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Endorphinergic Modulation of Neural Reward Systems Indicated by Behavioral Changes[1]

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DUM, J. AND A. HERZ. *Endorphinergic modulation of neural reward systems indicated by behavioral changes.* PHARMACOL BIOCHEM BEHAV 21(2) 259–266, 1984.—Experiments were performed in rats expecting and/or having received rewards to see if naloxone-antagonizable changes occur in behavior. It was found that rats expecting to receive candy display a naloxone-blockable increase in nociceptive thresholds and a naloxone-sensitive increase in rearing. Similarly, water-deprived rats expecting to receive water show a naloxone-blockable increase in rearing, whereas thirsty animals not expecting water show no changes in nociception or activity. Naloxone was also found to reduce the consumption of a highly palatable food and to diminish the performance of rats trained to wait in one place to receive candy. The latter indicates that the naloxone effect upon goal seeking is not dependent upon a decrease in general activity. Experiments in highly morphine-tolerant rats maintained on relatively constant morphine concentrations showed that these rats drink chocolate milk in the same way as placebo-treated animals. The same rats were found to fail to decrease their intake to the same extent as placebo-controls when water is substituted for the first time for chocolate milk given regularly every day. This points to the possibility that a negative action of endorphins, that is a decrease in release, decreasing consumption, upon a reduction in the palatability of an expected food, might be absent in highly tolerant animals. It is suggested that endogenous opiate(s) regulate mood as defined by the level of goal-seeking behavior, sensitivity to noxious stimuli and general activity.

Opioid peptides Reward Behavioral effects

THERE is a growing body of evidence that endogenous opiates (endorphins) influence not only nociception [18,20] and motor activity [22,24], but also appetitive behavior. The administration of the natural ligands of the opiate receptors stimulate food intake [12, 16, 21] and the blockade of opiate receptors with antagonists suppresses it [1, 5, 7, 15, 19, 23, 25]. The endogenous opioids also appear to be rewarding, since some of them and opiate agonists have been shown to be self-administrated [2,27]. There is also an indication that β-endorphin is released by incentive reward in the hypothalamus of rats [10]. Therefore, endogenous opiates probably play a role in neural reward systems. However, the exact nature of this role is still unknown.

This paper investigates the possibility that the endogenous opiate(s) released under rewarding conditions not only regulate consumption, but also trigger such opiate-like effects as analgesia and behavioral arousal. Investigations were primarily conducted under conditions of incentive reward to reduce any possible stress. To test for generality and to differentiate between a possible stimulation of endorphins by deprivation and reward, some experiments were done in thirsty animals which were or were not expecting water. To see if a development of opiate tolerance causes a malfunction of neural-reward systems, the consumption of a highly palatable food in dependent animals was also measured.

METHOD

Animals

In an effort to eliminate extraneous stimuli and stress to the animals (male, Sprague-Dawley rats), they were cared for exclusively by the experimenter in a separate, climatized room (20±1°C), where the experiments were also performed. For 2 weeks prior to the beginning of training, rats were habituated to the room and individually handled twice daily. Rats were kept 6/box (wire mest 30×50×20 cm) to avoid "isolation stress" and on a reversed day-light cycle (8:00–20:00, dim red light; 20:00–8:00, bright white light) so that procedures were done during the normal waking time of the animals. Except for experiments using water deprivation, lab chow and water were always available. Animals weighed 300±20 g at the time of testing.

Preferred Foods

As incentive rewards, highly palatable foods were given to the animals in the form of (1) sweatened chocolate milk,

[1]Part of the data appeared previously in preliminary form (J. Dum and A. Herz, Activation of endorphin(s) by reward. In: *Endogenous and Exogenous Opiate Agonists and Antagonists*, edited by E. L. Way. New York: Pergamon Press, 1980, pp. 431–434).

containing low fat milk with 6% chocolate powder, sugar and emulsifier added (Ostbayerische Milchwerke, AG, Passau, FRG) and (2) chocolate-covered waffled candy containing a minimum of 27% chocolate in the covering (Ronntree Mackintosh GmbH, Hamburg, FRG). Some of the preferred food was given to the rats in their home cages one day before the beginning of experiments, in order to familiarize them with it.

Drugs

Naloxone HCl (Endo Laboratories, Garden City, NY) was dissolved in saline solution (0.9%, 0.5 ml/rat). Tolerance to opiates was induced by the subcutaneous implantation of pellets [3], each containing 75 mg of morphine base (Merck, Darmstadt, FRG). By implanting 1 pellet on day 1, 2 pellets on day 2 and 3 pellets daily on days 3–9, a nearly complete tolerance to morphine was created by day 10 [3]. A rather complete cross-tolerance also existed towards the highly potent opiate agonist, etorphine [4], which is a rather non-selective ligand for different opiate receptor types [28], indicating a rather general insensitivity to opiates.

