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European Journal of Pharmacology 505 (2004) 75–82

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Intrathecal clonidine inhibits mechanical allodynia via activation of the spinal muscarinic M_1 receptor in streptozotocin-induced diabetic mice

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Received 17 June 2004; received in revised form 5 October 2004; accepted 12 October 2004
Available online 2 November 2004

Abstract

We examined the involvement of the spinal muscarinic receptors in the clonidine-induced antiallodynic effects. Mechanical sensitivity was assessed by stimulating the hind paw with von Frey filaments. In streptozotocin-treated (200 mg/kg, i.v.) diabetic mice, hypersensitivity to mechanical stimulation appeared 3 days after streptozotocin administration, and persisted for 11 days. This mechanical hypersensitivity (allodynia) was inhibited by the intrathecal (i.t.) injection of clonidine. The muscarinic receptor antagonist atropine (i.t.) and α_2-adrenoreceptor antagonist yohimbine (i.t. or subcutaneous injection) abolished the antiallodynic effect of clonidine. The effect was mimicked by the muscarinic M_1 receptor antagonist pirenzepine, but not by the muscarinic M_2 receptor antagonist methoctoramine or the muscarinic M_3 receptor antagonist 4-DAMP (4-diphenyl-acetoxy-*N*-methylpiperidine methiodide). In addition, the mechanical hypersensitivity in diabetic mice was reduced by the selective muscarinic M_1 receptor agonist McN-A-343 (4-(*m*-chlorophenyl-carbamoyloxy)-2-butynyltrimethylammonium chloride) (i.t.). These results suggest that spinal muscarinic M_1 receptors participate in the antiallodynic effect of clonidine in diabetic mice.

Keywords: Allodynia; Muscarinic receptor; α_2-Adrenoreceptor; Spinal cord; Diabetes

1. Introduction

Patients with diabetic neuropathy suffer from a variety of aberrant pain (Archer et al., 1983; Said, 1996). This painful peripheral neuropathy is one of the common neurological complications that are developed in the lower extremities in early stages of diabetes mellitus. Although hyperglycemia is considered as a major pathogenic factor in the development of diabetic neuropathy, the mechanisms are not yet fully understood. Moreover, the symptoms of diabetic painful neuropathy cannot be satisfactorily alleviated with non-steroidal anti-inflammatory drugs or opioids (Courteix et al., 1993; Kamei et al., 1992; Malcangio and Tomlinson, 1998).

Clinical and experimental studies have shown that systematic and intrathecal administration (i.t.) of α_2-adrenoreceptor agonists such as clonidine produced anti-nociceptive effects, both in humans and in animal models of pain (Danzebrink and Gebhart, 1990; Mendez et al., 1990; Millan et al., 1994, Malmberg et al., 2001). Moreover, the i.t. injections of cholinergic agonists and acetylcholinesterase inhibitors also caused antinociception and analgesia in animals and human (Hood et al., 1995; Iwamoto and Marion, 1993; Yaksh et al., 1985). Previously,

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doi:10.1016/j.ejphar.2004.10.033

we reported that the spinal cholinergic muscarinic receptors, especially the muscarinic M_3 receptor, were involved in formalin-induced nociception and that an acetylcholinesterase inhibitor neostigmine significantly suppressed this formalin-induced nociception (Honda et al., 2000). We also reported that intrathecal administration of the α_2-adrenoreceptor agonist clonidine had analgesic effects upon the response to mechanical stimulation via the spinal muscarinic M_1 receptor in naïve mice (Honda et al., 2002). It is known that blockade of spinal muscarinic receptors attenuates the antiallodynic effect of intrathecal clonidine in a rat model of neuropathic pain following peripheral nerve injury (Pan et al., 1999). These findings suggest that the interaction between descending noradrenergic neurons and spinal intrinsic cholinergic neurons may play an important role in the development of mechanical allodynia in neuropathic pain. However, the analgesic mechanism of α_2-adrenoreceptor agonists has not been fully elucidated in diabetic painful neuropathy. We decided to use the pancreatic β-cell toxin streptozotocin-induced diabetic mouse to examine the contribution of spinal muscarinic receptors to the antiallodynic effect of clonidine. This model was chosen firstly because the streptozotocin-induced diabetic mouse has been used as a model of diabetes mellitus, and secondly, because hyperalgesia and allodynia have also been demonstrated in this model (Calcutt and Chaplan, 1997; Courteix et al., 1993; Ohsawa and Kamei, 1999).

