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Effect of Morphine Self-Administration on Water and Food Intake in Rat

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Abstract

Objective

Some lines of evidences demonstrate that opioids are involved in water and food intake. On the other hand the dopaminergic mesolimbic system that consists of ventral tegmental area (VTA), nucleus accumbens (NAc) and medial prefrontal cortex is considered to be crucial in the rewarding actions of opiates. There are also reports showing that this system has some roles in appetite and drinking behaviors. The aim of this study was to investigate the effects of morphine self- administration on food and water intake in rats.

Materials and Methods

Male Wistar rats were first trained to receive small pellets of food by pressing active lever in self-administration apparatus. Rats were anaesthetized with ketamine and their jugular vein was cannulated. After recovery the animals were placed in self-administration apparatus and allowed to self-administer morphine (0.5 mg in 0.1 ml per infusion, in morphine group) or 0.1 ml saline (in saline group) during 10 consecutive days for 2 h /sessions. The amount of 24 h water and food intake during the last 3 days compared between saline and morphine groups.

Results

The results showed that water and food intake in morphine group in days 8, 9 and 10 was lower than saline group.

Conclusion

This study indicates that morphine self - administration alters food intake and drinking water but the exact mechanism(s) need to be more investigated.

Keywords: Drinking, Food, Morphine, Rat, Self-administration

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Introduction

The neural mechanisms underlying motivation to eat are still largely undetermined (1). Food intake is driven by several components, such as hunger, metabolic needs, orosensory reward, food palatability and incentive motivation (1). A large body of evidence has suggested a role of the endogenous opioids and their receptors in the regulation of appetite (2). On the other hand opioid receptors have a wide distribution in the nervous system and include a number of regions implicated in food intake such as the hypothalamic paraventricular nucleus and the central nucleus of amygdala (3). Morphine has been reported to stimulate feeding when administered into the ventral tegmental area and nucleus accumbence (4, 5). In other studies intracerebroventricular injections of morphine decreased food (6) and water intake (6 - 8). On the other hand some reports showed that morphine activation of the endogenous opioid system increased food intake in most species, including normal humans and rats, but decreased food intake in mice (9). Intraperitoneal administration of morphine in 24h food deprived rats, reduced levels of food and water intake (10, 11); in contrast a second experience showed that morphine increased levels of food and water intake in non-deprived animals (10, 11). Subcutaneous injections of morphine decreased food and water intake (10, 11). All of these findings are controversial regarding the effect of morphine on water and food intake. On the other hand the dopaminergic mesolimbic system that consists of ventral tegmental area (VTA), nucleus accumbens (NAc) and medial prefrontal cortex is considered to be crucial in the rewarding actions of opiates (12). There are also reports showing that this system has some roles in appetite and drinking behaviors (13 - 15). The aim of this study was to investigate the effects of morphine self- administration on food and water intake in rats.

Materials and Methods

Animals

Male Wistar rats weighing 200–250 g (Razi Institute, Tehran, Iran) were used. Animals were housed four to five per cage with access to food and water ad libitum, and they were maintained at 22.0 ± 2.0 °C with a 12-light/12-dark cycle (light period 07:00 and 19:00 h). All animals were allowed to adapt to laboratory conditions for at least 1 week before the studies. Experimental plan was approved by the Isfahan University Committee on Animal Research.

Self - administration apparatus

Briefly, to aid in acquisition of drug self-administration, rats were initially trained to press a lever using food as a reinforce before being surgically implanted with a chronic intravenous jugular catheter. Training and testing were done in standard operand conditioning cages (21cm×21cm×28cm) placed in a sound-attenuated room, ventilated with fans, as for the method of Alaei et al. (16), with minor modifications. The apparatus was equipped with active and passive levers, 2 cm above the floor, and a red light located 4 cm above the active lever. The intravenous cannula of the animal was connected to an infusion pump via a swivel, allowing the animal to move relatively freely. Pressing the active lever, marked by red light resulted in a 10-s infusion of 0.1 ml fluid via infusion pump. The fluid was saline in saline group and morphine (TEMAD Ltd., Teheran, Iran), with 5 mg/ml concentration in morphine group. Further pressing the active lever during this time would not infuse farther. Pressing the passive lever had no programmed consequences.

Surgical procedure

Animals were anaesthetized with ketamine (150 mg/kg) and rampon (0.1 mg/kg) and a cannula was inserted into the jugular vein. The cannula was guided subcutaneously up to the skull where it was fixed to acurred metal tube, which was secured on to the skull with small screws and fixed with dental acrylic cement.

After surgery, rats were given 300,000 units of procaine penicillin G (ip) and were allowed 7 days to recover from surgery.

