

# EFFECTS OF CHOLECYSTOKININ RECEPTOR ANTAGONISTS ON MORPHINE- AND COCAINE-INDUCED HYPOTHERMIA

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## ABSTRACT

The effects of cholecystokinin (CCK) receptor antagonists on hypothermia induced by cocaine or morphine have been studied in mice. In the present work, subcutaneous (SC) injection of cocaine (50-150 mg/kg) or morphine (125-500 mg/kg) induced hypothermia in mice. Administration of CCK<sub>A</sub> receptor antagonist MK-329 (0.5-1.5 mg/kg), CCK<sub>B</sub> receptor antagonist L-365, 260 (0.5-1.5 mg/kg) and CCK receptor antagonist proglumide (15-45 mg/kg) 60 min. prior to cocaine injection reduced hypothermia induced by cocaine. MK-329 or proglumide also reduced the morphine response. Single administration of MK-329 and L-365, 260 to mice decreased mice core body temperature. It is concluded that the hypothermic effect of cocaine and morphine may be mediated through CCK<sub>A</sub> and CCK<sub>B</sub> receptor mechanism(s).

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## INTRODUCTION

Cholecystokinin (CCK) is a major intestinal 33 amino acid peptide which has been shown to possess many of the characteristics of a neurotransmitter or neuromodulator in the CNS.<sup>1</sup> CCK receptors are generally divided into two classes, cholecystokinin-A (alimentary) and cholecystokinin-B (brain).<sup>2,3</sup>

CCK and dopamine (DA) are found to be located in certain mesencephalic DA neurons, including neurons in the posterior nucleus accumbens.<sup>4</sup> The CCK analog is selective for the CCK-A receptor which may cause an increase in potassium stimulated DA release.<sup>5,6</sup> CCK has been implicated as a modulator of dopamine neurotransmitter in the mesolimbic dopamine pathway, a primary pathway implicated in the effects of cocaine related to its abuse.<sup>7</sup> Involvement of dopamine receptor subtypes in mouse thermoregulation<sup>8</sup> has been shown.

CCK receptor mechanism has also been implicated in morphine dependence and tolerance.<sup>10</sup> Cholecystokinin receptor antagonists potentiate morphine antinociception<sup>11-13, 28, 29</sup> and elicit suppressive effects on  $\beta$ -endorphin-induced catalepsy in rats.<sup>14</sup>

Morphine affects body temperature in a variety of animal species; it elicits a biphasic effect in rats<sup>15-18</sup> and also causes hyperthermia and hypothermia in mice.<sup>19,20</sup>

Cocaine overdose may elicit seizure, cardiac dysfunction and elevate body temperature.<sup>21</sup> However, both hypothermia and hyperthermia have been reported following cocaine administration which may depend on environmental conditions, since ambient temperature has previously been shown to determine the effects of cocaine on body temperature.<sup>22</sup> In the present study, involvement of CCK receptor mechanism(s) on morphine and cocaine-induced hypothermia in mice has been investigated.

## MATERIAL AND METHODS

### Animals and set of measurement

Male albino mice (weight range 20-30 g) were used in

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all experiments. They remained in groups of 10 in their animal homes under conditional temperature ( $24 \pm 2^\circ\text{C}$ ) with free access to food and water, except during experiments. On the day of experiment, mice were housed individually in experimental cages and allow to rest for 1h before drug injection. The core body temperature was measured with a rectal thermistor probe (Light Labs England) inserted to a depth of 2 cm for a period of 2h. The data are shown as the mean temperature change ( $^\circ\text{C}$ ) from basal values. Basal values are those taken immediately before morphine or cocaine administration (time 0) after CCK antagonist injection. When vehicle, morphine, cocaine, and CCK antagonists were used alone, basal values were those taken immediately before the administration of vehicle or drug (time 0).

The experimental protocol was approved by the Research and Ethics Committee of the School of Pharmacy, Tehran University of Medical Sciences (no. p-50/94).

