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Possible mechanisms for insulin-induced attenuation of the antinociceptive effect of [D-Ala^2, *N*-MePhe^4, Gly-ol^5]enkephalin

Masahiro Ohsawa [a], Shyun-ichi Tanaka [b], Junzo Kamei [a,*]

[a] *Department of Pathophysiology and Therapeutics, Faculty of Pharmaceutical Sciences, Hoshi University, 4-41, Ebara 2-Chome, Shinagawa-ku, Tokyo 142-8501, Japan*

[b] *Department of Neurobiology of Aging Laboratories, The Mount Sinai School of Medicine, New York, NY 10029-6574, USA*

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Abstract

The effects of pretreatment with protein kinase C and protein kinase A inhibitors on the intraventricular insulin-induced attenuation of the antinociceptive effect of [D-Ala^2, *N*-MePhe^4, Gly-ol^5]enkephalin (DAMGO) were studied in mice. Intracerebroventricular (i.c.v.) pretreatment with insulin dose- and time-dependently attenuated the antinociceptive effect of i.c.v. DAMGO (5.6 ng) in mice. Intracerebroventricular pretreatment with a highly selective tyrosine kinase inhibitor, herbimycin A, at doses of 200 and 600 ng for 70 min, dose-dependently reversed the attenuation of the antinociceptive effect of DAMGO (5.6 ng, i.c.v.) caused by insulin. Furthermore, i.c.v. pretreatment with serine/threonin kinase inhibitor, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine hydrochloride (H7), at doses of 3–30 nmol for 60 min, dose-dependently reversed the attenuation of the antinociceptive effect of DAMGO (5.6 ng, i.c.v.) caused by insulin. Intracerebroventricular pretreatment with selective protein kinase C inhibitor, calphostin C, at doses of 1 and 3 pmol for 60 min, but not with a highly protein kinase A inhibitor, (8*R*, 9*S*, 11*S*)-(−)-9-hydroxy-9-*n*-hexyloxy-carbonyl-8-methyl-2, 3, 9, 20-tetrahydro-8, 11-epoxy-1*H*, 8*H*, 11*H*-2, 7*b*, 11*a*-triaqzadibenzo[*a*, *g*]cycloocta[*c*, *d*, *e*]-trinden-1-one (KT5720), at dose of 10 pmol for 60 min, reversed the attenuation of the antinociceptive effect of DAMGO (5.6 ng, i.c.v.) caused by insulin. These results suggest that the reduction of DAMGO-induced antinociception by insulin in mice may be, in part, due to the activation of protein kinase C followed by the activation of tyrosine kinase.

Keywords: Antinociception; μ-Opioid receptor; Protein tyrosine kinase; Protein kinase C; Calphostin C; (Mouse)

1. Introduction

Insulin's action is initiated when it binds to specific receptors on the plasma membrane of cells. The widespread distribution of insulin receptors in the brain (Baskin et al., 1986; Corp et al., 1986; Hill et al., 1986; Marks et al., 1988; Moss et al., 1990), suggests that insulin has important functions in the central nervous systems. Considerable evidence indicates that insulin may function as a central nervous system neurotrophic factor and neuromodulator (Baskin et al., 1988). It has been reported that insulin inhibits neuronal firing in the hippocampus (Palovick et al., 1984) and hypothalamus (Shibata et al., 1986). In addition, insulin modulates monoamine uptake in cultured neuronal cells (Boyd et al., 1985) and increases catecholamine turnover and release from central neurons (Sauter et al., 1983). Furthermore, we recently reported that intraventricular injection of insulin attenuates the antinociceptive effect of [D-Ala^2, *N*-MePhe^4, Gly-ol^5]enkephalin (DAMGO) in mice (Kamei et al., 1998).