2. Materials and methods

2.1. Animals

Male ddy mice (Kyudo, Kumamoto, Japan) weighing 25–30 g were used in our experiments. Mice were housed at 23±2 °C with a 12/12 h light/dark cycle (lights on at 07:00 h), and were given free access to commercial food and tap water. Experimental procedures were based on the Guidelines of the Committee for Animal Care and Use of Fukuoka University.

2.2. Preparation of diabetic mice

Diabetic mice were produced by an injection of streptozotocin (200 mg/kg body weight) dissolved in 0.05 M sodium citrate buffer (pH 4.6) into the tail vein. Age-matched non-diabetic control mice were injected with the same volume of citrate buffer only. One or 2 days after the injection of streptozotocin, diabetes was confirmed by measuring urinary glucose with a Test-tape A (Shionogi Pharmaceutical, Osaka, Japan), and by measuring glucose concentration in a blood sample obtained from the tail vein with a GLUTEST E (Sanwa Chemical). Mice with blood glucose levels above 400 mg/dl were used as the diabetic mice.

2.3. Intrathecal (i.t.) injection of drugs

For the i.t. injection of drugs, a 28-gauge needle was connected to a 25 μl Hamilton microsyringe, and inserted into the intervertebral space of a conscious mouse, between the lumbar 5 and 6 regions of the spinal cord, as previously described (Honda et al., 2000; Hylden and Wilcox, 1980). The accurate placement of the needle was confirmed by a quick "flick" of the mouse's tail. Drugs for i.t. injection were given slowly in a volume of 5 μl. Control mice received only artificial cerebrospinal fluid (ACSF).

2.4. Mechanical stimulation and allodynia test

Mechanical sensitivity was determined with von Frey filaments (semmes-weinstein monofilaments, Stoelting, IL, USA) with calibrated bending forces (g), as previously described (Honda et al., 2002). Briefly, mice were placed individually in a glass cage with a wire mesh bottom. After mice had adapted to the testing environment for 60 min, the von Frey filaments were pressed perpendicularly against the mid-planter surface of the hind paw from below the mesh floor and held for 3–5 s with it slightly buckled. Lifting of the paw was recorded as a positive response. Filaments were applied to the point of bending six times to the planter surface of the left and right hind paw for a total of 12 times per mouse at intervals of 5 s; the next lightest filament was chosen for each subsequent measurement. Paw withdrawal threshold (g) was taken as the lowest force that caused 100% withdrawals, and was considered as the mechanical nociceptive threshold. We used the non-noxious 0.07 g von Frey filament to assess mechanical allodynia, and the percent response frequency of paw lifts when the filaments was applied to the point of bending six times to the planter surface of the left (n=6) and right hind paw (n=6), for a total of 12 applications per mouse, was expressed as (100×number of positive responses/12).

2.5. Locomotor activity and rota-rod testing

To rule out the possibility of sedation and motor impairment by the i.t. injection of clonidine in diabetic mice, a open-field test and a rota-rod treadmill test (diameter, 3 cm; Muromachi Kikai, Tokyo, Japan) were carried out, respectively. All behavioral tests were performed during the light portion of the circadian cycle (9:00 a.m. to 5:00 p.m.). A mouse was placed on the center of the floor in the open-field chamber (60 cm in diameter and 50 cm in height; the floor was divided into 19 blocks), and observed for 1 min. Locomotor activity (ambulation) was expressed as the numbers of blocks traversed. Each mouse was subjected to the rota-rod treadmill test once a day for a total period of 3 days. The treadmill was set to a rotating speed of 10 rpm. Mice that stayed on the treadmill rotating at 10 rpm for 180 s were considered complete responders;

their latencies were recorded as 180 s. The results were presented as drop latency (s) measured 10 min after the i.t injection.

