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Short communication

Effects of a μ-opioid receptor agonist on G-protein activation in streptozotocin-induced diabetic mice

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Abstract

Many clinical and experimental studies have suggested that diabetes or hyperglycemia alter pain sensitivity, and sensitivity to several drugs. It has been reported that the antinociceptive potency of morphine is decreased in several rodent models of hyperglycemia, including streptozotocin-induced diabetes, an animal models of type I diabetes. The present study was designed to investigate in streptozotocin-induced diabetic mice the effect of the selective μ-opioid agonist [D-Ala^2, NMePhe^4, Gly-ol^5]enkephalin (DAMGO) on G-protein activation by monitoring guanosine-5′-*O*-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPγS) binding to pons/medulla membranes, which contain the key areas for opioid antinociception. In the tail-flick test, DAMGO (1–10 ng, intracerebroventricularly) produced a marked dose-dependent antinociception in non-diabetic mice. In streptozotocin-induced diabetic mice, the effect of DAMGO was significantly attenuated as compared to that in non-diabetic mice. In the [^{35}S]GTPγS binding assay, DAMGO (0.1–10 μM) increased the binding of [^{35}S]GTPγS to pons/medulla membranes from non-diabetic mice in a concentration-dependent manner, affording approximately 100% maximal stimulation at 10 μM. The maximal stimulation of [^{35}S]GTPγS binding by DAMGO (10 μM) in streptozotocin-induced diabetic mice (100.55 ± 3.12%), was similar to non-diabetic mice. The present results indicated that the antinociceptive effect of DAMGO given supraspinally was less potent in streptozotocin-induced diabetic mice than that in non-diabetic mice, whereas the μ-opioid receptor-mediated G-protein activation in pons/medulla was unaltered in streptozotocin-induced diabetic mice. Thus, the attenuation of DAMGO-induced antinociception in streptozotocin-induced diabetic mice is probably caused by dysfunction in cellular pathways after the activation of G-proteins.

Keywords: μ-Opioid receptor; G-protein; Antinociception; Pons/medulla; Diabetes

1. Introduction

It has been reported that the antinociceptive potency of morphine is decreased in several rodents models of hyperglycemia, including a spontaneously diabetic strain of mice and streptozotocin-induced diabetic mice, an animal model of type I diabetes (Simon and Dewey, 1981). We previously reported that the antinociceptive potency of intracerebroventricular (i.c.v.) administration of μ-opioid receptor agonists, such as morphine and [D-Ala^2, NMePhe^4, Gly-ol^5]enkephalin (DAMGO), in streptozotocin-induced diabetic mice were significantly less than those in non-diabetic mice (Kamei et al., 1994b; Ohsawa et al., 1998). It has been suggested that the reduction in the antinociceptive potency of μ-opioid receptor agonists in streptozotocin-induced diabetic mice is due to the dysfunction of supraspinal μ-opioid receptors (Kamei et al., 1992). However, there is little information available regarding the mechanism responsible for these changes.

The μ-opioid receptor has been recently cloned and this receptor belongs to the superfamily of seven-transmembrane domain receptors that are coupled to G-proteins

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(Chen et al., 1993). The agonist binding to μ-opioid receptors subsequently induce the activation of the G_i/G_o class of G proteins, which can be measured by assessing agonist stimulation of membrane binding of the non-hydrolyzable analog of GTP, guanosine-5′-*O*-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPγS)(Traynor and Nahorski, 1995; Narita et al., 1998). The increase of [^{35}S]GTPγS binding induced by the selective μ-opioid receptor agonist DAMGO has been reported in membranes from human neuroblastoma SH-SY5Y cells (Traynor and Nahorski, 1995), SK-N-SH cells (Selley et al., 1997a), C6 glioma cells (Emmerson et al., 1996), CHO cells (Selley et al., 1997b; Sim et al., 1995), rat striatum (Sim et al., 1995), rat locus coeruleus (Selley et al., 1997a), mouse spinal cord (Narita et al., 1998, 1999), and mouse pons/medulla (Mizoguchi et al., 1999). Such μ-opioid receptor agonist-stimulated [^{35}S]GTPγS binding in membrane preparations has provided dynamic measurements of agonist occupations of μ-opioid receptors and its efficacy for activation of G-proteins.

