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Effects of Morphine Injection Into the Parabrachial Area on Saccharin Preference: Modulation by Lateral Hypothalamic Neurons

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MOUFID-BELLANCOURT, S. AND L. VELLEY. *Effects of morphine injection into the parabrachial area on saccharin preference: Modulation by lateral hypothalamic neurons.* PHARMACOL BIOCHEM BEHAV **48**(1) 127–133, 1994.—The aim of the present study was to analyze the effects of morphine injected into the second relay station of the gustatory input pathways, the parabrachial area, on preference for saccharin over water. This study was carried out using both rats whose lateral hypothalamic neurons had been lesioned by ibotenic acid and sham-lesioned rats. As already shown, an 0.3 mM solution of the sweetener, which was clearly preferred over water by the sham-lesioned animals, was neutral for the lesioned rats. The injection of 50 ng of morphine into each parabrachial area transformed this neutral response of the lesioned rats to a clear preference for the sweetener, whereas the preference of sham-lesioned rats for the same solution was converted to an aversive response. Likewise, with a more palatable solution of saccharin (2.5 mM), the injection of 50 ng of morphine decreased the preference of the nonlesioned rats but increased the preference of the lesioned animals. Using the 2.5 mM solution of saccharin, the intraparabrachial injection of higher doses of morphine (100 and 500 ng) did not greatly modify the preference for the sweetener but induced a significant decrease in total fluid intake that was still observed 11 h after the injection of the opiate. These results are discussed: the morphine-induced aversion observed in the nonlesioned rats could be explained either by a specific influence on certain opioid receptors in the parabrachial area or, more probably, by the stimulation of pathways involved in taste or visceral aversive processes and relaying in the parabrachial area. The possible role of the intrinsic cells of the lateral hypothalamus on the responses to the morphine injection is discussed within the context of each of the above hypotheses.

Lateral hypothalamus Intrinsic neurons Ibotenic acid lesion Saccharin Intraparabrachial injection Morphine Rats

IN the course of our analysis of reward processes, we examined some years ago the modulatory role of intrinsic neurons located in the lateral hypothalamus on self-stimulation behavior recorded in the second relay station of the afferent gustatory signals, the medial part of the parabrachial area (17). We showed that self-stimulation in this area was almost completely suppressed following a bilateral ibotenic acid lesion of neurons localized in the middle and posterior parts of the lateral hypothalamus. This result suggested that self-stimulation in the parabrachial area is not a purely local process but also is under the control of descending hypothalamic influences.

Given that self-stimulation behavior in the parabrachial area could result from the artificial activation of a neural system underlying the rewarding properties of certain taste cues (39), we tested in a second experiment the role of intrinsic neurons of the lateral hypothalamus on gustatory preference-aversion functions for increasing concentrations of saccharin solution in the rat (18). Bilateral lesions of the lateral hypothalamic cells by ibotenic acid resulted in a significant shift to the right of the preference-aversion curve. In sham-lesioned rats, the preference threshold was observed with the 0.3 mM solution of saccharin, the best preference score was obtained with the 2.5 mM solution, whereas the 25 and 50 mM solutions were aversive. In the lesioned animals, the 0.3 mM solution was neutral, the best preference score being observed with the 7.5 mM solution, the 25 mM solution was preferred over water, while the 50 mM solution was not statistically aversive.

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Finally, the aversion threshold appeared in lesioned rats with a quinine concentration 5 times higher than the concentration inducing aversion in the control rats.

These results, showing that the rewarding and aversive values of saccharin are modulated by intrinsic hypothalamic neurons, suggested that this modulation forms part of the neural network implicated in the control of palatability (6).

Furthermore, there now exists substantial evidence to indicate that endogenous opioid systems are involved both in the rewarding effects of electrical brain stimulation [review in (41)] and, similarly, in reward mechanisms associated with taste [review in (13)]. Consequently, to test the hypothesis that some LH neurons are implicated in the opioid-mediated modulation of palatability, we compared the effect of the SC injection of morphine on saccharin preference in both LH-lesioned and sham-lesioned rats (43). The results obtained were complex: the aversion shown by normal rats for the most concentrated solution of the sweetener (50 mM) was converted by the opiate to a significant preference. Likewise, the same dose of opiate transformed the neutral response of the lesioned animals for this solution into a significant preference. However, with the 0.3 mM solution of saccharin that corresponds to the preference threshold for normal rats, the same dose of opiate induced an opposite effect in sham-lesioned and in lesioned rats. The preference of the normal rats was converted to a neutral response, whereas, in the lesioned animals, the neutral response was transformed into a significant preference. More recently we have demonstrated, in normal rats, that the decrease or the increase of the preference for saccharin by the same dose of morphine is dependent on the concentration of the sweetener solution and that a small dose of naloxone induces a paradoxical increase in preference for the 0.3 mM solution of saccharin (44).