Many investigators have indicated that insulin increases the activity of protein kinase C (for review, see Saltiel and Cuatrecasas, 1988). It has been reported that activation of protein kinase C is involved in mediating some of insulin's actions. Insulin activates a highly selective phospholipase C, which cleaves a unique glucophospholipid, resulting in the generation of diacylglycerol (an endogenous activator of protein kinase C) and inositol-glycan compounds in several tissues (Saltiel and Cuatrecasas, 1986). Phorbol esters, tumor-promoting agents that activate protein kinase C, mimic several of insulin's actions, including the regulation of fructose 2,6-bisphosphate levels (Bosca et al., 1985), stimulation of pyruvate dehydrogenase, amino acid uptake, glucose transport (Farese et al., 1985) and phosphorylation of ribosomal protein S6 (Trevillyan et al.,

* Corresponding author. Tel.: +81-3-5498-5030; Fax: +81-3-5498-5029; E-mail: kamei@hoshi.ac.jp

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1985). In cultured neurons, insulin activates the protein kinase C, especially μ-isoform (Heidenreich et al., 1990). Furthermore, protein kinase C inhibitor, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine hydrochloride (H7), completely counteracted the stimulation of endogenous dopamine release induced by insulin in rat tuberoinfundibular neurons (Amoroso et al., 1990). Thus, it is possible that the various physiological actions of insulin are mediated by the activation of protein kinase C.

The molecular analysis of the opioid receptors indicated that they conform to the structural motif of G-protein receptor family, including the seven conserved hydrophobic domains. All opioid receptors contain consensus protein kinase sites in the first and third intracellular loops, and in the C-terminal domain (Miotto et al., 1995). It is possible that the phosphorylation of opioid receptor by protein kinases desensitizes the function of opioid receptors. Indeed, it has been reported that incubation of NG108-15 hybrid cells with the phorbol ester, 12-*O*-tetradecanoylphorbol-13-acetate, abolished up to 45% of the opioid-induced inhibition of cyclic AMP accumulation in intact cells (Louie et al., 1990). Furthermore, it has been reported that the activation of protein kinase C by treatment of an oocyte with phorbol ester potentiated the desensitization of a μ-opioid receptor-induced current (Chen and Yu, 1993). We recently reported that the activation of protein kinase C by phorbol 12,13-dibutyrate leads to desensitization of μ-opioid receptor-mediated antinociception (Narita et al., 1996; Ohsawa and Kamei, 1997) and Straub tail reaction (Ohsawa and Kamei, 1998). Thus, the aim of this study was to investigate the possible involvement of protein kinase C in the insulin-induced reduction of the antinociceptive effect of DAMGO in mice.

2. Materials and methods

2.1. Animals

Male ICR mice weighing about 25 g were purchased from Tokyo Animal Laboratory, Tokyo, Japan. They had free access to food and water in an animal room which was maintained at 22 ± 1°C with a 12-h light–dark cycle. This study was carried out in accordance with the Declaration of Helsinki and/or with the guide for the care and use of laboratory animals as adopted by the committee on the care and use of laboratory animals of Hoshi University, which is accredited by the Ministry of Education, Science, Sports and Culture.

2.2. Antinociceptive assay

The antinociceptive response was evaluated by recording the latency in the tail-flick test using radiant heat as a stimulus. The intensity of the thermal stimulus was adjusted so that the animal flicked its tail in 2–4 s. A cut-off latency of 30 s was used to prevent injury to the tail. Animals which did not respond within 30 s were removed and assigned a score of 30 s. The percent maximum possible effect (%MPE) was calculated for each animal as %MPE = 100 × (post-drug latency − pre-drug latency)/(30 − pre-drug latency).

2.3. Intracerebroventricular (i.c.v.) injection

Intracerebroventricular administration was performed following the method described by Haley and McCormick (1957) using a 50-μl Hamilton syringe. The injection site was 1.5 mm from the midline, 0 mm from the bregma and 3.0 mm from the surface of the skull. Injection volumes were 5 μl for each i.c.v. administration.

