ELSEVIER

European Journal of Pharmacology 326 (1997) 31–36

ejp

The role of spinal δ_1-opioid receptors in inhibiting the formalin-induced nociceptive response in diabetic mice

Junzo Kamei [a,*], Takako Kashiwazaki [a], Hideki Hitosugi [a], Hiroshi Nagase [b]

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

[b] *Basic Research Laboratories, Toray Industries Inc., Kamakura 248, Japan*

Received 7 October 1996; revised 19 February 1997; accepted 25 February 1997

Abstract

Injection of formalin into the hindpaw of mice produced a biphasic nociceptive response consisting of immediate (first-phase) and tonic (second-phase) components. In diabetic mice, the flinching response of the first phase was increased while that in the second phase was decreased in diabetic mice relative to the results in non-diabetic mice. To examine the role of supraspinal and/or spinal endogenous δ_1-opioid receptors in inhibiting the formalin-induced nociceptive response in diabetic mice, we assessed the effect of 7-benzylidenenaltrexone, a selective δ_1-opioid receptor antagonist, and naltriben, a selective δ_2-opioid receptor antagonist, administered either i.c.v. or i.t., on the formalin-induced flinching response. The second-phase response appeared when diabetic mice were pretreated with 7-benzylidenenaltrexone (0.1 and 0.3 mg/kg, s.c.), but not with naltriben (0.3 and 1 mg/kg, s.c.). On the other hand, while 7-benzylidenenaltrexone (0.1, 0.3 and 1 μg/mouse) administered i.t. had no significant effect on the first phase, it significantly and dose-dependently increased the second phase of the formalin-induced flinching response in diabetic mice. 7-Benzylidenenaltrexone (1 and 3 μg/mouse) administered i.c.v. had no significant effect on either the first- or the second-phase response in both non-diabetic and diabetic mice. These results suggest that a spinal δ_1-opioid receptor-mediated endogenous antinociceptive system may inhibit the formalin-induced second phase of the nociceptive response in diabetic mice.

Keywords: Nociception, formalin-induced; δ_1-Opioid receptor; 7-Benzylidenenaltrexone; Naltriben; Diabetes; (Mouse)

1. Introduction

The formalin test has been accepted as a model of chemogenic pain (Dubuisson and Dennis, 1977; Hunskaar et al., 1985; Sugimoto et al., 1986; Murray et al., 1988; Ohkubo et al., 1990). Injection of diluted formalin into an animal's hindpaw produces a biphasic nociceptive response consisting of immediate (first-phase) and tonic (second-phase) components (Dubuisson and Dennis, 1977; Hunskaar et al., 1985; Sugimoto et al., 1986; Murray et al., 1988; Ohkubo et al., 1990). Kuraishi et al. (1989) and Kantner et al. (1986) showed that both substance P and somatostatin were released from the dorsal horn by the injection of formalin into the paw, indicating that both substance P and somatostatin participate in the transmission of the formalin-induced nociceptive response.

We previously demonstrated that although s.c. injection of formalin into the hindpaw produced the first phase of the nociceptive response, the second phase of the nociceptive response was hardly observed (Kamei et al., 1993a). Moreover, the time spent in the flinching response in the first phase was significantly longer in diabetic mice than in non-diabetic mice (Kamei et al., 1993a). The flinching response in the second phase became apparent in diabetic mice after pretreatment with naltrindole, a selective δ-opioid receptor antagonist (Kamei et al., 1993a). Recent studies have demonstrated the existence of two δ-opioid receptor subtypes, which have been referred to as δ_1-opioid and δ_2-opioid receptors (Jiang et al., 1991; Mattia et al., 1991; Sofuoglu et al., 1991a,b). In this regard, we previously demonstrated that the antinociceptive effect of either i.c.v. or i.t. administration of [D-Pen^{2,5}]enkephalin (DPDPE), which was antagonized by 7-benzylidenenaltrexone, a selective δ_1-opioid receptor antagonist, but not by naltriben, a selective δ_2-opioid receptor antagonist, was significantly increased in diabetic mice compared

