ELSEVIER

European Journal of Pharmacology 369 (1999) 319–323

ejp

Short communication

# Algogenic mediator-induced nociceptive response in diabetic mice

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Received 27 January 1999; accepted 5 February 1999

**Abstract**

The duration of the somatostatin-, bradykinin- or prostaglandin $F_{2\alpha}$-induced nociceptive response was significantly less in diabetic mice than in non-diabetic mice. Subcutaneous injection of 7-benzylidenenaltrexone (0.1, 0.3 and 1 mg/kg), an antagonist of $\delta_1$-opioid receptors, had no significant effect on either somatostatin-, bradykinin- or prostaglandin $F_{2\alpha}$-induced nociceptive responses in non-diabetic mice. 7-Benzylidenenaltrexone (0.1 and 0.3 mg/kg, s.c.) also had no significant effect on somatostatin- or prostaglandin $F_{2\alpha}$-induced nociceptive responses in diabetic mice. However, the bradykinin-induced nociceptive response in diabetic mice was dose-dependently and significantly increased when 7-benzylidenenaltrexone (0.1, 0.3 and 1 mg/kg, s.c.) was injected 10 min before the injection of bradykinin. These results suggest that a spinal $\delta_1$-opioid receptor-mediated endogenous antinociceptive system may inhibit the bradykinin-mediated nociceptive responses in the second phase of the formalin-induced nociceptive response in diabetic mice. 

*Keywords:* Nociception formalin-induced ; $\delta_1$-Opioid receptor; 7-Benzylidenenaltrexone; Naltriben; Diabetes; (Mouse)

## 1. Introduction

Injection of formalin into the hind paw of mice produced a biphasic nociceptive response consisting of immediate (first-phase) and tonic (second-phase) components. We previously demonstrated that although the duration of the first-phase response was significantly longer in diabetic mice than in non-diabetic mice, the second phase was significantly shorter in diabetic mice (Kamei et al., 1993a). When spantide, an antagonist of substance P, reduced the duration of the nociceptive response in the first phase to levels observed in non-diabetic mice, the second-phase nociceptive response appeared (Kamei et al., 1993a). Moreover, the second-phase nociceptive response also became apparent in diabetic mice after pretreatment with naltrindole, a selective δ-opioid receptor antagonist (Kamei et al., 1993a). Furthermore, we also reported that the second-phase nociceptive response also became apparent in diabetic mice after pretreatment with 7-benzylidenenaltrexone, a selective $\delta_1$-opioid receptor antagonist, but not with naltriben, a selective $\delta_2$-opioid receptor antagonist (Kamei et al., 1994a). On the other hand, we demonstrated that diabetic mice displayed greater swimming stress-induced antinociception, which is primarily mediated by δ-opioid receptors, than non-diabetic mice (Kamei et al., 1992c, 1994a). Furthermore, the normally redundant 'backup' antinociceptive system, which is also mediated by δ-opioid receptors, is enhanced in diabetic mice (Kamei et al., 1992b,c). Based on these results, although the detailed mechanism is not known, we proposed that $\delta_1$-opioid receptor-mediated endogenous antinociceptive systems may inhibit the second-phase of formalin-induced nociceptive response in diabetic mice.

Formalin induces a long-lasting nociceptive response in the mouse paw, and this effect has been widely used to model persistent tonic pain of moderate intensity which involves chemical irritation, some tissue damage, and the formation of edema due to the release of inflammatory mediators (Hunskaar et al., 1985; Hunskaar and Hole, 1987; Murray et al., 1988). This nociceptive model usually involves two distinct phases. It has been proposed that the early phase reflects the direct stimulation of nociceptors, while the late phase may be associated with the release of inflammatory mediators (Dubuisson and Dennis, 1977;

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0014-2999/99/$ - see front matter 
PII: S0014-2999(99)00076-X