Given the data summarized above, the purpose of the present study was to determine whether these unexpected effects of morphine on gustatory preference could be due to some action of the opiate on the medial part of the parabrachial area. Indeed, besides the fact that this area is reciprocally connected to the lateral hypothalamus (5,19,25,32,35,36,45), it also contains neurons and terminals immunoreactive for opioid neuropeptides (15,23,33) as well as a high density of μ receptors and a moderate density of κ receptors (2,28). Consequently, it is possible that at least a part of the complex influences of morphine on gustatory processes in normal rats could be due to a localized action of the opiate on the parabrachial area and that the loss of the lateral hypothalamic input consecutive to the ibotenic acid lesion could disturb the local effect of morphine. To test this hypothesis, we have studied the effect of intraparabrachial injections of morphine on saccharin preference of sham-lesioned and lateral hypothalamic lesioned rats.

METHOD

Animals

Male rats of the Sprague-Dawley strain (IFFA-CREDO, Lyon) were individually housed in wire-mesh cages and maintained on a regular 12 L : 12 D cycle (lights on at 0700 h) in a temperature-regulated (21–23°C) animal room.

Surgery

Under pentobarbital anesthesia (Nembutal 55 mg/kg IP), two chronic guide cannulae (outer diameter 0.45 mm) were stereotaxically implanted bilaterally, 2 mm above each medial parabrachial area (PBA) of 17 animals. The coordinates were 1 mm posterior to the interaural line, ± 1.8 mm lateral to the sagittal suture, and 5.3 mm ventral from the skull surface. The incisor bar was level with the interaural line. During the same operation, the intrinsic cells of the lateral hypothalamus (LH) were bilaterally destroyed in 10 of these animals by local injection of ibotenic acid (IBO). The coordinates were 6.1 mm anterior to the interaural line, ± 1.7 mm lateral to the sagittal suture, and 9.1 mm ventral to the skull surface. The cannula was connected with a micropump that delivered in each LH 4 μg of IBO in 0.5 μl of vehicle (phosphate buffer). Each injection lasted 6.6 min, and 10 more min elapsed before removal of the cannula. Using the same procedure the seven other rats were injected only with the vehicle of the neurotoxin. At the end of the operation one screw was fixed into the skull, and the guide cannulae were chronically fixed with dental cement.

Saccharin-Water Choice

Fourteen days after the operation all rats were placed on a schedule of restricted water access (7,18). Over 6 successive days rats were allowed only two daily periods for drinking, one beginning at 0830 h and lasting 2 h and one beginning at 1930 h and lasting 1 h. After habituation to the deprivation schedule, during the 2 h period of the morning session, rats were presented with two bottles 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 bottles were weighed at the beginning and at the end of the test to the nearest 0.01 g. The bottle containing saccharin solution was placed on a different side of the home cage every day. Two successive experiments were performed.

We tested first the 0.3 mM solution of saccharin. After the responses of lesioned and nonlesioned rats had stabilized (5–7 days), the solution was again presented during 3 consecutive days; the first 2 days no injection was performed, but the third day each rat was injected with the vehicle only. The following day a 50 ng dose of the opiate was injected.

In a second phase, the gustatory preference of the rats was tested with a highly preferred solution of saccharin, namely a 2.5 mM solution. After the preference for each rat was established, three different doses of morphine were injected, 50, 100, and 500 ng. To minimize the effect of some possible tolerance phenomena, these doses were tested in an ascending order and an interval of 3 days separated two successive injections of morphine. During this period, to limit mechanical damage to brain tissue, the vehicle was not injected, but rats were submitted daily to the preference test in the usual conditions. This test was also carried on during 3 consecutive days beginning the day following the injection of the 500 ng dose, to test the possible delayed influence of this dose of on saccharin and water intakes.

In the two experiments, the opiate (morphine sulfate) or the vehicle (NaCl 0,9%) were injected via two cannulae (outer diameter 0.24 mm introduced through the guides). A pump delivered to each side, over 38 s a 0.2 μl volume whatever the dose of morphine injected. Three parameters were recorded throughout these experiments, namely saccharin and water intakes during the choice test (2 h) and water consumption during the evening period of drinking (1 h). All rats were weighed daily from the fifth day before the operation to the day of sacrifice. Dry food was always available, during the two periods of water deprivation and during testing. At the

end of the experiments the rats were killed by an overdose of pentobarbital and the brains were frontally sectioned in a freezing microtome at 80 μm and stained with thionine. The extents of the LH lesions and the locations of the cannulae tips were reconstructed on the appropriate planes of the Paxinos and Watson atlas (38).

RESULTS

Histological Analysis

The 10 brains of the lesioned rats were analyzed. In eight cases, a complete loss of neurons associated with glial proliferation was observed in the LH. The anteroposterior extent of the lesions varied between 1280 and 2320 μm. In most cases, the frontal extent of lesion began at the caudal end of the paraventricular nucleus. The posterior limit was observed at the frontal level of the premammillary nuclei. In the mediolateral plane the lesions extended from the perifornical region to the internal capsule. In the dorsoventral plane the lesioned area extended from the base of the brain to the zona incerta (top part of the Fig. 1). In the two other brains the LH was not lesioned but the thalamus was greatly damaged. Thus, the data of these two rats were excluded from the analysis. Two sham-lesioned rats were also excluded because the tips of the cannulae were located dorsal to the PBA. For the 13 remaining brains, the tips of the cannulae were located in the medial part of PBA or immediately ventral to the nucleus (bottom part of Fig. 1).