### Statistical analysis

Comparison between groups were made with ANOVA following Newman Keul's test. Differences with  $p < 0.05$  between experimental groups of each point were considered statistically significant.

### Drugs

The following drugs were used: morphine sulphate (MacFarlan Smith Ltd., England) and cocaine (Sigma, England), proglumide (Sigma, USA), MK-329 (1-methyl-3-(2-indoloyl) amino-5-phenyl-3H-1,4-benzodiazepin-2-one) and L-365, 260 [3R(+)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-N-(3-methyl-phenyl) urea] (Merck Sharp & Dohme, England). All the drugs were dissolved in saline except MK-329 and L-365, 260 which were dissolved in dimethylsulfoxide and water (40 and 60% respectively). The doses of peptides and antagonists used were based on published studies using these drugs.<sup>10,20,23</sup>

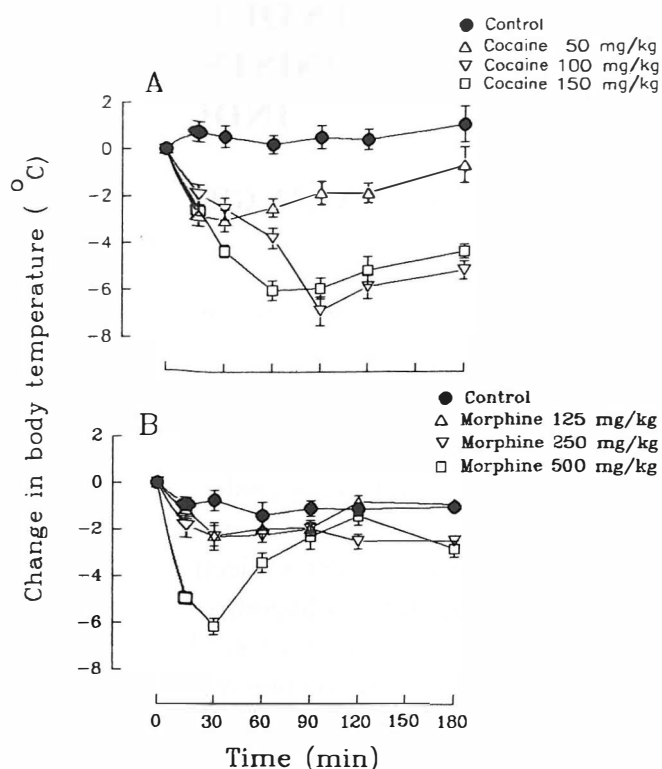
## RESULTS

### Effect of cocaine and morphine on mice core body temperature (Fig. 1)

Repeated measure one-way ANOVA with time as covariant indicates a significant difference between animals which were injected subcutaneously with different doses of cocaine (50, 100 and 150 mg/kg) or morphine (125, 250 and 500 mg/kg) as compared with saline controls. Further analysis indicated that cocaine and morphine induced hypothermia. The maximum response was obtained by 100 and 150 mg/kg of cocaine at 60-90 min and 500 mg/kg of morphine 30 min after injection.

### Effect of CCK antagonists on cocaine induced hypothermia (Table I)

Two way ANOVA indicates an interaction between ani-



**Fig. 1.** Morphine and cocaine effects on body temperature. (A) Mice were treated subcutaneously with saline control (●, 10 mg/mL) and different doses of cocaine (Δ, 50; ▽, 100; □, 150 mg/kg). (B) Mice were treated subcutaneously with saline control (●, 10 mg/mL) and different doses of morphine (Δ, 125; ▽, 250; □, 500 mg/kg). Body temperature was determined at 15, 30, 60, 90 and 180 min after drug administration. Each point is the mean  $\pm$  SEM of 9 animals.