Measurement of Antinociception

A hot plate fitted with a copper top heated (51°C) by water circulating directly under the surface was used to test paw-lick latencies. Animals were placed with all four paws on the surface of the hot plate, which had a clear Plexiglas cylinder (20 cm in diameter, 30 cm tall) resting on it. The time taken to lick a paw was measured with a stop watch. All rats paw-licked within 13 sec. Rats were tested only once.

Measurement of Drinking

A drinkometer was used to record the time course of drinking. It consisted of a Plexiglas box (30×30 cm base, 40 cm high) with two grey, opaque sides and two clear, colourless sides, open at the top and bottom and resting upon a stainless steel grid. The Plexiglas tip of the drinking bottle, suspended from the outside of one of the opaque sides, could be reached by the rat through a round hole (1 cm diameter) made 8 cm high in the side of the box. The metal grid and the tip of the drinking bottle were connected electrically to a cummulative recorder which registered the licks made on the bottle tip. The number of licks counted, "lick counts," was taken as an estimate of drinking activity. The drinkometer, except for the recorder, was contained in a soundproof, ventilated box (80×50 cm base×60 cm height), equipped with a single 15 W red light. A one way window was set in the door to allow for direct observation.

Measurement of Exploratory Behavior

Two types of exploration were measured. Rearing was measured in a clear Plexiglas cylinder (30 cm diameter×50 cm high) by giving rats a score made up of a point for each rear, for each rear lasting for more than 5 sec and for each 2.5 sec spent continuously standing up beyond 5 sec. Horizontal exploration was measured in a white open field box (100×80 cm base, 30 cm sides), divided into large squares (20×20 cm) by thin, black lines. The number of times a rat moved with all four paws into a new square was counted.

Procedure in Incentive Reward Experiments in Non-Pretreated Rats

In the *first* of these experiments, the time course of chocolate milk drinking was measured. Before experiments began the rats were placed every day in the drinkometer individually for 30 min, until a steady base-line of drinking was established. After this, rats were injected with saline every day for 5 days before placement in the drinkometer, during which time an initial suppression of drinking by the injections disappeared. Then a series of tests were performed in which naloxone injections were given instead of saline to rats once a week, and varied in a counter-balanced way. Saline given 5 days before and 1 day after naloxone was always injected according to the same schedule as naloxone that week.

In the *second* experiment, the nociceptive thresholds of rats expecting to receive candy was measured using the hot plate test. In order to habituate the rats to the testing situation, they were injected with saline (IP) and placed 10 min later, individually, on the disconnected (cold) hot plate once each morning for 14 days for 5 min and returned immediately to their home cages. In order to condition the expectancy of candy, half of the animals were given 3 pieces of candy (0.8 g/piece) to eat while on the hot plate. The other half were given 3 equally large pieces of lab chow on the hot plate and an equal amount of candy in their home cages during the afternoon, as a control for chronic candy intake. After 1 week, all animals consumed their daily ration of candy within 5 min. Control rats did not eat lab chow on the hot plate. On the day following the 14 days of conditioning, the hot plate was heated (51°C) and rats were injected with either naloxone or saline as usual, 10 min before placement on the hot plate. No food was placed on the hot plate on the day of testing.

In the *third* experiment, the amount of rearing of rats expecting to receive pieces of candy was measured in a cylinder. In order to habituate the rats to the testing situation, they were injected with saline (IP) and then placed 10 min later, individually, in the cylinder, every morning for 14 consecutive days for 35 min and returned to their home cages. In order to condition expectancy of candy, one group of rats was given 3 pieces of candy (0.8 g/piece) 10 min after placement in the cylinder and again 20 min later. Control rats were given 3 equally large pieces of lab chow at the same times and an equal amount of candy in their home cages during the afternoon as a control for the chronic intake of candy. Control rats did not eat lab chow in the cylinder. After 1 week, all rats consumed their portion of candy within 5 min. At the end of the 14 day period, the amount of rearing was stabilized. On the following day, half of the rats were given naloxone (1 mg/kg, IP) and on the next day, the other half, instead of the usual saline, and the amount of rearing was estimated.