The pons/medulla contains both the nucleus reticularis gigantocellularis and the raphe nuclei, which have been implicated in the induction of μ-opioid receptor agonist-induced antinociception (Takagi et al., 1978). It has been reported that locus coeruleus modulates the symptoms of μ-opioid withdrawal (Guitart and Nestler, 1993). Therefore, investigation of the coupling of μ-opioid receptors to G-proteins in this region provides significant information about μ-opioid receptor antinociception and intracellular signaling.

The present study was then designed to investigate the effect of μ-opioid receptor agonists on [^{35}S]GTPγS binding to pons/medulla membrane from streptozotocin-induced diabetic mice.

2. Materials and methods

2.1. Animals

Male ICR mice (Charles River Breeding Laboratories, Wilmington, MA) weighing 15–20 g at the beginning of the experiments were used. Animals were housed five per cage in a room maintained at 22 ± 0.5°C with an alternating 12-h light–dark cycle. Food and water were available ad libitum. Animals were used only once. Animals were rendered diabetic by an injection of streptozotocin (200 mg/kg, i.v.) prepared in saline adjusted to pH 4.5 in 0.1 N citrate buffer. Age-matched non-diabetic mice were injected with the vehicle alone. The experiments were conducted 2 weeks after the injection of streptozotocin or vehicle. Mice with serum glucose levels above 400 mg/dl were used as the diabetic mice. All experiments were approved by and conformed to the guidelines of the Medical College of Wisconsin Animal Care Committee.

2.2. Antinociceptive assay

Antinociception was determined by the tail-flick test (D'Amour and Smith, 1941). For measurement of the latency of the tail-flick response, mice were gently held by hand with their tail positioned in an apparatus (Model TF6, EMDIE Instrument, Maidens, VA) for radiant heat stimulation on the dorsal surface of the tail. The intensity of heat stimulus was adjusted so that the animal flicked its tail after 3–5 s. The intensity of the heat was not different between diabetic and non-diabetic mice. The inhibition of the tail-flick response was expressed as percent maximum possible effect, ''%MPE'', which was calculated as: $[(T_1 - T_0)/(T_2 - T_0)] \times 100$, where T_0 and T_1 were the tail-flick latencies before and after the injection of opioid agonist and T_2 was the cut-off time which was set at 10 s for the tests to avoid injury to the tail.

2.3. I.c.v. injection

The i.c.v. administration was performed as described by Haley and McCormick (1957) using a 10-μl Hamilton syringe. Injection volumes for i.c.v. administration were 4 μl.

2.4. Membrane preparations

Mice were killed by decapitation, the pons/medulla was rapidly excised at 4°C, and the tissue was homogenized using a Potter–Elvehjem tissue grinder with a Teflon pestle in 20 times volumes (w/v) of ice-cold Tris–Mg^{2+} buffer containing 50 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$ and 1 mM EGTA. The homogenate was centrifuged at 4°C for 10 min at $48{,}000 \times g$. The pellet was resuspended in ice-cold assay buffer containing 50 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$, 1 mM EGTA, and 100 mM NaCl, and centrifuged at 4°C for 10 min at $48{,}000 \times g$. The resultant pellet was resuspended in ice-cold assay buffer and stored at −70°C until used.

2.5. [^{35}S]GTPγS binding assay

The membrane suspensions (3–8 μg of protein/assay) were incubated 25°C for 2 h in assay buffer with various concentration of the DAMGO, 30 μM GDP, and 50 pM [^{35}S]GTPγS (1000 Ci/mmol; Amersham, Arlington Heights, IL) in a total volume of 1 ml. The reaction was terminated by filtering through Whatman GF/B glass filters, which had been previously soaked in 50 mM Tris–HCl (pH 7.4) and 5 mM $MgCl_2$. The filters were then washed three times with 50 mM Tris–HCl (pH 7.4) at 4°C and transferred to scintillation counting vials. Subsequently, 0.5 ml of Soluene-350 (Packard Instrument, Meriden, CT) and 4 ml of Hionic Fluor Cocktail (Packard

Instrument) were added to the vials. After a 12-h equilibration period, the radioactivity in the samples was determined with a liquid scintillation analyzer (Model 1600 CA, Packard Instrument). Non-specific binding was measured in the presence of 10 μM unlabeled GTPγS. Comparable results were obtained from more than three independent sets of experiments.

