise, the effect of naloxone is unlikely to be due to malaise: the doses of this antagonist necessary to induce conditioned taste aversion are about 10 times as great as the doses presently used (9,23). However, it was shown recently that the acquisition of preference was blocked by pretest injection of the 1 mg/kg of naloxone, but that this deficit appeared only after repeated daily injections (15). Thus given that the total dose of naloxone received by each rat was only 1.11 mg/kg, distributed over 6 days, the possibility that the suppression of the preference would be due to malaise is unlikely.

The increase in preference for saccharin produced by morphine and its suppression by naloxone is interpreted as suggesting that certain endogenous opioid systems play a role in the mediation of palatability [review in (6)]. The present results observed with morphine are in agreement with this possibility. However, the interpretation of the naloxone effects is more ambiguous given the well-documented suppressive effect of the antagonist on liquid intake [review in (5)]. Some authors observed that naloxone or naltrexone markedly attenuated the preference for saccharin while the consumption of water remained unaffected (6, 14, 15). The present results do not confirm these observations since in our experimental conditions the large decrease in saccharin consumption was associated with a moderate decrease in water intake. Thus it is difficult to conclude as to whether naloxone interacts with palatability or decreases consumption of saccharin by way of a nonspecific deficit manifest as a general decrease in fluid intake.

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