The effects of morphine and nicotine co-administration on body weight, food intake and appetite-regulating peptides in rats

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Summary

Previous studies in humans and animals have reported that nicotine administration decreases body weight and caloric intake. Opiate and cigarette have been used concomitantly as drug abuse. The aim of the present study was, therefore, to analyze the effect of chronic co-administration of nicotine and morphine on food intake, body weight and on some feeding-associated peptides. All experiments were carried out on male Wistar rats. Animals were randomly assigned to the free-fed and pair-fed control groups, nicotine- and morphine-treated and nicotine plus morphine groups. Morphine sulfate (20 mg/kg for 14 days s.c.) and nicotine (4 mg/kg for 14 days i.p.) were injected to the rats. The serum levels of leptin and neuropeptide Y (NPY) were measured by enzyme immunosorbant assay and radioimmunoassay, respectively. The results showed that nicotine had a greater suppressing effect on total food intake than morphine alone or nicotine plus morphine. Furthermore, chronic injection of nicotine significantly decreased body weight as compared with before injection, while body weight changes were not observed in morphine-treated rats. The mean body weight in the morphine-treated rats was lower than that in the free-fed control animals. The serum level of NPY was decreased just in the nicotine-injected group. A significant decrease in leptin levels was observed in the drug treated and pair-fed groups. In conclusion, morphine modulates the decreasing effect of nicotine on food intake, and it seems that the mechanism underlying the attenuating effects of morphine on the nicotine effects involves mediation, at least in part, by preventing the effect of nicotine on NPY levels.

Key words: Nicotine, Morphine, Feeding behavior, NPY, Leptin

Introduction

There are some reports indicating an inverse association between cigarette smoking and body weight (Albanes *et al*., 1987; Seo *et al*., 2009). This action of smoking on body weight appears to be nicotine mediated (Hajek *et al*., 1988). Previous studies in humans and animals have reported that nicotine administration decreases body weight and food intake (Hajek *et al*., 1988; Bellinger *et al*., 2003; Bishop *et al*., 2004; Kramer *et al*., 2007).

It has been suggested that endogenous opiate mechanisms are also involved in the physiological control of food and water intake. Morphine, as a mu-opioid agonist, causes a short-term increase in food intake (Gosnell and Krahn, 1993). In addition, Dhatt *et al*. (1988) reported that chronic intracerebroventricular injection of morphine significantly decreases body weight, food intake and water drinking in male rats. Furthermore, the endogenous opioid system has been suggested to be involved in some effects of nicotine

(Berrendero *et al*., 2002, 2005). Opioid agonists have been found to increase cigarette smoking in humans, and morphine has been shown to increase the potency and efficacy of nicotine in rats (Pomerleau, 1998).

There are traditional concepts without a scientific basis in some cultures. For instance, in Iran opiate and cigarette have been used concomitantly as drug abuse and addicted subjects believe that cigarette and opium smoking decrease body weight more than cigarette or opium alone. There is not an abundance of related research in the presented medical literature, but some researchers have studied the effects of these abused drugs separately. However, the interaction between opioid and nicotine receptors on the feeding behavior has not been yet elucidated.

The present study was designed with two goals in mind: first, to analyze the effect of morphine on nicotine-induced food intake and second, to evaluate interaction between morphine and nicotine on serum levels of appetite-regulating peptides.

Materials and Methods

Animals

All experiments were carried out on male Wistar rats, weighing 235.12 ± 12.4 g (Mean ± SD), that were housed one per cage under a 12 h light/dark cycle in a room with controlled temperature (22 ± 1°C). Food and water were available *ad libitum* except in the pair-fed group. Rats were divided randomly into five experimental groups, each comprising 6-8 animals. All experiments were approved by the Animal Experimentation Ethic Committee of Kerman Neuroscience Research Center (EC/KNRC/84/40).