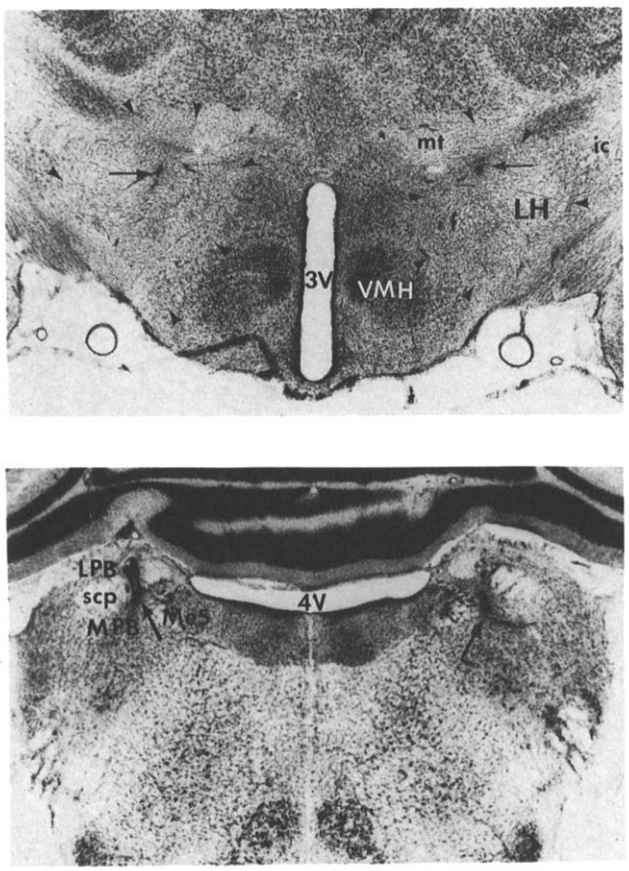

FIG. 1. Top: low-powered photomicrograph of the hypothalamic region showing an example of a bilateral lesion. Arrowheads show the limit of the lesioned area. The tips of the cannula tracks are indicated by arrows. Abbreviations: f—fornix; ic—internal capsule; LH—lateral hypothal. area; mt—mammillothalamic tract; VMH—ventromedial hypothal. nu.; 3V—third ventricle. Bottom: example of bilateral sites of injection into the parabrachial area. The tips of the cannula tracks are indicated by arrows. Abbreviations: LPB—lateral parabrachial nu.; Me5—mesencephalic trigeminal nu.; MPB—medial parabrachial nu.; scp—superior cerebellar peduncle; 4V—4th ventricle.

Effects of the LH Lesions on Body Weight and Daily Water Intake

As previously reported (18,30,43,48) the IBO lesioned rats initially showed a weight loss that was 16.6% of the preoperative body weight 4 days after the operation. The corresponding weight loss of the sham-lesioned rats did not exceed 1.9% 2 days after the operation. Despite the fact that the body weight of the lesioned rats increased regularly from the sixth day onward, this body weight remained clearly inferior to that of the unlesioned rats throughout the behavioral tests, and on the day of their sacrifice, the mean body weight of the lesioned rats was 436 g (±8.6) as compared to 514 g (±10.4) for the control animals, $t = 5.8$ $p < 0.001$).

In a similar manner, the LH lesion produced a significant and permanent deficit in water intake, and on the day of the sacrifice, the mean daily water intake of the lesioned animals was 21.9 ml (±2.1) as compared to 32.2 ml (±2.1) for the sham-lesioned rats, $t = 3.3$ $p < 0.01$).

Effect of the LH Lesion on the Preference for Saccharin Solution and on the Differential Influence of Intra-PBA Injection of Morphine

The top part of Fig. 2 summarizes the results of the sham-lesioned (left) and lesioned (right) rats for the saccharin-water

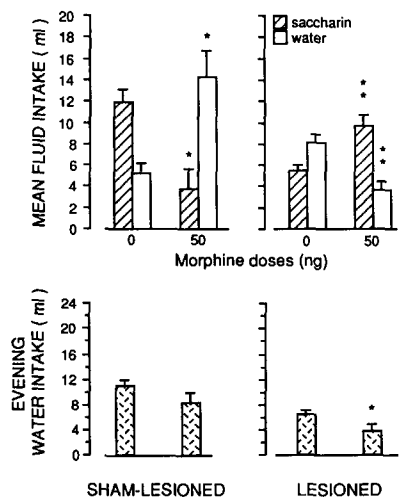

FIG. 2. Top: effects of a 50 ng dose of morphine injected into each PBA of sham-lesioned (left) and lesioned rats (right) on the intakes of the 0.3 mM solution of saccharin and water over 2 h. Asterisks show the significance between the consumption of saccharin solution or water after the injection of the opiate and the corresponding intakes without morphine indicated by O on the abscissa. These intakes are the mean values of three choice tests recorded during 3 consecutive days preceding the test with morphine. Bottom: corresponding intakes of water recorded over 1 h, 11 h after the beginning of the choice test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