Hunskaar et al., 1985, 1986; Tjolsen et al., 1992). In addition, formalin-induced persistent pain is thought to resemble clinical pain due to its tonic nature (Dennis and Melzack, 1979; Abbott et al., 1982; Abbott and Franklin, 1986). On the other hand, there is evidence that substance P and inflammatory mediators (e.g., somatostatin, bradykinin, and prostaglandins) participate in the first and second phases of the formalin-induced nociceptive response, respectively (Shibata et al., 1989; Ohkubo et al., 1990). Moreover, i.t. administration of substance P or inflammatory mediators, by themselves, produces a nociceptive behavioral response, consisting of scratching, biting and licking, in mice.

In the present study, to clarify the possible mechanisms of the δ-opioid receptor-mediated reduction in the second phase of the formalin-induced nociceptive response in diabetic mice, we examined the effects of 7-benzylidenenaltrexone, a selective $\delta_1$-opioid receptor antagonist, on the intrathecal inflammatory mediator (somatostatin, bradykinin and prostaglandin $F_{2\alpha}$)-induced nociceptive responses.

## 2. Materials and methods

### 2.1. Animals

Male ICR mice (Tokyo Animal Laboratory, Tokyo Japan), weighing about 20 g at the beginning of the experiment, were used. They had free access to solid food (MF; Oriental Yeast, Tokyo, Japan) and water in an animal room which was maintained at $24 \pm 1$°C with a 12-h light–dark cycle. Animals were rendered diabetic by an injection of streptozotocin (200 mg/kg, i.v.) prepared in 0.1 M citrate buffer at pH 4.5. Age-matched control mice were injected with the vehicle alone. The experiments were conducted 2 weeks after the injection of vehicle or streptozotocin. Mice with serum glucose levels above 4000 mg/l were considered diabetic. 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. Algogenic mediator-induced nociceptive responses

The experiment was performed according to the method described by Hylden and Wilcox (1981). Each mouse was acclimated to an acrylic observation chamber ($39 \times 26 \times 24$ $cm^3$) for at least 5 min before the injection of an algogenic mediator. Intrathecal injection was performed by the method described by Hylden and Wilcox (1980). Each i.t. injection was administered using a 30-gauge needle directly through the intact skin between the $L_5$ and $L_6$ vertebrae. Drugs were given in a volume of 5 μl/mouse. Immediately following the injection, the mice were placed in the observation chamber. The cumulative duration (s) of biting, paw licking and scratching episodes was measured for 30 min after the injection of somatostatin, bradykinin or prostaglandin $F_{2\alpha}$.

### 2.3. Drugs

Somatostatin and bradykinin were purchased from Peptide Institute, Osaka, Japan. Prostaglandin $F_{2\alpha}$ was purchased from Calbiochem-Novabiochem International, La Jolla, CA, USA. Prostaglandin $F_{2\alpha}$ was stored in ethanol solutions at −20°C. For injection, an aliquot of the desired stock $PGF_{2\alpha}$ solution was put into a borosilicate tube and the ethanol was removed by evaporation to dryness under nitrogen gas. Sterile saline was then added to dissolve the prostaglandin $F_{2\alpha}$. 7-Benzylidenenaltrexone was synthesized by Dr. Nagase (Toray Industries). 7-Benzylidenenaltrexone was dissolved in 0.9% and injected s.c. 10 min before the injection of an algogenic mediator.

### 2.4. Statistical analysis

Data are expressed as the mean with S.E. A one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test was used for the statistical evaluation.