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

PII S0014-2999(97)00157-X

to non-diabetic mice (Kamei et al., 1994b). On the other hand, there were no significant differences in the antinociceptive effects of the i.c.v. administration of [D-Ala^2]deltorphin II, which was antagonized by naltriben, but not by 7-benzylidenenaltrexone, between diabetic and non-diabetic mice (Kamei et al., 1994b). Based on these results, we proposed that mice with diabetes are selectively hyperresponsive to supraspinal and spinal δ_1-opioid receptor-mediated antinociception, but are normally responsive to the activation of δ_2-opioid receptors (Kamei et al., 1994b). Thus, these findings led us to propose the possibility that supraspinal and/or spinal δ_1-opioid receptors may play an important role in inhibiting the formalin-induced second-phase nociceptive response in diabetic mice. Therefore, in the present study, we assessed the effect of 7-benzylidenenaltrexone, administered either i.c.v. or i.t., on the formalin-induced flinching response in diabetic mice.

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 ± 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 N 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 400 mg/dl 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, Japan.

2.2. Formalin-induced flinching response

The experiment was performed according to the method described by Shibata et al. (1989). Each mouse was acclimated to an acrylic observation chamber ($32 \times 23 \times 17$ cm) for at least 5 min before the injection of formalin. 25 μl of a 0.5% solution of formalin in 0.9% saline was administered into the dorsal surface of the right hindpaw. Immediately after the injection, each animal was returned to the observation chamber and its flinching response was recorded for 30 min. The mouse licked and bit the injected paw; these responses were distinct and easily observed. The cumulative response time (s), i.e., the duration of licking and biting of the injected paw, was measured for each 5-min block.

2.3. Drugs

7-Benzylidenenaltrexone, naltriben and (−)-2-methyl-4*a*α-(3-hydroxyphenyl)-1,2,3,4,4*a*,5,12,12*a*α-octahydroquinolino [2,3,3,-*g*] isoquinoline ((−)-TAN-67) were synthesized by Dr. Nagase (Toray Industries, Co.). Both drugs were dissolved in 0.9% saline. Intracerebroventricular (i.c.v.) and intrathecal (i.t.) administrations, in a volume of 5 μl, were performed according to the methods of Haley and McCormick (1957) and Hylden and Wilcox (1980), respectively. Each antagonist was injected either s.c., i.c.v. or i.t. 10 min before formalin injection. (−)-TAN-67 was injected 30 min before i.t. injection of 7-benzylidenenaltrexone.

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

3.1. Effects of s.c. administration of δ_1- and δ_2-opioid receptor antagonists on the formalin-induced flinching response

In non-diabetic mice, s.c. injection of 0.5% formalin into the hindpaw caused an acute, immediate flinching response, i.e., licking and biting, which lasted about 5 min (first-phase response). The second-phase response then began and lasted about 20 min. In diabetic mice, s.c. injection of formalin into the hindpaw also produced biphasic flinching responses (Fig. 1). The time spent in the flinching response in the first phase was significantly longer in diabetic mice than in non-diabetic mice (Fig. 1). However, the time spent in the flinching response in the

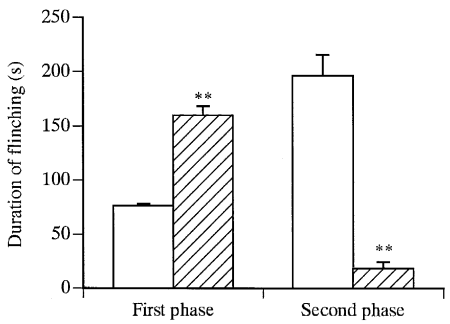

Fig. 1. Duration of the nociceptive response induced by the injection of formalin in non-diabetic (open column) and diabetic (hatched column) mice. Data are expressed as the total time spent in flinching (s) during the first (0–10 min) and second (10–30 min) phases. Each column represents the mean with S.E. ($n = 10$). ** $P < 0.01$ vs. non-diabetic mice.