Drugs

Morphine hydrochloride (TEMAD, Iran) was dissolved in physiological saline. Nicotine hydrogen tartrate (Sigma, USA) was dissolved in a fresh 0.14 M NaCl- 0.01 M sodium phosphate (pH = 7.4). Morphine was given in the volume of 250 µl (s.c.). Nicotine was injected intrapritoneally in the volume of 1 ml/kg. For drug treatment, the rats were divided into different groups, and the experiments were conducted according to protocol given as follows:

Experimental design

Animals were maintained in special feeding cages for 3 days prior to the beginning of behavioral testing and handled daily between 9:00-17:00 (every 2 h) in order to adapt them to manipulation and minimize nonspecific stress responses due to daily weighing and the injection protocol. The rats were randomly assigned to the following groups. In the nicotine-treated group, nicotine (4 mg/kg, i.p.) was given in five equal doses with an interval of 2 h (09.00-17.00) for 14 days. In the morphine-treated group, morphine (10 mg/kg, s.c.) was given at 09.00 and 17.00 and saline was given at 11.00, 13.00 and 15.00 for 14 days. Nicotine and morphine were given concomitantly in the third group according to the same schedule as mentioned above. In the free-fed control group, saline was injected five times a day from day 1-14. In the pair-fed control group, animals received food in amount to those in the nicotine-treated group and also were injected with saline.

Measurement of body weight and food intake

The average total food intake (g/rat/24 h) and also body weight were measured at 09:00 A.M every day during the time course of the experiments by a Sartarious measure with sensitivity of 0.001 g. The food consumed by individual rat was quantified by weighing leftover food in the hopper.

Leptin and neuropeptide Y assay

Before the experiment the blood sample was collected from the orbital sinus using microhematocric tubes under a light anesthesia (exposed to a CO_2 atmosphere). On the 14th day of the experiments, the rats were killed by decapitation between 9:00-10:00 A.M. and serum was obtained by centrifugation of blood at 2500 r.p.m (10 min). Samples were frozen immediately and stored until the time of assay at -80°C. Serum levels of neuropeptide Y (NPY) were measured by radioimmunoassay using a

commercial kit for rats (DRG International, Inc. USA). The sensitivity of assay was 0.25 pg/ml and the antibody cross-reacted 100% with rat NPY, 0.01% with substance P, and 0% with insulin.

Serum levels of leptin were measured by enzyme immunosorbant assay using a commercial kit for rats (DRG International, Inc. USA). The sensitivity of assay was 0.04 ng/ml and the antibody cross-reacted 100% with the rat leptin, <0.1% with porcine leptin and 0% with rat insulin, proinsulin and glucagons. The intra- and interassay coefficients of variation were 2.9 and 7.9%, respectively.

Statistical analysis

The results are expressed as mean ± SEM. The differences in body weight and amount of food intake between groups over the time course of the study were determined by repeated measurement analysis of variance (ANOVA) followed by the Newman-Keuls test. The differences in total food intake and hormone levels between the groups were determined by one-way analysis of variance (ANOVA) followed by the Newman-Keuls test. For statistical evaluation of mean differences between pre- and post-drug hormone levels, Student's t-test (paired) was used. $P<0.05$ was considered significant.

Results

The effect of nicotine and morphine on body weight

As shown in Fig. 1A, the body weight of free-fed rats was increased significantly during the 14 days of the experiment ($P<0.0001$). In contrast, chronic injection of nicotine (4 mg/kg i.p. for 14 days) significantly decreased body weight as compared with before injection ($P<0.0001$). In addition, chronic administration of morphine (20 mg/kg s.c. for 14 days) did not change body weight ($P=0.0713$).

In the group that received nicotine and morphine concomitantly, the mean body weight was not decreased during the first week of the experiment, but from the 7th day the mean of body weight started to decrease as compared with before treatment. In the pair-fed group, changes in body weight was similar to those in the nicotine-treated rats. In the experimental groups, there were no significant differences between the mean of body weight before the study.

Mean body weight on day 14 of the experiment has been shown in Fig. 1B. Chronic injection of nicotine, morphine and nicotine accompanied by morphine significantly decreased the mean of body weight as compared with the free-fed group. In addition, the mean body weight in the group that received chronic morphine was greater than those in the nicotine-treated and nicotine plus morphine-injected rats.

