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Short communication

Decreased opioid-induced antinociception but unaltered G-protein activation in the genetic-diabetic NOD mouse

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Abstract

Previous evaluation of antinociceptive action in experimental diabetes has been conducted almost exclusively in chemically induced diabetes mellitus. The purpose of the present study was to evaluate antinociceptive response and G-protein activation by μ-opioid receptor and δ-opioid receptor agonists in the genetic non-obese diabetic (NOD) mouse, a model of type I insulin-dependent diabetes mellitus (IDDM). Tail-flick latency before and after hyperglycemia was unaltered. Hyperglycemic NOD mice were hyporesponsive to intracerebroventricular (i.c.v.) injections of [D-Ala^2]deltorphin II but not to [D-Ala^2, *N*-MePhe^4, Gly-ol^5]enkephalin (DAMGO); however, G-protein activation in pons/medulla assessed by [^{35}S]GTPγS binding was not diminished. This suggests that a G-protein defect in signaling cannot account for the hyporesponsiveness of antinociception in this genetic model of IDDM.

Keywords: Opioid; Diabetes mellitus; Antinociception; G-protein; μ-Opioid receptor; δ-Opioid receptor

1. Introduction

Hypersensitivity to pain is a significant problem in the management of pain in diabetic patients. This problem may be manifested by diabetes-induced alteration of pain via peripheral and/or central mechanisms. Morphine, an opioid analgesic agent, is frequently used to modulate pain in patients. Morphine is believed to produce antinociception via activation of μ-opioid receptors. Previous studies have shown that supraspinal injection of μ-opioid receptor agonists elicits reduced antinociception in chemically induced diabetic animal models (Rady et al., 1998; Kamei et al., 1992b; Kamei et al., 1993). In contrast, response to a δ-opioid receptor agonist such as [D-Phen^{2,5}]enkephalin is unaltered in streptozotocin-induced diabetic mice (Kamei et al., 1992a).

Previous studies of diabetes-induced alterations in supraspinal analgesia achieved by opioid agonists have been performed exclusively in chemically induced diabetic animal models. In contrast, commercial sources for spontaneous genetic models of diabetes mellitus have become increasingly available for scientific evaluation of diabetes-associated disorders. Nevertheless, there has been essentially no experimental studies of supraspinal-antinociception in genetic models of type I insulin-dependent diabetes mellitus (IDDM). This is important to rule out any possible latent and peripheral toxic effects of diabetogenic chemical toxins on nociception and to verify that altered antinociception occurs in other experimental models of IDDM which are more relevant to this autoimmune disease.

Accordingly, the purpose of this study is to evaluate basal tail-flick response latency and agonist-induced antinociception in the non-obese diabetic (NOD) mouse, a genetic model of IDDM. We evaluated μ-opioid receptor-mediated antinociceptive responses using a selective μ-opioid receptor agonist ([D-Ala^2, *N*-Me Phe^4, Gly-ol^5]enkephalin, DAMGO). The μ-opioid receptor stimulated

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antinociception in this genetic model was also compared to responses elicited by the δ-opioid receptor agonist, [D-Ala^2]deltorphin II, as a control for these studies. Since G-protein activation is known to be coupled to μ-opioid receptor and δ-opioid receptor activation (Tsuji et al., 1999; Narita et al., 1999), we also determined [^{35}S]GTPγS binding as a functional measurement to assess G-protein activation in response to agonists from both control and diabetic animals.

2. Methods

2.1. Animals

Female diabetic-prone NOD mice (10 weeks of age) were obtained from Taconic Labs (Taconic, Germantown, NY, USA). Animals were housed, three 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. Glycemia was monitored using a drop of tail-vein blood, acquired by nicking the tail with a scalpel blade and assessed by glucose-test strips and glucometer (Medisense, Cambridge, MA, USA). Animals were considered to be hyperglycemic with a blood glucose value above 200 mg/dl. Antinociception was evaluated after 2 weeks of hyperglycemia. Normoglycemic controls were used for each day of testing.

2.2. Antinociception assessed by intracerebroventricular (i.c.v.) injection

The procedure involved a radiant heat tail-flick response established in the laboratory (Xu et al., 1995). For the 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, USA) for radiant heat stimulation on the dorsal surface of the tail. The intensity of the heat stimulus used for the chronic monitoring of basal latency was adjusted so that the animal flicked its tail after 3–5 s. Because of the possibility that latency might be increased following hyperglycemia, the normal heat intensity was adjusted lower for the studies involving agonist-induced antinociception. 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 injection of opioid receptor agonist and T_2 was the cut-off time which was set at 10 s. The peptides were administered i.c.v. according to the method of Haley and McCormick (1957), using a 4-μl injection volume.

