Adrenal Function Affects Morphine-Induced Feeding during Dark Period, but not during Light Period in Rats

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Abstract—The present study was undertaken to investigate the relationships between morphine-induced feeding and the adrenal functions. Morphine (5 mg/kg) was intraperitoneally administered at 10:45 (light period) or 18:45 (dark period). The orectic effects of morphine during the light period in normal rats were not influenced by adrenalectomy; however, the anorectic effects during the dark period in normal rats were attenuated by both adrenalectomy and adrenodemedullation. Corticosterone (10 mg/kg) itself had no effects on feeding during the light and dark period. Morphine did not alter blood insulin levels during the light period, but markedly decreased it during the dark period independently of feeding. These results show that morphine has two different effects on feeding by administration time, and they suggest that the adrenal affects morphine-induced feeding only during the dark period (hungry state), presumably through insulin release, but not during the light period (satiated state).

Many investigations using opioids agonists and antagonists have shown that opioids play important roles in feeding (1-4). However, it has been rarely studied that opioids such as morphine have different effects on food intake which are related to feeding conditions or administration time (5, 6). We previously reported that in non-fasted rats, morphine increased food intake for 2 hr following the injection during the light period, but decreased it during the dark period; and in fasted rats, morphine decreased food intake regardless of administration time (7). This report suggests that the two different effects of morphine disturb the baseline levels of feeding in naive rats; morphine could stimulate feeding when rats were in a satiated state (in non-fasted rats during the light period), whereas morphine could suppress feeding when rats were in a hungry state (in non-fasted rats during the light period and in fasted rats).

Glucocorticoids have been also shown to have different effects on feeding: low doses increased food intake and body weight (8-10), while high doses decreased food intake and led to weight loss (11, 12). It is well known that blood levels of glucocorticoids indicate a circadian rhythm; in rats, being nocturnal animals, the blood levels are low during the light period and high during the dark period (13). Morphine has been well demonstrated to elevate blood glucocorticoids levels through the stimulation of ACTH release from the pituitary (14, 15). From these reports, one possible explanation for the opposite effects of morphine on feeding can be made, that is, morphine elevates the blood glucocorticoids levels adequately to facilitate feeding during the light period, while morphine elevates glucocorticoids to a very high level, suppressing feeding during the dark period. Therefore, the present study was undertaken to investigate whether the adrenal gland, especially the adrenal cortex, really affects morphine-induced feeding during the light period (satiated state) or during the dark period (hungry state) in rats.
Materials and Methods

Animals and surgery: Male Sprague-Dawley rats (descended from Japan Charles River strain), weighing 260–290 g, were used. Each animal was housed individually in a hanging wire mesh cage (40 x 25 x 20 cm). The animal room was maintained at 22±2°C, with 55±5% relative humidity and on a 12 hr light-dark cycle (lights on 07:00 to 19:00). Powder chow (MF: Oriental Yeast Co., Tokyo) in a glass cylindrical vessel (8 cm diameter x 5 cm height) was used in the food intake study to minimize food spillage. The animals were freely given powder chow at least for 14 days prior to the experiments. Taking weight loss into consideration, the animals weighing about 290 g were used after performing adrenalectomy and adrenomedullation. Adrenalectomy was employed under pentobarbital anesthesia (40 mg/kg, i.p.), and the operated animals were given 0.9% NaCl solution instead of water. In the case of replacing glucocorticoids, corticosterone at a dose of 10 mg/kg in sesame oil was subcutaneously injected at 18:00 once a day after the surgery. Adrenomedullation was performed also under pentobarbital anesthesia, but the operated animals were not given 0.9% NaCl solution. Both operated animals were used about 7 days after the surgery.

Experimental designs: Animals were deprived of food only for 15 min after the drug administration to weigh the food vessels in all experiments. During the light period, saline or morphine (5 mg/kg) was intraperitoneally injected at 10:45. Food vessels were supplied to animals at 11:00 (0 time) and later weighed at 13:00 and 11:00 on the next day. During the dark period, morphine was injected at 18:45, and food vessels were supplied at 19:00 (0 time) when the lights of the animal room were gone off. Food vessels were later weighed at 21:00 and 19:00 on the next day. In both cases, food intake was calculated from the difference in the weight of the food vessel before and after the administration.

Repeated administration was performed by the following procedures: Morphine was injected 10 times at an interval of 3–4 days. Food intake was measured after the 9th injection, and blood was taken by decapitation 1 hr after the 10th injection.

