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Activation of Hypothalamic β-Endorphin Pools by Reward Induced by Highly Palatable Food

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DUM, J , CH GRAMSCH AND A HERZ *Activation of hypothalamic β-endorphin pools by reward induced by highly palatable food* PHARMACOL BIOCHEM BEHAV 18(3) 443–447, 1983 —Experiments were performed to find biochemical evidence of an activation of endogenous opiate peptides in the brain by incentive reward A method used to estimate specific in vivo opiate binding in rats using the labelled opiate agonist, ^{3}H-etorphine, indicated a considerable reduction in opiate binding exclusively in the hypothalamus of non-deprived animals given a highly palatable food to eat for 20 min Radioimmunoassay of the hypothalamus of rats under similar conditions found a pronounced drop in the concentration of β-endorphin, but not in dynorphin, in the hypothalamus, indicating a release and breakdown of β-endorphin Therefore, the reduction in opiate binding in the hypothalamus may at least be partially explained by an occupation of opiate receptors by β-endorphin, causing a reduced availability of receptors to etorphine A possibel role of hypothalamic β-endorphin in the facilitation of reward pathways in the brain is discussed

β-Endorphin Hypothalamus Reward Opiate binding Palatability

SINCE the discovery of engogenous ligands for opiate receptors, a possible regulatory function of these substances in reward systems of the brain, which also control consumatory behaviors, has been proposed [1] This proposal is supported by the fact that opiate agonists are self-administered and that appetitive behaviors and self-stimulation are increased by opiate agonists and suppressed by opiate antagonists [1,19] These effects appear to be opiate-specific, since they occur at low doses and some have been shown to be stereospecific [15,23] Nevertheless, such pharmacological experiments are not sufficient proof in themselves, since injected substances do not impinge on neural systems in the same way as naturally released transmitters Some experiments have been able to show more physiological links For example, the concentration of β-endorphin in the pituitary and blood of rodents with a hereditary tendency towards obesity and over-eating is increased [17,21] However, evidence indicates that β-endorphin, if causally involved, may be producing its effect in the periphery [17,21] An indication of a central effect was found in fasting rats, which have a reduction of β-endorphin in the hypothalamus [7], but this may be attributed to stress, since stress has been shown to reduce the concentration of β-endorphin in this structure [18]

The following investigations use methods designed to trace the activity of endogenous opiates in animals given a natural reward and kept as free from stress as possible The results indicate that the in vivo binding of a highly labelled opiate tracer to opiate receptors is reduced in the hypothalamus, but not in other brain structures, when non-deprived rats are given a highly palatable food in a familiar environment Under similar conditions, the total concentration of β-endorphin, but not of dynorphin, is reduced in the same structure, suggesting a release and break-down of the peptide Therefore, a good explanation for the reduction in the opiate binding in the hypothalamus of rats in a rewarding situation is that an increased number of opiate receptors are being occupied by β-endorphin To our knowledge, this is the first biochemical evidence for the activation of an endogeous opiate peptide by a pleasurable stimulus

METHOD

Two experiments were performed in non-deprived rats given preferred foods as incentive rewards Experiment 1 was done to measure the in vivo binding of a highly labelled opiate in different brain parts, and Experiment 2 was done to measure the concentration of two endogenous opiates in the hypothalamus Two, comparable, kinds of food were given as incentive rewards In Experiment 1, chocolate milk was made available to rats in a cage for 20 min providing uninterrupted contact with the incentive In Experiment 2, pieces of chocolate covered waffle candy were given randomly to the rats for the same length of time so that exploratory behavior could be observed between eating Previous behavioral ex-

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periments using naloxone [3] suggest that both types of rewarding situations activate endogenous opiate(s) and that an increase in exploratory behavior partially results from such an activation

Animals

In an effort to eliminate extraneous stimuli and stress to the animals in these experiments, the rats (male, Sprague-Dawley) were cared for exclusively by the experimenter in a separate, climatized room (20°±1°C) where the experiments were also conducted Killing, by decapitation, was performed in a curtained off section of the room For 2 wk prior to the beginning of training, rats were habituated to the room and individually handled twice daily Rats were kept four to a box (wire mesh 30×50×20) to avoid "isolation stress" and on an reversed day-light cycle (8 a m –8 p m , dim red light, 8 a m –8 p m , bright white light) so that procedure and experiments were done during the waking cycle of the animals Only two rats were used per box because the remaining rats might be stressed by the removal of rats at the end of the experiment Lab chow and water were always available