2.4. Drugs

The following drugs were used: [D-Ala^2, *N*-MePhe^4, Gly-ol^5]enkephalin (DAMGO; Peninsula Laboratories, San Carlos, CA, USA), hervimycin A (Calbiochem–Novabiochem International, San Diego, CA, USA), 1-(5-isoquinolinesulfonyl)-2-methylpiperazine hydrochloride (H7, Research Biochemicals, Natick, MA, USA), (8*R*, 9*S*, 11*S*)-(−)-9-hydroxy-9-*n*-hexyloxy-carbonyl-8-methyl-2, 3, 9, 20-tetrahydro-8, 11-epoxy-1*H*, 8*H*, 11*H*-2, 7*b*, 11*a*-triaqzadibenzo[*a*, *g*]cycloocta[*c*, *d*, *e*]-trinden-1-one (KT5720, Calbiochem–Novabiochem International, San Diego, CA, USA), calphostin C (Calbiochem–Novabiochem International, San Diego, CA, USA) and procine insulin (Biomedical Technologies, Stoughton, MA, USA). KT5720 and calphostin C were dissolved in ethanol 0.1% in saline (0.9% NaCl solution). Hervimycin A was dissolved in 0.6% dimethyl sulfoxide (DMSO) in saline. DAMGO, H7 and insulin were dissolved in saline.

2.5. Data analysis

The data are expressed as means ± S.E.M. The statistical significance of differences between groups was assessed with an analysis of variance (ANOVA) followed by the Bonferroni test.

3. Results

3.1. Effect of insulin on the antinociceptive effect of DAMGO

As shown in Fig. 1A, pretreatment with insulin (3 mU, i.c.v.) for 60 min, but not for 30 min, progressively attenuated DAMGO-induced antinociception. Furthermore, pretreatment with insulin (1 and 3 mU, i.c.v.) 60 min prior to i.c.v. challenge with DAMGO dose-dependently attenuated the antinociceptive effect of DAMGO (5.6 ng) (Fig. 1B).

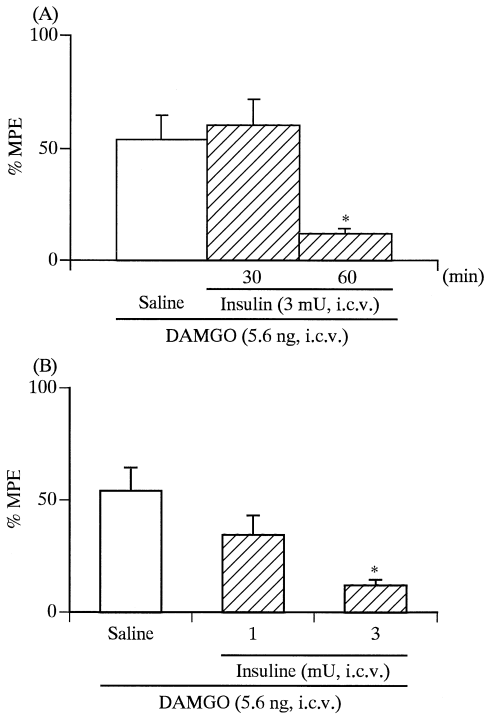

Fig. 1. (A) Effect of insulin (3 mU) on DAMGO-induced antinociception in mice after different duration of pretreatment time. (B) Effect of i.c.v. pretreatment with different doses of insulin on DAMGO-induced antinociception in mice. Insulin was injected i.c.v. 30 or 60 min (A) and 60 min (B) before the administration of DAMGO (5.6 ng, i.c.v.). The antinociceptive effect was examined 10 min after the injection of DAMGO using the tail-flick test. Each column represents the mean with S.E.M. for 9–10 mice in each group. $^{*}P < 0.05$ vs. respective saline-treated group.