2.3. Membrane preparation

Mice were sacrificed by decapitation, the pons/medulla was rapidly excised at 4°C, and the tissue was homogenized with 20 volumes (w/v) of ice-cold buffer containing 50 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$ and 1 mM EGTA using a Potter-Elvehjem tissue grinder with a Teflon pestle. 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 a second time. The resultant pellet was resuspended in assay buffer and stored at −70°C until used for analysis.

2.4. [^{35}S]GTPγS binding assay

Functional assay of opioid receptor activation was performed by a [^{35}S]GTPγS binding previously established in the laboratory (Narita et al., 1999). The membrane homogenate (3–8 μg protein/assay) was incubated at 25°C for 2-h in 1 ml of assay buffer with 10 μM opioid receptor agonists, 30 μM GDP and 50 pM [^{35}S]GTPγS (specific activity of 1000 Ci/mM, Amersham, Arlington Heights, IL, USA). The reaction was terminated by filtrations using a Brandel cell harvester (Model M-24, Brandel, MD, USA) and Whatman GF/B glass filters, presoaked in 50 mM Tris–HCl (pH 7.4) and 5 mM $MgCl_2$ at 4°C for 2-h. Filters were then washed three times with 5 ml of Tris–HCl buffer (pH 7.4), transferred to scintillation counting vials containing 0.5 ml of Soluene-350 (Packard Instrument, Meriden, CT, USA) and 4 ml of the scintillation cocktail Hionic Fluor (Packard Instrument), equilibrated for 12-h. The radioactivity in the samples was determined with a liquid scintillation analyzer (Model 1600CA, Packard Instrument). Non-specific binding was measured in the presence of 10 μM unlabelled GTPγS. Comparable results were obtained from at least three independent sets of experiments. Activation of μ- or δ-opioid receptors was achieved using maximum concentrations of DAMGO or SNC-80, ((+)-4-[(α*R*)-α-((2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide). [D-Ala^2]deltorphin II (a peptide agonist) and SNC-80 (a non-peptide agonist) are equieffective in activating δ-opioid receptors in vivo. Recent studies in our laboratory have shown that G-protein activation by δ-opioids in pons/medulla of the normal mouse was much weaker than that elicited by μ-opioid receptor activation (Mizoguchi et al., 2000). To maximize the response to δ-opioid receptor agonist, we elected to use SNC-80 because of the potential of destabilization of the peptide agonist, [D-Ala^2]deltorphin II, due to binding to glassware used in the in vitro reaction system.

2.5. Drugs

The drugs were DAMGO (Bachem California, Torrance, CA, USA), [D-Ala^2]deltorphin II (Molecular Re-

search Laboratories, Durhan, NC, USA), (+)-4-[(α*R*)-α-((2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide (SNC-80, Tocris Cookson, Ballwin, MO, USA), guanosine-5′-*O*-(3-thio)triphosphate tetralithium (GTPγS: RBI, Natick, MA, USA) and guanosine-5′-diphosphate (GDP, Sigma, St. Louis, MO, USA).

2.6. Statistics

Differences were compared by Student's *t* test or by analysis of variance followed by Newman-Keul's test for two group means or multiple group means, respectively. A *P* value of 0.05 was set to designate statistical significance.

