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The possible cross-tolerance between morphine- and nicotine-induced hypothermia in mice

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Abstract

In the present study, cross-tolerance between hypothermia induced by morphine and nicotine in mice has been investigated. Different doses of morphine or nicotine induced dose-dependent hypothermia. The sub-maximal doses of both drugs were used for interaction studies. Administration of mecamylamine either intracerebroventricularly (2–6 μg/animal icv) or intraperitoneally (0.5 and 1 mg/kg ip) decreased both morphine- or nicotine-induced hypothermia. Naloxone either intracerebroventricularly (2–6 μg/animal) or intraperitoneally (1 and 2 mg/kg) reduced the response to morphine, but not nicotine's response. Hexamethonium (5 and 10 mg/kg ip) caused a slight decrease in morphine's hypothermia, but not that of nicotine. Nicotine's response was decreased in the animals which were made tolerant to hypothermic effect of morphine. Pre-treatment of the animals with low doses of morphine (12.5 or 25 mg/kg), once daily for 3 days, did not cause significant tolerance to the hypothermic response to morphine or nicotine. However, the administration of low doses of morphine (12.5 or 25 mg/kg) plus nicotine (2 mg/kg), once daily for 3 days, increased levels of tolerance to hypothermia induced by either drug. It is concluded that nicotinic receptor mechanism may play a role in morphine-induced hypothermia and there is cross-tolerance between the two drugs.

Keywords: Morphine; Nicotine; Tolerance; Hypothermia; Mice

1. Introduction

Central nervous system and peripheral autonomic mechanisms are involved in the thermoregulation of mammals. Considerable evidence has also indicated a role for monoamines in the regulation of body temperature in animals (Cox, 1979; Lee et al., 1985).

Nicotine is widely used by humans and it is known to have multiple effects. The drug exhibits several pharmacological actions in the central and peripheral nervous systems and releases a number of neurotransmitters (Balfour, 1982; Damaj et al., 1996; Zarrindast et al., 1995b; Zarrindast and Farzin, 1996). It also is involved in activating opioid system(s) (Balfour, 1982; Davenport et al., 1990). Nicotinic receptor stimulation activates enkephalin release and biosynthesis in discrete brain nuclei and adrenal chromaffin cells (Eiden et al., 1984; Houdi et al., 1991). Thus, nicotine may alleviate at least some morphine abstinence signs. There is a report that nicotine suppresses naloxone-induced jumping in morphine-dependent mice (Brase et al., 1974). Recently, we also showed that nicotine can attenuate naloxone-induced jumping in the morphine-dependent mice (Zarrindast et al., 1995b). Chronic administration of opioids (Bhargava, 1994) and nicotine (Marks et al., 1983, 1985, 1986a,b, 1991; Pauly et al., 1992) for a long time may result in the development of tolerance to their pharmacological actions. However the role of several neurotransmitter receptor systems in morphine tolerance has been studied (Bhargava, 1994), the mechanisms underlying their tolerance and dependence are poorly understood. Previously, we have shown that there is a cross-tolerance between morphine and nicotine antinociception in mice (Zarrindast et al., 1999). There is also evidence showing that both morphine (Zarrindast et al., 1994) and nicotine (Zarrindast et al., 1995b) induced hypothermia. In the present study, tolerance to hypothermia induced by morphine and/or nicotine has been studied.

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2. Materials and methods

2.1. Animals

Male albino NMRI mice (weight range 20–30 g) were used in these experiments. They were housed in groups of 10 per cage ($45 \times 30 \times 15$ cm^3) at an environmental temperature of 24 ± 2°C on a 12-h light–dark cycle. Mice had free access to food and water. The animals were deprived of food 12 h before experiments, but water was available at all times. Each animal was used once only and was euthanized immediately after the experiments.

2.2. Drugs

The following drugs were used: morphine sulphate and morphine hydrochloride (MacFarlan, Smith, England), nicotine hydrogen (+)-tartrate (BDH Chemicals, England), mecamylamine (Research Biochemical, USA), hexamethonium bromide (Sigma, England) and naloxone hydrochloride (Dupont, Germany). Nicotine solutions were prepared in saline and the pH adjusted to 7.2 ± 0.1 with a small amount of NaOH. Other drugs were dissolved in saline immediately before use. Control animals received saline.

