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Modulatory Role of Oxytocin during Opioidergic Regulation of Food Intake in Rats

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ABSTRACT

Effects of acute and chronic administration of μ- and k-opioid receptor agonists, morphine (MOR) and ketocyclazocine (KCZ), on food intake and their interaction with neuro-hypophysial neuropeptide, oxytocin (OXY) have been investigated in rats. After single administration of MOR (1 μg/rat, icv) food intake was increased during the light phase (0- 6 h) as well as dark phase (6-24 h) in naïve rats. Similarly, single administration of KCZ enhanced the food intake during the light phase but with not much change in food intake during the dark phase. However, after chronic administration the responses were differentially modified, i.e. there was a further enhancement of hyperphagic effect of MOR during light phase (0-6 h), whereas tolerance developed to orexic effect of KCZ. Further, during dark phase, hyperphagic response was observed in response to both MOR and KCZ. During interaction studies with OXY, it was observed that pretreatment with OXY (0.1 μg/rat, icv) attenuated the hyperphagic response to single administration of both MOR and KCZ. OXY, per se, did not significantly affect the food intake response during light or dark phases of the diurnal cycle. However, on chronic treatment OXY (a) blocked the accentuation of hyperphagic response to MOR during both 0-6 h and 6-24 h and (b) blocked the

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hyperphagic response to KCZ during 6-24 h. Results are discussed in the light of complex opioid-oxytocin interaction during food intake in rats.

Keywords: Morphine, oxytocin, ketocyclazocine, food intake.

Introduction

Feeding or ingestive behavior is one of the many natural instincts possessed by both animals and men aimed at maintaining homeostasis. This is influenced by several cognitive and social factors, modulation in which can result in predictable changes in eating, body weight and energy output. Accordingly, an adequate control system for feeding seems mandatory. Both central and peripheral mechanisms have been implicated in food intake regulation (1-4).

Opioid peptides, initially known for their classical role in the regulation of pain sensitivity are now known to have important functions as mediator of various physiological processes involved in maintenance of bodily homeostasis, viz. cardiovascular responses, temperature regulation, endocrine responses, immune function, emotional responses, feeding control, etc. (5, 6). Being one of the important vegetative functions, the role of opioids in the control of ingestive behavior has been investigated (7, 8). For the first time Martin et al (9) reported stimulated feeding in free-fed rats following daily morphine (MOR) injection. Subsequent studies indicated that chronically administered (sc) MOR, heroin, codeine and levorphanol initially depressed feeding for 1 h, then stimulated it for 6 h. Although opioid antagonists have been constantly shown to produce hypophagia, opioid agonists are reported to produce variable effects, e.g. facilitation of food intake by methadone, MOR and pentazocine have been observed by Grandison and Guidotti (10), while Ostrowski et al (11) reported decreased consumption of food intake in food deprived rats in a dose dependent manner by MOR. However, Jackson and Cooper (12) demonstrated that MOR (0.3-10 mg/kg, ip) had no hyperphagic effect. The preferential k-agonist, ketocyclazocine (KCZ) was found to be more potent stimulators of food intake in rats. Morley et al (13) confirmed the orexic responses to highly specific k-agonist, U50-488H

and stereospecific effect of tifluadom. Contrary to this finding Ramarao and Bhargava (14) failed to demonstrate any effect of U50-488H, bremazocine and tifluadom on food consumption in free-fed rats, whereas in food deprived rats inhibition by bremazocine and facilitation by tifluadom was observed. They suggested that the differential responses may be related to either the existence of more than one population of receptors or their differential actions at opioid receptor type. Gulati et al (15, 16) demonstrated receptor-specific regulation of food intake by MOR and KCZ which is governed by diurnal variation and fasting status of rats. Similar modulation in food intake was observed after peripheral, icv or intrahypothalamic administration of MOR and KCZ in naïve and tolerant rats, suggesting central opioidergic regulation of the ingestive behavior with differential involvement of μ- or k-receptors (17-20). Demonstration of several endopioidergic receptors and their interactions with other neuropeptides lend further complexity to the picture as far as regulation of food intake is concerned.