2.6. Drugs

Atropine methyl sulphate was purchased from Nacalai Tesque (Kyoto, Japan). Streptozotocin, clonidine hydrochloride and yohimbine hydrochloride were purchased from Sigma (St. Louis, MO, USA). 4-DAMP (4-diphenylacetoxy-*N*-methylpiperidine methiodide), methoctramine tetrahydrochloride and McN-A-343 (4-(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride) were obtained from Research Biochemicals (RBI, Natick, MA, USA). Pirenzepine hydrochloride was a gift from Boehringer Ingelheim (Mannheim, Germany). Drugs were dissolved in ACSF or saline.

2.7. Statistical analysis

Date were expressed as means±S.E.M. For statistical analysis, comparisons of mechanical withdrawal thresholds were carried out using the non-parametric Kruskal–Wallis, or Friedman test, followed by the Dunn's test for multiple comparisons. The Mann–Whiney *U*-test was used to compare monofilament-induced withdrawal thresholds between two groups. Differences in the percentage response were assessed using the Student's *t*-test between two groups, and by using analysis of variance (ANOVA) followed by Dannett' s test or Turkey's test for multiple comparisons.

3. Results

3.1. Blood glucose levels

Streptozotocin-injected mice had glucosuria, an increase in blood glucose level and a decrease in body weight from 3rd on after the streptozotocin injection. Seven days after injection of streptozotocin, the mean blood glucose level of streptozotocin-injected mice (479.3±37.3 mg/dl, n=9) was significantly (P<0.01) elevated when compared with aged-

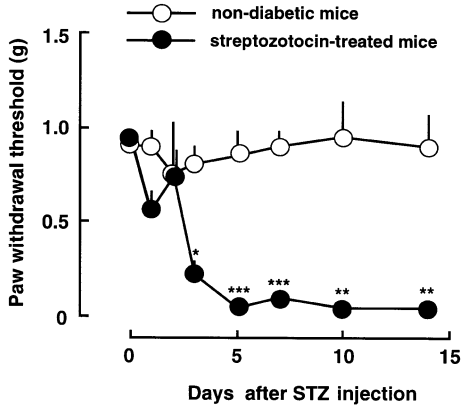

Fig. 1. Time course of mechanical nociceptive thresholds after injection of streptozotocin. Data are given as the paw withdrawal force to mechanical stimuli. All values are mean±S.E.M. for 7–9 mice in each group. *P<0.004, **P<0.002, ***P<0.001 compared to non-diabetic mice.