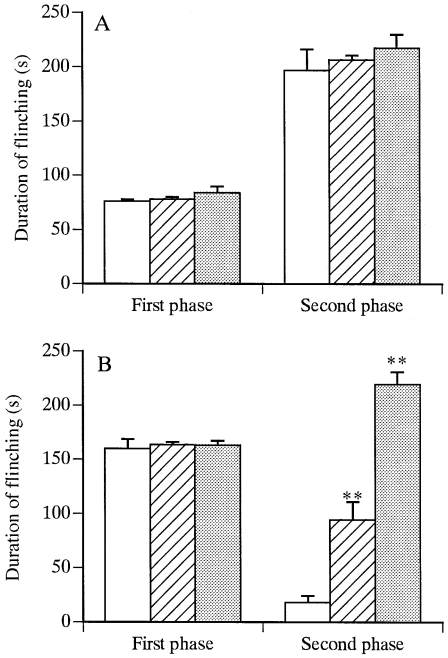

Fig. 2. Effects of s.c. administration of 7-benzylidenenaltrexone (0.1 mg/kg, hatched column; 0.3 mg/kg, dotted column) on the formalin-induced biphasic nociceptive response in non-diabetic mice (A) and diabetic mice (B). Data are expressed as the total time spent in flinching (s) during the first (0–10 min) and second (10–30 min) phases. Each column represents the mean with S.E. ($n = 9$). ** $P < 0.01$ vs. saline-treated group (open column).

second phase was significantly shorter in diabetic mice than in non-diabetic mice (Fig. 1).

Subcutaneous injection of 7-benzylidenenaltrexone (0.1 and 0.3 mg/kg), an antagonist of δ_1-opioid receptors, had no significant effect on either the first phase or the second phase of the formalin-induced flinching response in non-diabetic mice (Fig. 2A). 7-Benzylidenenaltrexone (0.1 and 0.3 mg/kg, s.c.) also had no significant effect on the first phase of the formalin-induced flinching response in diabetic mice (Fig. 2B). However, in contrast to the results in non-diabetic mice, the second phase of the formalin-induced flinching response in diabetic mice was dose-dependently and significantly increased when 7-benzylidenenaltrexone (0.1 and 0.3 mg/kg, s.c.) was injected 10 min before formalin injection.

In contrast, naltriben (0.3 and 1 mg/kg, s.c.), a selective δ_2-opioid receptor antagonist, had no significant effect on either the first or second phase of the formalin-induced flinching response in both non-diabetic and diabetic mice (Fig. 3A,B).

3.2. Effects of i.t. and i.c.v. administration of 7-benzylidenenaltrexone on the formalin-induced flinching response

Intrathecal injection of 7-benzylidenenaltrexone (0.1 μg) had no significant effect on either the first or second phase

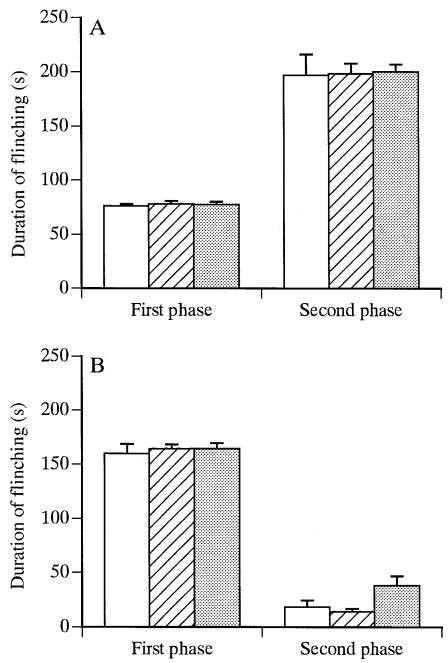

Fig. 3. Effects of s.c. administration of naltriben (0.3 mg/kg, hatched column; 1 mg/kg, dotted column) on the formalin-induced biphasic nociceptive response in non-diabetic mice (A) and diabetic mice (B). Data are expressed as the total time spent in flinching during the first (0–10 min) and second (10–30 min) phases. Each column represents the mean with S.E. ($n = 10$).