2.3. Development of tolerance by morphine

Tolerance to morphine hypothermia was achieved on the method based on our previous work (Rezayat et al., 1994). The mice were treated subcutaneously (sc) with morphine sulphate (12.5, 25 or 50, 75 or 100 mg/kg) or saline once daily (0800 h) for a period of 3 days. To assess the degree of tolerance, the hypothermic response to different test doses of morphine hydrochloride (20, 30 and 40 mg/kg sc) was measured on the 4th day after injection of the drug. Similarly in chronically morphine-administered animals, the hypothermia of nicotine (0.05, 1 and 2 mg/kg ip) was tested.

Another group of animals also was injected with subchronic treatment of morphine (12.5 or 25 mg/kg sc) plus nicotine (2 mg/kg ip) for 3 days and the hypothermia of morphine or nicotine was assessed on the 4th day.

2.4. Chronic guide cannula implantation

Stainless steel guide cannulae (23 gauge) were stereotaxically (David Koft Instruments, USA) implanted under anaesthesia with pentobarbital (60 mg/kg ip) 5–7 days before the experiments. The guide cannulae were implanted in the left lateral ventricle at the following coordinates based on the method of Jiang et al. (1990) with a minor modification: 2 mm lateral and 0.9 mm caudal to bregma at a depth of 3 mm. The drugs were injected in a volume of 1 μl in a period of 2 min by means of an internal cannula (30 gauge) connected by polyethylene tubing to a 10-μl Hamilton syringe and the injection cannula was left in place for a further 1 min before being slowly withdrawn.

2.5. Temperature recording

On the day of experiment, mice were placed individually in experimental cages and were allowed to rest for 1 h before drug injection. During this period, body temperature was measured at 10-min intervals in order to exclude the effect of handling on animal temperature. Body temperature was measured with a rectal thermistor probe (Light Lab, Brighton, UK, sensitivity 0.1°C). The probe was lubricated with petroleum before being inserted into the rectum to a depth of 2 cm. The data are presented as changes in rectal temperature from the basal values. Basal values are those taken immediately before the drug injection (time 0). The experiments took place between 0800 and 1400 h.

2.6. Analysis of data

Comparisons between groups were made with Tukey's test following ANOVAs. Differences with $P < .05$ between experimental groups at each point were considered statistically significant.

3. Results

3.1. Time course of hypothermia induced by morphine and nicotine

Fig. 1A shows the hypothermia induced by morphine. Repeated measures two-way ANOVA shows that subcutaneous administration of different doses of morphine (20, 30 and 40 mg/kg sc) [Factor A; $F(3,32) = 75.3$, $P < .0001$] to mice decreased the animals' body temperature at different times after the opioid administration (15, 30, 45, 60, 90 and 120 min) [Factor B; $F(5,160) = 48.9$, $P < .0001$] with interactions [Factor A × B; $F(15,160) = 9.7$, $P < .0001$]. The effect was dose-dependent. The maximum response was achieved between 30 and 45 min and with 40 mg/kg.

Fig. 1B indicates the hypothermia induced by nicotine. Repeated measures two-way ANOVA shows that intraperitoneal treatment of the animals with different doses of nicotine (0.5, 1 and 2 mg/kg ip) [Factor A; $F(3,24) = 80.6$, $P < .0001$] induced hypothermia at different times after nicotine injection [Factor B; $F(5,120) = 109.3$, $P < .0001$] with interactions [Factor A × B; $F(15,120) = 21.6$, $P < .0001$]. The greatest effect was achieved at 15 min after the drug injection and with 2 mg/kg of nicotine.

