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Role of Ca^{2+} channels on the hypothermic response produced by activation of κ-opioid receptors☆

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Abstract

The effect of nimodipine (NIM) and lercanidipine (LER) 1,4-dihydropyridine (DHP) calcium channel blockers (CCBs) on the hypothermic response of selective κ-opioid receptor agonists U50,488H (U50), PD117,302 (PD) and U69,593 (U69) was determined in rats by recording colonic temperature using digital telethermometer. Intraperitoneal (ip) injections of U50 (7.5, 15, 22.5 and 40 mg/kg), PD (7.5, 15 and 22.5 mg/kg) and U69 (5 and 20 mg/kg) produced a dose-dependent hypothermic response. However, higher doses of U50 (60 and 80 mg/kg) produced hypothermia, which is less when compared to that produced by 22.5-mg/kg dose of U50. NIM (1 mg/kg ip; 15 min prior) and LER (0.3 mg/kg ip; 15 min prior) did not produce any change in basal colonic temperature. Treatment of NIM and LER potentiated the U50 (7.5, 15, 22.5 and 40 mg/kg)-induced hypothermic effect. NIM did not potentiate hypothermia produced by U50 (60 mg/kg). On the other hand, PD (7.5, 15 and 22.5 mg/kg)- and U69 (5 and 20 mg/kg)-induced hypothermia was unaffected by the pretreatment of either NIM or LER. This differential modulation of κ-opioid agonist-induced hypothermia by CCBs suggest that there may be two mechanisms, Ca^{2+}-sensitive and Ca^{2+}-insensitive, involved in κ-opioid agonist-induced hypothermic response.

Keywords: Hypothermia; κ-opioid receptor agonists; Colonic temperature; 1,4-DHP calcium channel blockers

1. Introduction

Opioid receptors belong to the family of G-protein-coupled receptors and mediate the inhibition of adenylate cyclase, opening of voltage-gated (Ca^{2+}) calcium channels and promote the opening of potassium (K^+) channels (Childers, 1991). Change in body temperature upon acute opioid administration has been an important parameter to study. Opioid-induced thermal responses have been shown to follow a two-receptor model in rats (Geller et al., 1983). μ- and κ-opioid receptors are shown to mediate hyper- and hypothermia (Adler et al., 1988; Spencer et al., 1988), and their selective antagonists are known to block these actions, respectively (Cavicchinni et al., 1988; Handler et al., 1992). Several studies show selective κ-opioid agonists, such as U50,488H (U50) and PD117,302 (PD), to produce dose-dependent hypothermia in rats (Nemmani et al., 2001; Pillai and Ross, 1986a; Shukla et al., 1995; Spampinato et al., 1994; Spencer et al., 1988).

The movement of Ca^{2+} in discrete regions of hypothalamus can change the set point for thermoregulation (Myers, 1981, 1985). Subcutaneous (sc) administration of L-type Ca^{2+} channel blockers (CCBs), verapamil, diltiazem and nimodipine (NIM), which modulate $[Ca^{2+}]_i$, are shown to potentiate U50 (15 mg/kg sc)-induced hypothermia (Pillai and Ross, 1986a). On the other hand, verapamil (20 nmol) administered intracerebroventricularly (icv) was shown to antagonize dynorphin (1–17), (5 nmol icv)- and U50 (215 nmol icv)-induced hypothermic response (Spampinato et al., 1994). Despite the direct coupling of κ-opioid receptors to voltage-dependent Ca^{2+} channels (Attali et al., 1989; Gandhi and Ross, 1988), there are only few equivocal reports showing Ca^{2+}-related mechanisms in hypothermic effects of κ-opioid agonists (Pillai and Ross, 1986a; Spampinato et al., 1994). Hence, the purpose of the present study is to determine the effect of CCBs on various κ-opioid agonist-induced hypothermic response.