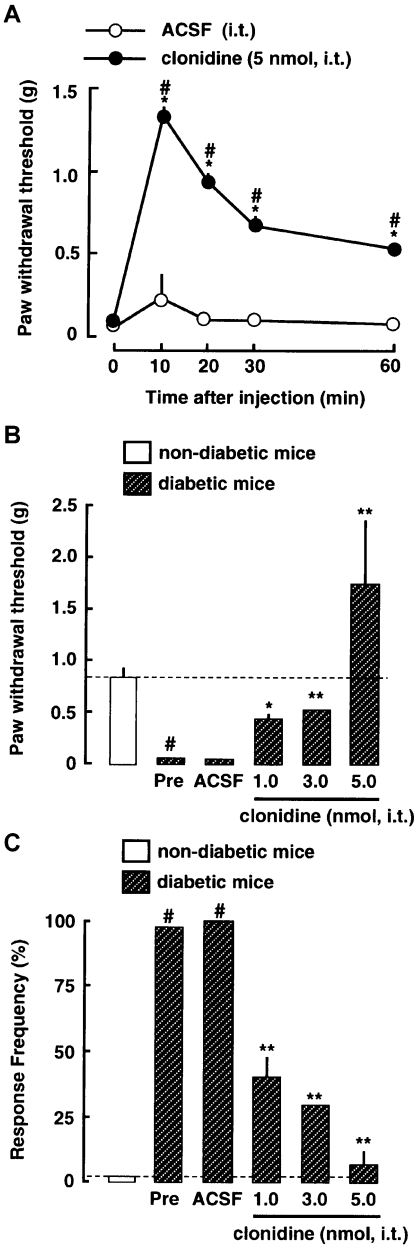

Fig. 2. Effects of i.t. injection of clonidine on mechanical nociceptive thresholds (A, B) and mechnical allodynia (C) in diabetic mice 7 days after the injection of streptozotocin. (A) Time course of effects of clonidine on mechanical nociceptive thresholds. (B) Dose dependency of effects of clonidine at 10 min after i.t. injection of clonidine. The dotted line indicates the mechanical nociceptive thresholds in non-diabetic mice. (C) Mechanical allodynia, as measured by response frequency of paw withdrawal to stimulation with 0.07 g von Frey filament stimulation. Further details are given in Materials and methods. The response was observed 10 min after i.t. injection of clonidine. All values are mean±S.E.M. for 6–10 mice in each group. *P<0.01, *P<0.02 compared to ACSF. #P<0.01 compared to non-diabetic mice (A) or pre-drug (Pre) values (B, C).

matched non-diabetic mice (143.8±13.8 mg/dl, n=8). However, the body weight of streptozotocin-induced diabetic mice (25.84±1.23 g, n=9) was significantly (P<0.01) reduced as compared with to aged-matched non-diabetic mice (37.90±1.01 g, n=8).

3.2. Changes of mechanical nociceptive thresholds after induction of diabetes

As shown in Fig. 1, the mechanical withdrawal threshold began to decrease significantly 3 days after the streptozotocin injection. The threshold of control and diabetic mice, 7 days after injection, was 0.90±0.10 and 0.10±0.03 g (P<0.001), respectively. Allodynia presenting in the diabetic mice persisted for 14 days after injection (Fig. 1). Therefore, experiments were carried out 7 days after the injection of streptozotocin or vehicle.

3.3. Effects of clonidine on mechanical nociceptive thresholds and allodynia in diabetic mice 7 days after injection of streptozotocin or vehicle

Intrathecally injected clonidine (1–5 nmol) increased the mechanical withdrawal thresholds in diabetic mice in a dose-dependent mannar (Fig. 2B). The response appeared 5 min and reached a peak 10 min after the clonidine injection (Fig. 2A). Therefore, experiments were carried out 10 min after the injection of clonidine. As shown in Fig. 2C, intrathecal clonidine (1–5 nmol) reduced mechanical allodynia to non-noxious stimuli in diabetic mice in a dose-dependent manner.

Since clonidine is thought to act not only on α_2-adrenoreceptors, but also on imidazoline receptors, we examined the effect of the α_2-adrenoreceptor antagonist yohimbine on the clonidine-induced antiallodynic effect. Treatment with yohimbine (0.5–2.0 mg/kg, subcutaneous injection or 5 nmol, i.t.) significantly attenuated the effect of clonidine (5 nmol, i.t.) in diabetic mice (Fig. 3). The subcutaneous (2.0 mg/kg) or i.t. (5 nmol) injection of yohimbine alone did not affect the mechanical withdrawal threshold in diabetic mice [the mechanical withdrawal threshold was 0.13±0.03 g (n=7) and 0.11±0.04 g (n=7) before and at 30 min after the subcutaneous injection of yohimbine, respectively; the mechanical withdrawal threshold was 0.11±0.02 g (n=7) and 0.12±0.04 g (n=7) before and at 10 min after the i.t. injection of yohimbine, respectively]. In addition, the injection of yohimbine alone also did not affect allodynia in diabetic mice (Fig. 3).

In diabetic mice, intrathecal clonidine (5 nmol) did not induce significant changes of motor activity according to Hall's open field test, and the same dose of clonidine had no influence on the rota-rod treadmill test (Fig. 4).