choice test with the 0.3 mM solution of the sweetener. As already observed (17,43), whereas this solution is significantly preferred to water by the nonlesioned animals, it is neutral for the LH lesioned rats. The preference ratio, i.e., saccharin solution taken/total fluid taken during the 2 h session of sham-lesioned rats, is significantly superior to the 0.5 value (no preference) (0.70 ± 0.06, $t = 3.5, p < 0.05$). The preference ratio of the lesioned rats was not different from the 0.5 value (0.41 ± 0.04, $t = 2.25$, NS). The intra-PBA injection of 50 ng of morphine induced an opposite effect in the gustatory responses of the two groups. A global ANOVA shows a significant multiple interaction (lesion × dose × saccharin), $F(1,11) = 27.28, p < 0.001$. In sham-lesioned rats, the preference for saccharin was converted to an aversive response by significantly decreasing sweetener intake and increasing water consumption (preference ratio 0.22 ± 0.04, $p < 0.05$). In contrast, in lesioned rats, morphine increased saccharin intake and decreased water intake, thus inducing a preference ratio superior to the 0.5 value (0.74 ± 0.05, $t = 4.6, p < 0.01$).

In the two groups of rats the injection of the opiate did not change the total liquid consumption during the choice test (sham-lesioned control: 17.1 ml ± 0.6, after morphine: 18.0 ml ± 1.6, $t = 0.7$, NS; lesioned, control: 13.5 ml ± 0.6, after morphine: 13.4 ml ± 1.3; $t = 0.1$, NS).

The bottom part of Fig. 2 shows, for each group of rats, the corresponding mean water intake over the 1 h evening session recorded 11 h after the intraparabrachial injection of 50 ng of morphine. Despite this long delay, the opiate induced a small decrease in water consumption but this decrease was significant only in the lesioned group.

The top part of Fig. 3 shows the effects of increasing doses of morphine on the preference for the 2.5 mM solution of saccharin of the sham-lesioned (left) and lesioned (right) rats. This solution of the sweetener was highly preferred to water by the sham-lesioned animals (preference ratio: 0.83 ± 0.01, $t = 23.00, p < 0.001$) and was also preferred by the lesioned rats (preference ratio: 0.69 ± 0.02, $t = 8.6, p < 0.001$).

As in the preceding experiment, the global ANOVA shows a significant multiple interaction (lesion × dose × saccharin), $F(3, 33) = 6.88, p = 0.001$. The 50 ng dose of morphine induced opposite effects on the choice test of the two groups of rats, namely in the sham-lesioned rats the opiate decreased saccharin intake and nonsignificantly increased water intake, whereas in lesioned rats, morphine significantly increased saccharin consumption and decreased water intake. In the lesioned rats, total liquid consumption was not modified by the 50 ng dose of the opiate (control: 16.2 ml ± 0.6; after morphine: 16.3 ml ± 0.9, $t = 0.05$, NS), whereas in the sham-lesioned animals a modest but significant decrease of total liquid consumption was produced by the same dose of morphine (control: 22.0 ml ± 1.1, after morphine: 18.7 ± 0.9, $t = 4.6, p < 0.01$). The injection of the two highest doses of morphine, 100 and 500 ng, induced a decrease of saccharin intake but, above all, a simultaneous and marked decrease in water consumption, particularly in the LH lesioned rats. Likewise, the evening water intakes were all significantly decreased by all doses of morphine (bottom part of Fig. 3). However, the saccharin and water intakes recorded the third day after each morphine injection, in lesioned as well as in sham-lesioned rats, were not significantly different from the corresponding intakes measured before the first injection of the opiate [lesioned rats: saccharin, $F(3, 21) = 0.97$, NS; water, $F(3, 21) = 2.4$, NS; evening water, $F(3, 21) = 0.18$, NS; sham-lesioned rats: saccharin, $F(3, 12) = 0.59$, NS; water, $F(3, 12) = 1.22$, NS; evening water, $F(3, 12) = 0.86$, NS].

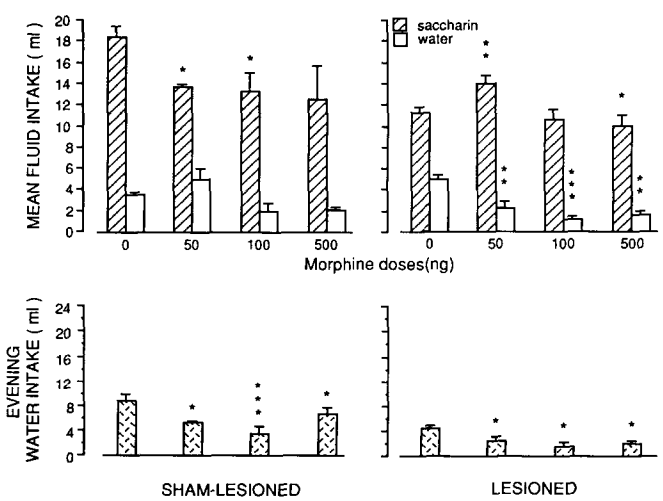

FIG. 3. Top: effects of different doses of morphine (abscissa) injected into each PBA of sham-lesioned (left) and lesioned (right) rats on the intakes of the 2.5 mM solution of saccharin and water during 2 h. Bottom: corresponding intakes of water recorded over 1 h, 11 h after the beginning of the choice test. For other details, see the caption of the Fig. 2.