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Moreover, several studies indicate the existence of subtypes of κ-opioid receptors (Attali et al., 1982; Clark et al., 1989; Horan et al., 1993; Nock et al., 1988; Zukin et al., 1988). Radioligand binding and pharmacological studies demonstrate that κ-opioid agonists PD and U69,593 (U69) selectively and specifically bind to κ_1-opioid receptor subtype with high affinity (Clark et al., 1988; Horan et al., 1991, 1993; Lahti et al., 1985; Nock et al., 1988; Zukin et al., 1988). In contrast, U50 was shown to bind to a high-affinity but low-density κ_1-receptor subtype and a low-affinity but high-density κ_2-receptor subtype in rat brain (Attali et al., 1989; Clark et al., 1989; Nock et al., 1988; Zukin et al., 1988). It has been demonstrated that CCBs potentiate U50-induced hypothermia in rats (Pillai and Ross, 1986a). However, the effect of CCBs on highly selective and specific κ_1-opioid agonists U69- and PD-induced hypothermic effect is not known.

In the present investigation, NIM, a cerebroselective CCB (Langley and Sorkin, 1989), and another new highly lipophilic 1,4-dihydropyridine (DHP) CCB, lercanidipine (LER) (Testa et al., 1997), were selected to determine the role of Ca^{2+} channels in κ-opioid agonist-induced hypothermia. U50, PD and U69 were used to find out the effect of CCBs on three arylacetamide κ-opioid agonists having different degrees of selectivity at κ-opioid receptor subtypes.

2. Materials and methods

2.1. Animals

Experiments were performed on male Sprague–Dawley rats weighing 150–200 g (Central Animal Facility, NIPER, India). Rats were housed six per cage in a room with controlled ambient temperature (23 ± 1 °C), humidity (50 ± 10%) and light (07:00–18:00 h). Food and water were made available ad libitum. In all experiments, the animals were used only once and received a single dose of the opioid drug. All experiments were performed between 09:00 and 17:00 h to minimize diurnal variations. All experiments were duly approved by the Institutional Animal Ethics Committee (IAEC/99/004).

2.2. Drugs

The κ-opioid agonists U50 {*trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl] benzene acetamide methane sulfonate}, U69 {(5α,7α,8β)-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro (4,5) dec-8-yl)] bezene acetamide} and PD {(±)-trans-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl] benzo [*b*] thiophene-4-acetamide} were gift samples of Pharmacia and Upjohn, Kalamazoo, MI, USA and Parke-Davis, Ann Arbor, MI, USA, respectively. The CCBs NIM and LER were gifted by USV (India) and Recordati Industria Chimica, Italy, respectively. The κ-opioids were dissolved in distilled water and CCBs were solubilized in 20% dimethylsulfoxide (DMSO) + 20% ethanol + 60% distilled water. The vehicle or drugs were administered intraperitoneally (ip) in a volume of 1-ml/kg body weight of the rat.

2.3. Measurement of colonic temperature in rats

The colonic temperature was recorded using telethermometer as described previously (Nemmani et al., 2001) in male rats, which were pretreated with either vehicle or NIM (1 mg/kg ip; 15 min prior). The animals were acclimatized and initial basal temperature was determined as an average of two readings measured in a 15-min gap. The colonic temperature was recorded with minimal stress (unrestrained) to the animal by inserting the lubricated probe to a definite (6 cm) length. Body temperature was recorded up to 3 h (0, 30, 60, 90, 120 and 180 min) after administration of κ-opioid or its vehicle. The change in colonic temperature was calculated from the basal values and a graph was plotted as change in colonic temperature versus time. The hypothermic [area under the curve $(AUC)_{0-180\ min}$] activity was expressed as the mean ± S.E.M.

2.4. Effect of NIM and LER on U50-, PD- and U69-induced hypothermia in rat

The change in colonic temperature produced by U50 (7.5, 15, 22.5, 40 and 60 mg/kg ip), PD (7.5,15 and 22.5 mg/kg ip) and U69 (5 and 20 mg/kg ip) was determined in rats pretreated with either vehicle or NIM (1 mg/kg ip; 15 min prior).

The effect of vehicle or LER (0.3 mg/kg ip; 15 min prior) on change in colonic temperature produced by U50 (7.5, 15 and 22.5 mg/kg ip) and PD (7.5,15 and 22.5 mg/kg ip) was also determined in rats.