3.4. Effects of the muscarinic antagonist atropine on the clonidine-induced antiallodynic effect in diabetic mice 7 days after the injection of streptozotocin or vehicle

To examine the involvement of muscarinic receptors in the clonidine-induced increase in the mechanical nociceptive thresholds and antiallodynic effect in diabetic mice, the muscarinic receptor antagonist atropine (273 or 546 pmol) was co-administered with clonidine (5 nmol). Intrathecal atropine completely abolished the clonidine-induced antiallodynic effect (Fig. 5). Injection of atropine alone did not change the mechanical withdrawal threshold (data not shown).

3.5. Effects of selective muscarinic M_1, M_2 and M_3 receptor antagonists on the clonidine-induced antiallodynic effect in diabetic mice 7 days after injection of streptozotocin or vehicle

To determine the muscarinic receptor subtype involved in the clonidine-induced anti-allodynic response, selective muscarinic M_1, M_2 and M_3 receptor antagonists (pir-

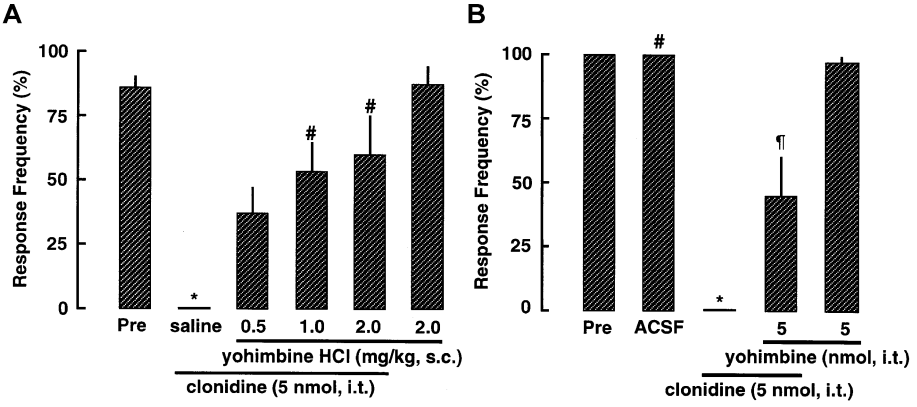

Fig. 3. Effects of i.t. or subcutaneous (s.c) injection of the α_2-adrenoreceptor antagonist yohimbine on the clonidine-induced antiallodynic effect 7 days after the injection of streptozotocin. Yohimbine was subcutaneously injected 30 min before the injection of clonidine, or was co-injected (i.t.) with clonidine. The response was observed 10 min after the i.t. injection of clonidine. All values are mean±S.E.M. for 8–12 mice in each group. *P<0.01 compared to pre-drug (Pre) values. $^{\#}P$<0.01 compared to clonidine.

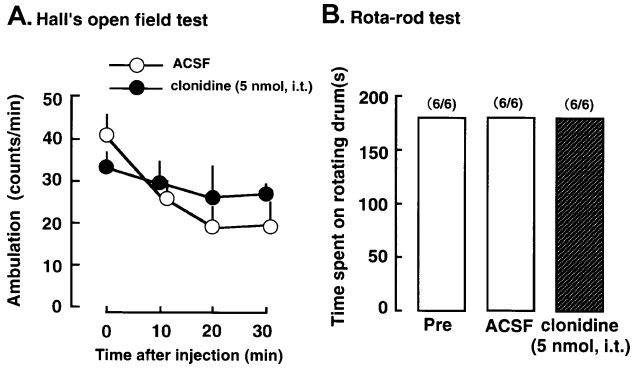

Fig. 4. Effects of i.t injection of clonidine on motor activity in diabetic mice 7 days after the injection of streptozotocin. (A) Hall's open field test. (B) Rota-rod test. Six mice were examined for 3 min. The number of animals that did not fall from the rotating drum at the indicated speeds during the period of examination is shown at the top of each column. All values are mean±S.E.M. for 6–8 mice in each group.

enzepine, methoctramine and 4-DAMP, respectively) (Eglen and Watson, 1996) were co-administered with clonidine. Intrathecal pirenzepine (27.3–546 pmol) inhibited the clonidine (5 nmol, i.t.)-induced antiallodynic effect in a dose-dependent manner (Fig. 6A). Injection of pirenzepine alone did not affect mechanical withdrawal (data not shown). In contrast, intrathecal muscarinic M_2 and M_3 receptor antagonists (methoctramine and 4-DAMP) did not attenuate the clonidine-induced responses (Fig. 6B,C).