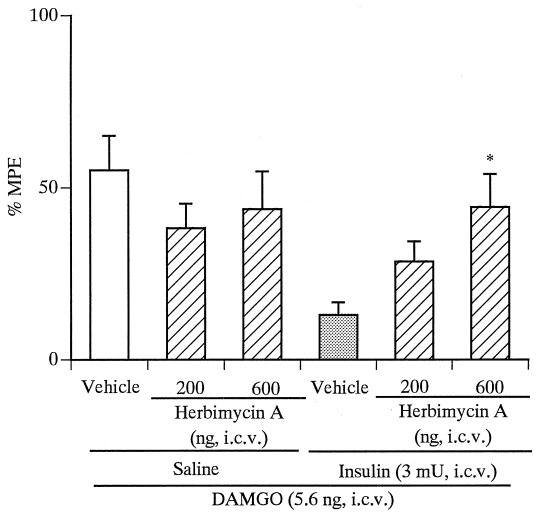

Fig. 2. The effects of a tyrosine kinase inhibitor, herbimycin A (200 and 600 ng, i.c.v.), on the insulin (3 mU, i.c.v.)-induced reduction of DAMGO (5.6 ng, i.c.v.)-induced antinociception. Insulin and herbimycin A were injected 60 min before the administration of DAMGO. The antinociceptive effect was examined 10 min after the injection of DAMGO using the tail-flick test. Each column represents the mean with S.E.M. for 9–12 mice in each group. $^{*}P < 0.05$ vs. respective saline-pretreated group. $^{\#}P < 0.05$ vs. respective vehicle-treated group.

The effects of herbimycin A on the antinociceptive effect of DAMGO in insulin-pretreated mice are shown in Fig. 2. Pretreatment with herbimycin A for 60 min, at doses of 200 and 600 ng, i.c.v. dose-dependently reversed the insulin-induced suppression of the antinociceptive effect of DAMGO (5.6 ng, i.c.v.). Intracerebroventricular pretreatment with herbimycin A did not affect DAMGO (5.6 ng, i.c.v.)-induced antinociception in saline-treated mice.

3.2. Effects of i.c.v. pretreatment with protein kinase A and C inhibitors on the insulin-induced attenuation of the antinociceptive effect of DAMGO

As shown in Fig. 3, i.c.v. pretreatment with a serine/threonin kinase inhibitor, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine hydrochloride (H7), at doses of 3–30 nmol, i.c.v., reversed the attenuation of DAMGO (5.6 ng, i.c.v.)-induced antinociception by insulin (3 mU, i.c.v.) (Fig. 3). Intracerebroventricular pretreatment with H7 did not affect the antinociceptive effect of DAMGO in saline-treated mice (Fig. 3). The effects of protein kinase C inhibitor, calphostin C, and protein kinase A inhibitor, (8*R*, 9*S*, 11*S*)-(−)-9-hydroxy-9-*n*-hexyloxy-carbonyl-8-methyl-2, 3, 9, 20-tetrahydro-8, 11-epoxy-1*H*, 8*H*, 11*H*-2, 7*b*, 11*a*-triazadibenzo[*a*, *g*]cycloocta[*c*, *d*, *e*]-trinden-1-one (KT5720), on the insulin-induced reduction of the antinociceptive effect of DAMGO (5.6 ng, i.c.v.) are shown in Fig. 4. Intracerebroventricular pretreatment with calphostin C, at doses of 1 and 3 pmol, dose-dependently