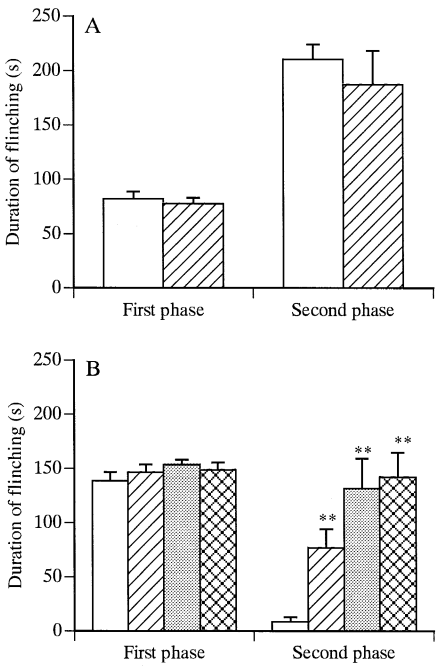

Fig. 4. Effects of i.t. administration of 7-benzylidenenaltrexone (0.1 μg, hatched column; 0.3 μg, dotted column; 1 μg, cross-hatched column) on the formalin-induced biphasic nociceptive response in non-diabetic mice (A) and diabetic mice (B). Data are expressed as the total time spent in flinching during the first (0–10 min) and second (10–30 min) phases. Each column represents the mean with S.E. ($n = 8$–10). ** $P < 0.01$ vs. respective saline-treated group.

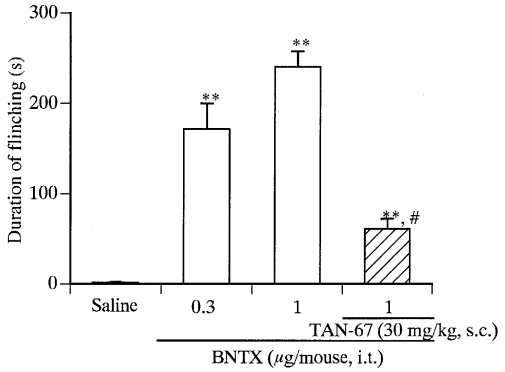

Fig. 5. Duration of and the effect of (−)-TAN-67 on the nociceptive response induced by the i.t. injection of 7-benzylidenenaltrexone (0.3 and 1 μg) in non-diabetic mice. (−)-TAN-67 (30 mg/kg) was injected s.c. 30 min before i.t. injection of 7-benzylidenenaltrexone (1 μg). Each point represents the mean with S.E. ($n = 10$) of the time spent in flinching per 30-min period. $^{**}P < 0.01$ vs. saline-treated group. $^{\#}P < 0.01$ vs. 7-benzylidenenaltrexone alone.

of the flinching response in non-diabetic mice (Fig. 4A). However, higher doses of 7-benzylidenenaltrexone (0.3 and 1 μg, i.t.), by itself, caused a marked flinching response, i.e., licking and biting, which lasted about 15 min (Fig. 5). The 7-benzylidenenaltrexone-induced flinching response was significantly reduced by pretreatment with (−)-TAN-67 (30 mg/kg, s.c.), a selective δ_1-opioid receptor agonist (Fig. 5). In diabetic mice, 7-benzylidenenaltrexone, at doses of 0.3 and 1 μg, i.t., had no effect on the first phase of the formalin-induced flinching response, as in non-diabetic mice (Fig. 4B). In contrast, the second phase of the formalin-induced flinching response in diabetic mice was dose-dependently and significantly increased when 7-benzylidenenaltrexone (0.1, 0.3 and 1 μg) was injected i.t. 10 min before formalin injection. However, 7-benzylidenenaltrexone (1 μg, i.t.), by itself, did not cause a flinching response in diabetic mice (saline, 1.0 ± 0.8 s/30 min, $n = 10$; 7-benzylidenenaltrexone, 0.0 ± 0.0 s/30 min, $n = 10$).

Intracerebroventricular injection of 7-benzylidenenaltrexone (1 and 3 μg) had no significant effect on either the first phase or the second phase of the formalin-induced flinching response in non-diabetic mice (Fig. 6A). 7-Benzylidenenaltrexone (1 and 3 μg, i.c.v.) also had no significant effect on either the first phase or the second phase of the formalin-induced flinching response in diabetic mice (Fig. 6B).