Opioid peptides are known to interact with several neurotransmitters including some neuropeptides during the expression of several centrally mediated behavioral paradigms. A number of studies have suggested complex interactions between opioids and oxytocin (OXY) and arginine-vasopressin (AVP), the two neurohypophyseal peptides which are found in the hypothalamus (21-24). Endogenous opioids, enkephalin and dynorphin have been shown to colocalize with OXY and AVP, respectively in the same neurons in the paraventricular and supraoptic nucleus and regulate each other's release (25). Moreover, OXY is reported to inhibit the development of tolerance to the analgesic effects of MOR, βendorphin, etc and AVP facilitates the rate of this tolerance development (21). However, no reports are available regarding such opioid-OXY interactions during ingestive behavior (21, 26, 27). The present work was thus designed to explore any possible opioid-OXY interactions during food intake in rats. Further since the physiological role of μ- and βreceptors are clearly delineated in other behaviors like antinociception, respiratory depression, cardiovascular control and euphoria, the effects of μ- and βdirected drugs were evaluated in such interactions during food intake.

Materials and methods

Male Wistar rats (200-250g) maintained under standard laboratory conditions of dark and light cycle of 18 h dark and 6 h light was used. Rats were housed individually and randomly allocated to four groups of 7 rats each and were given food ad libitum. For surgery, rats were anesthetized with pentobarbitone sodium (35 mg/kg ip) and secured in stereotaxic apparatus. Twenty three gauge stainless steel guide cannulae were placed into lateral ventricle using following coordinates: 1.5P (to bregma), 2L (to midline) and 4V (to dura), skull horizontal. The cannulae were secured in position and anchored to the skull by steel screws and dental acrylic. Oozing out of cerebrospinal fluid from the outer tip of cannula certified its placement in the lateral ventricle. After one week of postoperative recovery period and stabilization of basal food intake, they were administered icv vehicle, MOR (1µg/rat; Govt. Narcotics Lab, Ghazipur), OXY (0.1µg/rat; Sigma), OXY + MOR in separate groups. After 15 minutes of administration of vehicle or drugs, preweighed food pellets (Hindustan Lever, Bombay) were placed in the cage and quantity of food consumed was measured at 1, 3,6 (0-6 h light phase) and 6-24 h (dark phase) after commencing the experiment. All significant spillage was collected and deducted from the amount consumed. After completion of the acute study, the respective groups of animals were continued for seven days with saline or escalating doses of MOR (5 to 35 mg/kg, ip twice a day with an increment of 5 mg/kg/day). In the OXY interaction studies, the peptide was injected prior to each dose of MOR during the seven days treatment schedule. This was followed by a withdrawal period of 36 h. Food intake in response to test dose of MOR (1µg/rat, icv) was measured in these tolerant rats as was measured for naïve rats after single administration of MOR.

A similar set of experiments was done to study OXY (0.1µg/rat) and KCZ (1µg/rat, icv, Sterling Winthrop, Rensselaer, NY) interactions. For chronic studies, escalating doses of KCZ from 1 to 8 mg/kg, ip twice daily at 0900 and 1500 h were administered. The dose was doubled every third day upto eighth day. Food intake in response to test dose of KCZ (1 µg/rat) was measured on first and eighth day after 36 h of withdrawal period. All drugs were dissolved in saline, except KCZ which was dissolved

in 0.1 N HCl and then diluted with saline.

The data were analyzed by ANOVA followed by Student's paired 't' test to compare the food intake responses after acute and chronic drug administration. Post hoc Tukey's test was applied to compare drugs treated groups. A 'p' value of at least 0.05 was considered as the level of significance in all statistical tests.

Results

Acute treatment with MOR (1μg/rat, icv) resulted in a significant enhancement of cumulative food intake for 6 h ($p < 0.05$) as compared to vehicle treated group, the most remarkable increase being during 0-1 h, i.e. a 98% increase in food intake was observed (Table 1). Overall, for 0-6 h (light phase of the day) there was an increase in food intake by 23% from that observed in vehicle treated group. Prior treatment with naltrexone (5 μg/rat, icv) significantly blocked the hyperphagic response to MOR during all the time intervals (data not shown). Thus, suggesting that the response is specifically mediated through μ-receptors. After chronic administration with escalating doses of MOR, the test dose produced a significant accentuation of hyperphagic response during 0-1 h; the hyperphagic response was increased to 148% vs 98% in naïve rats. Overall for 0-6 h, there was an increase in food intake by 91% in tolerant rats compared to 23% in naïve rats (Table 1). OXY per se reduced the food intake only marginally, i.e. by 11% as compared to saline treated animals during 0-6 h and 6-24 h (Table 1). However, this did not attain level of statistical significance after both acute as well as chronic administration. There was no appreciable difference between reduction in food intake after single or repeated exposure to OXY. Pretreatment with OXY reduced the hyperphagic effect of single injection of MOR by approximately 10% during 0-6 h and 20% during 6-24 h. Chronic treatment with (central) OXY along with peripheral MOR significantly blocked the accentuation of hyperphagic response of 91% ($p < 0.05$) observed in animals treated with MOR alone was reduced to 25% in OXY+MOR group. Similarly, the 6-24 h (dark phase) food intake was also reduced significantly.