**Table I.** Effects of CCK receptor antagonists on hypothermia induced by cocaine (100 mg/kg).

Treatment 1	mg/kg	Treatment 2	
		Saline	Cocaine
Saline	10 mL/kg	-1.31 $\pm$ 0.33	-6.84 $\pm$ 0.45
Proglumide	15	-3.06 $\pm$ 0.52*	-0.74 $\pm$ 0.56**
Proglumide	30	-5.11 $\pm$ 0.69**	-0.61 $\pm$ 0.67**
Proglumide	45	-3.23 $\pm$ 0.35*	-0.18 $\pm$ 0.39**
Saline		-1.04 $\pm$ 0.218	-6.84 $\pm$ 0.45
MK329	0.5	-1.93 $\pm$ 0.23	0.06 $\pm$ 0.68**
MK329	1	-2.2 $\pm$ 0.44	1.53 $\pm$ 0.42**
MK329	1.5	-5.03 $\pm$ 0.64	0.88 $\pm$ 0.48**
Saline		-0.62 $\pm$ 0.439	-6.84 $\pm$ 0.45
L-365, 260	0.5	-2.955 $\pm$ 0.37**	0.711 $\pm$ 0.53**
L-365, 260	1	-4.17 $\pm$ 0.35**	1.3 $\pm$ 0.77**
L-365, 260	1.5	-3.188 $\pm$ 0.326**	0.233 $\pm$ 0.8**

Mice were treated intraperitoneally with saline (10 mL/kg), proglumide (15, 30 and 45 mg/kg), MK-329 (0.5, 1 and 1.5 mg/kg) or L-365, 260 (0.5, 1 and 1.5 mg/kg) 60 min before either saline or cocaine (100 mg/kg).

Each point is the mean  $\pm$  SEM of 10 experiments.

\* $p < 0.05$ , \*\* $p < 0.01$ , different from saline control groups.

mals which were treated subcutaneously either with cocaine (100 mg/kg) alone or with cocaine 60 min after administration of the CCK antagonists MK-329 (0.5, 1 and 1.5 mg/kg, intraperitoneally) [ $F(3,58)=25.97, p<0.0001$ ], L-365,260 (0.5, 1 and 1.5 mg/kg, intraperitoneally) [ $F(3,64)=9.3, p<0.0001$ ] or proglumide (15, 30 and 45 mg/kg, intraperitoneally) [ $F(3,64)=16.3, p<0.0004$ ]. Further analysis showed that the CCK receptor antagonists were able to decrease cocaine's hypothermic response. The antagonists themselves also decreased the core body temperature of mice as compared with saline treated animals.

#### Effects of CCK antagonists on morphine-induced hypothermia (Table II).

Two way ANOVA indicated an interaction between

**Table II.** Effects of CCK receptor antagonists on hypothermia induced by morphine (250 mg/kg).

Treatment 1	mg/kg	Treatment 2	
		Saline	Morphine
Saline	10 mL/kg	-1.49±0.32	-2.26±0.34
Proglumide	15	-2.39±0.54*	-0.48±0.55**
Proglumide	30	-4.52±0.48**	-1.99±0.2
Proglumide	45	-2.6±0.29*	-0.02±0.35**
Saline		-1.49±0.389	-2.26±0.34
L-365, 260	0.5	-2.34±0.37	-1.25±0.48
L-365, 260	1	-3.7±0.45**	-2.38±0.49
L-365, 260	1.5	-1.88±0.35	-1.68±0.57

Animals were treated intraperitoneally with saline (10 mL/kg), proglumide (15, 30 and 45 mg/kg) or L-365, 260 (0.5, 1 and 1.5 mg/kg) 60 min before either saline or morphine (250 mg/kg).

Each point is the mean±SEM of 10 experiments.