The effect of nicotine and morphine on food intake

The mean of 24 h food consumption has

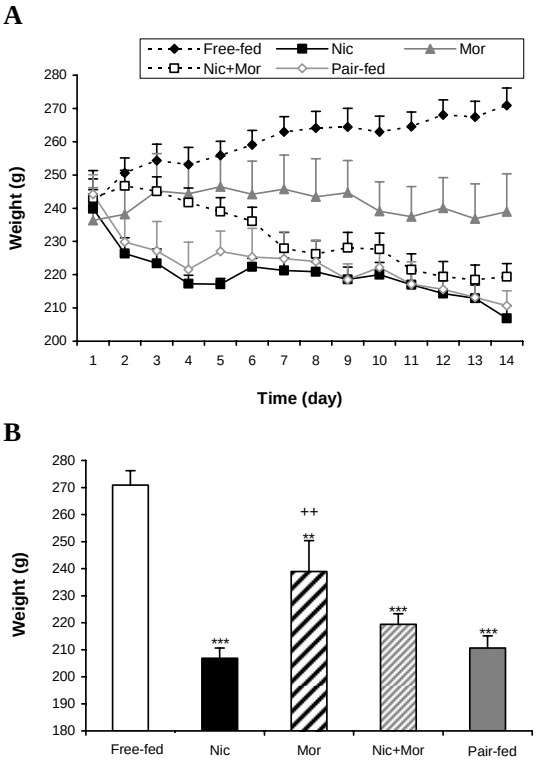

Fig. 1: Mean group daily body weight change (g) recorded from the beginning of daily administration of nicotine, morphine and nicotine plus morphine (A). At the start of injections, the body weights of all experimental groups were in the same level. Mean of body weight on the 14th day of the experiment (B). Values represent mean±SEM (n = 6-8). **: $P<0.01$ and ***: $P<0.001$ as compared with the free-fed group. $^{++}$: $P<0.01$ as compared with the nicotine-treated group and nicotine plus morphine group

been depicted in Fig. 2. Repeated-measure analysis showed that food intake was slightly higher in free-fed animals than other experimental groups (P<0.0001). Figure 3 shows total food intake during the 14 days of the test in experimental groups. Free-fed rats, as a control group, used 264.66 ± 5.38 g food during the time course of the study. Chronic injection of nicotine significantly decreased the amount of total food intake (P<0.001). In addition, the level of total food intake in groups that received chronic morphine or morphine accompanied with nicotine were lower than those in the free-fed ones (P<0.05 and P<0.01, respectively).

The results showed that nicotine had a lessening effect on total food intake than morphine alone or nicotine plus morphine (P<0.001). The level of total food intake in pair-fed rats was similar to those in the nicotine-treated animals.

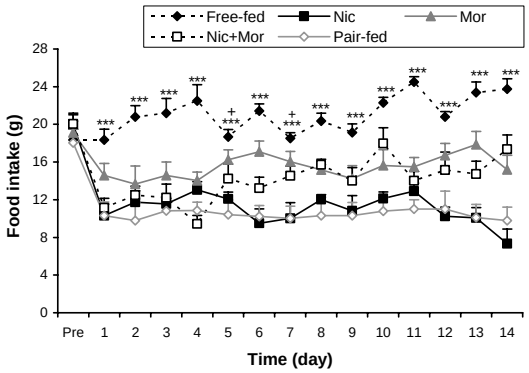

Fig. 2: Mean of 24-h food intake (g) recorded prior to, and during daily administration of nicotine, morphine and nicotine plus morphine. Values represent mean±SEM (n = 6–8 rats per group). Repeated-measure analysis showed that food intake was slightly higher in free-fed animals than other experimental groups. *: P<0.001 significantly different versus other experimental groups at the same time except versus morphine-treated animals in days 5 and 7. +: P<0.05 significantly different versus morphine-treated animals in days 5 and 7 at the same time

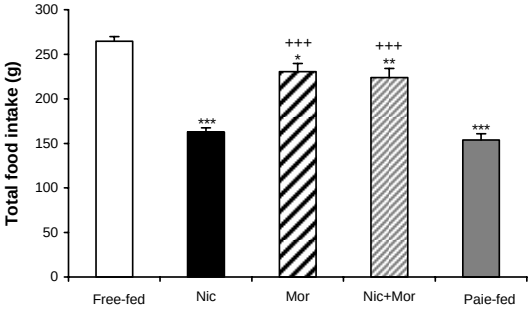

Fig. 3: Mean of total food consumption recorded during the 14 days of the study in free-fed and drugs-treated groups. Values represent mean±SEM (n = 6–8 rats per group). *: P<0.05, **: P<0.01 and ***: P<0.001 as compared with the free-fed group. +++: P<0.001 as compared with the nicotine-treated group and pair-fed group

The effect of nicotine and morphine on the serum levels of NPY and leptin

In this part of the study, changes in appetite-regulating peptides upon chronic exposure to nicotine and morphine were investigated. Chronic nicotine injection produced a significant decrease in the serum level of NPY as compared to before treatment. In contrast, the 14 day treatment of morphine and also nicotine plus morphine had no effect on the serum levels of NPY (Fig. 4).