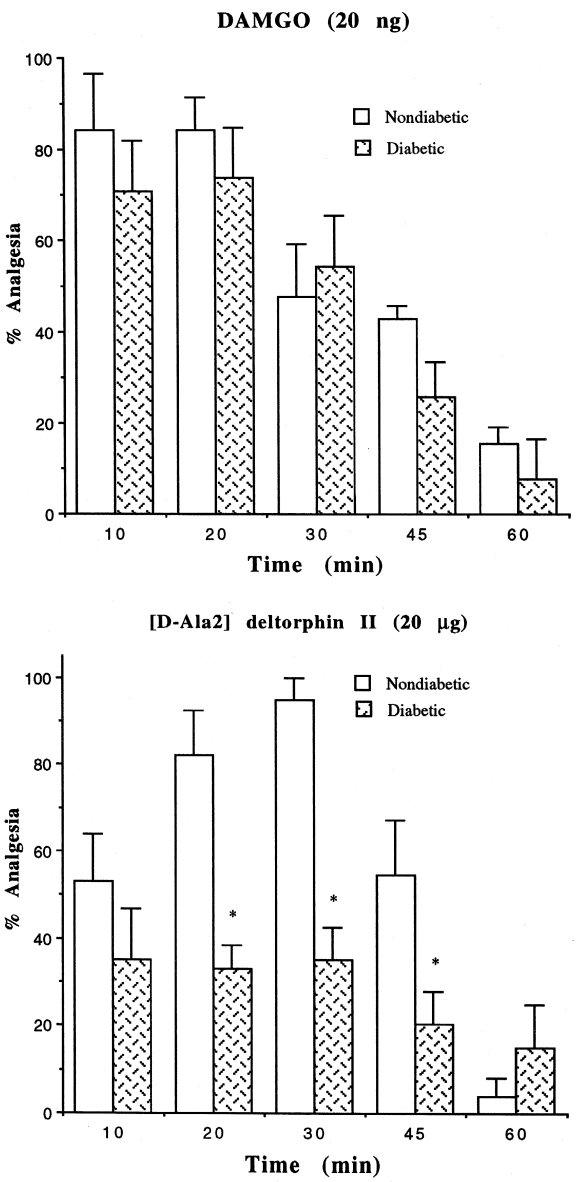

Fig. 1. Antinociceptive effect of i.c.v.-treated DAMGO or [D-Ala^2]deltorphin II in nondiabetic and diabetic NOD mice. Each bar represents the mean ± S.E.M. of $n = 8$–12 or $n = 7$–8 determinations, each for DAMGO or [D-Ala^2]deltorphin II, respectively. $^*P < 0.01$ vs. nondiabetic controls.

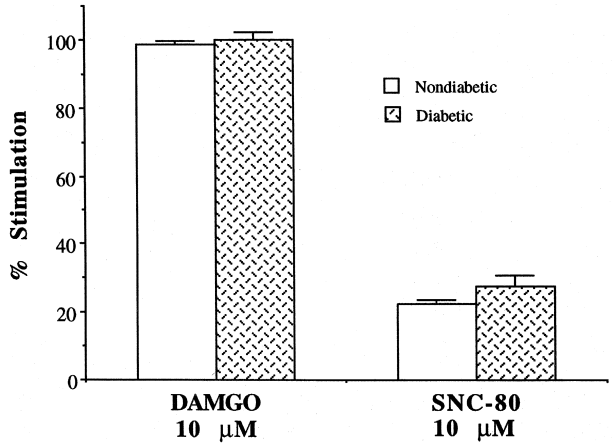

Fig. 2. The stimulation of [^{35}S]GTPγS binding by DAMGO and SNC-80 in pons/medulla membrane in nondiabetic and diabetic NOD mice. Each bar represents the mean ± S.E.M. of $n = 6$ determinations.

3. Results

Blood glucose levels at the time of agonist challenge was significantly ($P < 0.001$) higher in hyperglycemic NOD mice (414 ± 18 mg/dl) compared to normoglycemic NOD mice (96 ± 5 mg/dl) littermates. Body weights were significantly lower ($P < 0.01$) in hyperglycemic mice (hyperglycemic: 19.2 ± 0.3 g; normoglycemic: 22.1 ± 0.5 g).

In the absence of agonist challenge, the tail-flick latency for all animals was unaltered by diabetes (nondiabetic, $n = 55$: 6.1 ± 0.1 s; prediabetic, $n = 44$: 6.2 ± 0.1 s; post-diabetic, $n = 44$: 6.1 ± 0.1 s). In preliminary studies, we determined that 20 ng of DAMGO or 20 μg [D-Ala^2]deltorphin II were optimal doses for these studies. Thus, agonist-induced antinociception is reported at that dose. The percent analgesia induced by [D-Ala^2]deltorphin II (Fig. 1, lower panel), but not by DAMGO (Fig. 1, upper panel), was significantly decreased in diabetic compared to nondiabetic mice.

To evaluate G-protein activation coupled to opioid receptor activation, we also determined the increase in [^{35}S]GTPγS binding in pons/medulla membrane after stimulation with either DAMGO or SNC-80 (Fig. 2). DAMGO produced an increase in [^{35}S]GTPγS binding in both normoglycemic and hyperglycemic NOD mice; however, there was no difference in the stimulation between these two groups. For SNC-80, the magnitude of stimulation was less marked than for DAMGO with a similar magnitude in [^{35}S]GTPγS binding in the hyperglycemic compared to normoglycemic mice.