DISCUSSION

As indicated in the Introduction, the present experiment was an attempt to determine if a part of the opioid-mediated processes involved in the lateral hypothalamic modulation of the palatability level of saccharin solutions are located in the gustatory part of the parabrachial area. The results demonstrate that local infusion of a small dose of morphine (50 ng) into the PBA reproduced some of the unexpected effects previously observed following the subcutaneous injection of the opiate (43,44). In normal rats this dose of opiate suppressed the preference for the 0.3 mM solution of saccharin and attenuated the preference for a 2.5 mM solution of the sweetener, while the same dose of morphine converted the neutral response of the LH-lesioned rats to the 0.3 mM solution to a clear preference and increased the preference to the 2.5 mM solution.

The fact that a small dose of the opiate induced significant modifications of the preference for saccharin in the two groups of rats shows that opioidergic processes localized in the PBA are implicated in the control of gustatory signals. Several opioidergic pathways projecting to the PBA have been identified. Among the various neuropeptides involved in the efferent projections of the nucleus of the solitary tract on the PBA, enkephalin projection cells were the most numerous. A small number of dynorphin projection cells were also observed (27,40). Moreover, neurons immunoreactive to enkephalin were detected in the PBA (23). The neuropeptides implicated in the descending projection from the LH and projecting to the PBA were recently analyzed (31,49) (Touzani et al., in press). The only significant opioidergic projection consists of a cluster of cells immunoreactive to dynorphin, localized in the perifornical area. Finally, a high density of μ receptors and a moderate density of κ receptors were detected in the parabrachial area (28).

Although these different opioidergic pathways and, in par-

ticular, the ascending projections, do not relay exclusively gustatory information, it can be suspected that some of these pathways are implicated both in afferent gustatory transmission and in the descending lateral hypothalamic input. The influences of morphine locally injected into the PBA may generate different interpretations.

Do the present results concord with our working hypothesis according to which the LH input to the gustatory part of the PBA modulates the level of palatability of saccharin solutions? As already observed in our previous experiments (18,43), the ibotenic acid lesion of the LH transformed the preference of the normal rat for the 0.3 mM solution to a neutral response and attenuated the preference for the 2.5 mM solution, suggesting a general decrease of the incentive value of these solutions. Thus, we can suppose that the LH intrinsic cells are effectively implicated in the control of this value. Moreover, the present results demonstrate that some of the LH modulation processes take place within the PBA; of particular significance is the observation that the local injection of morphine into the PBA converted the neutral response of the lesioned rats to a significant preference for the 0.3 mM solution and increased the preference for the 2.5 mM. It is noteworthy that the 50 ng dose of the opiate did not change the total liquid intake of the lesioned rats, either with the 0.3 mM solution (Fig. 2), or with the 2.5 mM solution (Fig. 3) but only modified the respective consumptions of saccharin and water. The best explanation for these complex interactions between the LH lesion and the local infusion of the 50 ng of morphine is to suppose first, that the incentive value of preferred saccharin solutions is, at least in part, controlled by intrinsic cells of the LH, second, that this control takes place within the PBA, and third, that opioid processes located in the PBA are implicated in this control.

However, the results of the nonlesioned rats are difficult to integrate into the preceding explanations, because the same dose of morphine injected in the PBA suppressed the preference for the 0.3 mM solution of saccharin and significantly attenuated the preference for the 2.5 mM solution. A first explanation of this paradoxical response could be to take into account pharmacological data that have demonstrated that the activation level of the opioid systems increases as a function of the concentration of saccharin (26). Thus, with the 0.3 mM solution, which is around the preference-threshold value, it is possible that the activation of the opioid systems was very low and, as discussed previously (43,44), the suppressive effect of morphine, as well as the preference increase induced by a small dose of naloxone, might be due to the preferential stimulation of opioid autoreceptors. This hypothesis was formulated to interpret the analgesic properties of small doses of some opiate antagonists like naloxone (22,46), as well as the fact that these antagonists enhance the release of some opioid peptides (34,46). It is also possible that the suppressive effect of the opiate results from the stimulation of a particular subtype of opioid receptor located in the PBA and involved in suppressive processes. In agreement with this possibility it was shown that the central injection of a selective agonist (20) as well as the injection of selective antagonists of κ receptors (1,8,10,24) decrease intake of saccharin or of other palatable solutions, suggesting a role of these receptors in these suppressive processes. It is not sure, however, that this intake inhibition mediated by the κ receptors results from the modulation of palatability because it was recently shown that a selective κ antagonist reduces sucrose intake but not saccharin consumption (4). Experiments are currently in progress, in which the effects of intra-PBA injections of specific μ and κ agonists and antagonists on preference for saccharin are analyzed.