## 3. Results

Intrathecal injection of either somatostatin (1.0 nmol), bradykinin (1.0 nmol) or prostaglandin $F_{2\alpha}$ (2.8 nmol) elicited a behavioral syndrome consisting of reciprocal hindlimb scratching directed towards caudal parts of the body, and biting or licking of the hind legs in both

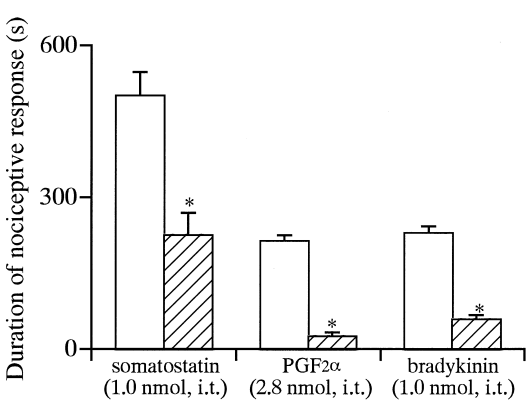


Fig. 1. Duration of the nociceptive response induced by the intrathecal administration of somatostatin (1.0 nmol), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 2.8 nmol) or bradykinin (1.0 nmol) in non-diabetic (open column) and diabetic (hatched column) mice. Data are expressed as the total duration of the response during the 30-min period after injection. Each column represents the mean with S.E. ($n = 10$). $^*P < 0.05$ vs. non-diabetic mice.

non-diabetic mice and diabetic mice. The duration of these somatostatin-, bradykinin- or prostaglandin $F_{2\alpha}$-induced nociceptive responses was significantly less in diabetic mice than in non-diabetic mice (Fig. 1).

Subcutaneous injection of 7-benzylidenenaltrexone (0.1, 0.3 and 1 mg/kg), an antagonist of $\delta_1$-opioid receptors, had no significant effect on either somatostatin-, bradykinin- or prostaglandin $F_{2\alpha}$-induced nociceptive responses in non-diabetic mice. 7-Benzylidenenaltrexone (0.1 and 0.3 mg/kg, s.c.) also had no significant effect on somatostatin- or prostaglandin $F_{2\alpha}$-induced nociceptive responses in diabetic mice. However, the bradykinin-induced nociceptive response in diabetic mice was dose-dependently and significantly increased when 7-benzylidenenaltrexone (0.1, 0.3 and 1 mg/kg, s.c.) was injected 10 min before the injection of bradykinin. On the other hand, s.c. injection of 7-benzylidenenaltrexone (0.1, 0.3 and 1 mg/kg), by itself, did not produced any nociceptive responses, i.e., licking and biting, in both non-diabetic and diabetic mice (Fig. 2).

