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Morphine-Elicited Feeding: Diurnal Rhythm, Circulating Corticosterone and Macronutrient Selection

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BHAKTHAVATSALAM, P AND S F LEIBOWITZ *Morphine-elicited feeding Diurnal rhythm, circulating corticosterone and macronutrient selection* PHARMACOL BIOCHEM BEHAV 24(4) 911–917, 1986 —The present study examined the feeding response elicited by morphine, injected either intraperitoneally (IP), or into the paraventricular nucleus (PVN), as a function of diurnal cycle and also in adrenalectomized rats with or without peripheral corticosterone replacement In animals maintained on a single diet of chow, milk and sugar, a diurnal rhythm in both the peripheral and central morphine-induced feeding responses was observed, with a stronger eating effect occurring in the early dark hours compared with the responses obtained in the early light period Adrenalectomy significantly reduced the feeding induced by morphine injected IP or into the PVN, and acute corticosterone replacement restored the response Rats maintained on a self-selection feeding paradigm, with carbohydrate, protein and fat simultaneously available, exhibited a significant increase in total caloric intake after morphine injected IP, along with a preferential increase in the consumption of protein and fat Adrenalectomy nearly abolished this stimulatory effect of morphine on total intake and altered the diet preference pattern These findings underscore the importance of corticosterone in the feeding response of morphine injected peripherally or specifically into the PVN The present findings suggest that corticosterone plays an important role in determining the diurnal rhythm of opiate-induced feeding and the function of endogenous opioids in the regulation of energy balance

Paraventricular nucleus Morphine α-Noradrenergic receptors Feeding rhythm Corticosterone

THE brain opiate system is well recognized to play a major role in the physiological regulation of food and water intake, energy balance and body weight [37, 41, 50]. One important anatomical site of this opiate system appears to be the hypothalamus, specifically the paraventricular, ventromedial and perifornical nuclei, where the opiate peptides and receptor systems are dense (see [41,60]). When administered peripherally, or centrally into the medial hypothalamus, morphine strongly stimulates food intake in rats [35, 50, 56, 60]. This feeding response is naloxone reversible [50] and stereospecific [32,57], suggesting that morphine acts directly with the endogenous opiate receptors, apparently of the μ subtype, to elicit this response. With respect to the function of opiates in the physiological control of feeding, most studies agree that they are basically stimulatory in their action and that they may be specifically involved in stress-related feeding [2] in the sensory and nutrient regulation of feeding [14,34] and possibly in the development of obesity [22,46].

It is well established that opiates and monoamines have a variety of modulatory interactions within the central nervous system. Norepinephrine (NE), through the mediation of α2-noradrenergic receptors [11], is recognized to be an important hypothalamic factor in the regulation of short-term [25] and long-term feeding behavior [27,30] Moreover, there is considerable neuroanatomical and biochemical evidence to provide support for an interaction between noradrenergic and opiate systems in the expression of their functions [17, 28, 38, 56]. A possible nature of this interaction in the control of feeding is that the hypothalamic noradrenergic feeding system is a specific mediatory link for the effects of the opiates

Recent reports from this laboratory have shown that the α-noradrenergic feeding response exhibits a diurnal rhythm, which peaks at the onset of the dark cycle when circulating cortocosterone (CORT) also raises [4]. Other studies have demonstrated a close link between CORT, the α-noradrenergic receptor eating response [18], and the α2-receptor binding capacity in the PVN. A variety of studies have shown that adrenal steroid hormones are critical in several pharmacological actions of opiates, such as analgesia [36] and hypothermia [16]. Further, adrenal steroids alter the potency of opiates [1, 7, 16] and affect the uptake of morphine into the CNS cells [15]. Morphine and other opiates are also reported to affect the neuronal activity of hypothalamic centers, e.g , the PVN, which control the secretion of corticotropin releasing hormone [5, 42, 45]

Taken together, these findings underscore the interaction between the adrenal corticoids and brain opiate systems in eliciting their actions. In light of the evidence that the PVN noradrenergic feeding response is CORT dependent and may in some manner be related to the opiate feeding system, it is possible that the opiate feeding response may also be affected by fluctuating levels of corticosteroid hormone In the present study, therefore, we examined this possibility by

testing centrally (PVN) and peripherally injected morphine at different times of the light-dark cycle and by analyzing the effect of adrenalectomy and CORT replacement in different feeding paradigms The findings of this study suggest a possible interaction between the hypothalamic α-adrenergic and opiate feeding systems in relation to adrenal secretion of CORT