2.5. Statistics

The fall in colonic temperature and hypothermic response as represented by the $AUC_{0-180\ min}$ were compared using one-way analysis of variance (ANOVA) followed by the Tukey's test at a 5% level of significance ($P < .05$).

3. Results

3.1. Effect of NIM on U50-, PD- and U69-induced hypothermic response in rats

NIM (1 mg/kg ip) did not produce any change in colonic temperature when compared to vehicle-treated control group. The effect of NIM (1 mg/kg ip; 15 min prior) on

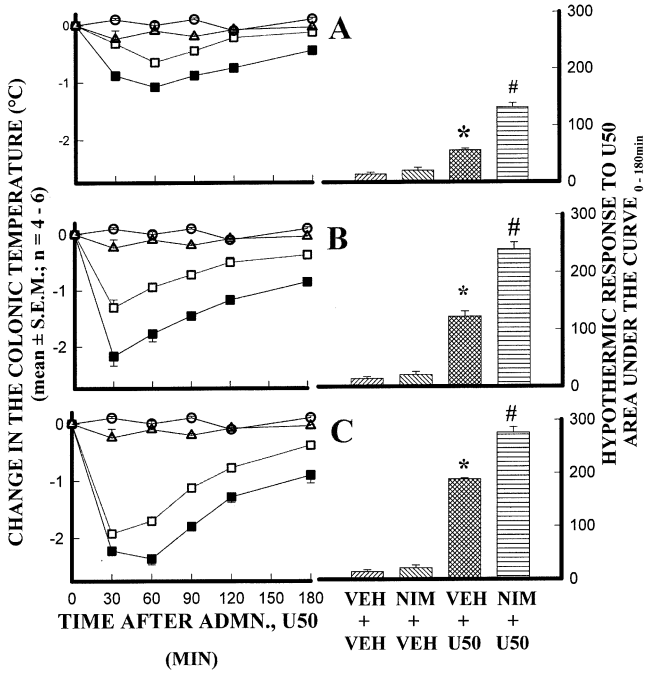

Fig. 1. Time course of action and $AUC_{0-180\ min}$ of change in colonic temperature response of U50 administered by intraperitoneal route to male Sprague–Dawley rats pretreated with either vehicle (VEH) or NIM (1 mg/kg ip). VEH + VEH (○), NIM + VEH (△), VEH + U50 (□) and NIM + U50 (■). Panel A 7.5 mg/kg, Panel B 15 mg/kg and Panel C 22.5 mg/kg. * $P < .05$ vs. vehicle control and NIM, # $P < .05$ vs. U50-injected group.

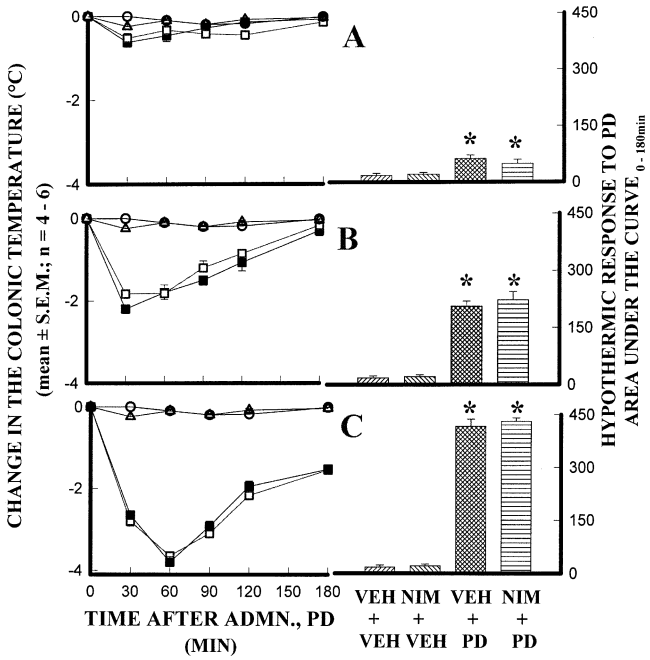

Fig. 3. Time course of action and $AUC_{0-180\ min}$ of change in colonic temperature response of PD administered by intraperitoneal route to male Sprague–Dawley rats pretreated with either vehicle (VEH) or NIM (1 mg/kg ip). VEH + VEH (○), NIM + VEH (△), VEH + PD (□) and NIM + PD (■). Panel A 7.5 mg/kg, Panel B 15 mg/kg and Panel C 22.5 mg/kg. * $P < .05$ vs. vehicle control and NIM.