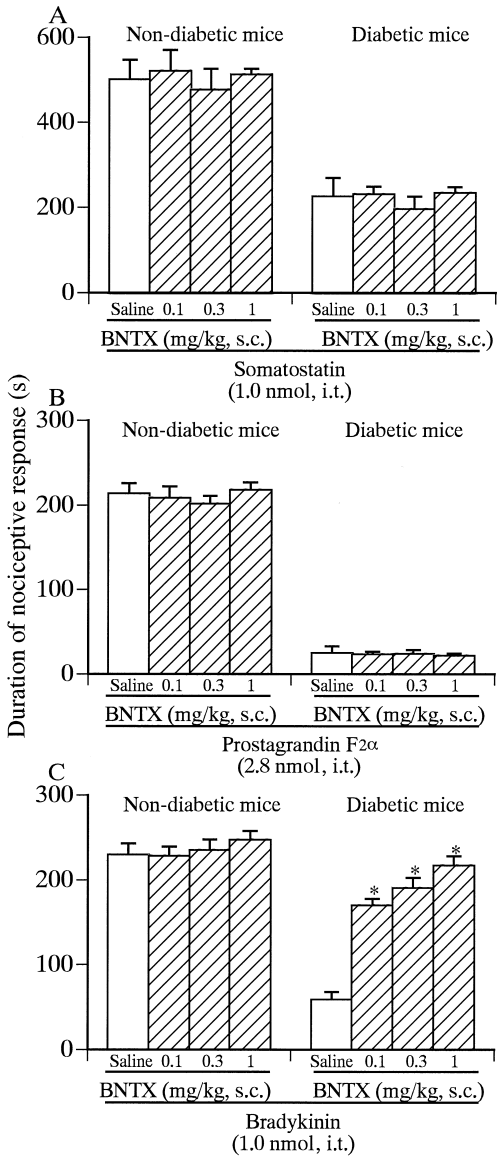


Fig. 2. Effects of the subcutaneous administration of 7-benzylidenenaltrexone (BNTX, 0.1, 0.3 and 1.0 mg/kg) on the somatostatin (1.0 nmol, i.t., A)-, prostaglandin $F_{2\alpha}$ (2.8 nmol, i.t., B)- or bradykinin (1.0 nmol, i.t., C)-induced nociceptive response in non-diabetic mice and diabetic mice. Data are expressed as the total duration of the response during the 30-min period after injection. BNTX was injected s.c. 10 min before the injection of each algogenic mediator. Each column represents the mean with S.E. ($n = 10$). $^{*}P < 0.05$ vs. saline-treated group (open column).

## 4. Discussion

In the present study, we demonstrated that the somatostatin-, bradykinin- and prostaglandin $F_{2\alpha}$-induced nociceptive responses were significantly less intense in diabetic mice than in non-diabetic mice. The finding that the intensity of somatostatin-induced nociceptive responses in diabetic mice was significantly less than that in non-diabetic mice is consistent with our previous finding (Kamei et al., 1992a). On the other hand, an unexpected but important finding in this study is that not only somatostatin-induced but also bradykinin- and prostaglandin $F_{2\alpha}$-induced nociceptive responses in diabetic mice were significantly reduced in diabetic mice compared to those in non-diabetic mice. These results suggest that diabetic mice are not as sensitive to inflammatory mediators in processing nociception. We previously demonstrated that although s.c. injection of formalin into the hindpaw of mice with diabetes produced an excess first phase of the nociceptive response, the second phase of the nociceptive response was barely observed (Kamei et al., 1993a). Although the data are not shown, the second phase, but not the first phase, of the formalin-induced nociceptive response was dose-dependently and significantly reduced by pretreatment with Hoe-140 (D-Arg–[Hyp$^3$, Thi$^5$, D-Tic$^7$, Oic$^8$]bradykinin), a selective bradykinin $B_2$ receptor antagonist. Therefore, it seems likely that a lower sensitivity to bradykinin in processing nociception may be responsible, at least in part, for the reduction in the second phase of the formalin-induced nociceptive response in diabetic mice.

We previously demonstrated that the duration of the second phase of the formalin-induced nociceptive response in diabetic mice was significantly increased when mice were pretreated with 7-benzylidenenaltrexone, a selective $\delta_1$-opioid receptor antagonist, but not with naltriben, a selective $\delta_2$-opioid receptor antagonist (Kamei et al., 1997). Therefore, it seems likely that $\delta_1$-opioid receptors, rather than $\delta_2$-opioid receptors, are involved in these δ-opioid receptor-mediated endogenous antinociceptive systems (Kamei et al., 1997). The first phase of the formalin-induced nociceptive response may represent a direct effect on nociceptors, whereas the second phase may represent an enhanced response of sensitized dorsal horn neurons resulting from low-level neuronal input due to peripheral inflam-