Determination of serum 11-hydroxycorticosteroids (11-OHCS), glucose and insulin: Serum 11-OHCS was fluorometrically measured by the modified method of Mattingly (16). Serum glucose was assayed by an enzymatic method (17) and the data was converted into whole blood levels by using a 48.3% hematocrit value. Serum insulin was immunoenzymatically determined with a commercial kit (INSULOTEC MOCHIDA®, Mochida Pharmaceutical Co., Ltd., Tokyo).

Statistics: The data obtained in this study were statistically analyzed by Student’s t-test or the Cochran-Cox test. They were considered significant when the P value was less than 0.05 as compared with the saline control group. To clarify the interactions between morphine treatment and the operation (adrenalectomy and adrenomedullation), the animals were divided into equal size groups, and a two factor analysis of variance (ANOV) was employed.

Results

Roles of the adrenal in morphine-induced feeding during the light period: Figure 1 shows the effects of morphine on food intake during the light period in normal, adrenalectomized and adrenomedullated rats. In normal rats, morphine significantly increased the 2 hr feeding as compared with that of saline treated animals, indicating very low levels. This increasing effect in normal rats was not altered by any of the surgical treatments. In saline-treated animals, the 24 hr feeding of normal rats was not altered by adrenomedullation, but was significantly decreased by adrenalectomy, regardless of receiving corticosterone. However, corticosterone itself increased the 24 hr-feeding of saline-treated animals in adrenalectomized rats. On the other hand, morphine significantly decreased the 24 hr-feeding in normal rats. This decreasing effect of morphine was abolished by adrenalectomy, but not by adrenomedullation.

Roles of the adrenal in morphine-induced feeding during the dark period: Figure 2 shows
the effects of morphine on food intake during the dark period in normal, adrenalectomized and adrenodemedullated rats. In saline-treated animals, the levels of 2 hr-feeding of normal rats were significantly decreased by all surgical treatments. Injection of morphine to normal rats produced a significant decrease in the 2 hr-feeding. This decreasing effect of morphine in normal rats was attenuated by both adrenalectomy and adrenodemedullation. When these data were analyzed by a two factor ANOVA, there were significant interactions (P<0.05) between morphine treatment and the operation: F(1,36)=10.46 and F(1,36)=5.31 were shown in rats with adrenalectomy and adrenodemedullation, respectively. However, morphine tended to decrease the 2 hr-feeding in adrenalectomized rats receiving corticosterone which itself increased the 2 hr-feeding.

The 24 hr-feeding of normal rats was significantly decreased by morphine. This decreasing effect of morphine was attenuated by all three surgical treatments.

Relationships between morphine-induced feeding and blood 11-OHCS levels in normal rats: Figure 3 shows the changes in food intake and serum 11-OHCS levels by repeated administration during the light period. Morphine increased the 2 hr-feeding after a single administration and tended to potentiate it in proportion to administration frequency. In contrast, morphine significantly elevated serum 11-OHCS levels by single injection; however, morphine had no effects on serum 11-OHCS levels by repeated injection.

Figure 4 shows the results during the dark period. Morphine significantly decreased food

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**Fig. 1.** Roles of the adrenal in morphine-induced feeding during the light period. Morphine was injected at 10:45. Food intake was measured for 2 hr (upper figures) and for 24 hr (lower figures) after 11:00. Open and hatched columns indicate the saline- and morphine-treated group, respectively. Data are shown as means±S.E.M. The number of animals used is represented in parenthesis. Normal: normal rats, ADx: adrenalectomized rats, ADMx: adrenodemedullated rats, S: saline, Mor: morphine (5 mg/kg), Cor: corticosterone (10 mg/kg). *P<0.05 compared to the saline-treated group in each figure. †P<0.05 compared to normal rats among the 4 saline-treated groups. xP<0.05 compared between the saline-treated groups of ADx and ADx+Cor treated rats.
intake by single injection; however, the decreasing effect of morphine was attenuated by repeated injection. The saline-treated animals had 2-3 times higher serum 11-OHCS during the dark period than during the light period. Morphine showed no significant changes in serum 11-OHCS levels by both single and repeated injection.

Effects of corticosterone on feeding in normal rats: Corticosterone at a dose of 10 mg/kg did not alter the 2 hr-feeding during the light period and tended to increase it during the dark period (Fig. 5). During the dark period, corticosterone also tended to increase the 24 hr-feeding, but these changes were not significant.