3.2. Effects of nicotinic and opioid receptor antagonists on hypothermia induced by morphine or nicotine

Table 1 shows hypothermia induced by morphine or nicotine in the presence or absence of naloxone, mecamylamine or hexamethonium. Two-way ANOVA indicated a

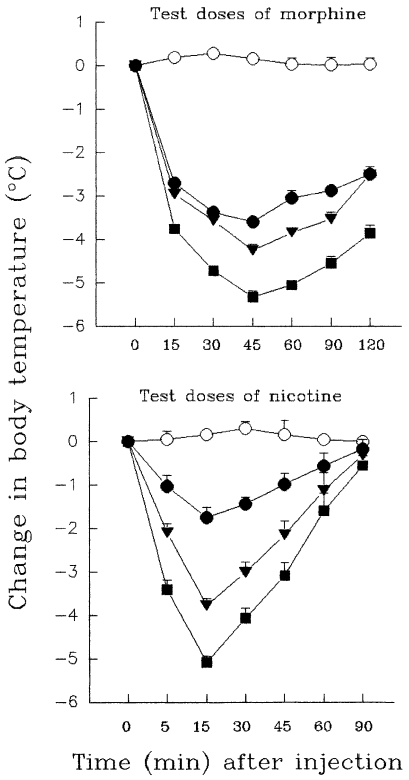

Fig. 1. Effect of morphine and nicotine on mice body temperature. Mice were injected subcutaneously with saline (○) or morphine (●) 20, (▼) 30 and 40 (■) mg/kg. Hypothermia induced by the drugs was recorded 15, 30, 45, 60, 90 and 120 min after drug administration. Nicotine (●) 0.5, (▼) 1 and (■) 2 mg/kg was administered intraperitoneally and hypothermia was recorded 5, 15, 30, 45, 60 and 90 min after nicotine injection. Each point is the mean ± S.E.M. of change in body temperature for at least seven mice. Both drugs reduced the animals' body temperature (see Results).

significant difference between animals administered nicotine (2 mg/kg ip) alone with those injected naloxone (1 and 2 mg/kg ip) 1 min [$F(2,54)=3.59$, $P<.05$], mecamylamine (0.5 and 1 mg/kg ip) 30 min [$F(2,54)=178.4$, $P<.001$] or hexamethonium (5 and 10 mg/kg ip) 30 min before nicotine injection [$F(2,54)=6.1$, $P<.01$]. Further analysis showed that mecamylamine, but not naloxone or hexamethonium, reduced the hypothermia induced by nicotine.

Two-way ANOVA also showed that same doses of naloxone [$F(2,54)=86.6$, $P<.0001$], mecamylamine [$F(2,54)=59.07$, $P<.0001$] and hexamethonium [$F(2,54)=32.54$, $P<.0001$] reduced hypothermia induced by morphine (40 mg/kg sc). Naloxone, mecamylamine or hexamethonium itself [one-way ANOVA; $F(6,63)=1.72$, $P>.05$] did not have any effect on body temperature.

Table 2 shows hypothermia induced by nicotine or morphine in the presence or absence of intracerebroventricular administration of naloxone or mecamylamine. Two-way ANOVA indicated a significant difference between animals which received nicotine or morphine alone with those which received nicotine [$F(3,48)=5.0$, $P<.05$] or morphine [$F(3,48)=22.5$, $P<.0001$] 1 min after intracerebroventricular injection of naloxone (2, 4 and 6 μg/mice).

Table 1
Effect of intraperitoneal administration of naloxone and mecamylamine on hypothermia induced by nicotine or morphine

Treatment 1 (mg/kg)	Treatment 2 (mg/kg)		
	Saline	Nicotine 2	Morphine 40
Saline	0.3 ± 0.1	− 5.1 ± 0.2	− 4.8 ± 0.1
Naloxone 1	0.1 ± 0.1	− 4.1 ± 0.3	− 2.1 ± 0.2*
Naloxone 2	0.1 ± 0.1	− 4.2 ± 0.2	− 1.3 ± 0.2*
Mecamylamine 0.5	0.0 ± 0.0	− 2.5 ± 0.1*	− 3.1 ± 0.2*
Mecamylamine 1	− 0.1 ± 0.1	− 1.8 ± 0.1*	− 2.5 ± 0.1*
Hexamethonium 5	0.1 ± 0.1	− 4.6 ± 0.2	− 3.0 ± 0.3*
Hexamethonium 10	0.0 ± 0.0	− 4.1 ± 0.4	− 2.7 ± 0.2*

Mice were pretreated intraperitoneally with saline (1 mg/kg), naloxone (1 and 2 mg/kg) 1 min, mecamylamine (0.5 and 1 mg/kg) or hexamethonium (5 and 10 mg/kg) 30 min before nicotine (2 mg/kg ip) or morphine (40 mg/kg sc) administration. Each point is the mean ± S.E.M. of hypothermic response ($n=10$).