In the *fourth* experiment, the performance of rats trained to stay 90 sec in a clear shallow Petrie dish (20 cm diameter, 1 cm high) in the middle of an open field (see measurement of exploratory behavior) to receive candy was measured during a 30 min session. Over a period of two weeks, rats were trained daily in 30 min sessions to stay in the Petrie dish using a variable interval 10 sec reward schedule. If candy was removed from the dish to be eaten elsewhere, the rat was returned to its home cage and the session ended. If the rat left the dish before eating, the candy was removed from the dish. The schedule was then gradually stretched to a fixed interval 90 sec, so that, at the end of training, a single piece of candy (0.8 g) was given every 90 sec, including the time spent eating, if the rat did not leave the dish. If the rat left and re-entered the dish, candy was given again after the

rat had spent 90 continuous sec in the dish. The rats were then habituated to injections by being given saline (0.9%) before the beginning of each session (0.5 ml, IP, at 30, 15 and 0 min before placement in the box), which caused a slight suppression of the learned behavior which disappeared within 3 days. Training was then continued until, after 3 weeks, all rats spent at least 50% of the total time, minus the time spent eating, in the Petrie dish. On the following day, half of the rats were given naloxone (3×1 mg/kg, IP) and on the next, the other half, instead of saline.

Procedure in the Experiments With Non-Pretreated, Water-Deprived Rats

In the *first* of these experiments, the paw-lick latencies of water-deprived rats were measured using the hot plate test. The rats were first habituated on 14 consecutive days to the test situation in a non-deprived state by injecting them every morning with saline (IP), 10 min before they were placed individually for 5 min on the disconnected hot plate and then returned immediately to their home cages. The water bottles were removed from the cages of half of the rats after they had had their time on the hot plate on the 14th day. Twenty-four hr later, the hot plate was heated (51°C) and half of the rats were injected with naloxone instead of the usual saline 10 min before placement on the hot plate.

In the *second* of these experiments, the rearing of different groups of water-deprived rats was estimated in a Plexiglas cylinder. Non-deprived rats were habituated, on 14 consecutive days, to injections given every morning with saline (0.9%, IP) at 30, 15 and 0 min, before individual placement in a cylinder for 40 min. During habituation, the amount of rearing reached a steady baseline. The water-bottles were then removed from the cages of one group of rats after they had had their time in the cylinder. Twenty-four hr later, half of this group of rats was injected with naloxone instead of saline. A second group of rats was given the usual saline injections and time in the cylinder after 24 hr water deprivation and half given naloxone instead of saline 24 hr later, i.e., after 48 hr water deprivation. To test for the effects of water-expectancy, a third group of rats was also deprived of water for 24 hr but then given water via a water bottle hung in the cylinder (spout 8 cm high) for 7 min starting 10 min after the usual placement in the cylinder. Controls received water for an equal length of time in their home cages at this time and were otherwise placed in the cylinder for observation as usual. Twenty-four hr later, half of both groups of rats were given naloxone instead of saline before placement in the cylinder, where no water was available.

Procedure in the Experiments With Non-Deprived, Morphine-Pretreated Rats Given Chocolate Milk to Drink

In the *first* of these experiments, the time course of chocolate milk drinking of non-deprived rats was measured during the development of nearly complete tolerance to morphine. Rats were first familiarized with chocolate milk and put into the drinkometer every day until a steady baseline of drinking was established as described for the first experiment under "Procedure in incentive reward experiments in non-pretreated rats." Tolerance was established by the implantation of morphine pellets over 10 days, as described under "Drugs." Chocolate milk drinking was measured every morning and pellets were implanted every evening so that the animals had time to recover from the implantation by the time of testing, 14 hr later. Control animals were implanted in the same way with the same number of placebo pellets, which contained only the carrier substances.

In the *second* of these experiments, one group of the placebo- and of the morphine-pretreated animals (on the 10th day after the start of pretreatment) were given water for the first time instead of chocolate milk for the first 15 min of the session in the drinkometer after which chocolate milk was again given.

Data Analysis

Simple two-tailed *t*-tests were used to compare groups, except for the following. In the third and fourth incentive reward experiments, a matched-pairs analysis, two-tailed *t*-test, was applied in the comparison of naloxone and saline-treatment effects. Injections were done in a counterbalanced way and naloxone was injected only once in these experiments. The correlation between the rate of exploration and passive approach in the fourth incentive reward experiment was calculated with a Pearson product-moment correlation coefficient and significance calculated with a two-tailed *t*-test. In the experiments done on non-deprived morphine-pretreated rats, two-tailed, Wilcoxon rank tests were applied.

RESULTS

Incentive Reward Experiments in Non-Pretreated Rats

The following experiments were performed to investigate the possible behavioral effects of endogenous opiate(s) released in non-deprived rats receiving or expecting to receive a highly palatable food.