Procedure of Experiment 1

In this experiment which measured in vivo opiate binding, animals were first familiarized with the preferred food, chocolate milk (Ostbayerische Milchwerke AG, Passau, F R G , containing low fat milk with 6% chocolate powder, sugar and emulsifier), by giving it to them in their home cages (in four drinking bottles per cage) for 1 hr/day on 2 consecutive days After this, rats were put through a procedure to familiarize them with the experimental situation Every morning for 14 consecutive days, they were alternately placed in a restraining tube for 10 sec, in which IV injections were performed on the day of the experiment, and then immediately thereafter, for 20 min in either of two clear acrylic boxes (20×30×15), containing wood shavings and equipped with wire mesh covers and food through filled with lab chow The boxes sat side by side and the drinking bottles were filled with water or chocolate milk for the control and experimental animals, respectively During the 14 days, the amount of chocolate milk drunk during each session gradually increased and finally stabilized As a control for the chronic intake of chocolate milk, control rats continued to received chocolate milk everyday in their home cages for 20 min, starting at 16 00 Control rats took in the same amount of chocolate milk/rat as the experimental animals On the day of the experiment, following the last day of training, the same procedure was followed, except that IV injections of ^{3}H-etorphine were given in the restraining tube and decapitation was performed following the usual 20 min in the acrylic box Both control and experimental rats weighed 190±10 g at the end of the experiment

Procedure of Experiment 2

In this experiment, which measured the concentration of dynorphin and β-endorphin in the hypothalamus, a similar procedure was followed Pieces of chocolate-covered waffled candy (Kitkat, minimal chocolate content 27%, Rowntree Mackintosh GmbH, Hamburg) were used as a preferred food To familiarize the rats with it, several pieces of the candy were placed in the home cages of the rats for 2 consecutive days Thereafter, the rats were familiarized with the experimental situation every morning for 14 consecutive days by placing them alternately for 20 min in one of two clear acrylic cylinders (20 cm × 60 cm), placed side by side on top of clean, vinyl-coated lab paper Three pieces (4 g/piece) of candy or lab chow were dropped at random into the cylinders of the experimental and control animals, respectively, during the entire period of time the animals were in the cylinders As a control for the chronic intake of candy control rats were given the same amount of candy everyday at 16 00 in their home cages during this time On the day following completion of training, the same procedure was followed, except that the animals were decapitated upon removal from the cylinder The rate of vertical exploratory behavior was estimated on the day of the experiment between eating, while animals were in the cylinder by counting the number of rearings Control and experimental rats weighed 250±20 g at the time of killing

Estimation of In Vivo Opiate Receptor Binding

The estimation was done by measuring the radioactivity in different brain parts 20 min after an IV injection (taking 10 sec) of 100 μl physiological saline containing a trace dosage of ^{3}H-etorphine (2 μCi/rat, 41 Ci/mmole, The Radiochemical Center, Amersham, England) into the rat tail Etorphine was used as an opiate receptor ligand because it is a potent narcotic analgesic with a relatively non-selective action on different types of receptors [24], making the detection of a change in the binding in some population of opiate receptors more likely Since the cerebellum is known to contain almost no opiate receptors, this structure can be used as an internal standard for the amount of radioactivity not specifically bound As shown in previous experiments [12], this method traces opiate receptor binding with characteristics similar to those found using in vitro methods Rats were killed by decapitation and the brains immediately removed and dissected as described previously [8] Brain parts were weighed and combused in a Packard Tri-Carb sample oxidizer and the radioactivity measured by scintillation counting in a Packard Tri-Carb Scintillation Spectrometer with 40% efficiency

Estimation of Concentration of Dynorphin and β-Endorphin

The concentrations of immunoreactive dynorphin and β-endorphin were measured in the hypothalamus after extraction using a highly sensitive and specific RIA as previously described [9,11] Brain dissection was performed in the same way as for the measurement of in vivo receptor binding

Data Analyses

Statistical differences were calculated using the two-tailed *t*-test

RESULTS

All of the rats given chocolate milk in the acrylic box avidly drank it by the end of two weeks of training Drinking started immediately after placement in the box and continued at a decreasing rate during the rest of the 20 min A total of 15± 2 ml/rat was drunk This corresponds to observations made in previous experiments in which the time course of chocolate milk drinking by rats was measured using a drink-o-meter [3] In contrast, the control rats, which had water in the drinking bottle, drank nothing and spent the majority of the time exploring on the floor of the box Similarly, the rats given pieces of chocolate-covered candy in a cylinder con-

TABLE 1

AVERAGE NUMBER OF REARINGS/MINUTE IN EACH FIVE MINUTE PERIOD DURING TWENTY MINUTES OBSERVATION IN A CYLINDER AND DURING THE TOTAL TWENTY MINUTE PERIOD, EXCEPT FOR TIME SPENT EATING