* $P<.001$, different from respective nicotine or morphine control animals.

Further analysis showed that naloxone reduced morphine but not nicotine hypothermic effect. Naloxone did not elicit any response by itself [$F(3,48)=0.78$, $P>.05$]. Two-way ANOVA indicated that pretreatment of animals with mecamylamine (2, 4 and 6 μg/mice) 1 min prior to nicotine or morphine reduced effect of nicotine [$F(3,48)=51.2$, $P<.0001$] or morphine [$F(3,48)=36$, $P<.0001$]. Mecamylamine itself caused a slight hypothermic effect [$F(3,48)=3.2$, $P<.05$].

3.3. Hypothermic response to morphine or nicotine in morphine-tolerant mice

Animals received morphine chronically (50 mg/kg, Fig. 2A; 75 mg/kg, Fig. 2B or 100 mg/kg, Fig. 2C) once daily

Table 2
Effect of intracerebroventricular injection of naloxone and mecamylamine on hypothermia induced by nicotine or morphine

Treatment 1 (μg or μl/mice)	Treatment 2 (mg/kg)		
	Saline	Nicotine 2	Morphine 40
Saline 1	0.5 ± 0.1	− 4.6 ± 0.1	− 3.9 ± 0.1
Naloxone 2	0.1 ± 0.1	− 4.1 ± 0.3	− 2.7 ± 0.4
Naloxone 4	0.1 ± 0.1	− 4.0 ± 0.1	− 1.7 ± 0.4***
Naloxone 6	0.2 ± 0.1	− 3.7 ± 0.3	− 1.5 ± 0.2***
Mecamylamine 2	− 0.7 ± 0.1	− 3.0 ± 0.2***	− 2.3 ± 0.3**
Mecamylamine 4	− 0.9 ± 0.1*	− 2.4 ± 0.3***	− 2.1 ± 0.3***
Mecamylamine 6	− 1.0 ± 0.8*	− 2.0 ± 0.2***	− 1.8 ± 0.1***

Animals were pretreated intracerebroventricularly with saline (1 μl/mice), naloxone (2, 4 and 6 μg/mice) or mecamylamine (2, 4 and 6 μg/mice) 2 min before nicotine (2 mg/kg ip) or morphine (40 mg/kg sc) administration. Each point is the mean ± S.E.M. of change in body temperature for eight mice.

* $P<.05$, different from respective nicotine or morphine control animals.

** $P<.01$, different from respective nicotine or morphine control animals.

*** $P<.001$, different from respective nicotine or morphine control animals.

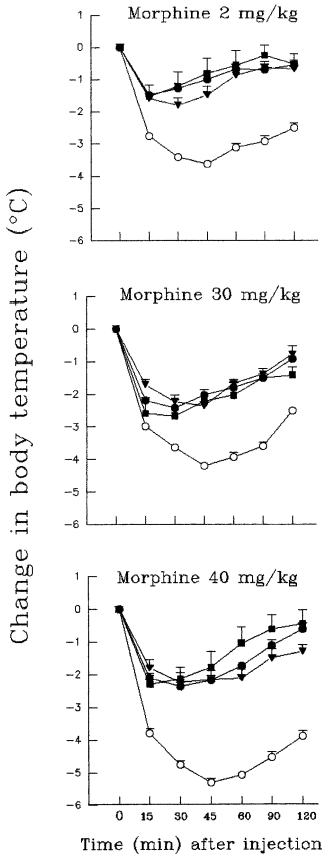

Fig. 2. Comparison of hypothermic effect of morphine in tolerant and non-tolerant mice. Animals were pre-treated subcutaneously with saline (○) or morphine (●) 50, (▼) 75 and (■) 100 mg/kg, once daily for 3 days, in order to induce tolerance to morphine response. Hypothermia of the different test doses of morphine 20, 30 and 40 mg/kg was recorded 15, 30, 45, 60, 90 and 120 min after the test dose of morphine. Each point is the mean ± S.E.M. of change in body temperature for at least eight mice. Tolerance was induced to all doses of the drug (see Results).

