

The Change of Food Intake in Morphine Treated Rat

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몰핀이 흰쥐의 Food Intake 에 미치는 영향

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몰핀의 투여량, 몰핀을 주사하는 시간, 쥐의 나이에 따라서 몰핀이 food intake 에 미치는 효과 유무를 Sprague-Dawley 쥐를 이용해 조사하였다. 또한 ^3H -naloxone 을 이용한 opiate receptor binding assay 에 의해서 정상상태에서 몰핀이나 식염수를 주사한 쥐의 뇌에서 opiate receptor binding 의 변화를 조사하여 아래와 같은 결과를 얻었다.

Food intake 에 미치는 몰핀의 효과는 2시간에 유의차를 나타냈으며 몰핀 투여량이 증가할수록 효과는 감소한 것으로 나타났다. 또 어릴수록 효과가 많이 나타났으며 female 이 male 보다 효과가 컸으나 지속적이지 못했다.

몰핀을 주사하는 시간을 달리하는 실험에서 male 이나 female 모두 10:00 에 주사한 경우에 몰핀 효과가 16:00 에 주사한 경우보다 크게 나타났다. Opiate receptor binding assay 결과, 몰핀을 주사한 쥐에서 ^3H naloxone binding 이 유의있게 감소했으며 오전에 몰핀을 주사한 쥐에서 naloxone binding 이 더 많이 감소한 것으로 나타났다.

이상의 실험에서, 몰핀이 food intake 에 미치는 효과는 몰핀의 opiate receptor binding 을 통해서 작용한 것으로 생각된다.

ABSTRACT

The effect of morphine on food intake on freely fed Sprague-Dawley rats was examined. Opiate receptor binding assay was used to investigate the possibility of the opioid system involved in food

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intake regulation of normal rats.

When rats were treated with 5mg morphine per kg body weight, subcutaneously, the food intake of the rats for the first 2 hours was increased 125% of the control rats. The effect of morphine on food intake of male and female rats were greater when the morphine was injected at 10:00 a.m. than that

in the rats administered the morphine at 4:00 p.m. The morphine effect was not significant in older rats and female was more responsive than male rats. In morphine treated rats, opioid receptor density has exhibited 33% reduction as measured by the ^3H -naloxone binding assay with whole brain homogenate.

These results indicate that the increase of food intake by morphine for 2 hours after the injection may be mediated through the opioid system in rat brain.

INTRODUCTION

The daily or weekly food intake of animals maintained on a diet of homogenous composition under constant ambient temperature is relatively consistent. The existence of regulation of food intake, both short term and long term has been recognized for long time¹⁾. Homeostatic controls, such as glucostatic, lipostatic, thermostatic and gastrointestinal control are the classically discussed area of concern in food intake regulation. The homeostatic hypothesis often suppose the existence of a reference value in the regulated variable compared. Nonhomeostatic theories, such as ecological, psychological and computable theory assert that the primary control of food intake originate in the configuration of the environment and the genetic adaptation. It seems, however, that the simplistic explanations such as dual centers in the brain or primary control by a single organ such as the stomach or liver can not satisfactorily explain the multiplicity of signal and sites involved in the control of food intake.

Since the relatively recent discovery of endogenous opioid peptide with opiate like activity²⁾ in the central nervous system, considerable research has been processed toward identifying physiological roles of the opioid peptide, such as central regulation of respiration³⁾, analgesia⁴⁾⁵⁾, nociception and neuroendocrine regulation and complex mood and behavior⁶⁾⁷⁾⁹⁾, regulation of catecholamine synthesis and gonadotropine regulation¹⁰⁾. Margules¹¹⁾ first

hypothesized that the opioid system may be involved in the regulation of food intake. The evidence supporting this suggestion has come mainly from investigation of the effect of opiate antagonist. Several studies have shown that the opioid antagonist naloxone reduces the food intake of freely fed animals as well as food deprived animals¹²⁾¹⁶⁾. Such suppressing effect of naloxone on food and water intake in rats has been shown to be mediated through effects at opiate receptors¹⁵⁾¹⁷⁾¹⁸⁾. Therefore, the administration of exogenous opiate would thus be expected under certain condition to induce food and water intake. Several studies have shown that morphine can increase food and water intake¹⁹⁾²⁰⁾²¹⁾ and injection of β -endorphine into the intracerebroventricle can induce eating²²⁾. And stabilized enkephaline analogue (Rx 783030) and other agonist ethylketocyclazocin induced food and water intake of freely-fed rats²³⁾.