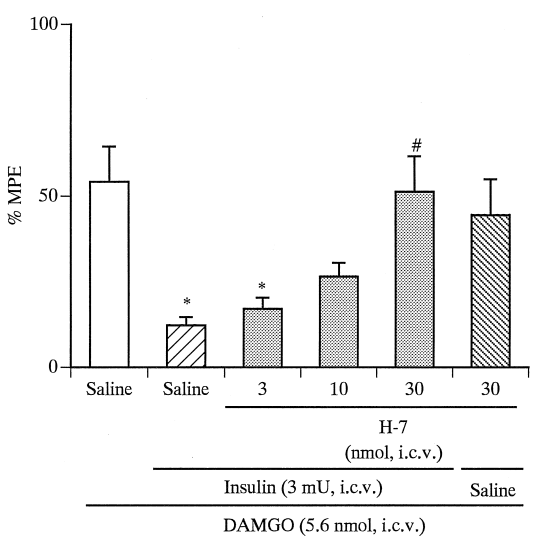

Fig. 3. The effect of a serine/threonin kinase inhibitor, H7 (3–30 nmol, i.c.v.), on the insulin (3 mU, i.c.v.)-induced reduction of DAMGO (5.6 ng, i.c.v.)-induced antinociception. Insulin and H7 were injected 60 min before the administration of DAMGO. The antinociceptive effect was examined 10 min after the injection of DAMGO using the tail-flick test. Each column represents the mean with S.E.M. for 9–12 mice in each group. $^{*}P < 0.05$ vs. respective saline-pretreated group. $^{\#}P < 0.05$ vs. respective saline-treated group.

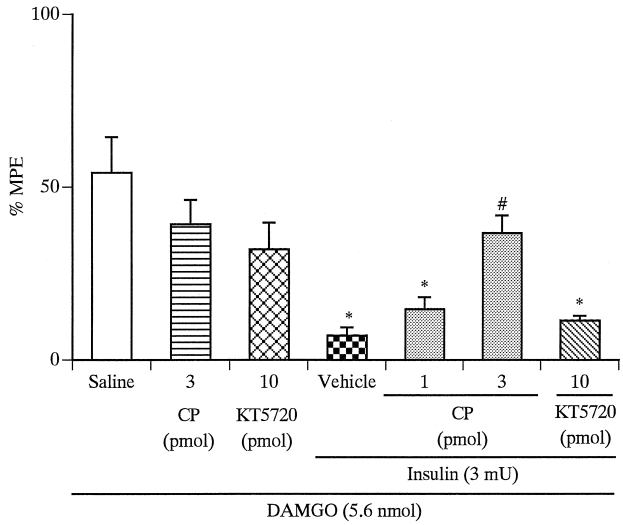

Fig. 4. The effects of a protein kinase C inhibitor, calphostin C (CP, 1 and 3 pmol, i.c.v.), and a protein kinase A inhibitor, KT5720 (10 pmol, i.c.v.), on the insulin (3 mU, i.c.v.)-induced reduction of DAMGO (5.6 ng, i.c.v.)-induced antinociception. Insulin, calphostin C and KT5720 were injected 60 min before the administration of DAMGO. The antinociceptive effect was examined 10 min after the injection of DAMGO using the tail-flick test. Each column represents the mean with S.E.M. for 9–12 mice in each group. $^{*}P < 0.05$ vs. respective saline-pretreated group. $^{\#}P < 0.05$ vs. respective vehicle-treated group.

reversed the reduction of the antinociceptive effect of DAMGO by insulin (3 mU). On the other hand, i.c.v. pretreatment with KT5720, at a dose of 10 pmol, did not affect the insulin-induced reduction of DAMGO-induced antinociception. Furthermore, DAMGO (5.6 ng, i.c.v.)-induced antinociception, by itself, was not affected by the i.c.v. pretreatment with calphostin C (3 pmol) and KT5720 (10 pmol) in saline-treated mice.

4. Discussion

The results of this study indicated that i.c.v. pretreatment with insulin attenuated the antinociceptive effect of i.c.v. DAMGO in mice. This reduction in DAMGO-induced antinociception by insulin was reversed by i.c.v. pretreatment with herbimycin A, a selective protein tyrosine kinase inhibitor (Omura et al., 1979; Uehara and Fukagawa, 1991). These results are consistent with our previous results (Kamei et al., 1998). In our previous study (Kamei et al., 1998), we observed that i.c.v. pretreatment with lavendustin A, another selective protein tyrosine kinase inhibitor (Onoda et al., 1989; O'Dell et al., 1991), reversed the attenuation of DAMGO-induced antinociception by insulin. Thus, it is possible that the insulin-induced attenuation of DAMGO-induced antinociception in mice is particularly mediated by the activation of tyrosine kinase.