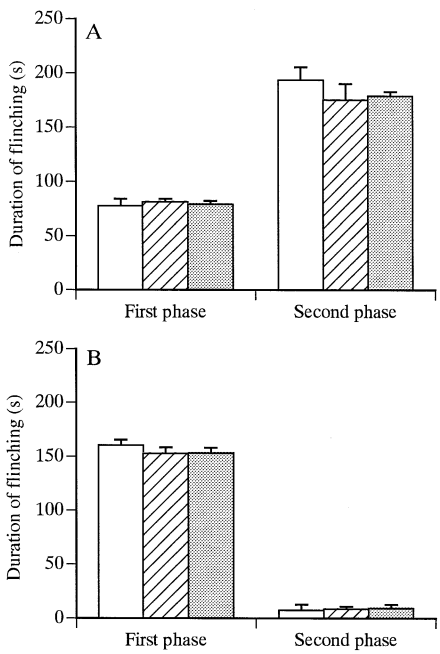

Fig. 6. Effects of i.c.v. administration of 7-benzylidenenaltrexone (1 μg, hatched column; 3 μg, dotted column) on the formalin-induced biphasic nociceptive response in non-diabetic mice (A) and diabetic mice (B). Data are expressed as the total time spent in flinching during the first (0–10 min) and second (10–30 min) phases. Each column represents the mean with S.E. ($n = 8$–10).

4. Discussion

The first phase of the formalin-induced flinching 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 inflammatory 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 which acts to limit the intensity of the nociceptive signal. Furthermore, they reported that this inhibitory tone appears to be mediated via activation of δ- and κ-opioid receptors. We recently proposed that mice with diabetes are selectively hyper-responsive to both supraspinal and spinal δ_1-opioid receptor-mediated antinociception. However, we did not observe a significant difference in either supraspinal or spinal κ-opioid receptor-mediated antinociception between diabetic and non-diabetic mice (Kamei et al., 1992a,b). In the present study, we demonstrated that the time spent in the second phase of the formalin-induced flinching response in diabetic mice was significantly increased when the mice were pretreated with 7-benzylidenenaltrexone, a selective δ_1-opioid receptor antagonist, but not with naltriben, a selective δ_2-opioid receptor antagonist. Thus, it is possible that the δ-opioid receptor-mediated tonic effect on the second phase of the formalin-induced flinching response, and activated δ_1-opioid receptor-, rather than δ_2-opioid receptor-mediated antinociceptive systems, may account for the selective reduction of the second phase of the formalin-induced flinching response in diabetic mice.

It has been suggested that substance P is involved in nociceptive transmission during the first phase of the formalin-induced nociceptive response in mice (Ohkubo et

al., 1990). When the first phase of the formalin-induced nociceptive response was decreased to the level observed in non-diabetic mice by pretreatment with spantide, a substance P antagonist, the second phase appeared (Kamei et al., 1993a). It has been well established that exposure to a stressful experience induces pronounced antinociception in animals. In this regard, we previously demonstrated that forced swimming stress significantly reduced the duration of the second phase, but not that of the first phase, of the formalin-induced flinching response (Kamei et al., 1993b). Furthermore, forced swimming stress-induced antinociception of the second phase of the formalin-induced flinching response was antagonized by pretreatment with naltrindole (Kamei et al., 1993b). Moreover, we recently proposed that the enhanced forced swimming stress-induced antinociception in diabetic mice may be due the activation of δ_1-opioid receptors (Kamei et al., 1994a). In connection with the formalin-induced flinching response, Aloisi et al. (1995) reported that a higher formalin concentration induced a stronger flinching response, and was associated with higher levels of plasma β-endorphin in rats. In contrast, a low concentration of formalin did not induce a significant increase in plasma β-endorphin levels. On the other hand, we recently demonstrated that the antinociceptive effect of β-endorphin in diabetic mice is markedly greater than that in non-diabetic mice (Kamei et al., 1993c). Furthermore, the antinociceptive effect of β-endorphin in diabetic mice is more responsive to naltrindole than that in non-diabetic mice (Kamei et al., 1993c). In the present study, we demonstrated that although i.c.v. administration of 7-benzylidenenaltrexone had no significant effect on the second phase of the formalin-induced flinching response, i.t. administration of 7-benzylidenenaltrexone significantly increased the second phase of the formalin-induced flinching response in diabetic mice. Accordingly, we considered that excessive nociceptive transmission in diabetic mice during the first phase of the formalin-induced nociception could represent a stressful experience, which activates the endogenous spinal δ_1-opioid receptor-mediated antinociceptive system.