In the *first* experiment, the importance of endogenous opiate(s) to the consumption of an incentive food reward was investigated by measuring the effect of an opiate antagonist, naloxone, upon the drinking of chocolate milk in non-deprived rats using a drinkometer. Rats treated with saline drank a total of 18±2 ml of chocolate milk at a decreasing rate during the 30 min period of time in the drinkometer. Preliminary experiments showed naloxone to be most effective when injected at least 30 min before the beginning of drinking. Injections of naloxone (1 mg/kg, IP), given immediately before or 10 min before placement of animals in the drinkometer caused reductions in drinking which only became visible during the second half of a drinking session, when drinking was already lessened, making measurement difficult. Therefore a dose-response curve for naloxone was performed with naloxone injected 30 min before testing, with booster doses of the drug given 15 min and 30 min later to maintain the concentration of the drug, which is known to have rapid kinetics. The results, seen in Fig. 1, showed a dose-dependent decrease in drinking between 3×0.1 mg/kg and 3×1 mg/kg naloxone.

In the *second* of the incentive reward experiments, the nociceptive thresholds of non-deprived rats trained over 2 weeks to expect to receive candy on a cold hot plate was measured using the hot plate test by turning on the hot plate (51°C) on the day following the completion of training (see the Method section). As shown in Fig. 2, rats expecting chocolate candy had an increased paw-lick latency over saline controls. This increase was completely reversed by naloxone (1 mg/kg and 10 mg/kg, IP), given 10 min before the test. Naloxone had no significant effect upon the paw-lick latencies of control rats not expecting to receive candy.

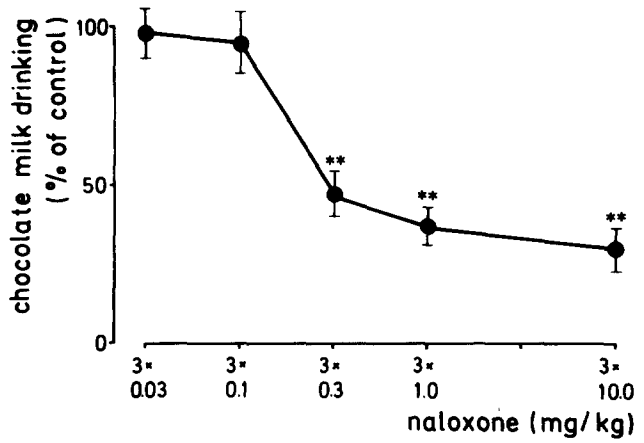

FIG. 1. The effect of different repeated doses of naloxone (IP), administered at 0, 15, 30 min before the start of drinking, upon the amount of chocolate milk drunk in 30 min, in non-deprived rats. Rats injected with saline drank 18±2 ml. Values are means±S.E.M. N=6. **Significantly different from controls ($p<0.01$).

In the *third* of the incentive reward experiments, the amount of rearing of rats expecting to receive pieces of candy was measured. Rats were trained over 2 weeks to expect to receive candy 10 min and 30 min after placement in an observation cylinder (see the Method section). As shown in Fig. 3, no change could be seen in the amount of rearing during the first 5 min among the animals expecting candy, but a large increase could be observed during the next 5 min period, immediately preceding the first candy, and during the 15 min period following the consumption of this candy and reception of the next. No naloxone effect upon the rearing of the control or expectant rats during the first 5 min could be detected, but a large, significant decrease in the amount of rearing of the expectant rats could be seen during the other observation periods. The increased rearing in these animals was reversed by an average of 63%.

In the *fourth* of the incentive reward experiments, the effect of naloxone upon the performance of rats trained to wait inside a shallow Petrie dish in the middle of a large open field to receive pieces of candy was measured. The experiment was designed this way in an effort to differentiate a possible direct effect of naloxone upon activity from one upon motivation to receive candy. Thus, if the primary effect of naloxone were to quiet the animals, then it should improve performance and increase the length of time spent waiting in the dish. If the primary effect of naloxone were to decrease motivation to receive candy, without affecting activity, then it should have the opposite effect. In addition, another measure of activity was gathered by observing the amount of horizontal exploratory behavior outside of the dish, in the open field. As can be seen in Table 1, naloxone caused a significant decline (about 27%) in the length of time spent waiting in the dish, after the time spent eating was subtracted from both groups. This reduction in waiting time is accounted for by tendencies (not statistically significant) of the naloxone-treated rats to both enter the dish less often and to stay in it for shorter timespans. The total amount of horizontal exploring done by the naloxone-treated rats was slightly increased over that of the saline-treated rats (not statistically significant), but, since the naloxone-treated animals spent more time in the open field, the rate of exploration was de-