\* $p<0.05$ , \*\* $p<0.01$ , different from saline control groups.

morphine (250 mg/kg, subcutaneously) in combination with proglumide (15-45 mg/kg, intraperitoneally) [ $F(3, 32)=8.4, p<0.001$ ] but not with L-365, 260 (0.5-1.5 mg/kg, intraperitoneally) [ $F(3, 32)=7.3, p>0.05$ ]. Further analysis indicated that the response of morphine was reduced by proglumide administration.

#### DISCUSSION

In the present study, administration of cocaine induced hypothermia in mice. Cocaine has been shown to reduce the core temperature of guinea-pigs.<sup>21</sup> Our data showed that morphine also induced hypothermia. This is in agreement with studies which show that opioid receptors may be involved in thermoregulation<sup>24</sup> and opioid receptor agonists are able to affect body temperature.<sup>25,26</sup> Morphine has been shown to influence body temperature in a variety of animal species; it elicits a biphasic effect in rats<sup>15-18</sup> and causes hypothermia and hyperthermia in mice.<sup>19,20</sup>

Our data indicate that CCK receptor antagonists

proglumide, MK-329 and L-365, 260 reduced the hypothermia induced by cocaine and morphine. Therefore, the present effects of cocaine and morphine may be mediated through CCK receptor mechanism(s).

CCK and dopamine are colocalized in certain dopaminergic neurons, including neurons in the posterior nucleus accumbens.<sup>4</sup> CCK has been shown to potentiate dopamine-stimulated adenylate cyclase activity in the posterior nucleus accumbens and to inhibit dopamine-stimulated adenylate cyclase activity in the anterior nucleus accumbens, suggesting that CCK modulates the effects of dopamine at  $D_1$  receptors.<sup>27</sup> The dopaminergic system may be involved in thermoregulation<sup>8</sup> and the CCK receptor mechanism may be implicated in cocaine abuse.<sup>7</sup>

Our previous results showed that CCK receptor mechanism(s) may be involved in morphine dependence<sup>9</sup> and morphine tolerance.<sup>10</sup> There are also reports showing that CCK receptor antagonists potentiate morphine antinociception<sup>11-13, 28, 29</sup> and elicit a suppressive effect on  $\beta$ -endorphine-induced catalepsy in rats.<sup>14</sup>

There are at least two types of CCK receptors designated  $CCK_A$  and  $CCK_B$ ; however, the  $CCK_B$  receptor predominates in the CNS and  $CCK_A$  is localized more in the periphery.<sup>2,30</sup> The  $CCK_A$  and  $CCK_B$  receptors are distributed within the CNS, although the distribution of  $CCK_A$  receptors are limited to regions of the mid and hindbrain in rodents.<sup>30-31</sup>

The hypothermic response induced by cocaine has been reduced by CCK receptor antagonists. One possibility is that the CCK receptor mechanism is involved in the cocaine response. However, this effect may be induced through other neurotransmitters such as dopamine, which affects thermoregulation.<sup>8</sup> CCK has been shown to have a role in the dopamine pathway in cocaine abuse.<sup>7</sup> There may be a possibility that the cocaine response is reduced by CCK antagonists via the dopaminergic system, although to clarify this possibility more experiments are required. Since all the CCK receptor antagonists used were able to prevent cocaine's response, therefore involvement of both receptors seems likely.

Our present results also showed that morphine reduced body temperature in mice. The results are in agreement with other studies.<sup>19,20</sup> CCK receptor mechanism(s) have also been implicated in morphine dependence<sup>9</sup> and morphine tolerance.<sup>10</sup> Proglumide, but not L-365, 260 also reduced morphine-induced hypothermia, which may show that CCK receptor mechanism(s) are involved. However, since CCK receptor agonist L-365, 260 did not affect the morphine response, the involvement of this mechanism is unlikely. **The present study also showed that single administration of CCK receptor antagonists proglumide and L-365, 260 reduced body temperature. This effect of the antagonists may be mediated through CCK or other neurotransmitters. More experiments are needed to evaluate the possible involvement of CCK receptors.**

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