2.6. Drugs

The drugs used were: streptozotocin (Sigma, St. Louis, MO). DAMGO (Bachem California, Torrance, CA), GTPγS (RBI, Natick, MA), and guanosine-5′-diphosphate (GDP; Sigma).

2.7. Statistical analysis

The data are expressed as the mean ± S.E.M. The statistical significance of differences between groups was assessed with an analysis of variance (ANOVA) followed by the Newman–Keuls test and the potency ratio (Tallarida and Murray, 1987).

3. Results

The dose–response curve for DAMGO-induced antinociception is shown in Fig. 1. DAMGO injected i.c.v. produced a dose-dependent increase in the inhibition of the tail-flick response in non-diabetic mice and streptozotocin-induced diabetic mice. Mice with diabetes were significantly less sensitive to DAMGO-induced antinociception. The ED_{50} values and (95% confidence limits) for the antinociceptive effect of DAMGO were 5.24 (2.60–9.01) ng in non-diabetic mice and 10.69 (4.00–28.59) ng in diabetic mice. The dose–response curve for DAMGO-induced antinociception was shifted to the right 2.3-fold (95% confidence limits; 1.56–2.69) (Fig. 1).

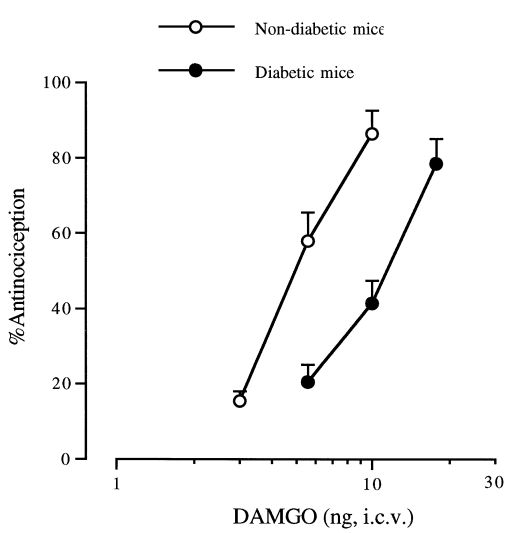

Fig. 1. Dose–response curve for the DAMGO-induced antinociception in streptozotocin-induced diabetic (filled circle) and non-diabetic (empty circle) mice. The tail-flick response was assessed 10 min after the injection of DAMGO. Each point represents the mean ± S.E.M. for 10 mice in each group.