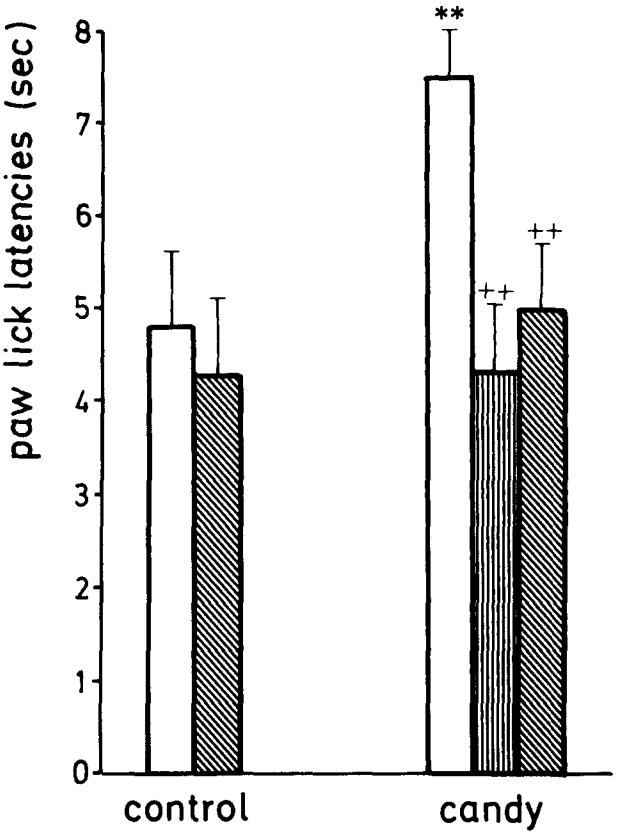

FIG. 2. The antagonism of naloxone of an increase in pawlick latencies, on a hot plate (51°C), of rats expecting to receive a highly palatable food (candy). Open box=saline; diagonal striped box=10 mg/kg naloxone; vertical striped box=1 mg/kg naloxone. Injections were 10 min before test. N=8–16/group. **Significantly different from saline controls ($p<0.01$). ††Significantly different from saline candy ($p<0.01$).

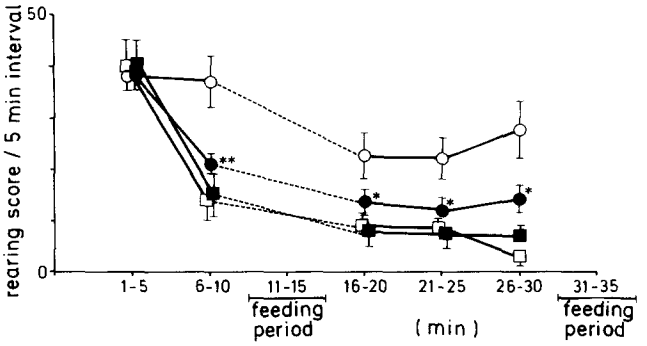

FIG. 3. The reduction by naloxone (1 mg/kg), injected 10 min before the start of observation, of an increase in rearing in non-deprived rats, at various times after placement in a cylinder before and between regularly scheduled feedings of candy. Controls (fed lab chow, which was not eaten): □=saline; ■=naloxone; Candy-fed rats (fed candy, which was eaten): ○=saline; ●=naloxone. Mean±S.E.M. N=12/group. *$p<0.02$; **$p<0.01$ significantly different from saline treated candy-fed rats.

TABLE 1

THE EFFECT OF NALOXONE UPON THE BEHAVIOR OF RATS TRAINED TO WAIT IN A SHALLOW PETRIE DISH IN THE MIDDLE OF AN OPEN FIELD IN ORDER TO RECEIVE PIECES OF CANDY

	Saline	Naloxone (3×1 mg/kg, IP)
Number of candies received	7.4 ±0.8	6.6 ±0.8
Rate of horizontal exploration	0.31 ±0.03	0.23 ±0.02*
Waiting time (sec)	1058 ±44	768 ±89†
Coefficient of correlation of exploration rate and waiting	0.45	0.79†

Rate of horizontal exploration = the number of squares (20×20 cm) entered/min in the open field outside of the dish. Waiting time = the time spent in the dish minus that spent eating candy. Pearson product-moment correlation coefficient. Naloxone injected at 30, 15 and 0 min before the beginning of session. Mean ± S.E.M. N=12/group. *$p<0.05$, †$p<0.01$, two-tailed t-test. Matched pairs analysis for exploration rate and waiting time.

creased by about 25%. In order to test the possibility that animals spending more time in the open field, and therefore less in the dish, have a lower rate of exploration, the correlation between the time in the open field and the rate of exploration was calculated. It was found to be rather low for the saline group (−0.45, not statistically significant), but substantial for the naloxone group (−0.79, $p<0.01$).

Experiments With Water-Deprived, Non-Pretreated Rats

The following experiments were performed to determine if water-deprivation could produce some of the same naloxone-sensitive changes caused by incentive reward, since water deprivation, like incentive reward, also motivates a form of consumption.