Procedure

Self- administration

Training phase: One week before starting the experiments, the animals were transferred to a special room and the day–night cycle was reversed (lights on at 19.00 hs) before tests, and the animals were recorded during the dark phase of the cycle. Before surgery, the training program was started after 24 h food restriction. The animals were placed in the self-administration apparatus where a lever filled with food pellets was available. Pressing the lever resulted in the delivery of a 100 mg pellet on a fixed ratio (FR) schedule. Each rat allowed self-training and pressing for 40 pellets before being returned to *ad libitum* food. Following acquisition of lever pressing behavior, rats were returned to ad libitum food and allowed to gain their weight for 3 days and then the surgery was performed.

Self -administration phase: Seven days after recovery the rats were placed in the operand chambers, the jugular cannula of rats was connected to an infusion pump and the animals were placed in the self-administration apparatus for 2 h each day on an FR-1 schedule for 10 days. The trained animals allowed pressing the active and passive levers freely. With pressing the active lever, rats received 0.1 ml of morphine or saline. Pressing the passive lever did not deliver fluid or food. Catheters were flushed daily with 0.1 ml saline containing heparin sulfate (50 IU/ml) during the recovery period as well as before and after the self-administration sessions. All operand sessions were conducted during the animals' dark cycle. Catheter patency was tested by injection of 0.1 ml solution of sodium pentobarbital (10 mg/ml) into the catheter and observation of the animal behavior. Animals with patent catheters exhibit prominent signs of anesthesia (loss of muscle tone) few seconds after administration.

Experimental design

To evaluate the effects of morphine –self administration on water and food intake, 18 male rats were divided into two groups: (1) saline group, which received saline in the self-administration sessions; (2) morphine group, which received 0.1 ml of morphine in saline solution (concentration 5 mg/ml) during the self-administration sessions. The water intake volume (ml) and the food intake amount (g) was measured during 24 hs.

Statistical analysis

Data are presented as mean ± SEM. The mean of active and passive lever pressing number in the last 3 days was compared in each group with using paired *t* test. The comparison of the number of active lever pressing between two groups accomplished with using un-paired *t* test. Water intake volume and food intake amount in 3 days were compared by using repeated measure ANOVA. The criterion for statistical significance was $p<0.05$.

Results

In the saline group, which received saline, there was no significant difference between the number of active and passive lever pressing. In the morphine group, where animals received morphine during 10 days,

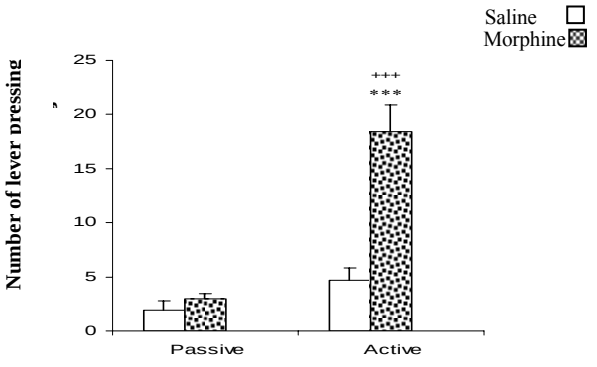

Figure 1. Comparison of active and passive lever pressing in each group and between saline and morphine groups. Data are mean ± SEM. $^{***}p<0.001$ compared to active lever pressing number of saline group, $^{+++}p<0.001$ compared to passive lever pressing of morphine group.

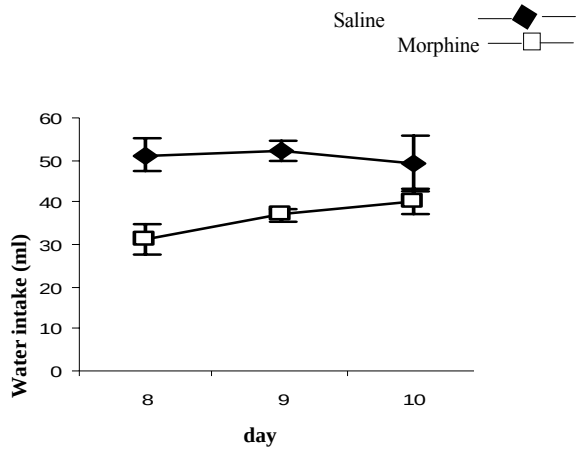

Figure 2A

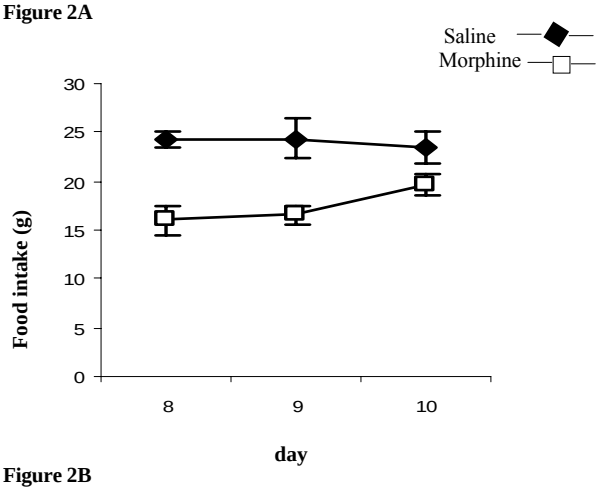

Figure 2B

Figure 2. Comparison of water (2A) and food intake between saline and morphine groups. Data are mean ± SEM. Data were compared using repeated measure ANOVA. Food and water intake in morphine group was significantly lower than saline group ($p<0.01$).