Time Period (min)	1–5	6–10	11–15	16–20	1–20
Candy	8 1 ± 0 9*	4 1 ± 0 2	3 6 ± 0 2*	3 5 ± 1 2	4 8 ± 0 5†
Rat Chow	5 3 ± 0 7	2 6 ± 0 7	1 8 ± 0 6	1 2 ± 0 7	2 7 ± 0 4

Candy Rats given three pieces of candy at random during the twenty minute period
Rat Chow Rats given three pieces of rat chow at random to eat
Eight rats/group Mean±S E M Difference from rat chow controls statistically significant *($p<0$ 05) †($p<0$ 01)

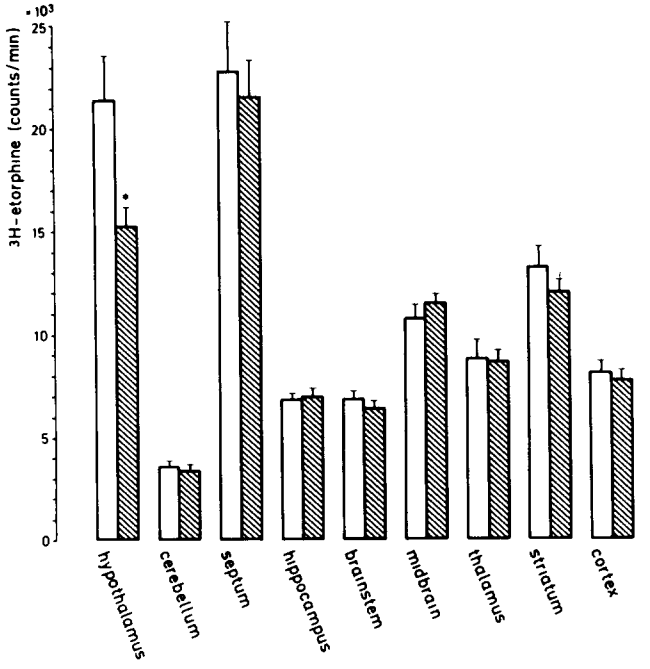

FIG 1 Radioactivity in different brain parts of rats twenty minutes after injection (IV) with ^{3}H-etorphine (2 μCi/rat) All rats were non-deprived and were given either chocolate milk (cross-hatched columns) or water (open columns) to drink freely for twenty minutes before sacrifice (8 rats/group) Mean±S E M *Statistically significant difference from controls ($p<0$ 05)

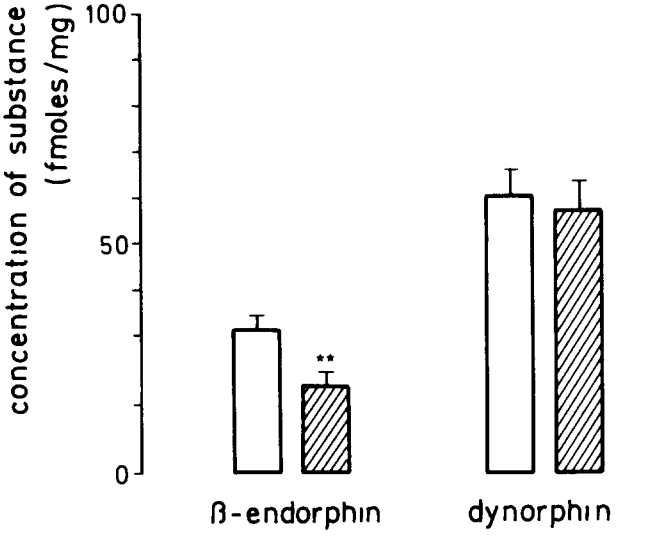

FIG 2 Concentration of dynorphin and β-endorphin in the hypothalamus of non-deprived rats after having received three pieces of either candy (cross-hatched columns) or rat chow (open columns) to eat during a twenty minute period (10–23 rats/group) Mean±S E M **Statistically significant difference from controls ($p<0$ 01)

sumed all three pieces, whereas the control rats ate nothing Except for the periods spent eating, the amount of vertical exploring, i e , rearing, performed by the rats given candy was increased by 77% over that of the controls (Table 1) This increase was also observed among rats before receiving chocolate (not shown) and therefore seemed to be keyed to "expectancy" conditioned to the environment