Acute administration of KCZ significantly enhanced food intake during all the time intervals, i.e. 0-1, 3-6 and 0-6 h during light phase ($p < 0.05$, Table 2), but it was not increased during dark phase. Pretreatment with βreceptor antagonist,

Table 1: Effects of morphine (MOR, 1 µg/rat, icv) and oxytocin (OXY, 0.1 µg/rat, icv) on food intake in naive and tolerant rats.

Treatment	n	Food intake (g) ± SE									
		0-1 h		1-3 h		3-6 h		0-6 h		6-24 h	
		Naive	Tolerant	Naïve	Tolerant	Naive	Tolerant	Naive	Tolerant	Naive	Tolerant
Vehicle	7	2.04±0.28	2.74±0.21	1.76±0.28	2.36±0.26	2.14±0.29	1.17±0.14	5.94±0.45	6.27±0.27	13.34±0.69	12.71±0.94
MOR	7	4.03±0.77 *	6.80±0.62 *#	2.20±0.12	2.85±0.40	1.42±0.16	2.35±0.20 *	7.30±0.59 *	12.00±0.79 *#	18.50±0.92*	15.65±0.82*
OXY	7	2.92±0.17	2.72±0.26	1.25±0.12 *	1.42±0.18	1.13±0.25	2.37±0.24	5.30±0.27	6.50±0.50	11.80±0.75	13.68±1.04
OXY+ MOR	7	3.28±0.38	4.83±0.37	2.20±0.10	1.63±0.16#	1.27±0.14	1.77±0.55	6.75±0.47	7.82±0.55	14.60±1.13	10.03±0.80

* P < 0.05 compare to respective vehicle treated group; # P < 0.05 compared to respective naïve group

Table 2: Effects of ketocyclazocine (KCZ, 1 µg/rat, icv) and oxytocin (OXY, 0.1 µg/rat, icv) on food intake in naive and tolerant rats.

Treatment	n	Food intake (g) ± SE									
		0-1 h		1-3 h		3-6 h		0-6 h		6-24 h	
		Naive	Tolerant	Naïve	Tolerant	Naive	Tolerant	Naive	Tolerant	Naive	Tolerant
Vehicle	7	2.06±0.29	2.34±0.36	2.50±0.38	2.38±0.33	2.08±0.36	1.84±0.28	6.64±0.92	6.56±0.65	13.00±1.41	14.10±0.78
KCZ	7	3.70±0.30 *	1.95±0.24 #	2.38±0.24	2.41±0.17	3.73±0.40 *	1.49±0.28 #	9.80±0.78 *	5.85±0.37 #	16.30±1.32	19.60±1.02*
OXY	7	1.53±0.42	2.05±0.17	1.88±0.17	2.25±0.16	2.95±0.44	3.30±0.28	5.47±0.36	6.60±0.24	12.28±0.79	14.30±0.88
OXY+ KCZ	7	2.47±0.40	2.15±0.37	2.37±0.26	1.35±0.21	2.92±0.38	2.58±0.28	7.75±0.59	7.08±0.50	15.48±1.06	17.30±1.21

* P < 0.05 compare to respective vehicle treated group; # P < 0.05 compared to respective naïve group

Mr2266 (0.003 μg/rat, icv) significantly blocked the hyperphagic response (0-6 h) to KCZ (data not shown). After chronic administration, unlike MOR, the light phase-hyperphagic response to KCZ was significantly reduced as compared to that of naïve rats, i.e. tolerance developed to this effect ($p < 0.05$, Table 2). However, there was an accentuation in food intake during 6-24 h, i.e. reverse tolerance was observed. Prior administration of OXY reduced the hyperphagic response to KCZ by 41% as compared to KCZ alone group. Chronic treatment with OXY further attenuated the hyperphagic response to KCZ, i.e. facilitated the development of tolerance during the light phase and blocked hyperphagic response during the dark phase (Table 2).