U50 (7.5, 15, 22.5, 40 and 60 mg/kg ip)-induced hypothermia is shown in Figs. 1 and 2. U50 (7.5–40 mg/kg) produced significant hypothermia with a peak fall in temperature of 0.65, 1.30, 1.92 and 1.90 °C, respectively. A maximal fall of 1.90 °C was observed at 22.5- and 40-mg/kg doses of U50, whereas U50 (60 mg/kg) produced significantly less hypothermic response with a peak fall of only 1.67 °C (Fig. 2). In addition, U50 (80 mg/kg) produced

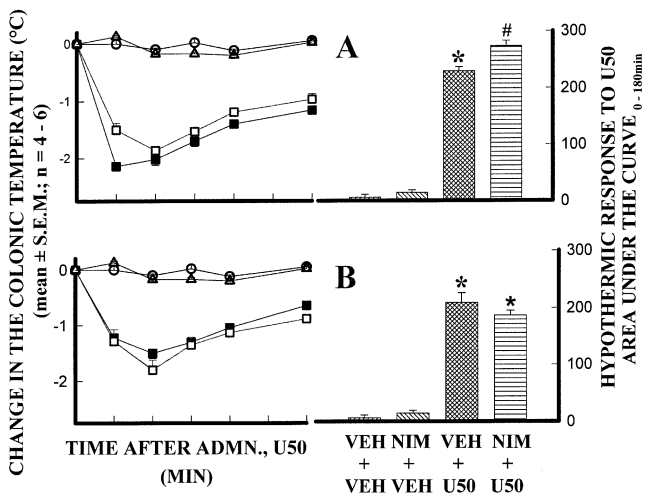

Fig. 2. Time course of action and $AUC_{0-180\ min}$ of change in colonic temperature response of U50 administered by intraperitoneal route to male Sprague–Dawley rats pretreated with either vehicle (VEH) or NIM (1 mg/kg ip). VEH + VEH (○), NIM + VEH (△), VEH + U50 (□) and NIM + U50 (■). Panel A 40 mg/kg and Panel B 60 mg/kg. * $P < .05$ vs. vehicle control and NIM, # $P < .05$ vs. U50-injected group.

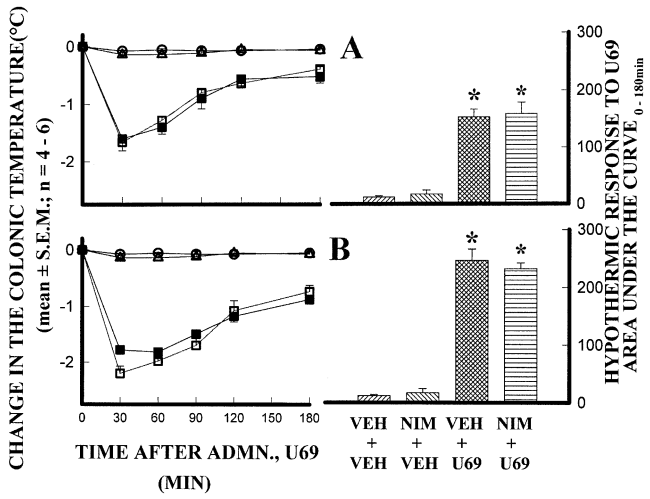

Fig. 4. Time course of action and $AUC_{0-180\ min}$ of change in colonic temperature response of U69 administered by intraperitoneal route to male Sprague–Dawley rats pretreated with either vehicle (VEH) or NIM (1 mg/kg ip). VEH + VEH (○), NIM + VEH (△), VEH + U69 (□) and NIM + U69 (■). Panel A 5 mg/kg and Panel B 20 mg/kg. * $P < .05$ vs. vehicle control and NIM.

much less fall in colonic temperature (1.47 °C) when compared to lower doses (data not shown). Pretreatment of NIM significantly potentiated the hypothermia produced by U50 (7.5–40 mg/kg) with a peak maximal fall of 0.88, 2.17, 2.36 and 2.20 °C, respectively (Figs. 1 and 2). However, NIM (1 mg/kg) did not alter the U50 (60 mg/kg)-induced hypothermic response (Fig. 2).