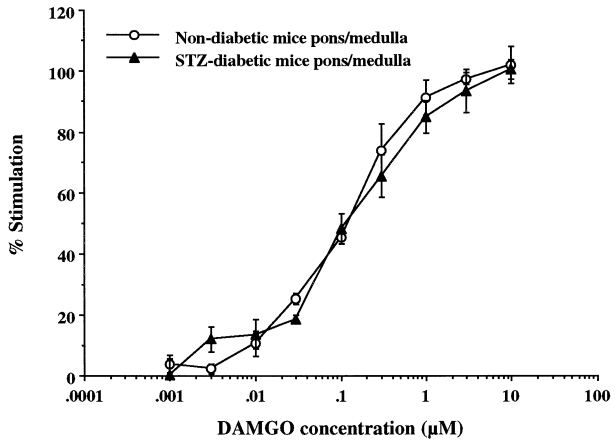

Fig. 2. Concentration–effect curve for DAMGO-stimulated [^{35}S]GTPγS binding to the pons/medulla membranes obtained from streptozotocin-induced diabetic and non-diabetic mice. Membranes were incubated with 50 pM [^{35}S]GTPγS and 30 μM GDP with and without various concentration of DAMGO for 2 h at 25°C. The data are expressed as the percentage of basal [^{35}S]GTPγS binding measured in the presence of GDP, and represent the mean ± S.E.M. from at least three independent experiments. The basal [^{35}S]GTPγS binding activity in diabetic mice was not different from that in the non-diabetic mice (44.94 ± 2.09 fmol/mg protein for diabetic mice, 50.75 ± 3.22 fmol/mg protein for non-diabetic mice).

The concentration–effect curve for DAMGO-stimulated [^{35}S]GTPγS binding to the pons/medulla membranes from non-diabetic and streptozotocin-induced diabetic mice is shown in Fig. 2. DAMGO increased [^{35}S]GTPγS binding in a concentration-dependent and saturable manner in non-diabetic and streptozotocin-induced diabetic mice. DAMGO at 10 μM produced a maximal stimulation of [^{35}S]GTPγS binding of 101.77 ± 6.01 and 100.55 ± 3.12 in non-diabetic and streptozotocin-induced diabetic mice, respectively. There was no significant difference in the concentration-effect curve for DAMGO-stimulated [^{35}S]GTPγS binding between non-diabetic and streptozotocin-induced diabetic mice.

4. Discussion

In agreement with previous studies showing a decreased sensitivity for μ-opioid receptor agonist-induced antinociception in diabetic mice (Simon and Dewey, 1981; Kamei et al., 1992, 1994b; Ohsawa et al., 1998). The potency of DAMGO-induced antinociception was significantly, but weakly, attenuated in diabetic mice. In contrast to the behavioral experiment, there was no differences in

DAMGO-stimulated [^{35}S]GTPγS binding to pons/medulla membrane between non-diabetic and streptozotocin-induced diabetic mice. μ-Opioid receptor agonist-stimulated [^{35}S]GTPγS binding in membrane preparation has provided dynamic measurements of agonist occupation of μ-opioid receptors and its efficacy for activation of G-proteins. Therefore, the present results suggest that there are no differences in the μ-opioid receptors-mediated G-protein activation between non-diabetic and streptozotocin-induced diabetic mice. Recently, it has been reported that the K_D and B_{max} values for [^{3}H]DAMGO binding to cerebral μ-opioid receptor was not altered by diabetes (Courteix et al., 1998). Thus, coupling of μ-opioid receptors to G-protein may not be altered in diabetic mice.

In the present study, we have shown that μ-opioid receptor-mediated activation of G-proteins may not be a key component for the attenuation of μ-opioid receptor agonist-induced antinociception in diabetic mice. Recently, we reported that an enhancement of protein kinase C activity in streptozotocin-induced diabetic mice may be involved in the attenuation of DAMGO-induced antinociception, since the protein kinase C inhibitor calphostin C reversed the attenuation of DAMGO-induced antinociception in streptozotocin-induced diabetic mice (Ohsawa and Kamei, 1998). Moreover, we previously reported that the enhancement of intracellular calcium concentration and the dysfunction of ATP-sensitive K^+ channel may be involved in the attenuation of μ-opioid receptor agonist-induced antinociception in streptozotocin-induced diabetic mice (Kamei et al., 1994a; Ohsawa et al., 1998). The above evidence strongly supports the contention that the attenuation of μ-opioid receptor agonist-induced antinociception in streptozotocin-induced diabetic mice is caused by the alterations in second/third messengers or ion channels.

In conclusion, the present data indicate that functional coupling of μ-opioid receptors and G-proteins were not affected in streptozotocin-induced diabetic mice. Therefore, the attenuation of μ-opioid receptor mediated antinociception in streptozotocin-induced diabetic mice may not be caused by the dissociation of μ-opioid receptor–G protein coupling. The attenuation of μ-opioid receptor-mediated antinociception in streptozotocin-induced diabetic mice may be caused by the alterations of second/third messengers and/or ion channels.

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