METHOD

Animals

The animals were one hundred and forty albino male Sprague-Dawley rats (Charles River Breeding Laboratory) weighing 300 g at the beginning of the experiment They were housed individually, tested in their home cages, and maintained in a 12 12 hr light.dark cycle, with the lights on at 7 00 a m Tests in the dark were carried out under a dim red lighting They had free access to either tap water or 0 9% saline (for adrenalectomized animals) and either a mash diet (Groups 1 and 2) or separate sources of each of the 3 macronutrients, carbohydrate, protein and fat (Group 3)

Surgery

Each rat (Group 1) was stereotaxically implanted, under Nembutal anesthesia (40 mg/kg, IP), with a chronic unilateral guide cannula (stainless steel 23 g tubing with a screw on top) The coordinates, which have consistently been found to aim the cannula tip toward the dorsal aspect of the PVN [25] were 0 2 mm caudal to bregma, 0 3 mm lateral to midline, 8 2 mm vertical to the skull surface, with the incisor bar positioned 3 1 mm above the interaural line The cannula was secured on top of the skull with stainless steel hooks penetrating the bone and fixed with acrylic cement At the end of the tests, approximately one third of the rats were randomly chosen, and their brains were histologically prepared for confirmation of the cannula placement (frozen sections were cut at 50 μm and stained with cresyl violet) Eighty-six percent of these subjects had the cannula tips within a 0 3 mm radius of the PVN

Adrenalectomy (ADX) was performed on the rats under Metofane anesthesia by means of two dorsal incisions caudal to the costal margin. Each adrenal gland was dissected out carefully and removed in its capsule SHAM adrenalectomy involved bilateral dorsal incisions and manipulation of the adrenals Adrenalectomized animals were maintained on a 0 9% saline solution Completeness of ADX was verified at the end of the experiment, 5 weeks post-surgery, by direct assessment of ether stress-induced serum CORT levels The blood samples were obtained in the late afternoon by means of cardiac puncture, and were centrifuged, the serum separated, frozen and later assayed for CORT by radioimmunassay according to the method of Krey *et al* [23] Those animals found to have CORT titres below 0 1 μg% were considered adrenalectomized, as compared with an average of 10 0 μg% of CORT in SHAM animals

Chemicals

Morphine sulphate (Merck and Company) and CORT (Sigma) were used in this study Morphine was dissolved in bacterostatic physiological saline just prior to injection and administered in a volume of 1 0 μl (25 nmoles, for PVN injection) or 0 1–0 2 ml (2 5 mg/kg, for IP injection) Corticosterone was dissolved in propylene glycol and injected subcutaneously in a volume of 0 1–0.3 ml (2 mg/kg)

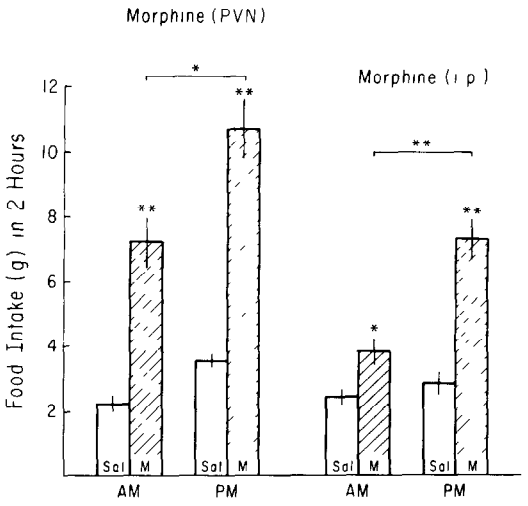

FIG 1 Effect of PVN injection of morphine and IP morphine on the eating behavior exhibited in the tests conducted during early light (a m) and early dark (p m) periods Values are mean±sem $*p>0$ 01, $**p>0$ 001 for comparisons between saline (sal) scores, or between a m and p m drug scores