It is not sure, however, that the suppression of preference observed in nonlesioned rats results from the stimulation of either opioid autoreceptors or some particular opioid receptor subtype. The present results do not agree with our previous studies using SC injections of the opiate (43,44). With this route of injection, in normal rats, morphine transformed the preference for the 0.3 mM solution to a neutral response, suggesting the disappearance of the preference but not an aversion, while in the present study, the intra-PBA injection of the opiate converted the preference to a significant aversive response. Likewise, whereas the preference for a highly preferred solution of saccharin (2.5 mM) is increased by the SC injection of morphine (7), the intra-PBA injection of the opiate produced a weak but significant decrease in preference. The best way to explain these discrepancies is to suppose that they result from the different routes of opiate administration used. As already indicated, the medial part of the PBA is the main relay station of the afferent gustatory signals, and a significant part of these pathways coming from the nucleus of solitary tract are opioidergic. Consequently, it is likely that the aversive response induced in normal rats by local injection of morphine may result from a direct disturbance of the afferent gustatory signals. The present data do not permit to conclude as to the origin of this aversion, which could be due to an increase by the opiate of the aversive properties of saccharin in such a way that the 0.3 mM and 2.5 mM solutions would be perceived of as bitter.

It is also possible that the opiate injected into the PBA becomes itself aversive. It is known that the peripheral injection of repeated high doses of morphine produces aversive responses and can induce taste aversion (3,9,16,47). Because signals coming from the visceral regions project to the PBA, it is possible that the local injection of morphine, by a direct action on the opioid receptors of these projections, produces some kind of taste aversion by mimicking a peripheral malaise.

Whatever the origin of the aversion, the transformation of the taste qualities of saccharin or centrally induced taste aversion, the magnitude of the aversive response would be dependent on the concentration of the saccharin solution, such that the aversion would be maximum with the 0.3 mM solution but would be highly attenuated with the 2.5 mM solution.

If, as suggested by the above observations, the response of the sham-lesioned rats to morphine is aversive, how might we explain the effect of the LH lesion, namely the transformation of the morphine-induced aversion observed in nonlesioned rats for the 0.3 mM solutions to a significant preference? As indicated in the Introduction, the ibotenic acid lesion of the LH produced a shift toward higher concentrations of gustatory stimuli along the preference-aversion continuum. The lesion decreased not only the preference for the palatable solutions of saccharin but also the aversion to the bitter solutions of the sweetener as well as the aversion to the quinine solutions. Thus, it seems that the LH-PBA input is implicated not only in rewarding mechanisms but also in the aversive mechanisms associated to gustatory stimuli and, therefore, that the LH lesion suppressed the preference for the 0.3 mM solution as well as the aversion induced in the normal rats by the intra-PBA injection of morphine. The present data do not enable conclusions as to whether the opiate-induced increase of preference for the 0.3 and 2.5 mM solutions is a direct consequence of the lesion or results from a different process.

Some pharmacological data show that aversiveness and preference of tastants are differentially affected by drugs such as pimozide (37). Thus, it is possible that despite the fact that LH intrinsic cells control the gustatory signals along a preference-aversion continuum, the LH modulation of rewarding and aversive gustatory stimuli is not symmetrical. At the neural level, this differential influence could possibly be due to some disturbance consecutive to the LH lesion in the organization of the opioid receptor populations located in the PBA. For example, the opioid-induced increase of preference observed in lesioned rats could be due to a significant loss of a subpopulation of opioid receptors involved in the aversive processes while another population of opioid receptors that participate in the enhancement of sweeteness properties could be spared by the lesion.

A second significant result of the present experiment must be briefly discussed. In disagreement with other data showing an increase in preference for very palatable solutions of saccharin following SC or intraventricular injection of opiate agonists [(7,14,20,44, review in (13)], intra-PBA injection of the two highest doses of morphine, 100 and 500 ng, did not enhance the preference for the 2.5 mM solution of saccharin, neither in sham-lesioned, nor in lesioned rats. This lack of increase in preference may be due to different factors.

First, it can be suspected that the repeated injections into the parabrachial area produced a significant brain lesion in the target area, or that the repeated infusion of increasing doses of morphine induced tolerance. Thus, the absence of preference enhancement could be due to a decrease of the response to the opiate, consecutive either to tissue damage or to the development of opiate tolerance.

A second possibility to explain the lack of increased preference by high doses of morphine is to take into account the systematic inhibition of drinking by increasing doses of the opiate. This result confirms the well-known observations that morphine, as well as opioid selective agonists injected subcutaneously (16), intraventricularly (42), and into the hypothalamus (11,12), suppress liquid intake.

Thus, it can be suspected that the increase in preference for palatable solutions frequently observed following SC or intraventricular injection of morphine, is masked by the strong and global inhibition of drinking induced by the intra-parabrachial injection of the opiate.

However, the present results do not allow to choose unequivocally between these different possibilities, and further data are needed to better understand why injection of morphine into the PBA does not induce a preference increase for a palatable solution of saccharin.