Time-course changes in blood 11-OHCS, glucose and insulin levels associated with morphine-induced feeding in normal rats: Table 1 shows the results during the light period. For 15 min after morphine injection (at 11:00), morphine significantly elevated blood 11-OHCS levels, but did not change blood glucose and insulin levels. For 75 min after the injection (at 12:00), most of the morphine-treated animals did not begin to eat, and the blood 11-OHCS levels were still significantly elevated by morphine. For 135 min (at 13:00), morphine significantly increased food intake and elevated blood 11-
Fig. 3. Food intake and blood 11-OHCS levels by single or repeated administration of morphine during the light period. Morphine (5 mg/kg) was injected at 10:45 on a single or a 3-4 days repeated administration schedule. Food intake was measured for 2 hr after the injection, and animals were killed by decapitation 1 hr after the injection to collect their blood. Data are shown as means±S.E.M. of 5 animals. *P<0.05 compared to the saline-treated group.

Fig. 4. Food intake and blood 11-OHCS levels by single or repeated administration of morphine during the dark period. Morphine (5 mg/kg) was injected at 18:45 on a single or a 3-4 days repeated administration schedule. Food intake was measured for 2 hr after the injection, and animals were killed by decapitation to collect their blood. Data are shown as means±S.E.M. of 5 animals. *P<0.05 compared to the saline-treated group.

Table 1. Time-course changes in blood 11-hydroxycorticosteroids (11-OHCS), glucose and insulin levels associated with morphine-induced feeding during light period in normal rats

| Clock time | Time after injection (min) | Treatment | Food intake (g) | 11-OHCS (µg/dl) | Glucose (mg%) | Insulin (µU/ml) |
|------------|-----------------------------|-----------|----------------|----------------|--------------|-----------------|
| 11:00      | 15                          | Saline    | 11.2±1.8       | 1210±3.7       | 32.1±6.5     | 19.4±6.6       |
|            |                             | Morphine  | 26.7±3.0*      | 126.3±8.2      | 23.3±1.9     | 19.4±6.6       |
| 12:00      | 75                          | Saline    | 0.3±0.1        | 7.6±0.9        | 126.8±1.8    | 34.1±4.8       |
|            |                             | Morphine  | 0.6±0.3        | 15.1±3.0*      | 123.5±2.7    | 37.6±5.5       |
| 13:00      | 135                         | Saline    | 0.3±0.2        | 3.8±0.3        | 115.9±2.7    | 39.5±7.2       |
|            |                             | Morphine  | 2.5±1.0*       | 14.4±2.9*      | 117.6±2.0    | 69.6±19.4*     |

Animals were freely given food and water except for 15 min after the drug administration on the experimental day; Morphine was intraperitoneally injected at 10:45, and food was supplied to the animals at 11:00. They were killed at 11:00, 12:00 and 13:00 by decapitation to collect their blood. Data are shown as means±S.E.M. of 5 animals. *P<0.05 compared to the saline-treated group.
OHCS and insulin levels, but did not change blood glucose levels.

Table 2 shows the results during the dark period. Although the animals were not given food for 15 min after the administration, morphine markedly decreased blood insulin levels without changing the blood levels of 11-OHCS and glucose. For 75 min after the injection (at 20:00), the saline-treated animals ate 3.3 g for 1 hr and showed higher blood levels of glucose and insulin than those at 19:00. Compared to the saline-treated animals, morphine significantly decreased food intake and reduced the blood levels of glucose and insulin. For 135 min after the injection (at 21:00), morphine significantly decreased the total 2 hr food intake (19:00–21:00); however, for 1 hr (20:00–21:00), morphine administered animals showed comparable levels of food intake to those of the saline-treated animals. Morphine increased blood insulin levels at this time.

**Discussion**

The present study revealed that the orectic effects of morphine during the light period in normal rats were elicited even by adrenalectomy, suggesting that the adrenal had no effects on morphine-induced feeding during the light period (Fig. 1). This is supported by the results that the repeated administration of morphine failed to elevate blood 11-OHCS levels, but significantly increased food intake; during the light

![Table 2](image)

| Clock time | Time after injection (min) | Treatment | Food intake (g) | 11-OHCS (µg/ml) | Glucose (mg%) | Insulin (µU/ml) |
|------------|---------------------------|-----------|-----------------|----------------|--------------|----------------|
| 19:00      | 15                        | Saline    | 24.0±1.7        | 139.9±3.6      | 51.8±8.1     |                 |
|            |                           | Morphine  | 30.7±3.7        | 143.0±3.3      | 16.3±1.6*    |                 |
| 20:00      | 75                        | Saline    | 3.3±0.6         | 15.5±2.2       | 150.8±13.7   | 78.0±10.0      |
|            |                           | Morphine  | 1.7±0.4*        | 20.1±2.7       | 122.7±5.7*   | 32.2±10.2*     |
| 21:00      | 135                       | Saline    | 5.4±0.3         | 17.3±5.7       | 124.8±1.9    | 74.3±18.3      |
|            |                           | Morphine  | 3.9±0.7*        | 23.9±1.7       | 125.9±3.7    | 94.1±28.4      |