3.6. Effects of the selective muscarinic M_1 receptor agonist McN-A-343 on mechanical allodynia in diabetic mice 7 days after the injection of streptozotocin or vehicle

Intrathecally administered muscarinic M_1 receptor agonist McN-A-343 (Davies et al., 2001) increased mechanical nociceptive thresholds in both non-diabetic mice and diabetic mice in a dose-dependent manner (Figs. 7 and

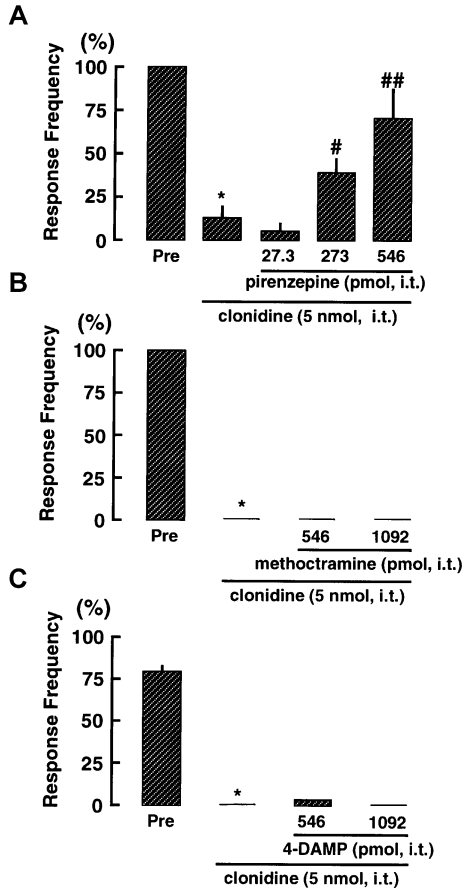

Fig. 6. Effects of i.t. injection of muscarinic antagonists on the clonidine-induced anti-allodynic effects. Muscarinic antagonists were co-injected i.t. with clonidine 7 days after the injection of streptozotocin. The response was observed 10 min after the i.t. injection of drug injection. All values are mean±S.E.M. for 6–10 mice in each group. *P<0.01 compared to pre-drug values. *P<0.01, compared to pre-drug (Pre) values. $^{\#}P$<0.01, $^{\#\#}P$<0.05 compared to clonidine.