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REFERENCES

1. Arjune, D.; Bodnar, R. J. Suppression of nocturnal, palatable and glucoprivic intake in rats by the κ opioid antagonist norbinaltorphimine. Brain Res. 534:313-316; 1990.
2. Atweh, S. F.; Kuhar, M. J. Autoradiographic localization of opiate receptors in rat brain. II. The brainstem. Brain Res. 129: 1-12; 1977.
3. Bechara, A.; Van Der Kooy, D. Opposite motivational effects of endogenous opioids in brain and periphery. Nature 314:533-534; 1985.
4. Beczkowska, I. W.; Koch, J. E.; Bodnar, R. J. Differential involvement of central opioid receptor subtypes in saccharin intake in Rats. Soc. Neurosci. Abstr. 18:1430; 1992.
5. Berk, M. L.; Finkelstein, J. A. Efferent connections of the lateral hypothalamic area of the rat: An autoradiographic investigation. Brain Res. Bull. 8:511-526; 1982.
6. Berridge, K. C.; Grill, H. J. Alternating ingestive and aversive consummatory responses suggest a two-dimensional analysis of palatability in rats. Behav. Neurosci. 97:563-573; 1983.
7. Calcagnetti, D. J.; Reid, L. D. Morphine and acceptability of putative reinforcers. Pharmacol. Biochem. Behav. 18:567-569; 1983.
8. Calcagnetti, D. J.; Calcagnetti, R. L.; Fanselow, M. S. Centrally administered antagonists Nor-Binaltorphimine, 16-Methyl cyprenorphine and MR2266 suppress intake of a sweet solution. Pharmacol. Biochem. Behav. 35:69-73; 1990.
9. Cappell, H.; Le Blanc, A. E.; Endrenyi, L. Aversive conditioning by psychoactive drugs: Effects of morphine, alcohol and chlordiazepoxide. Psychopharmacologia 29:239-246; 1973.
10. Carr, K. D.; Aleman, D. O.; Bak, T. H.; Simon, E. J. Effects of parabrachial opioid antagonism on stimulation-induced feeding. Brain Res. 545:283-286; 1991.
11. Chance, W. T.; Rosecrans, J. A. Inhibition of drinking by intrahypothalamic administration of morphine. Nature 270:167-168; 1977.
12. Cooper, S. J. Evidence for opioid involvement in controls of drinking and water balance. In: Rogers, R. J.; Cooper, S. J., eds. Endorphins, opiates and behavioural processes. Chichester: John Wiley and Sons; 1988:187-216.
13. Cooper, S. J.; Jackson, A.; Kirkham, T. C.; Turkish, S. Endorphins, opiates and food intake. In: Rogers, R. J.; Cooper, S. J., eds. Endorphins, opiates and behavioural processes. Chichester: John Wiley and Sons; 1988:143-186.
14. Czirr, S. A.; Reid, L. D. Demonstrating morphine's potentiating effects on sucrose intake. Brain Res. Bull. 17:639-642; 1986.
15. Fallon, J. H.; Leslie, F. M. Distribution of dynorphin and enkephalin peptides in the rat brain. J. Comp. Neurol. 249:293-336; 1986.
16. Farber, P. D.; Gorman, J. E.; Reid, L. D. Morphine injections in the taste aversion paradigm. Physiol. Psychol. 4:365-368; 1976.
17. Ferssiwi, A.; Cardo, B.; Velley, L. Electrical self-stimulation in the parabrachial area is depressed after ibotenic acid lesion of the lateral hypothalamus. Behav. Brain Res. 25:109-116; 1987.
18. Ferssiwi, A.; Cardo, B.; Velley, L. Gustatory preference-aversion thresholds are increased by ibotenic acid lesion of the lateral hypothalamus in the rat. Brain Res. 437:142-150; 1987.
19. Fulwiler, C. E.; Saper, C. B. Subnuclear organization of the efferent connections of the parabrachial nucleus in the rat. Brain Res. Rev. 7:229-259; 1984.
20. Gosnell, B. A.; Majchrzak, M. J. Centrally administered opioid peptides stimulate saccharin intake in non deprived rats. Pharmacol. Biochem. Behav. 33:805-810; 1989.
21. Jalowiec, J. E.; Panksepp, J.; Zolowick, A. J.; Najam, H.; Herman, B. H. Opioid modulation of ingestive behavior. Pharmacol. Biochem. Behav. 1:477-484; 1981.
22. Kayser, V.; Besson, J. M.; Guilbaud, G. Paradoxical effects of low doses of naloxone in experimental model of inflammatory pain. Prog. Brain Res. 77:301-312; 1988.
23. Khachaturian, H.; Lewis, M. E.; Watson, S. J. Enkephalin systems in diencephalon and brainstem of the rat. J. Comp. Neurol. 220:310-320; 1983.
24. Levine, A. S.; Grace, M.; Billington, C. J.; Portoghese, P. S. Norbinaltorphimine decreases deprivation and opioid-induced feeding. Brain Res. 534:60-64; 1990.
25. Luiten, P. G. M.; Ter Horst, G. J.; Steffens, A. B. The hypothalamus, intrinsic connections and outflow pathways to the endo-