In the present study, we indicated that i.c.v. pretreatment with serine/threonin kinase inhibitor, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine hydrochloride (H7), completely reversed the attenuation of DAMGO-induced antinociception by insulin. Furthermore, the insulin-induced reduction of DAMGO-induced antinociception was reversed by i.c.v. pretreatment with selective protein kinase C inhibitor, calphostin C, but not with a selective protein kinase A inhibitor, (8*R*, 9*S*, 11*S*)-(−)-9-hydroxy-9-*n*-hexyloxy-carbonyl-8-methyl-2, 3, 9, 20-tetrahydro-8, 11-epoxy-1*H*, 8*H*, 11*H*-2, 7*b*, 11*a*-triaqzadibenzo[*a*, *g*]cycloocta[*c*, *d*, *e*]-trinden-1-one (KT5720). These results suggest that the insulin-induced attenuation of DAMGO-induced antinociception may be due to the activation of protein kinase C. Many investigators have indicated that insulin promotes the activation of protein kinase C (Cooper et al., 1987; Heidenreich et al., 1993). It has been reported that when neurons were treated with insulin prior to cell fractionation, protein kinase C activity was higher than that in control cells in both the cytosolic and membrane fractions (Heidenreich et al., 1990). Furthermore, the stimulation of protein kinase C activity was maximal within 5–10 min of exposure to insulin (Heidenreich et al., 1990). In the present study, at least 60 min of pretreatment were required for insulin to attenuate DAMGO-induced antinociception. We previously reported that at least 30–60 min of pretreatment time was required for phorbol ester, a protein kinase C activator, to desensitize μ-opioid receptor-mediated responses (Narita et al., 1996). Therefore, it is possible that the attenuation of DAMGO-induced antinociception by insulin may be due to desensitization of μ-opioid receptor induced by the activation of protein kinase C.

Many investigators have indicated that insulin activates protein kinase C in several tissues. Furthermore, several mechanisms apparently underlie the insulin-induced activation of protein kinase C. Insulin receptors regulate cellular processes through the activation of cytoplasmic domains containing intrinsic tyrosine kinase activity. After receptor autophosphorylation, several cytoplasmic proteins associate with the membrane-bound receptors though src homology 2 (SH2) domains to propagate second-messenger signaling (White et al., 1985; Ullrich and Schlessinger, 1990; Pawson and Grish, 1992). It has been reported that the signaling intermediates, which consist of phospholipase Cγ, $p21^{ras}$-GTPase-activating protein, and phosphatidylinositol 3-kinase, bind to the insulin receptor substrate-1 peptide upon stimulation with insulin (White et al., 1985; Myers et al., 1994; Wang et al., 1994; White and Kahn, 1994). The activation of phospholipase Cγ leads directly to an increase in the activity of downstream components of phosphatidylinositol metabolism, i.e., to changes in protein kinase C enzyme activity. On the other hand, it is indicated that the insulin-induced increases in membrane protein kinase C activity were due to an increase in diacylglycerol, resulting from de novo phosphatidic acid synthesis (Bosca et al., 1985). In the present study, pretreatment with calphostin C reversed the insulin-induced attenuation of

DAMGO-induced antinociception. Calphostin C particularly inhibits the binding of diacylglycerol to the regulatory domain of protein kinase C (Kobayashi et al., 1989). Thus, it is possible that the insulin-induced activation of protein kinase C may be due to an increase in diacylglycerol levels followed by the activation of tyrosine kinase.

5. Conclusion

Insulin attenuates μ-opioid receptor-mediated antinociception in mice. Furthermore, this insulin-induced reduction of μ-opioid receptor-mediated antinociception may be due to the desensitization of μ-opioid receptors by the activation of protein kinase C, followed by the activation of tyrosine kinase.

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