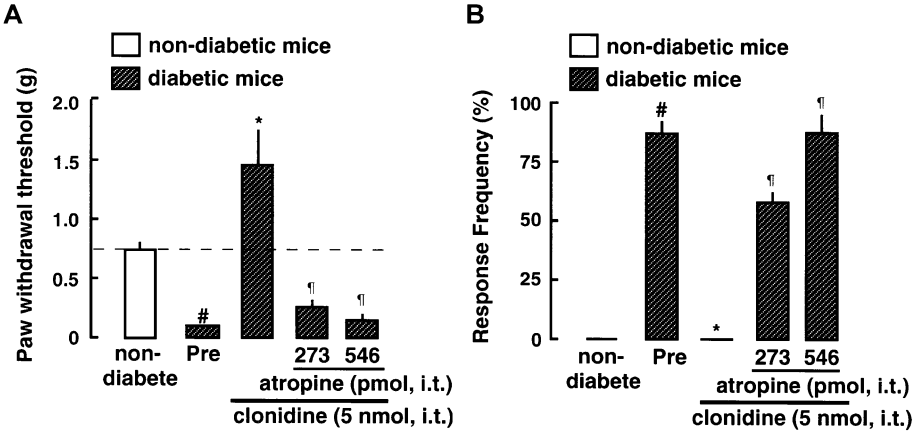

Fig. 5. Effects of i.t. injection of the muscarinic antagonist atropine on the clonidine-induced mechanical nociceptive thresholds (A) and antiallodynic effects (B) 7 days after the injection of streptozotocin. Atropine was co-injected i.t. with clonidine. The effects were observed 10 min after the i.t. injection of drug injection. The dotted line indicates the mechanical nociceptive thresholds in non-diabetic mice. All values are mean±S.E.M. for 6–11 mice in each group. *P<0.01 compared to pre-drug (Pre) values. $^{\#}P$<0.01 compared to non-diabetic mice. $^{¶}P$<0.01 compared to clonidine.

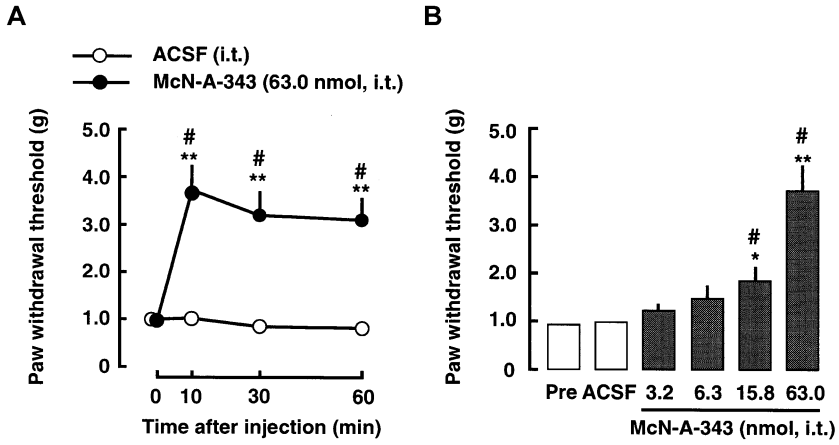

Fig. 7. Effects of i.t. injection of the muscarinic M_1 agonist McN-A-343 on mechanical nociceptive thresholds in non-diabetic mice 7 days after the injection of vehicle (non-diabetic mice). (A) Time course of McN-A-343-induced antinociceptive effect on mechanical nociceptive thresholds. (B) Dose dependency of the effect of McN-A-343 10 min after the i.t. injection. All values are mean±S.E.M. for 5–12 mice in each group. *P<0.05, **P<0.01 compared to ACSF. $^{\#}P$<0.01 compared to pre-drug (Pre) values.

8A), and alleviated mechanical allodynia to non-noxious stimuli in diabetic mice (Fig. 8B).

4. Discussion

Previous studies have demonstrated that streptozotocin-induced diabetic animals show decreased thresholds to mechanical stimulation (Ahlgren and Levine, 1993; Courteix et al., 1993; Malcangio and Tomlinson, 1998), and tactile allodynia (Calcutt and Chaplan, 1997). In addition, it has been reported that intrathecal administration of an α_2-adrenergic agonist inhibited tactile allodynia in the streptozotocin-induced diabetic rat (Calcutt and Chaplan, 1997). In the present study, a decreased mechanical nociceptive thresholds and mechanical allodynia in streptozotocin-induced diabetic mice were found, and this allodynia was alleviated with the administration of intrathecal clonidine as previously reported. Intrathecal clonidine had no effect on locomotion activity and motor function. We concluded that the clonidine-induced antiallodynic effect in diabetic mice was not due to sedation or motor dysfunction.

Treatment with an α_2-adrenoreceptor antagonist yohimbine (s.c. or i.t. administration) significantly inhibited the clonidine-induced antiallodynic effect. Therefore, the clonidine-induced antiallodynic effect was mediated by α_2-adrenoreceptors in the spinal cord.