The concentration of ^{3}H-etorphine, as measured by the level of radioactivity in the various brain parts of the rats is shown in Fig 1 As found in previous investigations [4,12], the amounts of labelled opiate found in the brain parts of the control rats varied with the concentration of opiate receptors For example, the concentrations of opiate in the hypothalamus and septum which high densities of opiate receptors, are about seven times greater than that in the cerebellum which has virtually no such receptors The concentration of opiate in the brains of rats which drank chocolate milk was not significantly different from controls except in the hypothalamus, where a drop of 33% in the specifically bound substance was measured, when the concentration of radioactivity in the cerebellum is taken as a measure of substance not specifically bound

In rats given pieces of candy to eat, the concentration of dynorphin in the hypothalamus was not significantly different from that in controls (Fig 2) The overall average concentration of dynorphin in all groups, 59±4 pmoles/g, is in agreement with that measured in previous experiments in rats at night [22] In contrast to dynorphin, there was a large drop, 38%, in the concentration of β-endorphin ($p<0$ 01) in the same rats in the hypothalamus The concentrations of β-endorphin measured are within close range of that previously found to be in the rat hypothalamus using the same assay [10]

DISCUSSION

Experiment 1 showed a reduction in the concentration of ^{3}H-etorphine in the hypothalamus of rats given chocolate milk to drink. This is probably due to a drop in the binding of the drug to specific opiate receptors in this structure because of an occupation of more of the receptors by endogenous opiate. It is unlikely to be caused by a change in the absorption or elimination of the drug since the change was only observed in this one area of the brain. It is also improbable that the reduction in radioactivity is due to a reduction in the total number of opiate receptors in the hypothalamus, since the time available for such a change, 20 min, is very short. This finding is similar to that of Pert and Bowie [20], who found that the opiate receptor binding was reduced in the brain homogenate of brains taken from acutely stressed rats. They indicated that this reduction was also probably due to an increase occupation by endogenous ligand. It is not possible to conclude from the data presented here which type or types of opiate receptor(s) might be involved since etorphine is a rather non-selective ligand for different opiate receptor types [24]. Experiment 2 found that rats given a comparable preferred food, candy, to eat have a large drop in the concentration of β-endorphin and not of dynorphin in the hypothalamus. This result is unlikely to be due to a reduction in the synthesis of β-endorphin, since such a change would probably only appear after a longer time. Instead, the change can be better explained by an increased release and breakdown of β-endorphin. This would also be consistent with the findings of Experiment 1, since an increased release would be expected to cause an increased occupancy of opiate receptors. Thus, both experiments imply an activation of endogenous opiate. It is not yet known if other opiate peptides besides β-endorphin are involved. As reported earlier [3], the rats waiting to receive candy in these experiments showed a considerable increase in rearing. Since this behavioral change has previously been shown to be partially naloxone sensitive [3], it is probably a further indication of opiate peptide activity. Since the increase in exploration occurred in rats before chocolate was given, it appears to reflect an expectancy of chocolate, which is conditioned to the environment.

It is interesting to speculate about the function of the β-endorphin apparently released in these experiments and the nature of the stimuli causing it. Various reports in the literature point to widely different, natural stimuli seemingly able to activate endogenous opiate(s): food-deprivation, which causes a large drop in hypothalamic β-endorphin [7], leads to naloxone-antagonizable eating and increase in pain threshold [19], aggression in rodents, which is sometimes naloxone-sensitive causes a naloxone-antagonizable increase in nociceptive thresholds [14, 16], and the expectancy of water in water-deprived rats, causes a naloxone-antagonizable increase in exploratory behavior [5]. It is clear that, although some of the conditions eliciting a release of endogenous opiate(s) involve stress, stress is not a necessary condition for the activation of endogenous opiate(s) since β-endorphin was apparently released under the largely stress-free conditions used in this investigation. It is quite possible that different neural pathways containing different endogenous opiates are activated by the various conditions mentioned, although hypothalamic β-endorphin is apparently activated by both starvation and the incentive-reward situation presented here. A major source of commonality among the different conditions seems to be that they are highly motivating. There is evidence that this motivation may be accompanied by a sensitization of reward systems in the brain, since food deprivation [2] has been reported to facilitate brain stimulated reward. Since the opiate agonist, morphine, is reported to lower the threshold of lateral hypothalamic self-stimulation [6], it is suggested that one role of endogenous opiate(s), and particularly of hypothalamic β-endorphin, may be to facilitate the activation of reward pathways by rewarding stimuli. This would also explain why some of the situations reported to activate endogenous opiate(s) are not themselves rewarding, but only potentiating rewarding behavior. Thus, endogenous opiate(s) may be modulators of neural reward centers, but not "reward transmitters" themselves. Opiate agonists may thus be self-administered because they lower the thresholds of reward pathways to such an extent that previously neutral stimuli, or even the normal activity of the neurons involved, may become rewarding.

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