The present study was carried out to investigate the effect of opioid agonist, morphine, on food intake in freely-fed rats. The change in food intake of these rats when administered with morphine has been further investigated directly with the change in ^3H -naloxone binding sites density of the rat brain.

MATERIALS AND METHODS

Male and female Sprague-Dawley rats weighing 174–320g were used in this study. Animals were housed individually in wire mesh cages and maintained with standard pellet and tap water.

Experiment I: morphine effect on food intake

1) Effect of various levels of morphine on food intake

Male Sprague-Dawley rats were randomly allocated to four experimental groups of 7 rats each.

At the beginning of the experiment, control group was intraperitoneally injected with 0.9% saline solution and the experiment group animals were injected with morphine hydrochloride subcutaneously at one of three dose—3.5, or 10mg/kg body weight.

Carefully preweighed foods were given to rats immediately after injection. The quantities of food consumed were measured for 2, 4, and 6 hours after the start of the experiment which was begun at 10:00 a.m. The spilled foods were also collected from each cage, weighed and taken into account in calculating food intake.

2) Effect of morphine administered at different time of a day on food intake

We wanted to know the effect of morphine on food intake at different physiological state, so we injected morphine at different time (10:00 a.m., 4:00 p.m.). We selected those times for 4:00 p.m. as feeding time and 10:00 a.m. as non-feeding time. Male and female rats were randomly allocated to eight experimental groups of 7 rats each.

Control group was intraperitoneally injected with 0.9% saline solution. Experimental group animals were injected with morphine hydrochloride subcutaneously at dose of 5 mg/kg body weight. The experiments started by injecting the morphine at 10:00 a.m. and 4:00 p.m. The food intake were measured by using the same method as described above.

3) Morphine effect on cumulative food intake of 5 weeks, 10 weeks and 15 months old male and female rats

We wanted to know the morphine effect on food intake depending sex and age. Therefore, we grouped the rats into three, that is pre-puberty, post-puberty and post-menopouse rats. And, male and female rats were randomly allocated to 12 experimental groups of 7 rats each.

The control groups were, intraperitoneally, injected with 0.9% saline solution and the experimental group animals were injected with morphine HCl, subcutaneously, at dose of 5mg/kg B.W. The morphine was administered at 10:00 a.m. and the food intake was measured for 2 hours after injection as described previously.

Experiment II: Morphine effect on opiate receptor binding in vivo

Sixteen animals (4 group) were administered with morphine (5mg/kg B.W.) or 0.9% saline solution at

10:30 a.m. and 4:00 p.m. All subjects were sacrificed by cervical dislocation after an hour from the injection and their brain were rapidly removed²⁴⁾ For whole brain opioid binding assay, the cerebellum which is devoid of receptor activity²⁵⁾ was excised. Each sample was homogenized in 0.95M Tris HCl buffer of pH 7.4 and homogenate was diluted to 110 times of tissue with the cold Tris HCl buffer.

The method of Pert & Snyder²⁶⁾²⁷⁾ was used, for opiate receptor binding assay. [N-allyl-2,3-³H-naloxone (final concentration 5nM) purchased from the Radiochemical Center, Amersham, was used as opiate ligand. Radioactivity was determined by liquid scintillation spectrometry at a counting efficiency of 32%.

Brain protein content was measured in triplicate for each sample by the method of Lowry with bovine serum albumin as standard²⁸⁾.

Means of morphine treated groups were compared with that of the control group using student t-test²⁹⁾.

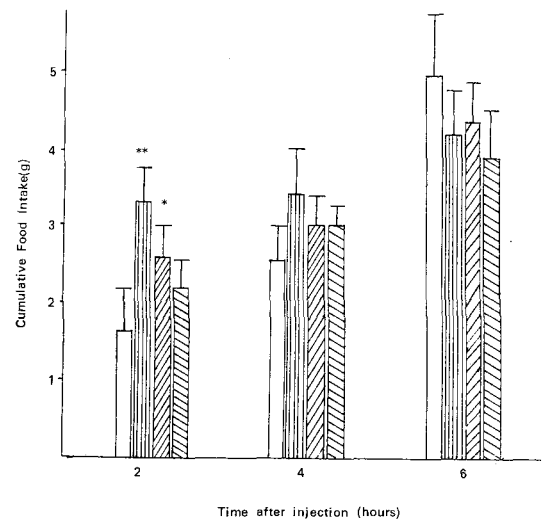


Fig. 1. Effects of morphine on cumulative food intake of freely fed rats during a 6 hours period after injection. (Values present Mean±S.E.M., n=7 for each group, B.W. 220–271g, male rat) Each histobar represents mean cumulative food intake for : 0mg/kg, : 3mg/kg, : 5mg/kg, : 10mg/kg. Vertical lines indicate S.E.M. Asterisks indicate points that are significantly different from control.