for 3 days. The hypothermia induced by different test doses of morphine (20, 30 and 40 mg/kg sc) was tested on the 4th day, 24 h after the last dose of morphine. Repeated measures two-way ANOVA shows that different test doses of morphine [Factor A; $F(3,30)=18.8$, $P<.0001$], at different times [Factor B; $F(5,150)=35.9$, $P<.0001$], induced hypothermia with interactions [Factor A × B; $F(15,150)=3.4$, $P<.0001$] in the animals which received 50 mg/kg of morphine in order to induce tolerance (Fig. 2A). Repeated measures two-way ANOVA also showed that different test doses of morphine [Factor A; $F(3,30)=11.97$, $P<.0001$] induced hypothermia, at different times [Factor B; $F(5,150)=38.2$, $P<.0001$] with interactions [Factor A × B; $F(15,150)=5.8$, $P<.0001$], in the animals which received 75 mg/kg to induce tolerance to morphine (Fig. 2B). However, the ANOVA indicates that different test doses of morphine [Factor A; $F(3,30)=26.3$, $P<.0001$] induced hypothermia. The drug response at different times [Factor B; $F(5,150)=1.9$, $P>.05$] did not show any difference, with no interactions [Factor A × B; $F(15,150)=1.4$, $P>.05$], in the animals which received 100 mg/kg to induce tolerance to morphine (Fig. 2C). Further analysis shows that tolerant animals in the three groups had become tolerant to morphine as compared with saline control groups and showed only a small hypothermic effect to test doses of 20, 30 and 40 mg/kg of morphine, respectively.

Fig. 3 indicates the hypothermic effect in chronically morphine-treated mice. In the animals which received saline or morphine (100 mg/kg sc, once daily for 3 days), repeated measures two-way ANOVA shows a significant difference between hypothermia induced by different doses of nicotine 0.5 mg/kg [$F(1,13)=7.7$, $P<.05$], at the different ti-mes [$F(4,52)=13.96$, $P<.0001$] with interactions [$F(4,52)=2.9$, $P<.05$], 1 mg/kg [$F(1,13)=12.8$, $P<.01$] at different times [$F(4,52)=48.8$, $P<.0001$] with interactions [$F(4,52)=5.2$, $P<.01$] and 2 mg/kg [$F(1,13)=29.2$, $P<.001$], at different times [$F(4,52)=64.5$, $P<.0001$] with no interactions [$F(4,52)=1.6$, $P>.05$] in tolerant and non-tolerant animals. Further analysis shows that nicotine

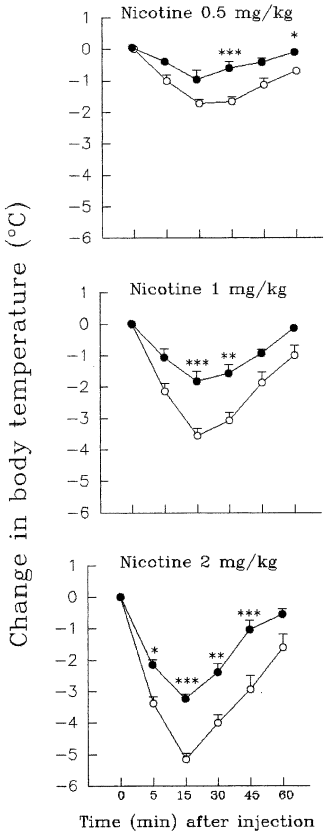

Fig. 3. Hypothermic response of nicotine in tolerant and non-tolerant mice. Animals were pre-treated intraperitoneally with saline (○) or morphine (●) 100 mg/kg in order to induce tolerance. Hypothermia induced by different test doses of nicotine 0.5, 1 and 2 mg/kg was recorded 5, 15, 30, 45 and 60 min after drug administration in tolerant and non-tolerant mice. Each point is the mean ± S.E.M. of change in body temperature of at least seven mice. * $P<.05$, ** $P<.01$, *** $P<.001$, different from respective saline-treated animals.