The results of the present study were unexpected, but important. I.t. administration of 7-benzylidenenaltrexone, at doses of 0.3 and 1 μg, by itself, produced hyperalgesia in non-diabetic mice, but not in diabetic mice. This conclusion is based on the finding that 7-benzylidenenaltrexone given i.t. produced a flinching response, i.e., scratching and biting. Furthermore, the 7-benzylidenenaltrexone-induced flinching response in non-diabetic mice was significantly reduced by pretreatment with (−)-TAN-67, a selective δ_1-opioid receptor agonist (Kamei et al., 1995, 1997; Tseng et al., 1997). The doses of 7-benzylidenenaltrexone which produced a flinching response in non-diabetic mice were the same as those which significantly increased the second phase of the formalin-induced flinching response in diabetic mice. The exact mechanism which produces hyperalgesia is not clear at this time. One possibility is that in non-diabetic mice, a δ_1-opioid receptor-mediated tonic inhibitory tone is not only the consequence of a nociceptive stimulus, but is also present in the normal state.

In conclusion, the data presented in this paper provide strong evidence that formalin-induced nociception may activate spinal, but not supraspinal, endogenous δ_1-opioid receptor-mediated antinociceptive systems which selectively reduce the second phase of the formalin-induced flinching response in diabetic mice.

Acknowledgements

We thank Ms. Y. Shinohara and Ms. R. Shoji for their expert technical assistance.

References

Aloisi, A.M., Sacerdote, P., Albonetti, M.E., Carli, G., 1995. Sex-related effects on behavior and β-endorphin on different intensities of formalin pain in rats. Brain Res. 699, 242–249.

Dubuisson, D., Dennis, S.G., 1977. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain 4, 161–174.

Haley, T.J., McCormick, W.G., 1957. Pharmacological effects produced by intracerebral injections of drugs in the conscious mouse. Br. J. Pharmacol. 12, 12–15.

Hunskaar, S., Hole, K., 1987. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 30, 103–114.

Hunskaar, S., Fasmer, O.B., Hole, K., 1985. Formalin test in mice, a useful technique for evaluating mild analgesia. J. Neurosci. Methods 14, 69–76.

Hylden, J.L., Wilcox, G.L., 1980. Intrathecal morphine in mice: a new technique. Eur. J. Pharmacol. 67, 313–316.

Jiang, Q., Takemori, A.E., Sultana, M., Portoghese, P.S., Bowen, W.D., Mosberg, I.H., Porreca, F., 1991. Differential antagonism of opioid δ antinociception by [D-Ala^2,Leu^5,Cys^6]enkephalin and naltrindole 5-isothiocyanate: Evidence for δ receptor subtypes. J. Pharmacol. Exp. Ther. 257, 1069–1075.

Kamei, J., Kawashima, N., Kasuya, Y., 1992a. Paradoxical analgesia produced by naloxone in diabetic mice is attributable to supersensitivity of δ-opioid receptors. Brain Res. 592, 101–105.

Kamei, J., Ohhashi, Y., Aoki, T., Kawashima, N., Kasuya, Y., 1992b. Streptozotocin-induced diabetes selectively alters the potency of analgesia produced by μ-opioid agonists, but not by δ- and κ-opioid agonists. Brain Res. 571, 199–203.

Kamei, J., Hitosugi, H., Kasuya, Y., 1993a. Formalin-induced nociceptive responses in diabetic mice. Neurosci. Lett. 149, 161–164.

Kamei, J., Hitosugi, H., Misawa, M., Nagase, H., Kasuya, Y., 1993b. δ-Opioid receptor-mediated forced swimming stress-induced antinociception in the formalin test. Psychopharmacology 113, 15–18.

Kamei, J., Kawashima, N., Hitosugi, H., Misawa, M., Nagase, H., Kasuya, Y., 1993c. Effects of diabetes on the antinociceptive effect of β-endorphin. Brain Res. 619, 76–80.