* $P < 0.025$, ** $P < 0.005$.

Table 1. The effect of morphine (5mg/kg) administered at different period of a day (10:00 a.m., and 4:00 p.m.) on food intake of male and female rats (n=7 for each group, male B.W.:231–320, female B.W. 174–235g) for 2 hours

Injection time		Food intake(g)			
		Male		Female	
10:00(a.m.)	Control	1.392±0.25	(180%)	Control	2.098±0.49
	Morphine	2.513±0.37 *		Morphine	4.710±0.45 ** (224%)
4:00(p.m.)	Control	4.415±0.69	(75%)	Control	3.866±0.54
	Morphine	3.292±0.38		Morphine	5.284±0.35* (136%)

Values represent mean±S.E.M.

* P < 0.025, ** P < 0.005

(): Percent values in parenthesis represent the ratio of the food intake of morphine administered group to that of control group.

RESULTS AND DISCUSSION

The effect of morphine on cumulative food intake up to 6 hours in the normally fed rats is shown in Fig. 1. The increase in food intake of the morphine treated rats continued for the first 2 hours. There was statistically significant effect of morphine at two lower doses (3mg/kg B.W., 5mg/kg B.W.) on food intake for 2 hours after the administration. The average food intake of the morphine treated rat (3.5 mg/kg) were 3.38 ± 0.57 , 2.64 ± 0.36 (mean±S.E.M.) as compared to 1.639 ± 0.590 of the control group for 2 hours. Morphine at dose 10mg/kg didn't cause a significant increase in food intake. The food intake of morphine treated rat decreased with increase in dose level of morphine. Sanger¹⁹⁾ has shown that the morphine effect on food consumption was significantly greater when measured for two hours after the injection, but no such response was observed when measured for an hour after the morphine administration and the lower dose was significantly more effective than the higher one. By 6 hours after injection, cumulative food intake was substantially below control in animal injected with 3, 5, and 10mg/kg B.W. However, Simantov²⁹⁾ reported that enkephaline and opiate content (morphin) after acute morphine (15mg/kg B.W.) injection reached a maximum within an hour.

Table 1 shows that the morphine effect on food intake of rat when administered at different time of a day. The effect of morphine injected at 10:00 a.

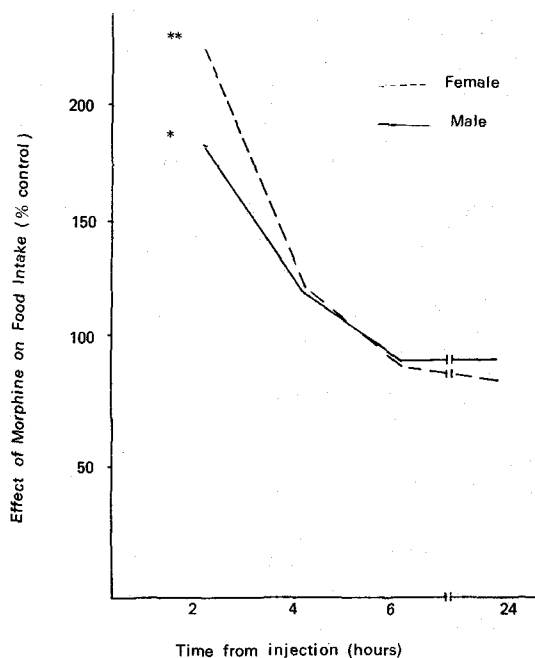


Fig. 2. The effect of morphine (5mg/kg) and food intake in male (B.W.: 231–303) and female (B.W.: 174–210) rats for 24 hours after morphine administration. (Value presents Mean±S.E.M., n=7 for each group) These data are presented as a percent of the control level of food intake in order to facilitate comparisons of morphine effect.

* P < 0.025, ** P < 0.005.

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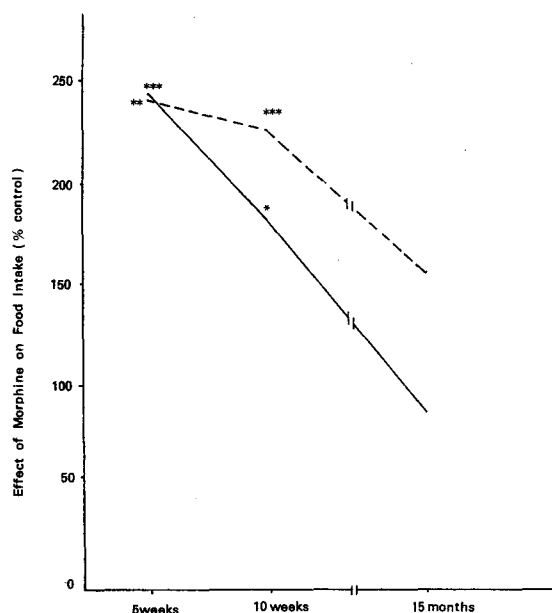