Table 3
Effect of concomitant administration of nicotine and morphine on tolerance to morphine

Treatment (mg/kg; 3 days)	Test doses (mg/kg)		
	Saline	Nicotine 2	Morphine 40
Saline	0.3 ± 0.1	− 5.0 ± 0.2	− 4.8 ± 0.1
Nicotine 2	0.3 ± 0.1	− 4.7 ± 0.1	− 2.9 ± 0.2
Morphine 12.5	0.3 ± 0.1	− 4.3 ± 0.3	− 3.0 ± 0.3
Morphine 25	0.3 ± 0.2	− 4.1 ± 0.3	− 3.2 ± 0.2
Morphine 12.5 + nicotine 2	0.2 ± 0.1	− 3.0 ± 0.4 $^{+}$	− 2.4 ± 0.5
Morphine 25 + nicotine 2	0.4 ± 0.1	− 3.0 ± 0.4 *	− 1.7 ± 0.4 **

Mice were injected subcutaneously with different doses of morphine (12.5 and 25 mg/kg) plus intraperitoneal administration of nicotine (2 mg/kg) for three consecutive days in order to induce tolerance to morphine or nicotine hypothermia. Each point is the mean ± S.E.M. of change in body temperature for at least eight mice.

$^{+}$ $P < .05$, different from morphine 12.5.

* $P < .05$, different from morphine 25.

** $P < .01$, different from morphine 25.

induced less hypothermic response in the tolerant animals as compared with non-tolerant animals.

3.4. The development of tolerance by combination of morphine and nicotine

Table 3 indicates the hypothermic effect of the single test dose of morphine or nicotine in animals which received repeated doses of morphine and/or nicotine for 3 days. A three-way ANOVA indicates no significant interaction in the response of pretreatment of nicotine (2 mg/kg ip, once daily for 3 days) with morphine (12.5 and 25 mg/kg, once daily for 3 days) [$F(2,128) = 0.2$, $P > .05$]. However, pretreatment of animals with nicotine [$F(2,128) = 22$, $P < .0001$] or morphine alone [$F(2,128) = 3.6$, $P < .01$] reduced the effect of test doses of the drugs with interactions. Further analysis showed that tolerance to hypothermia induced by the test dose of nicotine (2 mg/kg) or morphine (40 mg/kg sc) in animals which received morphine plus nicotine for 3 days was increased as compared with those which received morphine alone for 3 days.

4. Discussion

Nicotine is a lipophilic compound which rapidly enters and accumulates in the central nervous system (Clarke, 1990). It causes the release of catecholamines from central and peripheral adrenergic neurons and chromaffin cells (see Introduction). Moreover, opiate interaction with both dopaminergic, cholinergic and serotonergic systems has been demonstrated. There appears to be a close association between opiate receptors, dopaminergic cell bodies and nerve endings in the substantia nigra (Llorens-Cortes et al., 1979) and striatum (Yonehara and Clouet, 1984). Opioids also modulate dopaminergic neurotransmission in mesolimbic (Iyenger et al., 1987), mesocortical (Kim et al., 1986), and nigro-striatal (Wood et al., 1980) pathways. Morphine has been shown to stimulate synthesis of 5-HT in different brain areas (Brase, 1979) and may play a facilitatory role in striatal serotonin release (Parenti et al., 1983). The serotonin system seems to be involved in morphine dependence or tolerance (Neal and Sparber, 1990; Samanin et al., 1980; Zarrindast et al., 1995a). In the present work, cross-tolerance and interactions between hypothermia induced by morphine and nicotine were studied.

The present data indicate that administration of nicotine to mice induced hypothermia, which agrees with previous studies (Clement, 1991; Zarrindast et al., 1995a) showing that the drug causes hypothermic response in mice. Since intraperitoneal or intracerebroventricular administration of mecamylamine, which is a central nicotinic receptor antagonist (Martin et al., 1989), but not peripheral nicotinic receptor antagonist hexamethonium, reduced the hypothermic effect of nicotine, this points to a possible central nicotinic component in the hypothermia induced by nicotine. Nicotinic receptor stimulation may also increase levels of endogenous opioid peptides (Eiden et al., 1984; Houdi et al., 1991). Since peripheral or intracerebroventricular administration of the opioid receptor antagonist, naloxone, did not alter hypothermic response of nicotine, the involvement of endogenous opioid peptide in the nicotine's effect seems unlikely.