General Test Procedures

The animals in the central injection study rested for a fortnight following cannulation During this period, they were handled and mock-injected daily in order to adapt them to the test procedure Water and food (37% Purina lab-chow mixed with 30% Carnation evaporated milk and 30% sugar) were available ad lib. The non-cannulated animals in the self-selection feeding paradigm (Group 3) were given at least 2 weeks to adapt to the dietary conditions The food for this group was supplied in 3 separate dishes, containing carbohydrate (37% sucrose, 28% dextrin and 28% corn starch), protein (casein), and fat (lard), each fortified with 4% minerals and 3% vitamins Calculation of caloric density was based on a caloric coefficient of 3 7 kcal/g for carbohydrate and protein and 7 7 kcal/g for fat These diets were provided in glass cups, braced with a metal bar against the side of the cage to minimize spillage On the few occasions where spillage did occur, food lost under the cage was collected and added to the unconsumed total The placement of food cups within the cage was changed daily to prevent position preferences Fresh food was provided daily Body weight and food intake measurements were taken daily in order to determine whether the rats were exhibiting a normal growth pattern and balanced nutrient selection In all but a few cases, the rats were found to gain at least 1 5 g/day and to consume at least 10–15% of their total food intake from each of the seperate diets offered

The experiments were conducted on 3 separate groups one with PVN cannulas and maintained on mash diet (Group 1) and two with no cannulas, maintained either on a mash diet (Group 2) or pure macronutrients (Group 3) At the beginning of every testing was a 1-hr satiation period, during which freshly prepared food was supplied to the animals Following this, the baseline feeding responses to morphine versus saline injections were first obtained prior to ADX or SHAM surgery, and then similar tests were repeated after

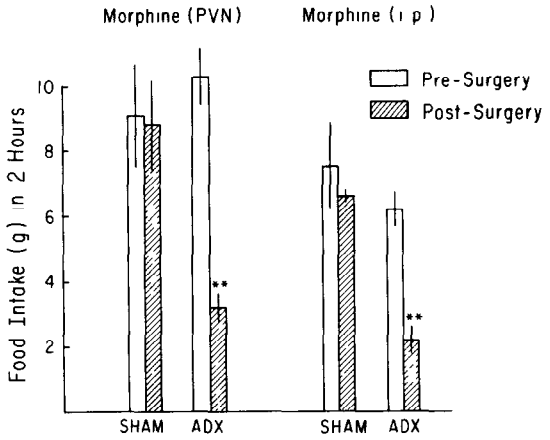

FIG 2 Effect of adrenalectomy (ADX) or sham surgery (SHAM) on the feeding responses to morphine injected into the PVN or IP, in the tests conducted during the early dark period Values are mean±sem **$p>0$ 001 for comparisons between pre- and post-surgery scores

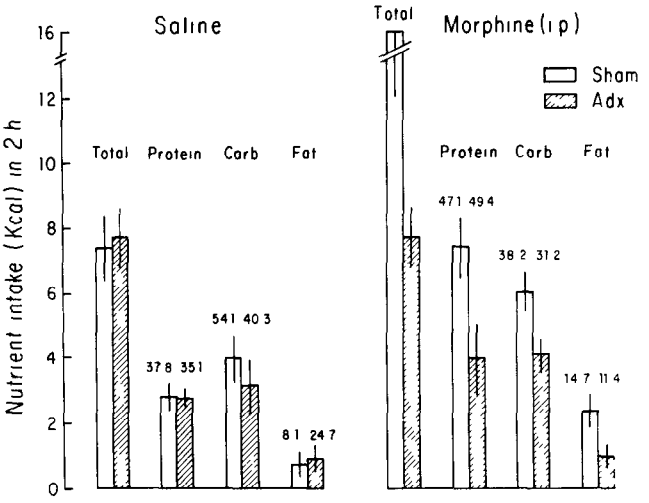

FIG 3 The effect of saline and morphine, injected IP, on the self-selection pattern of the three different macronutrients, protein, carbohydrate (Carb) and fat in Sham and Adx animals The values are kcal±sem The numbers on the top of each bar represent the percent of one diet eaten relative to the total diet eaten for the Sham and Adx groups separately

surgery These tests were conducted in the evening (5 to 9 p.m.), with the exception of the morning tests (8 to 11 p m.) performed for Group 1. During the evening tests, after the satiation period, the drug injections were given at the tail-end of light period just prior to 7.00 p m., when the lights go off Effect of morphine on food intake was studied for 2 hr, from 7.00 to 9 00 p.m