Fig. 3. The effect of morphine (5mg/kg) on food intake (percent of saline baseline) of male and female rats with 5 weeks, 10 weeks, and 15 months old for 2 hours after morphine administration (n=7 per group) male rat, female rat.
* $P < 0.025$, ** $P < 0.01$, *** $P < 0.005$.

m. on food intake in male and female rats was greater than that in the rats received the morphine at 4:00 p.m. It has been reported that opioid activity in human plasma and monkey cerebrospinal fluid (C.S.F.) has a diurnal rhythm. Wesch³¹⁾ showed by radioimmuno assay that met-enkephaline level increased significantly almost twice in the afternoon (15:30). The present study is in good agreement with previous reports³⁰⁾³¹⁾.

Fig. 2 presents the effect of morphine on cumulative food intake in male and female rats for 24 hours. Effect of morphine on food intake in female rat was greater than that of male rat, but such morphine effect in female didn't continue for 24 hours.

The effect of morphine on cumulative food intake up to 2 hours in male and female rat of different ages is shown in Fig. 3. The morphine effect on food intake in both sexes of 5 weeks old was similar, but the morphine effect in female matured rats was more effective than that of male matured rats. Kato³⁰⁾ showed that the effect of opiate might be

Table 2. Opiate receptor radioactivity of male rat brain. It was obtained 1 hour after the injection of morphine (5mg/kg body weight) at 10:30 a.m.

	Body weight (g)	Brain weight (g)	total brain protein (mg)	cpm/mg protein	³ H-naloxone bound (fM/mg protein)
Control group	311.2±5.1	1.423±0.027	78,760±1.048	1177.7±65.8	32.17±1.80
Morphine treated group	308.7±7.6	1.632±0.058	92,139±1.883	794.64±25.60	21.71±0.19

Values represent Mean±S.E.M.

Table 3. Opiate receptor radiobinding activity of male rat brain obtained 1 hour after the administration of morphine (n=4 for each group, triplicate run)

Administered time	treatment	³ H-naloxone bound (fmol/mg protein)
10:30 (a.m.)	Control	32.17±1.89
	Morphine (5mg)	21.71±0.19 ** (67%)
4:00 (p.m.)	Control	30.36±1.58
	Morphine (5mg)	23.44±1.14 * (77%)

* $P < 0.01$, compared with the control group.

** $P < 0.005$, compared with the control group.

Percent values in parenthesis represent the ratio of radiobinding activity of morphine administered group to that of control group.

sex-related as morphine administration decreased the content of cytochrome P-450 and the hydroxylation of testosterone and progesterone in male rats but not in female. Morphine effect on cumulative food intake decreased with age (Fig. 3).

Table 2 shows that body weight, brain weight, total brain protein, cpm/mg protein and opiate receptor radiobinding activity of rat brain which treated with morphine at 10:30 a.m. Table 3 shows opiate receptor radiobinding activity of rat brain which treated with morphine at two different time. This decrease of ^3H -naloxone binding to opiate receptor in morphine treated rat brain may indicate that the exogenous opiate agonist morphine has occupied the endogenous opiate receptor binding sites leaving less binding sites for the ^3H -naloxone. And decrease of ^3H -naloxone binding of rat brain obtained an hour after the administration of morphine at 10:30 a.m. was greater than that at 4:00 p.m. When these data are compared with those of experiment I, it seemed that the effect of morphine on food intake may be mediated by means of effect on opiate receptors.

SUMMARY

The effect of morphine on food intake in freely fed Sprague-Dawley rats was examined. Opiate receptor binding assay was used to investigate the possibility of the opioid system may be involved in food intake regulation of normal rats.

1) The lower dose (3mg, 5mg/kg B.W.) of morphine was more effective than higher one (10mg/kg B.W.).

2) The morphine effect on food intake of female rats was greater than that of the male rats.

3) The effect of morphine on food intake was greater when the morphine was injected at 10:00 a.m. than that in the rats administered at 4:00 p.m.

4) In morphine treated rats, opioid receptor density has exhibited 33% reduction as measured by the ^3H -naloxone binding assay with whole brain homogenate. These results indicate that the increase of food intake by morphine for 2 hours after the

injection may be mediated through the opioid system in rat brain.

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