the number of active lever pressing was significantly higher than that of passive lever pressing ($p<0.001$; Figure1). The number of active lever pressing in morphine group in the last three days of experiments was higher than saline group ($p<0.001$; Figure 1).

The results also showed that water intake in morphine group on days 8, 9 and 10 was 31±3.7, 37 ±1.3 and 40.3±2.9 ml, respectively and was lower than saline group (51.2±3.8, 52.4±2.5 and 49.2±6.3 ml) (Figure 2A). The food intake in morphine group, during days 8, 9 and 10(16±1.5, 16.6±0.1 and 19.6±1.1 g respectively) also reduced, compared to saline group (24.2±0.8, 24.4 ± 1.9 and 23.5± 1.7 g) (Figure 2B).

Discussion

The results of present study showed that morphine self- administration reduced daily water and food intake in non deprived rats. These results agree with the other studies which showed that systemic morphine administration decreased water intake in water restricted male rats and male NMRI mice deprived of water (10, 17). Other researchers showed that intracerebroventricular injections of morphine suppressed and decreased water drinking of rabbits and water deprived rats respectively (6, 7). In those studies the animals were deprived of water but the results of this study are from non deprived rats. In another study subcutaneous treatment with morphine failed to significantly affect water intake, while food intake was significantly reduced only by the higher dose of morphine (11). In contrast, Bondar et al. (2003) showed that icv injection of morphine increased food intake in both male and female rats (18). Other studies showed that intracranial injection of μ, δ, or Κ receptor-specific opioid agonists also increased food intake in lean rats, and central injection of general

or μ, δ, or Κ receptor-specific opioid antagonists decreased feeding in lean and obese rats (19 - 21). In another investigation subcutaneously and intraperitoneally administration of a kappa opiate receptor agonist, Tifluadom, increased food intake in rats without altering water intake (14). These results were against the results of this study but in the present study water and food intake decreased after morphine – self administration while in other studies the animals were injected opioids and they weren't volunteer to receive morphine. Other researchers showed that morphine activation of the endogenous opioid system increases food intake in most species, including normal humans and rats, but decreases food intake in mice (8, 9, 21). Some of investigators believe that applying an opioid antagonism, naltrexone, influenced preferred and non-preferred food consumption, depending on the site of administration (3). In other studies it has been shown that injection of opioid agonists to the nucleus accumbens, VMH, MPOA, paraventricular nucleus of the hypothalamus, ventral tegmentum, or hindbrain elicits food intake (19). These areas overlap with the distribution of β-endorphin, dynorphin, and enkephalin fibers, terminals, and the three opioid receptor subtypes (23 – 25). Bilateral micro infusion of morphine in nucleus accumbens shell also increased feeding (5). Administration of morphine into ventral tegmental area also stimulated feeding (4). The prefrontal cortex receives projections from cells in the ventral tegmental area that express opiate receptors (27) and opiate function is persistently altered in the ventral tegmentum after chronic opiate experience in isolation (28). Mu opioid agonists (MOA) acting in the NAc robustly enhance consumption of palatable foods. In contrast, kappa opioid agonists (KOA) have variable effects on feeding and KOA agonists have MOA opposing behavioral actions when microinjected at several brain sites. NAcc MOA and KOA receptors have robust and opposing role in palatability based food choice and consumption and raise the possibility that an endogenous KOA agonist acting in the NAc contributes to the phenomenon of sensory specific satiety (29). The effect of morphine self - administration on water and food intake in the present study may be due to endogenous opioids. Studies showed that effects of morphine on ingestive behaviors are related to deprivation or restriction to food and water intake and doses of morphine (30). In the present study the animals self- administered 0.5 mg of morphine by pressing the active lever and they were nondeprived of food and water.

The previous studies showed that opioids increase the dopaminergic turnover in nucleus striatum and NAc of mice, causing change in food and water intake (31). The results of another study showed that food restriction decreased dopamine level in NAc and systemic morphine administration or meal could restore dopamine level (32).The results of present study may be related to activation of dopaminergic mesolimbic system after morphine self- administration. This system has important role in food and water intake (13 - 15) and also rewarding properties of morphine (33 - 35). The exact mechanism (s) needs to be more investigated.

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