crine system in the control of feeding and metabolism. Prog. Neurobiol. 28:1–54; 1987.
26. Lynch, W. C. Opiate blockade inhibits saccharin intake and blocks normal preference acquisition. Pharmacol. Biochem. Behav. 24:833–836; 1986.
27. Maley, B. E.; Panneton, W. N. Enkephalin-immunoreactive neurons in the nucleus tractus solitarius project to the parabrachial nucleus of the cat. Brain Res. 442:340–344; 1988.
28. Mansour, A.; Khachaturian, H.; Lewis, M. E.; Akil, H.; Watson, S. J. Anatomy of CNS opioid receptors. Trends Neurosci. 11:308–314; 1988.
29. Mantyh, P. W.; Hunt, S. P. Neuropeptides are present in projection neurones at all levels in visceral and taste pathways: From periphery to sensory cortex. Brain Res. 299:297–311; 1984.
30. Markowska, A.; Bakke, H. K.; Walther, B.; Ursin, H. Comparison of electrolytic and ibotenic acid lesions in the lateral hypothalamus. Brain Res. 328:313–323; 1985.
31. Moga, M. M.; Saper, C. B.; Gray, T. S. Neuropeptide organization of the hypothalamic projection to the parabrachial nucleus in the rat. J. Comp. Neurol. 295:662–682; 1990.
32. Moga, M. M.; Herbert, H.; Hurley, K. M.; Yasui, Y.; Gray, T. S.; Saper, C. B. Organization of cortical, basal forebrain and hypothalamic afferents to the parabrachial nucleus in the rat. J. Comp. Neurol. 295:624–661; 1990.
33. Murakami, S.; Okamura, H.; Yanaihara, C.; Yanaihara, N.; Ibata, Y. Immunocytochemical distribution of met-enkephalin-Arg^6-Gly^7-Leu^8 in the rat lower brainstem. J. Comp. Neurol. 261: 193–208; 1987.
34. Nikolarakis, K. E.; Almeida, O. F. X.; Herz, A. Feedback inhibition of opioid peptide release in the hypothalamus of the rat. Neuroscience 23:143–148; 1987.
35. Norgren, R. Taste pathways to hypothalamus and amygdala. J. Comp. Neurol. 166:17–30; 1976.
36. Norgren, R.; Leonard, C. M. Ascending central gustatory pathways. J. Comp. Neurol. 150:217–238; 1973.
37. Parker, L. A.; Lopez, N., Jr. Pimozide enhances the aversiveness of quinine solution. Pharmacol. Biochem. Behav. 36:653–659; 1990.
38. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. San Diego: Academic Press; 1986.
39. Phillips, A. G. Brain reward circuitry: A case for separate systems. Brain Res. Bull. 12:195–201; 1984.
40. Riche, D.; De Pommery, J.; Menetrey, D. Neuropeptides and catecholamines in efferent projections of the nuclei of the solitary tract in the rat. J. Comp. Neurol. 293:399–424; 1990.
41. Schaefer, G. J. Opiate antagonists and rewarding brain stimulation. Neurosci. Biobehav. Rev. 12:1–17; 1988.
42. Spencer, R. L.; Deupree, D.; Hsiao, S.; Mosberg, H. I.; Hruby, V.; Burks, T. F.; Porreca, F. Centrally administered opioid selective agonists inhibit drinking in the rat. Pharmacol. Biochem. Behav. 25:77–82; 1986.
43. Touzani, K.; Velley, L. Ibotenic acid lesion of the lateral hypothalamus increases preference and aversion thresholds for saccharin and alters the morphine modulation of taste. Pharmacol. Biochem. Behav. 36:585–591; 1990.
44. Touzani, K.; Akarid, K.; Velley, L. Modulation of saccharin preference by morphine and naloxone. Inversion of drug effects as a function of saccharin concentration. Pharmacol. Biochem. Behav. 38:37–41; 1991.
45. Touzani, K.; Ferssiwi, A.; Velley, L. Localization of lateral hypothalamic neurons projecting to the medial part of the parabrachial area of the rat. Neurosci. Lett. 114:17–21; 1990.
46. Ueda, H.; Fukushima, N.; Kitao, T.; Ge, M.; Takagi, H. Low doses of naloxone produce analgesia in the mouse brain by blocking presynaptic autoinhibition of enkephalin release. Neurosci. Lett. 65:247–252; 1986.
47. Van Der Kooy, D.; Phillips, A. G. Temporal analysis of naloxone attenuation of morphine-induced taste aversion. Pharmacol. Biochem. Behav. 6:637–641; 1977.
48. Winn, P.; Tarbuck, A.; Dunnett, S. B. Ibotenic acid lesions of the lateral hypothalamus: Comparison with the electrolytic lesion syndrome. Neuroscience 12:225–240; 1984.
49. Zardetto-Smith, A. M.; Moga, M. M.; Magnuson, D. J.; Gray, T. S. Lateral hypothalamic dynorphinergic efferents to the amygdala and brainstem in the rat. Peptides 9:1121–1127; 1988.