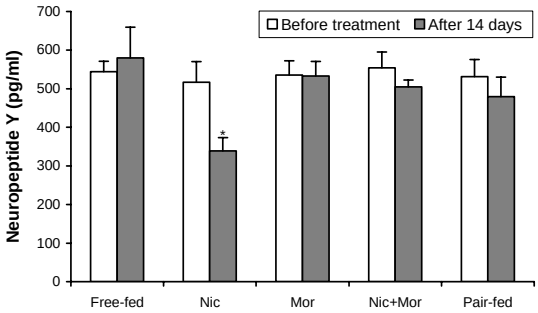

Fig. 4: Serum neuropeptide Y concentration before and after drug treatment. Each bar represents mean±SEM (n = 6-8). *: P<0.05 significantly different versus before treatment in the same group

Figure 5 depicts the effects of drugs on leptin concentration in experimental groups. Leptin concentration was similar in all experimental groups before treatment [F (4,32) = 0.4263, P=0.7885]. The serum level of leptin was dramatically increased in free-fed rats (P<0.001), while chronic administration of nicotine, morphine and nicotine plus morphine had a significant lessening effect on the serum level of leptin as compared to before treatment. In addition, leptin levels were decreased in pair-fed animals (P<0.01).

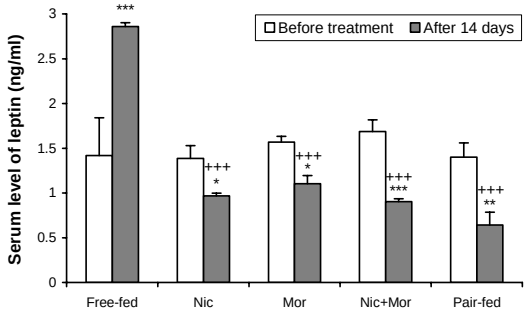

Fig. 5: Serum leptin concentration before and after drug treatment. Each bar represents mean±SEM (n = 6–8). *: P<0.05, **: P<0.01 and ***: P<0.001 significantly different versus before treatment in the same group. $^{+++}$: P<0.001 significantly different versus free-fed rats (after 14 days)

Discussion

Although it has been shown that chronic administration of nicotine can modulate feeding behavior and reduce food intake and body weight, the effect of nicotine plus morphine (which are common drug abuse) on feeding behavior has not been fully identified.

The data showed that chronic nicotine had a prominent inhibitory effect on total food intake and reduced body weight. The role of nicotine receptor in feeding behavior has been examined in numerous reports but the mechanisms underlying the alterations in body weight related to smoking are not well-understood. Several possible mechanisms have been proposed for the effects of nicotine on body weight (Li *et al*., 2000b). Some human and animal studies suggest that nicotine suppresses food intake (Grunberg *et al*., 1986; Chen *et al*., 2007), while others argue that nicotine increases the metabolic rate (Sztalryd *et al*., 1996). In any case, the effects of nicotine on appetite and body weight cannot be completely explained by either mechanism exclusively. In this study the level of weight loss in nicotine-treated rats was similar to those in pair-fed animals. Therefore, it supports the idea that weight changes induced by nicotine result from changes in food intake.

The results show that chronic morphine could not change body weight as compared with before treatment but it prevents accessing weight. It is documented that opioid receptors are involved in the physiological control of food and water intake. The effects of morphine on food intake have been controversial. Both activation (Gosnell and Krahn, 1993) and inhibition (Dhatt *et al*., 1988) have been reported.

The effect of co-administration of morphine and nicotine on food intake and body weight has not yet been elucidated. This study showed that chronic injection of morphine plus nicotine decreases body weight during the time course of study. It also slightly decreases total food intake. We found that the amount of decrease in food intake in nicotine + morphine treated rats was significantly lower than those observed in nicotine treated animals. Therefore, we can conclude that morphine has a modulating effect on decreased food intake than that induced by nicotine.

The interaction between morphine and nicotine on food intake is in opposition with other interactions of morphine and nicotine. For example, Pomerleau *et al*. (1998) have reported that morphine increases the potency and efficacy of nicotine in rats. Furthermore, nicotine improves morphine-induced amnesia (Ahmadi *et al*., 2007).