The effect of NIM (1 mg/kg ip) on hypothermic response of PD (7.5, 15 and 22.5 mg/kg ip) and U69 (5 and 20 mg/kg ip) is depicted in Figs. 3 and 4, respectively. PD produced a dose-dependent hypothermic response with a fall of 0.52, 1.83 and 3.65 °C in colonic temperature, respectively (Fig. 3). However, NIM did not modify the hypothermic response of PD at all the doses tested (Fig. 3). The highly selective and specific κ_1-opioid agonist U69 (5 and 20 mg/kg) produced a dose-dependent fall of 1.6 and 2.2 °C in colonic temperature, respectively. NIM failed to alter the U69 (5 and 20 mg/kg)-induced hypothermic response (Fig. 4).

3.2. Effect of LER on U50- and PD-induced hypothermic response in rats

The effect of LER (0.3 mg/kg ip; 15 min prior) on U50 (7.5, 15 and 22.5 mg/kg ip)- and PD (7.5, 15 and 22.5 mg/kg ip)-induced hypothermic response is shown in Figs. 5 and 6, respectively. LER significantly potentiated the dose-dependent hypothermic response of U50 (Fig. 5). In contrast, LER failed to alter the hypothermic response produced by PD (Fig. 6).

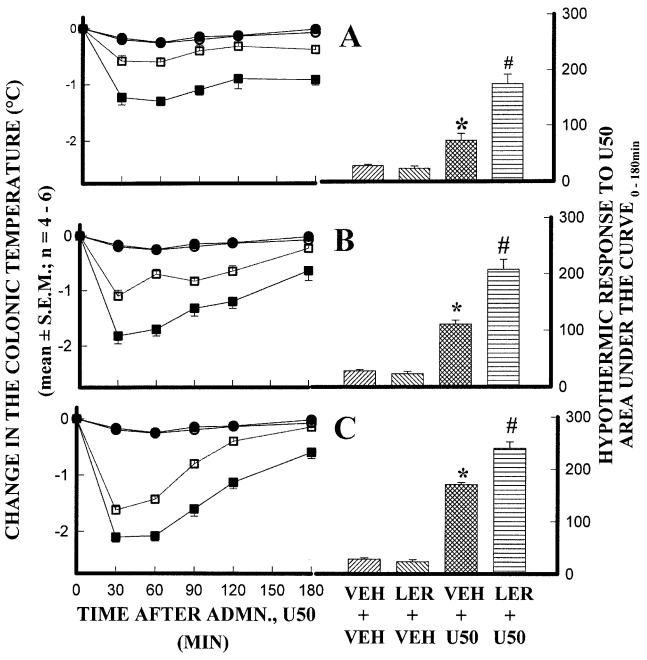

Fig. 5. Time course of action and $AUC_{0-180\ min}$ of change in colonic temperature response of U50 administered by intraperitoneal route to male Sprague–Dawley rats pretreated with either vehicle (VEH) or LER (0.3 mg/kg ip). VEH + VEH (○), LER + VEH (△), VEH + U50 (□) and LER + U50 (■). Panel A 7.5 mg/kg, Panel B 15 mg/kg and Panel C 22.5 mg/kg. $^{*}P<.05$ vs. vehicle control and LER, $^{\#}P<.05$ vs. U50-injected group.