In the *first* of these experiments, the paw-lick latencies of water-deprived rats were measured using the hot plate test (51°C). Rats which had been deprived of water for 24 hr had an average paw-lick latency of 4.4±1 sec (N=8), which was not significantly different from that of control rats, 4.8±0.8 sec (N=8). Naloxone, at a dose of 10 mg/kg (IP), injected 10 min before the test as in the incentive reward experiments, did not significantly alter the paw-lick latencies of either the deprived or non-deprived rats, whose thresholds were then 4.1±0.5 sec (N=8) and 4.3±0.8 sec (N=8), respectively.

In the *second* of the water-deprivation experiments, the amount of rearing in a cylinder of water-deprived rats was compared to that of normal rats and water-deprived rats which had previously received water to drink in the cylinder. As seen in Fig. 4, naloxone had no significant effect upon the rearing of the deprived rats not expecting water. Rats deprived of water for 48 hr (not shown) displayed less rearing (significant $p<0.02$, rearing score 35±3/30 min, N=8), which was also not significantly affected by the same treatment with naloxone (rearing score 42±9/30 min, N=8). The rats which had been deprived of water for 24 hr, been given water to drink during observation in the cylinder, then deprived

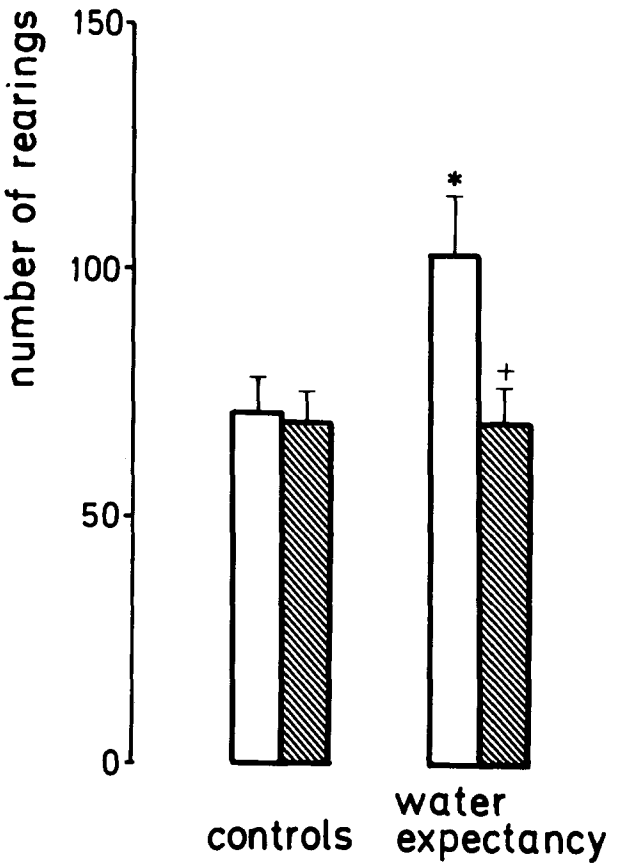

FIG. 4. The effect of naloxone (3×1.0 mg/kg, IP), injected at 30, 15 and 0 min before the beginning of observation, on the rearing of water-deprived rats (24 hr deprivation) expecting to receive water as compared to rats deprived to the same length of time, but not expecting water. Open box=saline-injected, diagonal striped box=naloxone-injected. Mean±S.E.M. N=12/group for controls, N=9/group for water-expectancy. *$p<0.05$ as compared to saline-injected controls, †$p<0.05$ as compared to saline-injected water-expectancy, two tailed t-test.

another 24 hr and observed again in the cylinder showed an increased amount of rearing which was completely reversed by naloxone.

Experiments With Non-Deprived, Morphine-Pretreated Rats Given Chocolate Milk to Drink

The following experiments were performed in highly morphine-tolerant rats made tolerant by the subcutaneous implantation of slow-releasing depot pellets containing morphine. The constant occupation of opiate receptors by exogenous opiate in these animals most likely prevents changes in the level of stimulation to opiate receptors which would normally be caused by changes in the release of endorphins.

In the *first* experiment, the consumption of chocolate milk was measured over a 10 day period in which rats were gradually made nearly completely tolerant to morphine. If the consumption of a preferred food is increased by the release of endorphins, animals would be expected to consume less and less as tolerance develops. As seen in Table 2, no consistent or statistically significant changes could be seen in

TABLE 2

A COMPARISON OF THE TOTAL CHOCOLATE MILK DRINKING (TOTAL LICK COUNTS) OF RATS AT VARIOUS STAGES OF THE DEVELOPMENT OF MORPHINE TOLERANCE AND PLACEBO TREATMENT DURING DAILY 30 MIN SESSIONS IN A DRINKOMETER