Kamei, J., Iwamoto, Y., Hitosugi, H., Misawa, M., Nagase, H., Kasuya, Y., 1994a. Differential mediation of cold water swim stress-induced antinociception by δ-opioid receptor subtypes in diabetic mice. Life Sci. 54, PL425–430.

Kamei, J., Iwamoto, Y., Misawa, M., Nagase, H., Kasuya, Y., 1994b. Streptozotocin-induced diabetes selectively enhances antinociception mediated by δ_1- but not δ_2-opioid receptors. Life Sci. 55, PL121–126.

Kamei, J., Saitoh, A., Ohsawa, M., Suzuki, T., Misawa, M., Nagase, H., Kasuya, Y., 1995. Antinociceptive effects of the selective non-peptidic δ-opioid receptor agonist TAN-67 in diabetic mice. Eur. J. Pharmacol. 276, 131–135.

Kamei, J., Kawai, K., Mizusuna, A., Saitoh, A., Morita, K., Narita, M., Tseng, L.F., Nagase, H., 1997. Supraspinal δ_1-opioid receptor-mediated antinociceptive properties of (−)-TAN-67 in diabetic mice. Eur. J. Pharmacol. 322, 27.

Kantner, R.M., Goldenstein, B.D., Kirby, M.L., 1986. Regulatory mechanisms for substance P in the dorsal horn during a nociceptive stimulus: axoplasmic transport vs. electrical activity. Brain Res. 385, 282–290.

Kuraishi, Y., Hirota, N., Sato, Y., Hanashima, N., Takagi, H., Satoh, M., 1989. Stimulus specificity of peripherally evoked substance P release from the rabbit dorsal horn in situ. Neuroscience 30, 241–250.

Mattia, A., Vanderah, T., Mosberg, H.I., Porreca, F., 1991. Lack of antinociceptive cross-tolerance between [D-Pen^2,D-Pen^5]enkephalin and [D-Ala^2]deltorphin II in mice: evidence for δ receptor subtypes. J. Pharmacol. Exp. Ther. 258, 583–587.

Murray, C.W., Porreca, F., Cowan, A., 1988. Methodological refinements to the mouse paw formalin test. J. Pharmacol. Methods 20, 175–186.

Ohkubo, T., Shibata, M., Takahashi, H., Inoki, R., 1990. Roles of substance P and somatostatin on transmission of nociceptive information induced by formalin in spinal cord. J. Pharmacol. Exp. Ther. 252, 1261–1268.

Ossipov, M.H., Kovelowski, C.J., Wheeler-Aceto, H., Cowan, A., Hunter, J.C., Lai, J., Malan, T.P., Porreca, F., 1996. Opioid antagonists and antisera to endogenous opioids increase the nociceptive response to formalin: demonstration of an opioid kappa and delta inhibitory tone. J. Pharmacol. Exp. Ther. 277, 784–788.

Shibata, M., Ohkubo, T., Takahashi, H., Inoki, R., 1989. Modified formalin test: characteristic biphasic pain response. Pain 38, 347–352.

Sofuoglu, M., Portoghese, P.S., Takemori, A.E., 1991a. Differential antagonism of δ opioid agonists by naltrindole (NTI) and its benzofuran analog (NTB) in mice: evidence for δ receptor subtypes. J. Pharmacol. Exp. Ther. 257, 676–680.

Sofuoglu, M., Portoghese, P.S., Takemori, A.E., 1991b. Cross-tolerance studies in the spinal cord of b-FNA-treated mice provides further evidence for δ opioid receptor subtypes. Life Sci. 49, PL153–156.

Sugimoto, M., Kuraishi, Y., Satoh, M., Takagi, H., 1986. Involvement of medullary opioid-peptidergic and spinal noradrenergic systems in the regulation of formalin-induced persistent pain. Neuropharmacology 25, 481–485.

Tseng, L.F., Narita, M., Mizoguchi, H., Kawai, K., Mizusuna, A., Kamei, J., Suzuki, T., Nagase, H., 1997. δ_1-Opioid receptor-mediated antinociceptive properties of a nonpeptidic δ-opioid receptor agonist, (−)TAN-67, in the mouse spinal cord. J. Pharmacol. Exp. Ther. 280, 600–605.