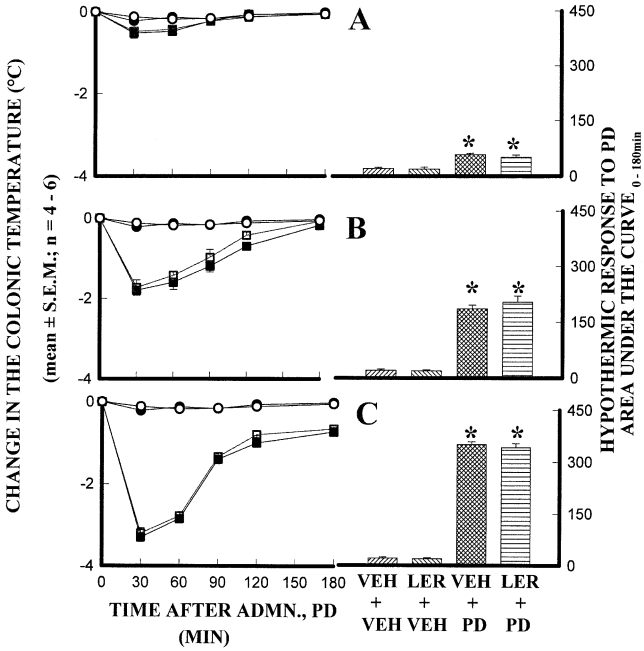

Fig. 6. Time course of action and $AUC_{0-180\ min}$ of change in colonic temperature response of PD administered by intraperitoneal route to male Sprague–Dawley rats pretreated with either vehicle (VEH) or LER (1 mg/kg ip). VEH + VEH (○), LER + VEH (△), VEH + PD (□) and LER + PD (■). Panel A 7.5 mg/kg, Panel B 15 mg/kg and Panel C 22.5 mg/kg. $^{*}P<.05$ vs. vehicle control and LER.

4. Discussion

The present findings for the first time provided the evidence that CCBs differentially modulate the hypothermic responses produced by κ-opioid agonists. NIM (1 mg/kg ip) and LER (0.3 mg/kg ip) did not affect the basal colonic temperature. The κ-opioid agonists U50, PD and U69 produced dose-dependent hypothermia in rats (Figs. 1–6) but CCBs selectively potentiated only U50-induced hypothermia, indicating differential modulation of κ-opioid agonist-induced fall in colonic temperature by these agents.

U50 (7.5–40 mg/kg) and PD (7.5–22.5 mg/kg) produced dose-dependent hypothermia. These findings are in line with our previous study (Nemmani et al., 2001) and other reports (Pillai and Ross, 1986a; Shukla et al., 1995; Spampinato et al., 1994; Spencer et al., 1988). Pretreatment of NIM (1 mg/kg ip; 15 min prior) potentiated the U50-induced hypothermia in rats (Figs. 1 and 2). Similarly, Pillai and Ross (1986a) demonstrated that CCBs potentiate the U50-induced hypothermia. In contrast, in the present study, NIM failed to modify the hypothermic response of PD in rats (Fig. 3). To confirm these findings, the effect of LER (0.3 mg/kg ip),

another CCB on U50- and PD-induced hypothermic response, was studied. Similar to NIM, LER selectively potentiated U50-induced hypothermia but not that of PD (Figs. 5 and 6). The present results suggest that κ_1-opioid receptor-induced hypothermia is insensitive to modulation by CCBs (Ca^{2+}-insensitive). The findings indicate that NIM differentially modulates the hypothermia produced by κ-opioid agonists. This differential effect of NIM on κ-opioid agonists may be due to their differing selectivity to subtypes of κ-opioid receptors. Several studies indicate that PD selectively bind to κ_1-opioid receptors with high affinity (Clark et al., 1988; Horan et al., 1993; Nock et al., 1988). In contrast, U50 was shown to bind κ_1- and κ_2-receptor sites in biochemical and pharmacological studies (Attali et al., 1989; Nock et al., 1988; Zukin et al., 1988). Thus, the differential modulation of U50- and PD-induced hypothermia by NIM may be due to their differing selectivity to subtypes of κ-opioid receptors. To confirm this hypothesis, we have studied the effect of NIM on highly selective and specific κ_1-opioid receptor agonist U69-induced hypothermic response (Horan et al., 1993; Lahti et al., 1985; Nock et al., 1988; Zukin et al., 1988). Interestingly, NIM also did not potentiate hypothermic response produced by U69 (Fig. 4). The results show that PD- and U69-induced hypothermia is insensitive (Ca^{2+}-insensitive) and U50 hypothermia is sensitive (Ca^{2+}-sensitive) to modulation by CCBs. Further, it may be suggested that κ_1-opioid receptor-mediated hypothermia is insensitive to modulation by CCBs.