Animals were freely given food and water except for 15 min after the drug administration on the experimental day. Morphine was intraperitoneally injected at 18:45, and food was supplied to the animals at 19:00. They were killed at 19:00, 20:00 and 21:00 by decapitation to collect their blood. Data are shown as means±S.E.M. of 5 animals. *P<0.05 compared to the saline-treated group.
period, there was no correlation between morphine-induced feeding and the changes in 11-OHCS levels (Fig. 3). In contrast, the anorectic effects of morphine during the dark period in normal rats were attenuated by adrenalectomy (Fig. 2). These results suggest that the adrenal plays important roles for morphine-induced feeding during the dark period. Furthermore, since morphine tended to decrease 2 hr-feeding in adrenalectomized rats receiving corticosterone (Fig. 2), the adrenal cortex (glucocorticoids) is an undeniable factor for the anorectic effects of morphine during the dark period. However, a high dose of corticosterone had no effects in normal rats during both the light and dark period (Fig. 5). Therefore, it is possible to estimate that during the dark period, glucocorticoids may not have direct effects on morphine-induced feeding (permissive effects). On the other hand, the anorectic effects of morphine during the dark period in normal rats were abolished by adrenomedullation (Fig. 2). This result suggests that the adrenal medulla (catecholamines) plays important roles for morphine-induced feeding during the dark period.

Van Puttern et al. (11) and Panksepp (12) reported that low doses of glucocorticoids increased food intake, while high doses decreased it in normal rats. Panksepp also explained that low doses of glucocorticoids stimulate insulin release to facilitate feeding, and high doses induced very high levels of blood glucose to depress feeding because the animals mistakenly interpret this as a satiated state. However, the present study revealed that morphine did not alter blood insulin levels during the light period, although blood 11-OHCS levels were markedly elevated (Table 1). In contrast, morphine significantly reduced blood insulin levels and did not significantly elevate blood 11-OHCS levels during the dark period (Table 2). These results show that there were no relationships between blood insulin and 11-OHCS levels in morphine-induced feeding. The anorectic effects of glucocorticoids at very high doses and prolonged treatments reported by Van Puttern et al. and Panksepp may result from toxic actions such as extreme catabolism and behavior disturbances (18). Because insulin well documented to increase food intake, insulin may play important roles in the anorectic effects of morphine during the dark period.

It is well known that there are the satiation and feeding centers in the ventromedial hypothalamus (VMH) and lateral hypothalamus (LH), respectively (19, 20). By the injection of norepinephrine (NE) into the VMH, feeding was produced through strictly α-adrenergic effects (20, 21). McLean and Hoebel reported interesting results that changes in the pituitary-adrenal axis by dexamethasone or adrenalectomy had no effects on feeding elicited by the microinjection of D-Ala-Met-endorphinamide, a synthetic endorphin, into the paraventricular nucleus of the hypothalamus, but it did alter NE-induced feeding (22). Sawchenko et al. also reported that NE-induced feeding was blocked by coeliac vagotomy or vagal efferents with methyl atropine (23). The above two reports show that opioids-induced feeding differs from NE-induced feeding; the latter may be affected by glucocorticoids and insulin, but the former may not be. These results are also coincident with our data that morphine-induced feeding during the light period. Even the anorectic effects of morphine during the dark period in the present study may be related to NE, glucocorticoids and insulin-sensitive feeding because during the dark period, morphine did not alter food intake in adrenomedullated rats (Fig. 2) and markedly reduced blood insulin levels independently of feeding (Table 2). However, the microinjection of morphine into the VMH enhanced feeding during the light period, and this feeding was blocked by an α-adrenergic blocker, phentolamine (24). The report suggests that NE modifies morphine-induced feeding even during the light period. There are complicated mechanisms for the morphine-induced feeding, and more extensive study on the relationships between morphine-induced feeding and α-adrenergic systems will be required.

In summary, morphine had two different effects on feeding; one is the orectic effects during the light period (satiated state) and the other is the orectic effects during the
dark period (hungry state). The former effects were even elicited by repeated administration and not influenced by adrenal functions. In contrast, the latter effects were attenuated by repeated administration and influenced by adrenal cortex and adrenal medulla functions, presumably through insulin release.

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