4. Discussion

In the present study, i.c.v injection of a δ-opioid receptor agonist produced diminished antinociception in the

genetic diabetic NOD mouse. To our knowledge, this is the very first report of altered antinociceptive action of any i.c.v.-injected δ-opioid receptor agonist in any genetic model of IDDM. Previous studies of antinociception in experimental diabetic animals have been performed almost exclusively in chemically induced diabetic models. Since IDDM is an autoimmune disease, it could not previously be assured that prior reports in streptozotocin-induced diabetic animals models were representative of IDDM, or to peripheral toxic effects of the diabetogenic agent which might be peculiar to streptozotocin. In contrast to our study of supraspinal administration of opioid agonists in the NOD mouse, the study of antinociception in genetic diabetic animals has been limited to studies using the *db/db* mouse, a model of non-insulin-dependent (NIDDM) diabetes mellitus (Kamei et al., 1998; Takeshita and Yamaguchi, 1998). These studies have also been limited to subcutaneous administration of the μ-opioid receptor agonist. In this model, conflicting findings have been observed. For example, morphine-induced antinociception was either unaltered using the formalin test (Takeshita and Yamaguchi, 1998), or the sensitivity to morphine was decreased using the tail-flick test (Kamei et al., 1998). The reason for this discrepancy is not clear, but may result from activation of different nociceptive pathways by individual tests.

Previous studies have indicated a reduced potency of morphine-induced antinociception in streptozotocin-induced and genetic *db/db* diabetic mice. This defect could not be accounted for by decreased μ-opioid receptor numbers or decreased affinity for μ-opioid receptor (Simon and Dewey, 1991; Brase et al., 1987). This study conducted in a model of NIDDM suggests that this type of diabetes mellitus does not produce a generalized alteration in μ-opioid receptor number or agonist affinity. Because of this finding, we performed alternative experiments to determine antinociception and G-protein activation using DAMGO (a selective μ-opioid receptor agonist) vs. SNC-80 (a δ-opioid receptor agonist). To our understanding, this would be the first evaluation of G-protein activation by opioid receptor agonists in a genetic model of IDDM.

G-protein activation is coupled to μ-opioid receptor and δ-opioid receptor activation (Tsuji et al., 1999; Narita et al., 1999). G-protein function in neuronal tissue is unknown in diabetes mellitus but is known to be altered in non-neuronal tissue (Bushfield et al., 1990). Our studies utilizing [^{35}S]GTPγS binding assay reveal no alteration in G-protein activation, produced by DAMGO or SNC-80, in hyperglycemic NOD mice compared to normoglycemic NOD mice littermates controls. These results are consistent with recent findings in our group, showing unaltered G-protein function in response to DAMGO in streptozotocin-induced diabetic mice (Ohsawa et al., 1999). Thus, we conclude under the present conditions in genetic diabetic NOD mice that there is no down-regulation of either μ-opioid receptor-mediated antinociception or G-protein activation in this model. In contrast, our studies suggest that a defect in δ-opioid receptor agonist-mediated antinociception likely arises by a defect in the signal transduction pathway defect, which is distal to G-protein activation in supraspinal sites. For ganglionic neurons of the spontaneous diabetic Bio-Breeding/Wistar (BB/W) rat (Hall et al., 1996), opioid-mediated calcium signaling via G-proteins results in diminished calcium signaling. It is possible, but untested, that decreased calcium signaling distal to G-protein activation in the spinal cord might explain the altered supraspinal analgesia of δ-opioid receptor agonist in the genetic diabetic NOD mice.

In streptozotocin-induced diabetic mice, the antinociceptive effect of a δ-opioid receptor agonist, [D-Pen^{2,5}]enkephalin, was unaltered (Kamei et al., 1992a). In the present study using a δ-opioid receptor agonist, [D-Ala^{2}]deltorphin II, we observed that the antinociceptive effect was decreased in the genetic NOD mouse. Thus, it appears that information derived in chemically induced diabetic models cannot always be extrapolated to genetic models of IDDM.

In summary, the present study is novel in at least two instances. First, it is the first report documenting hyposensitive action of δ-opioid receptor-mediated antinociception in a genetic model of IDDM. Secondly, it is also novel in showing that this defect in by δ-opioid receptor-mediated antinociception cannot be accounted for by defective G-protein activation by δ-opioid receptor agonist, but more likely from some undefined signal transduction step distal to G-protein activation in the supraspinal site.

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