Treatment day	Day before start	Day 1	Day 3	Day 6	Day 8	Day 10
Morphine treated	1295 ±200	1235 ±200	1295 ±240	1420 ±210	1415 ±190	1335 ±200
Placebo treated	1350 ±170	1295 ±150	1170 ±210	1125 ±240	1135 ±190	1160 ±210

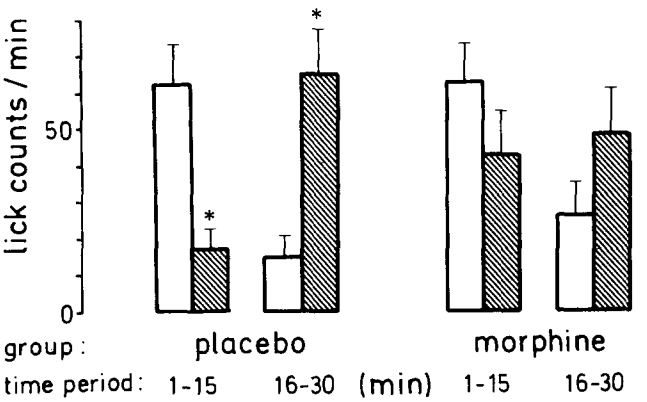

FIG. 5. A comparison of the drinking of non-deprived morphine-tolerant and placebo rats when water is substituted for the usual chocolate milk during the first 15 min in a drinkometer. Open box=chocolate milk given during both periods. Diagonal striped box=water given during the first 15 min and chocolate milk during the second 15 min. Mean±S.E.M. N=8/group. *$p<0.05$ significantly different from controls.

chocolate milk drinking over the time of treatment, as compared to placebo. The time course of drinking for rats treated with morphine and treated with placebo were also similar during and after 10 days of treatment.

In the *second* of these experiments, water was substituted for chocolate milk during the first 15 min of the usual 30 min session in the drinkometer on day 10 of morphine treatment, when tolerance was fully developed. This is equivalent to reducing the amount of expected reward given to the animal, which is adversive. If the reduction in consumption seen in normal animals under such conditions is related to a decrease in the release of endorphins, the reduction of consumption might be lessened in tolerant animals. The results are seen in Fig. 5. As expected, placebo-treated rats drank very little of the water presented during the first 15 min of the session. In contrast, the morphine-tolerant rats continued to drink a considerable, though somewhat reduced (not statistically significant), quantity. When chocolate milk was again given to the animals, during the second half of the session, the placebo rats showed a large increase in drinking (significant $p<0.05$), whereas the morphine-pretreated rats showed only a tendency (not significant) to drink more. However, since the morphinized group had already taken in more water in the first half, it is difficult to interpret the meaning of this last finding.

DISCUSSION

The administration of naloxone to non-deprived rats receiving, or expecting to receive, a highly palatable food was found to produce a variety of behavioral changes.

The opiate antagonist caused a large, dose-dependent reduction in the consumption of chocolate milk in non-deprived rats. This is in accordance with previous studies [1, 5, 7, 15, 19, 23, 25] which show that opiate antagonists are able to inhibit appetitive behavior in both deprived and non-deprived animals. In this case, the inhibition of the consumption of an incentive reward under relatively stress-free conditions emphasizes the importance of endogenous opiate(s) to behaviors motivated primarily by pleasure. The effect of naloxone in these experiments cannot easily be explained by non-opiate actions of the drug since relatively low, non-discriminable [14,26] doses were effective and higher doses did not increase this effectiveness greatly. Surprisingly, naloxone was found to be much more effective in inhibiting consumption when administration of the drug was started 30 min before the beginning of testing than when injected once either immediately before or 10 min before. A more rapid onset of action would be expected in view of the well-known rapid pharmokinetics of naloxone.

In the second experiment, the nociceptive thresholds of non-deprived rats expecting pieces of candy were found to be raised as judged from paw-lick latencies on a hot plate. This increase was completely blocked by a low dose of naloxone which had no visible effect upon the paw-lick latencies of control rats. This fits with the suggestion of Le Magnen *et al.* [17] that endorphin regulation of reward systems and nociception may be closely related. Since no candy was actually eaten on the day of testing, it appears that, in this case, the conditioned stimuli, not the candy itself, or the consumption of it, triggered the activation of endogenous opiates.

In the third exerpiment, a similar approach found a naloxone-sensitive increase in rearing in non-deprived rats expecting and/or having received candy. In agreement with previous reports [22,24], naloxone had no measurable effect upon the rearing of control rats. It is difficult to judge from this experiment if the increase in rearing reflects an increase in general arousal or more directly goal-directed activity, i.e., looking or sniffing for candy. It is tempting to suggest that it is at least partially due to a generally increased arousal, since a similar increase is seen in rats after low doses of opiate agonist and endogenous opiates [6,9], where no single goal object is immediately obvious.