In the present study, U50 produced a maximum of 1.90 °C fall in colonic temperature at 22.5- and 40-mg/kg dose of U50 (Figs. 1 and 2), whereas U50 (60 mg/kg) produced significantly less hypothermia with a 1.67 °C fall in colonic temperature (Fig. 2). In addition, U50 (80 mg/kg) produced still less fall (1.47 °C) in colonic temperature (data not shown). These observations suggest that U50 produce opposing effects on colonic temperature. U50 (10–100 μg icv) produced dose-dependent hypothermia (Spencer et al., 1988). However, U50 (300 μg icv) produced an early hypothermia followed by a strong hyperthermic phase (Spencer et al., 1988). In our studies, the maximum observable fall in colonic temperature produced by U50 (7.5–80 mg/kg ip) was only 1.90 °C (Figs. 1, 2 and 5). In contrast, PD (22.5 mg/kg) produced significantly greater fall (3.65 °C) in colonic temperature (Figs. 3 and 6). These results suggest that the overall response at each dose of U50 may possibly a combination of hypothermic and hyperthermic responses. Hence, the potentiation of U50-induced hypothermia by CCBs may be due to the blockade of possible hyperthermic phase produced by U50's action on subtypes of κ-opioid receptors. The potentiation of hypothermic response by NIM (1 mg/kg) was observed to be maximal at low doses of U50 (Figs. 1 and 5) than at high doses (Fig. 2). The lack of potentiation may be due to the inability of NIM (1 mg/kg) to block the opposing effects of U50 at higher dose. Based on the available literature, it can be attributed that the complex response of U50 may be due to its effects on different subtypes of κ-opioid receptors. In addition, U50 (300 μg icv) was shown to produce a slowly developing hyperthermia preceded by an early hypothermia (Spencer et al., 1988). The qualitative differences in temperature effects because of the route of administration are common. Morphine administered systemically is known to produce both hyperthermia and hypothermia in rats depending on the dose, whereas it produces only hyperthermia when administered intracerebroventricularly (Adler and Geller, 1993; Burks, 1991). The hyperthermic phase produced by U50 (300 μg icv) may be due to differences in neuroanatomical distribution of the κ-opioid receptor subtypes especially in hypothalamic and thalamic areas (Robson et al., 1985; Zukin et al., 1988). Both κ_1- and κ_2-opioid receptors of rat were of moderate density in the dorsal hypothalamus and more κ_2-opioid receptors were found in ventromedial nucleus of the hypothalamus (Zukin et al., 1988). Moreover, κ_2-opioid receptors were detected in high density throughout the thalamic region compared to selective localization of κ_1-opioid receptors in thalamus (Zukin et al., 1988). It may be speculated that the late hyperthermic phase produced by U50 (300 μg icv) is possibly due to its effect on low-affinity but high-density κ_2-opioid receptors in hypothalamic region of rats. Although the radioligand studies show U50 to bind κ_1- and κ_2-receptor sites (Attali et al., 1989; Zukin et al., 1988), the latest studies suggest that κ_2 receptor is actually a heterodimer of κ- and δ-receptors (Jordan and Devi, 1999). This may indicate general loss of selectivity of U50 for κ-opioid vs. other opioid receptors. Moreover, studies show evidence for two U50-sensitive κ_1-subtypes of receptors (Clark et al., 1989). Further studies using subtype-selective agonists aid in better understanding of the pharmacological responses mediated by κ-opioid receptor subtypes. Based on the present results, it can only be suggested that κ_1-opioid receptor activation lead to hypothermic response, which was insensitive to CCBs (Ca^{2+}-insensitive), whereas, U50-induced hypothermia is sensitive (Ca^{2+}-sensitive) to modulation by CCBs.