In the present study, morphine also caused hypothermia, which was decreased by intraperitoneal or intracerebroventricular injection of naloxone and mecamylamine or intraperitoneal administration of hexamethonium. These data may indicate that response induced by morphine may be elicited through central opioid and central/peripheral nicotinic receptor mechanisms. Mecamylamine may block other receptor systems (e.g., glutamate); thus, the involvement of other systems in the nicotine or morphine response should be considered.

Drug tolerance can be defined as a reduction of response to a challenged dose following repeated administration of that drug. Tolerance to effects of morphine, including hypothermia (Bhargava, 1994), has been demonstrated before. Several mechanisms have been proposed to mediate morphine tolerance. The opioid μ (Sanchez-Blazquez et al., 1996; Wang et al., 1994) and even delta receptors (Kest et al., 1996) have been implicated in the development of tolerance to and dependence on morphine. Moreover, regulation of μ opioid receptor gene expression in the mouse following in vivo treatments that produce tolerance has been shown (Sehba et al., 1997). In the present study, repeated doses of morphine induced tolerance to hypothermic effect of the both morphine and nicotine, which may show a cross-tolerance between the two drugs. When combination of both morphine and nicotine was used daily for 3 days, a potentiated tolerance to hypothermia induced by the test dose of either drug was obtained. One may suggest a tolerance or a common pathway for their development of tolerance. The

development of tolerance to morphine and nicotine is complex. There is suggestion that translocation and activation of protein kinase C may be a critical step in the development of opiate tolerance and dependence (Mayer et al., 1995). Chronic morphine treatment has been shown to alter the number of μ-opioid receptors. However, it has been shown that the μ-opioid receptors are downregulated in the brain of morphine-tolerant and -dependent rats (Bhargava and Gulati, 1990); others indicated that μ-opioid receptors are upregulated in both mice and rats (Abdelhamid and Takemori, 1991; Rothman et al., 1991). Furthermore, several studies have demonstrated that tolerance develops to the effects of nicotine when mice received chronic treatment of nicotine (Marks et al., 1983, 1985, 1986a,b, 1991; Martin et al., 1989; Rezayat et al., 1994). Chronic treatment with nicotine may induce tolerance to the drug effect which appears to be either stress-related and does not affect nicotinic receptor levels (Rezayat et al., 1994), or does not require handling and induce changes in receptor sites (Marks et al., 1983, 1985, 1986a,b, 1991; Robinson et al., 1996). Suppression of naloxone-induced jumping behaviour in morphine-dependent mice by nicotine and cross-tolerance to analgesic responses induced by nicotine and morphine (see Introduction) may indicate a unique pathway or similar mechanism(s) for tolerance to morphine or nicotine effects. However, our results may show that at least part of morphine's hypothermia can be elicited through nicotinic receptor mechanism, while that of nicotine cannot be due to opioid receptors. Both drugs can release neurotransmitters, including dopamine. Since both nicotine (Zarrindast et al., 1995b) and morphine (Zarrindast et al., 1994) caused hypothermia through dopaminergic mechanism(s), one pathway involved in the mechanism related to tolerance induced by the both drugs could be the dopamine receptor system.

Tolerance can be modulated by agents that influence cellular calcium homeostasis (Damaj et al., 1996); therefore, involvement of this mechanism in cross-tolerance between the two drugs should be considered.

One may also propose that potentiation of tolerance induced by combination of nicotine and morphine may be mediated through interaction between the pharmacokinetics of two drugs. However, at least cellular changes have been shown to be an important factor in regulating the development of tolerance to nicotine and no significant changes in nicotine metabolism following chronic nicotine treatment have been indicated (Hatchell and Collins, 1977). To clarify the exact mechanism(s) involved, more experiments may be needed.

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