matory insult (Hunskaar and Hole, 1987). Peripheral inflammatory processes may elicit changes in spinal levels of endogenous opioids. Recently, Ossipov et al. (1996) demonstrated the presence of an opioid inhibitory tone that acts to limit the intensity of the nociceptive signal. Furthermore, they reported that this inhibitory tone appears to be mediated via the activation of δ- and κ-opioid receptors. It has been proposed that [D-Pen$^{2,5}$]enkephalin (DPDPE) is a selective agonist for $\delta_1$-opioid receptors, while [D-Ala$^2$]deltorphin II is a selective agonist for $\delta_2$-opioid receptors (Mattia et al., 1991; Sofuoglu et al., 1991a,b). We previously demonstrated that the antinociceptive effect of i.c.v. administration of DPDPE was significantly greater in diabetic mice than in non-diabetic mice, whereas there was no significant difference in the antinociceptive effect of i.c.v. [D-Ala$^2$]deltorphin II between diabetic and non-diabetic mice (Kamei et al., 1994b). Furthermore, pretreatment with 7-benzylidenenaltrexone, but not with naltriben, significantly antagonized the antinociceptive effect of DPDPE in both diabetic and non-diabetic mice (Kamei et al., 1994b). In contrast, the antinociceptive effect of [D-Ala$^2$]deltorphin II was significantly antagonized by naltriben, but not by 7-benzylidenenaltrexone, in both non-diabetic and diabetic mice (Kamei et al., 1994b). We also observed that the antinociceptive effect of DPDPE administered i.t. is significantly increased in diabetic mice compared to that in non-diabetic mice (Kamei et al., 1993b). Based on these results, we proposed that mice with diabetes are selectively hyper-responsive to both supraspinal and spinal $\delta_1$-opioid receptor-mediated antinociception. Furthermore, the δ-opioid receptor-mediated tonic opioid influence on the second phase of the formalin-induced nociceptive response and activated $\delta_1$-opioid receptor-mediated antinociceptive systems may account for the selective reduction of the second phase of the formalin-induced nociceptive response in diabetic mice (Kamei et al., 1993a). In the present study, we observed that the bradykinin-induced, but not the somatostatin- or prostaglandin $F_{2\alpha}$-induced nociceptive response in diabetic mice was dose-dependently and significantly increased when mice were pretreated with 7-benzylidenenaltrexone (0.1, 0.3 and 1 mg/kg, s.c.). This finding suggests that enhanced $\delta_1$-opioid receptor-mediated endogenous antinociceptive systems in diabetic mice may account for the selective reduction in bradykinin-mediated nociceptive transmission. Although the mechanism is not clear, it is possible that the enhancement of the second phase of the formalin-induced nociceptive response in diabetic mice after pretreatment with 7-benzylidenenaltrexone may result from improved bradykinin-mediated nociceptive transmission.

On the other hand, the somatostatin- or prostaglandin $F_{2\alpha}$-induced nociceptive response in diabetic mice were not increased when mice were pretreated with 7-benzylidenenaltrexone (0.1, 0.3 and 1 mg/kg, s.c.). Welch et al. (1991) reported that intrathecal injection of $Ca^{2+}$ produced antinociceptive effect in the *p*-phenylquinon stretching test. Furthermore, pretreatment with κ-opioid receptor antagonist, but not with δ-opioid receptor antagonist, significantly antagonized the antinociceptive effect of i.t. $Ca^{2+}$ in *p*-phenylquinon stretching test (Welch et al., 1991). It has been suggested that chronic excessive intracellular $Ca^{2+}$ overload might induce cardiac dysfunction in chronic diabetes (Heyliger et al., 1987; Nishio et al., 1990). Furthermore, it has been suggested that the diabetic state may change intracellular $Ca^{2+}$ levels in neuron and various other tissues (Lowery et al., 1990; Hall et al., 1995; Kostyuk et al., 1995). Thus, it seems likely that the enhancement of intracellular calcium levels in the spinal cord may activate the κ-opioid receptor-mediated endogenous antinociceptive systems for the inflammatory mediator-induced nociceptive responses in diabetic mice. Thus, it is possible that the reduction in the somatostatin- or prostaglandin $F_{2\alpha}$-mediated nociceptive transmission in diabetic mice may be due to the activation of κ-opioid receptor-mediated endogenous antinociceptive systems caused by an increase in intracellular $Ca^{2+}$ levels. However, further studies are necessary before this possibility can be established with greater certainty.

## Acknowledgements

We thank Ms. H. Ohtsuka and Ms. K. Yanagisawa for their expert technical assistance.

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