In order to prove that Ca^{2+}-sensitive mechanisms modulate the hypothermic effects of U50 by activation of subtype of κ-opioid receptor other than κ_1 type, further studies were done in guinea pigs. It was reported that more than 85% of κ-opioid receptors in guinea pig were of U69-sensitive κ_1-subtype with the negligible population of κ_2-opioid receptors (Zukin et al., 1988). In addition, our results show that κ_1-opioid agonist U69-induced hypothermia was not potentiated by NIM in rats. NIM should not potentiate U50-induced hypothermia in guinea pigs since κ-opioid receptors are mainly κ_1-subtype. As expected, our preliminary studies show that NIM (1 mg/kg ip) did not modify U50 (22.5 mg/kg ip)-induced hypothermia in guinea pigs (data not shown). These results further confirm that κ_1-opioid receptor-mediated hypothermia is insensitive to CCBs (Ca^{2+}-insensitive) and Ca^{2+}-sensitive mechanisms modulate the

hypothermic effects of U50 by activation of subtype of κ-opioid receptor other than κ_1 type.

The exact mechanisms responsible for hypothermic effect of κ-opioid receptors are not clearly known. It was shown that U50-induced hypothermia was due to changes in intracellular calcium $[Ca^{2+}]_i$ levels in hypothalamic region (Pillai and Ross, 1986a). The hypothermic effect of U50 in rats was accompanied by an enhanced Ca^{2+}/Mg^{2+} –ATPase activity in synaptosomal fractions obtained from hypothalamus (Pillai and Ross, 1986a). This enzyme is responsible for maintaining $[Ca^{2+}]_i$ levels in nerve terminals by extrusion of Ca^{2+} from intracellular to extracellular sites (Pillai and Ross, 1986a). Pretreatment with CCBs, NIM, diltiazem and verapamil potentiated the hypothermic effect of U50 by stimulating Ca^{2+}/Mg^{2+} –ATPase activity in hypothalamus and thus resulting in decrease in $[Ca^{2+}]_i$ levels (Pillai and Ross, 1986a). The present study suggests that blockade of Ca^{2+} influx through L-type channels by CCBs result in potentiation of U50 but not PD- and U69-induced hypothermic response. Moreover, Ca^{2+} channel agonist BAY K8644 (3 mg/kg) upon systemic administration produced potent hypothermia in freely moving rats (Pillai and Ross, 1986b).

On the other hand, verapamil (ICU) was reported to antagonize the U50 (ICU)-induced hypothermia (Spampinato et al., 1994). This may be due to different route of administration of verapamil employed in the study. It was proposed that Ca^{2+} movements in discrete regions of the hypothalamus can change the set point for thermoregulation (Myers, 1985). It was shown that verapamil when perfused within anterior hypothalamic/preoptic area produce a dose-dependent hypothermia (Beleslin et al., 1985; Rezvani et al., 1986). Conversely, verapamil perfused into posterior hypothalamus (PH) evoked an intense dose-dependent hyperthermia (Beleslin et al., 1985; Rezvani et al., 1986), whereas excess Ca^{2+} (25-mM $CaCl_2$) perfused into PH evoked a hypothermic response (Beleslin et al., 1985). Moreover, Ca^{2+} channel agonist BAY K8644 (3 mg/kg) upon systemic administration (subcutaneous) produced potent hypothermia in freely moving rats (Pillai and Ross, 1986b). Thus, it is extremely difficult to interpret the sequence of events underlying this differential response produced by drug treatments through different routes of administration. In addition, Spampinato et al. (1994) proposed that peripheral vasodilation induced by CCBs might be playing role in the potentiation of κ-opioid receptor agonist-mediated hypothermia. If so, CCBs should have potentiated the hypothermia produced by all the three κ-opioid receptor agonists studied. The differential modulation of κ-opioid agonist-induced hypothermia by CCBs indicate that peripheral vasodilation may not be the possible mechanism.

In conclusion, the above findings suggests that CCBs differentially modulate κ-agonist-induced hypothermia, suggesting that Ca^{2+}-sensitive and -insensitive mechanisms are involved in the κ-opioid receptor-induced changes in body temperature.

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