Discussion

The results of the present study showed that icv administration of μ- as well as β receptor agonists, MOR and KCZ, respectively enhanced food intake during light phase (0-6 h). The involvement of specific μ - and β receptors in this hyperphagic effect is evident from blockade of the response by respective antagonists, naltrexone and Mr2266 (data not shown). These findings are in line with those suggesting role of both μ- and β receptors in ingestive behavior (12, 28).

Interestingly, after repeated administration with MOR the test dose of MOR markedly enhanced the hyperphagic response during 0-6 h as compared to that after acute injection. The observation of lack of tolerance to this effect is in contrast to the analgesic response and is similar to that of lowering of self-stimulation-threshold following chronic administration of opiate agonists (29). In fact, Morley et al (12) also reported similar enhancement of food intake following repeated injections of opioids and termed it as 'reverse tolerance'. It may be due to different type/subtypes of opioid receptors and central sites involved in feeding behavior from those involved in analgesia.This is supported by the fact that no correlation was found between antagonists' potency in reducing eating and blocking analgesia (30, 31). Alternatively, it could be due to sensitization of receptors mediating excitatory responses after chronic administration (29).

The present results clearly show that the βagonist, KCZ produces differential effects on food intake after acute and

chronic administration. KCZ lead to increased food intake during 0-6 h with not much change during 6-24 h. After repeated administration of KCZ tolerance developed to the acute hyperphagic effect during the light phase (0-6 h). This could be due to reduced levels of endogenous β-ligand as β-agonists are known to act on autoreceptors and inhibit the release of dynorphin which has definite role in enhancing food intake (32). However, an increase in food intake was observed during 6-24 h as compared to that in naïve rats. The facilitation in the dark phase (6-24 h) food intake after chronic KCZ administration is interesting and some reports have termed such a trend as "reverse tolerance" as has been with MOR in the present and earlier studies (7, 13).

The differential temporal adaptive changes in food intake in response to chronic administration of KCZ and MOR may be due to the diurnal variation in the levels of endogenous μ- and β-directed ligands. Moreover, Bhargava et al (33) also demonstrated an up-regulation of brain and spinal cord β opioid receptors in rats with downregulated μ-receptors following repeated treatment with MOR.

These feeding inhibitory effects of OXY on both the μ- and k-opioid agonists, MOR and KCZ get credence from the results of Olszewski et al (34) who also observed an inhibition of food consumatory behaviour by OXY. Anorexic effect of OXY also get support from studies where OXY-null mice were observed to ingest enhanced amount of sweet solution during both light and dark cycles of the day (35) and clinical findings of hyperphagia and morbid obesity reported in patients of Prader-Willi syndrome who (besides showing mild mental retardation, short stature, abnormal body composition, muscular hypotonia, and distinctive behavioural features) also exhibit low levels of OXY, growth hormone, insulin and insulin-like growth factor alongwith hyperghrelinaemia (36).

Neuropeptides are reported to interact with each other during expression of several centrally mediated behavioral paradigms (21-25, 37). The hypothalamus, which is crucial for physiological regulation of food intake, is rich in both opioidergic and oxytocinergic nerve terminals. Further, colocalization of endogenous opioids and OXY in the same terminal is also

reported, suggesting that the regulation of the release/effect of one by the other is possible (37). Our results show that OXY is effective in attenuating MOR-induced food intake responses during the light and dark phases after both acute and chronic administration. Another notable aspect of our study is the complex interactions of KCZ with OXY during feeding behavior. The (acute) hyperphagic effect of KCZ during the light phase (0-6 h) is markedly attenuated by OXY. In addition this neurohypophyseal peptide prevented the tolerance development to the acute effects of KCZ. Further, both endogenous opioids and neurohypophyseal peptides are known to interact with classical neurotransmitters like NA, DA, 5-HT, etc. (38-42), and the net outcome of such interactions could have contributed to the present results. The probable mechanism of such interaction is hard to define on the basis of the present data. Nevertheless, the concept of such an interaction may be of some physiological significance particularly because both groups of neuropeptides are found in those areas of the CNS, like hypothalamus, amygdale, etc., which are seemingly crucial for feeding behavior.

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