In order to help clarify this question a fourth experiment was done. It was found that naloxone worsened the performance of rats trained to wait passively in a shallow dish in the middle of an open field to receive candy. This suggests that the antagonist effect upon behavior directed towards food is more closely related to a specific decline in interest in food rather than to a general reduction in arousal, since an independent decrease in arousal would be expected to improve the ability of the rats to wait. However, as might be expected from the third experiment, the rate of horizontal exploration of the naloxone-treated rats, when they were in the open field outside of the dish, was also decreased. This type of activity is probably not related to the seeking of candy since no candy was ever received in the open field. Since a signifi-

cant linear correlation could be found between the rate of exploration and time spent in the open field, i.e., not waiting in the dish, among the naloxone-treated rats, the drug is probably affecting both food-motivation and general activity concomitantly, causing the greatest changes in exploration and goal-seeking in the same rats. This may result from a generality of a naloxone effect upon reward mechanisms, since exploration, which occurs spontaneously, appears to be itself rewarding.

Similar to the incentive reward experiments, a naloxone-blockable increase in rearing could be observed in thirsty rats in a cylinder where they had previously been given water to drink. In contrast, water-deprived rats which were not expecting water showed no detectable changes in rearing (naloxone-antagonizable or not) in a cylinder or in paw-lick latencies on the hot plate. Thus, it appears as if endogenous opiate(s) are only activated in thirsty rats when they are expecting water, as in non-deprived rats when they are expecting an incentive reward.

In the final experiments, non-deprived, highly morphine-tolerant rats, maintained on relatively constant concentrations of morphine by subcutaneous depot pellets, consumed a similar amount of chocolate milk according to a similar time course as placebo rats during a 30 min period. This result is quite different from that found when animals are maintained by single, large, daily doses of morphine, which cause alternate periods of opiate stimulation and withdrawal, resulting in alternate periods of increased and decreased appetitive behavior, respectively [13]. The apparent ability of highly tolerant rats in the present experiment to react normally to highly palatable food is difficult to reconcile with a vital function of endorphins in incentive reward. Possibly other neural systems are able to compensate for the crippling of endorphin systems during chronic morphine treatment under these experimental conditions. Nevertheless, the morphine-treated rats seem to react differently when the palatability of the expected solution is reduced. In contrast to placebo rats, they show only a tendency (not statistically significant) to drink less when water is substituted for 15 min for chocolate milk at the beginning of a drinking session. Considering that the adaptive changes taking place during tolerance development are still not fully understood, it is impossible to know exactly what neural mechanism might be behind this finding. However, one logical explanation is that, normally, the reduction in consumption, which occurs when an expected food is less palatable than anticipated, is partially caused by a sudden decrease in the release of endorphins. Such a decrease would be expected to have no effect in highly tolerant animals because of the high concentration of exogenous opiate in their bodies. Normally, the "disappointment" accompanying the unfulfillment of a positive expectancy might be related to a kind of natural "endorphin withdrawal," which depresses food intake.

The above explanation agrees well with the suggestion, presented earlier [10], that endogenous opiate(s) are modulators of neural reward systems. In view of the findings indicating that the release of β-endorphin is increased in the hypothalamus by incentive reward [10] and that the opiate agonist, morphine, lowers self-stimulation thresholds in the lateral hypothalamus [11], it is possible that an increased release of β-endorphin in this structure normally facilitates the activation of neural reward systems there, whereas a decreased release would do the opposite. Other endogenous opiates may also be involved. The fact that endogenous opiate(s) seem to be activated by stimuli connected with incentive reward and water in thirsty rats, before anything is even consumed, also speaks in favor of a role of endogenous opiate(s) in a facilitation of an activation of reward pathways and apporach behavior, rather than in the satiation and sedation which follows consumption. These experiments cannot exclude the possibility that water-deprivation alone also activates the same endogenous opiate peptide(s). No behavioral evidence for such an activation was found, but if the effects measured result from a modulation of the sensitivity of reward pathways, they might not be seen if the pathways are not stimulated.

It is difficult to say whether or not the naloxone-sensitive increase in nociceptive thresholds seen in a rewarding situation is directly due to endorphin activity or more indirectly through an effect upon reward systems. Electrical stimulation of reward pathways in the hypothalamus is reported to produce antinociception [8], but such stimulation might also release endogenous opiate peptide(s). The fact that the effect found here in a rewarding situation is completely blocked by naloxone speaks in favor of a direct effect. In any case, the finding that three wellknown accompaniments of a positive mood in man, namely increased response to rewarding stimuli, decreased sensitivity to pain and increased motor activity, also appear together in naloxone-sensitive or -blockable forms in rats expecting or receiving an incentive reward, opens up the possibility that endogenous opiate(s) play a basic role in the regulation of mood.

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