In addition, chronic nicotine could decrease the serum level of NPY. There is an inverse association between nicotine use and food intake, and NPY is a stimulator of feeding, therefore, it is predictable that the NPY level is decreased by nicotine treatment.

Frankish and collaborators found that nicotine administration reduced food intake by 30%, and lowered NPY and NPY mRNA levels in the Arc nucleus (Frankish *et al*., 1995). In contrast, Li *et al*. (2000a) found that chronic nicotine suppressed food intake and elicited a dose-dependent increase in hypothalamic NPY mRNA levels and increased NPY immunoreactivity. Furthermore, there are reports indicating no change in hypothalamic NPY gene expression following nicotine administration (Kramer *et al*., 2007) and no change in hypothalamic NPY concentration after cigarette smoking (Chen *et al*., 2005).

NPY is highly concentrated in the hypothalamus and synthesized largely in the arcuate nucleus, which sends projections into the surrounding hypothalamic structure

(Allen *et al*., 1983). In addition, it is documented that NPY is also produced in peripheral location, such as the adrenal glands and the peptide passes the brain-blood barrier, and can be distributed in the central nervous system (Higuchi *et al*., 1988; Kastin and Akerstrom, 1999). Since almost all previous reports have evaluated the effect of nicotine on NPY level in hypothalamus nucleuses here, we decided to analyse the effect of nicotine on serum levels of NPY.

The data indicate that chronic morphine could not affect the serum levels of NPY (Fig. 4). Pages *et al*. (1991) also demonstrated that chronic treatment of morphine did not change plasma NPY in rats. In addition, morphine could prevent the lessening effect of nicotine on serum NPY level. It seems that this phenomenon could be helpful in attenuating the effect of nicotine on food intake. Recently, it has been clarified that the NPY receptor in the hypothalamus has an important role in nicotine effects on the feeding behavior, so that nicotine-induced anorexia is antagonized by pre-treatment with NPY Y1 agonist, and potentiated by its antagonist (Nakhate *et al*., 2009). However, the mechanism(s) underlying the effect of morphine on nicotine-induced NPY suppression needs to be clarified by further investigation.

The data indicated that administration of chronic nicotine reduced the levels of serum leptin (Fig. 5). This phenomenon also occurred in morphine-treated and in the group that received morphine plus nicotine (Fig. 5). Leptin is primarily secreted by adipocytes in proportion to adipose tissue mass in human and animals (Maffei *et al*., 1995; Considine *et al*., 1996). Hence, it is logically apparent that leptin levels increase in free-fed rats and decrease in pair-fed animals. In humans, rodents and dogs, the blood leptin concentration is known to positively correlate with increasing body weight and body fat content (Ishioka *et al*., 2002; Sagawa *et al*., 2002). In our study, leptin level was increased in free-fed animals (Fig. 5). Furthermore, food intake and body weight of free-fed animals were also higher than those in other groups (Figs. 1A and 2). Therefore, we can speculate that the dramatic increase in leptin levels in free-fed rats is due to their increased body weight.

Consistent with most human studies (Wei *et al*., 1997; Reseland *et al*., 2005), significantly lower leptin plasma concentration has been observed in nicotine-treated rats (Li *et al*., 2000a; Sanigorski *et al*., 2002). Some researchers believe that nicotine usage increases the sensitivity to leptin and therefore, a negative feed-back may lower leptin levels (Hodge *et al*., 1997; Li *et al*., 2000a). In addition, Sanigorski *et al*. (2002) have demonstrated that functional leptin signaling is not required for the suppression of body weight by nicotine.

According to these results, morphine also had an inhibitory effect on serum leptin concentration (Fig. 5). Other researchers have also found that chronic morphine treatment reduces plasma leptin concentration (Houshyar *et al*., 2003, 2004). It has been documented that chronic morphine is associated with increased noradrenergic and catecholaminergic activity in the brain and hypothalamus (Fuertes *et al*., 2000). Catecholamines are known to reduce leptin expression *in vitro* and *in vivo* (Carulli *et al*., 1999). Therefore, it seems possible that morphine exerts its effect on leptin through catecholaminergic pathway.

Taken together, chronic morphine modulates the decreasing effect of chronic nicotine on food intake and body weight and seems the mechanism underlying the attenuating effects of morphine on the nicotine effects in body weight and food intake involves mediation, at least in part, by preventing the effect of nicotine on NPY levels.

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