In all the 3 groups of animals, ADX or SHAM surgery was performed after 2 weeks of baseline testing. Only those animals which showed strong feeding responses after the drug treatment (>7.5 g for PVN morphine injection; >2.5 g of mash intake after IP morphine and >7.0 kcal of macronutrient diet intake after IP morphine) were selected for surgery. In order to compare directly the responses of ADX and SHAM animals, both groups were tested simultaneously under precisely the same conditions. Post-surgery testings were started the next day after surgery and conducted for about 4 weeks in the same manner as described for pre-surgery testings. After this, tests were conducted to confirm whether the effect of ADX could be reliably normalized by CORT replacement. The ADX animals were subcutaneously injected with vehicle or corticosterone (2 mg/kg) according to a Latin Square sequence, 30 min prior to morphine This dose of CORT has been found in our earlier studies [48] to be particularly effective in restoring the attenuated responses in ADX animals The SHAM animals were injected with a corresponding volume of vehicle (propylene glycol) prior to drug injections The response to morphine after CORT treatment was compared against the response to vehicle injection of the same animals

Experiment 1 examined the feeding responses to morphine, injected into the PVN (Group 1) or IP (Group 2) as a function of testing time, i.e., in the morning and in the evening. In Experiment 2, the effect of ADX and SHAM surgery on the diurnal feeding responses of morphine injected into the PVN or IP was analyzed (Group 1 and 2). Experiment 3 analyzed the effect of IP injected morphine on the intake patterns of carbohydrate, protein and fat, maintained in a self-selection paradigm, prior to and after ADX or SHAM surgery (Group 3). In Experiment 4, feeding responses to morphine (Group 1 and 2) were tested after CORT replacement, in order to determine the effectiveness of the restoration

Saline or morphine was injected in a Latin Square design, either intraperitoneally (2 5 mg/kg) or directly into the PVN (25 nmoles in 1 μl) In other studies, it has been found that, with daily injections of morphine either into the PVN or IP, the increase in food intake induced by this opiate does not exhibit any tachyphylaxis Therefore, the tests were conducted 3–4 times per week.

The results were statistically evaluated by analysis of variance, with Student's t-test for individual mean comparisons

RESULTS

Feeding Responses to Morphine as a Function of Diurnal Cycle

In intact animals (N=36), injection of morphine, either IP (2 5 mg/kg) or into the PVN (25 nmoles), significantly increased food intake ($p<0$ 001) in both the morning and evening tests. This increase was measured against the low saline baseline scores, of 2.2–3.7 g, observed for these two different test periods (Fig 1). Direct comparisons between the drug scores of light and dark tests showed that, with PVN as well as IP injections, morphine produced a significantly larger feeding response with evening tests than in the morning ($p<0$ 01 for PVN, $p<0.001$ for IP). Whereas the differences in the saline scores for the two test periods was only 1 2 g (PVN) and 0.7 g (IP), with drug injections this difference was increased to 3 7 g and 3.0 g, respectively.

Effect of ADX on the Feeding Responses to Morphine

In the present study, the effects of ADX on the feeding

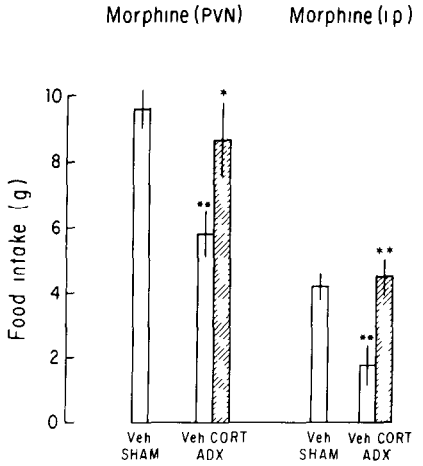

FIG 4 The effect of corticosterone (CORT) or vehicle (Veh) injection on the feeding response elicited by morphine injected into the PVN or IP, in adrenalectomized (ADX) and sham operated (SHAM) animals Values are mean±sem **$p>0$ 001 for comparisons between Veh scores of SHAM and ADX The stars represent the comparisons made between vehicle and SORT scores $p>0$ 05 for PVN, $p>0$ 001 for IP groups

induced by central (PVN) and IP injection of morphine were examined The tests were conducted only in the evening. Results of this study (Fig 2) showed that the morphine-elicited feeding responses in SHAM animals (N=13) remained relatively stable during pre- and post-surgery testings and were significantly greater (+5.7 to 5.8 g) than their respective saline baseline values ($p<0$ 001). This contrasts with the PVN and IP injected ADX groups, which were significantly attenuated ($p<0$ 001) when compared with their respective pre-ADX values or with the values of the SHAM groups

Effect of ADX on Morphine-Induced Increase in Macronutrient Selection

The present experiment analyzed the effect of morphine on the self-selection pattern of the three different macronutrients, in SHAM (N=6) and ADX animals (N=8) The tests were conducted only in the evening. Figure 3 shows, for the saline and morphine tests, the baseline pattern of nutrient selection in kcal, as well as in percent scores, i e , percentage of one diet eaten relative to the total diet eaten After the injection of morphine (2.5 mg/kg, IP), in contrast to saline injections, the total caloric intake was increased more than two fold (from 7.4 to 15.7 kcal, $p<0$ 001) Morphine significantly increased the eating of all the three nutrients ($p<0$ 001), although a preferential increase in consumption of protein diet (+164%, $p<0.001$) and fat (+283%, $p<0$ 001) was seen Morphine had some stimulatory effect on carbohydrate intake (+50%), but this effect was significantly smaller ($p<0.001$) compared to protein and fat intake In terms of the scores for percentage of total diet, morphine increased the proportion of protein from 37 8 to 47 1%, fat from 8 1 to 14.7% and decreased the percentage of carbohydrate from 54 1 to 38 2%

Whereas the saline baseline scores in ADX and SHAM did not differ significantly for any of the diets, ADX caused a profound attenuation of the stimulatory effect of morphine on total diet consumption (from 15 7 to 7 9 kcal, $p<0$ 001) This represents a near total abolition of morphine's action on diet intake, with no reliable increase in the consumption of any of the 3 macronutrient diets relative to the saline baseline scores

Morphine Feeding Response After CORT Replacement in ADX Rats

In the present study, we tested the effect of CORT replacement on the feeding response to morphine, in order to test the effectiveness of restoration Figure 4 shows the results obtained in the ADX (N=18) and SHAM (N=33) animals The pre-surgery scores of both the ADX and SHAM were similar to each other, as well as to the SHAM post-surgery scores, and therefore are not represented in this figure In the post-surgery tests, propylene glycol treatment did not significantly alter the food intake compared to the responses without this vehicle treatment In the ADX animals, morphine-induced feeding, obtained with either IP or PVN drug administration, was significantly reduced (40 to 62%, $p<0$ 001 relative to the pre-surgery or SHAM feeding scores) and was almost completely restored by a single injection of CORT, as opposed to the vehicle injection

DISCUSSION

The main purpose of this study was to analyze the periodicity in the feeding response of rats to centrally and peripherally injected morphine, and secondly, to understand the role of adrenal corticosteroids in modulating the feeding response to morphine When injected intrahypothalamically into the PVN or intraperitoneally, morphine produced a significant feeding response in both the morning and evening tests The magnitude of the feeding responses was significantly greater with the tests in the dark compared to that in the light Morphine-induced feeding, with mash diet as well as with specific macronutrients, was attenuated by ADX and rapidly and reliable restored after a single injection of CORT These findings emphasize the dependency of morphine-elicited feeding on circulating CORT These findings are in line with our earlier reports which demonstrated a similar periodicity to the feeding of α-noradrenergic drugs injected into the PVN or IP, demonstrating the permissive role of CORT in eliciting the α-noradrenergic feeding response [4,48]

It is well known that the normal feeding pattern in rats is circadian in nature, with 60–90% of eating occuring in the dark [3] Several investigators have reported that opiate-induced feeding also exhibits a diurnal rhythm For example, when administered into animals on ad lib feeding schedules, centrally (PVN) and peripherally injected opiates stimulated feeding in the light period [24, 28, 35, 49, 50], but suppressed the feeding in the dark [24, 40, 49] It may be noted that, in dark, the baseline eating remains high, suggesting that the decrease in food intake reported by these studies may be due to this increased feeding baseline However, even in the light period, when morphine was injected into food-deprived rats, a suppression of eating was recorded [24,49] In the present study, when morphine was injected in satiated animals, an increase in eating was observed both in the light and dark periods, with greater responses during dark tests, which finding is in agreement with earlier reports [19,20] The inconsistency in these results is due to the differences in the experi-

mental procedures used, especially with regard to the time of testing, dose of morphine and the baseline control responses

A circadian rhythm in opiate activity has been well recognized. A diurnal rhythm of β-endorphin immunoreactivity in hypothalamus has been reported [39,54], in which higher levels are observed in the dark compared to generally low levels in the light and lowest levels at the onset of light period Opiate-mediated pain sensitivity responses exhibit a similar diurnal variability, namely, stronger in the late afternoon as opposed to early morning [8, 9, 10, 34]. This diurnal fluctuation of pain sensitivity parallels the β-endorphinergic activity in the hypothalamus. The morphine-elicited feeding rhythm as shown in the present study, and the natural feeding rhythm, are closely in phase with each other, in addition to their close correlation with the β-endorphinergic rhythm and the α-noradrenergic rhythm in the hypothalamus [61]. A similar diurnal feeding rhythm, with stronger feeding responses at night compared to day, has also been recently reported with different doses of morphine injected peripherally [19,20] These findings strongly suggest the PVN and IP morphine-induced feeding, as well as that of natural feeding, are interrelated

The functional interaction between opiate-induced responses and adrenal hormones in rats is well known. A large number of studies have suggested that the adrenal steroids are critical in several pharmacological actions of opiates, such as catelepsy [59] analgesia [10, 36, 58] and hypothermia [15]. Adrenal steroids are known to specifically alter the metabolism and consequently change the potency of opiate drugs [1, 7, 16] as well as affect thier uptake into brain cells [7,15] On the other hand, morphine is reported to increase hypothalamic content of CRF [5], the cell bodies of which are particularly dense within the PVN [55]. Furthermore, morphine as well as β-endorphin are reported to stimulate corticosteroid release in a dose-dependent manner [13] It has also been demonstrated that the endogenous opiate activity [21], naloxone reversible pain sensitivity [34], and even the lethal effects of morphine [6] exhibit a diurnal rhythm, which is in phase with the natural corticosterone diurnal rhythm The above evidence strongly supports the proposal that CORT plays an important role in the opiate-related feeding and its diurnal rhythm

There is overwhelming evidence to suggest a relationship of catecholaminergic (CA) and opiate systems at anatomical, biochemical, and behavioral levels. For instance, brain areas that contain CA cell bodies and terminal fields also receive a marked innervation of opiate peptidergic axon terminals, with actual synapsing occurring between them (see [43]). Biochemical studies show that opiate agonists and antagonists can affect the concentration of CA in a specific manner at various parts of the brain ([17, 31, 47] and see [43]) These reports indicate that opiate and noradrenergic feeding responses act via similar mechanisms, probably via the medial hypothalamic sites

For the present study, the PVN was chosen as the site of injection, since this area is a sensitive brain site to both the feeding-stimulatory effects of opiate agonists and the feeding suppressive effects of opiate-antagonists [28, 35, 56, 60]. Further, PVN lesions appear to attenuate the feeding-stimulatory effects of peripherally injected morphine [52]. The findings that the PVN is moderately to densely innervated by various opiate systems [44,53] provide clear anatomical support for the proposed existence of PVN opiate receptor sites involved in energy balance.

The PVN has been shown to be the most sensitive site for the feeding stimulatory effects of NE [25] and the α-noradrenergic agonists [33]. The noradrenergic mechanism in the PVN has also been shown to play a physiological function in the stimulation of feeding [26, 43, 45]. A close link between opiate and α-noradrenergic feeding at this site has been reported by several workers The eating induced by β-endorphin injection into the PVN is positively correlated in magnitude with the NE induced eating response [28], and PVN lesions attenuate the feeding response to peripherally injected morphine [52] Furthermore, the feeding response to PVN injected opiate agonists is antagonized by α-adrenergic receptor antagonists [28] Taken together, these findings strongly support the hypothesis that opiate and noradrenergic systems at the PVN are closely linked with regard to the feeding behavior

Recent investigations, in which ad lib fed animals are permitted to choose their nutrient intake from three separate sources of protein, carbohydrate and fat, have revealed that peripherally injected morphine exhibits a selective increase in protein ingestion, leaving carbohydrate ingestion either unaffected or even suppressed [52] In food-deprived animals, in contrast, morphine preferentially increases fat ingestion, the opposite to that observed with naloxone [38,52] These findings argue for a more specific role of the opiate receptors in feeding control, a role that involves a selective change in appetite for certain nutrients rather than just a change in quantity of food ingested The noradrenergic system of the PVN has also been shown to regulate the specificity in nutrient selection However, in this case, carbohydrate ingestion is selectively stimulated, while fat or protein intake are either unaffected or suppressed [29] These findings may reveal an underlying difference between the opiate and noradrenergic systems. For instance, firstly, the eating stimulatory effects of delta and kappa agonists, in contrast to the μ agonist morphine (as in the present study), and NE [27,28], are unaffected by ADX [35,41] Secondly, electrolytic PVN lesions, which abolish feeding induced by α2-noradrenergic stimulation [20,33], leave partially intact the eating response elicited by peripheral morphine injection [52] Finally α2-noradrenergic and opiate stimulations have differential effects on diet selection [51,52].

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