Recently, we reported that intrathecal clonidine induced antinociception, at least in part, via the activation of spinal muscarinic receptors (Honda et al., 2002, 2003). In the present study, intrathecal atropine and the muscarinic M_1 receptor antagonist completely abolished the clonidine-induced antiallodynic effect in diabetic mice. Similarly, it has been reported that intrathecal atropine inhibited clonidine-induced antiallodynic effects in the animal model of peripheral nerve injury (Pan et al., 1999). These findings indicate that the antiallodynic effect of α_2-adrenoreceptor agonists is related to the activation of muscarinic receptors

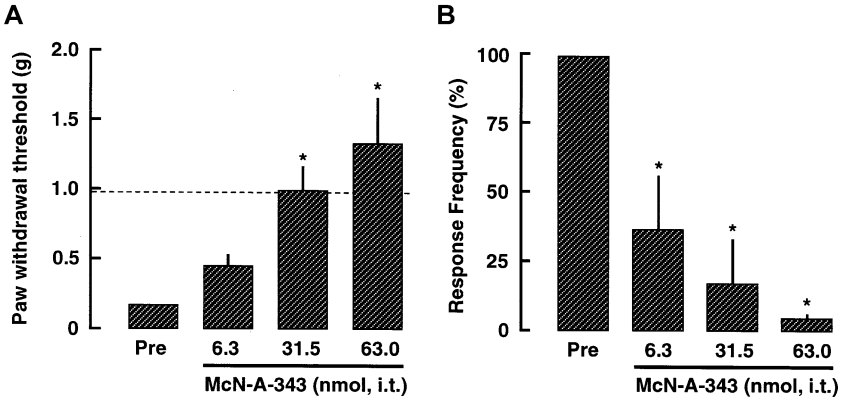

Fig. 8. Effects of i.t. injection of the muscarinic M_1 agonist McN-A-343 on mechanical nociceptive thresholds (A) and allodynia (B) in diabetic mice 7 days after injection of streptozotocin. (A) Dose dependency of the effect of McN-A-343 on mechanical nociceptive thresholds 10 min after the i.t. injection. The dotted line indicates the mechanical nociceptive thresholds in non-diabetic mice. (B) The effect of McN-A-343 on mechanical allodynia 10 min after i.t. injection. All values are mean±S.E.M. for 6–12 mice in each group. *P<0.01 compared to pre-drug (Pre) values.

in the spinal cord. There is some evidence to suggest that intrathecally administered muscarinic receptor agonists do inhibit nociceptive responses to noxious heat stimuli (Iwamoto and Marion, 1993; Naguib and Yaksh, 1997). Previously, we demonstrated that spinal muscarinic receptors, especially muscarinic M_3 receptors, were involved in formalin-induced nociception (Honda et al., 2000). In addition, it has been reported that intrathecal administration of a cholinesterase inhibitor caused antiallodynic effects via the spinal muscarinic M_1 receptor in a model of neuropathic pain produced by spinal nerve ligation injury (Hwang et al., 1999).

In the present study, to determine the subtypes of muscarinic receptor involved in the clonidine-induced antiallodynic effect, three muscarinic receptor antagonists, pirenzepine (selective muscarinic M_1 receptor antagonist), methoctramine (selective muscarinic M_2 receptor antagonist), and 4-DAMP (selective muscarinic M_3 receptor antagonist), were intrathecally administered. The clonidine-induced antiallodynic effect was inhibited by pirenzepine, but not by methoctramine or 4-DAMP. Furthermore, we demonstrated that intrathecal administration of the selective muscarinic M_1 receptor agonist McN-A-343 caused an increase in mechanical nociceptive threshold in both non-diabetic mice and diabetic mice. The increase in mechanical nociceptive threshold in diabetic mice induced by intrathecal muscarinic M_1 receptor agonist resulted in rescue from the allodynia in diabetic mice. These results suggest that the spinal muscarinic M_1 receptors, at least in part, participate in clonidine-induced antiallodynia in diabetic mice.

Several lines of evidence suggest that the descending noradrenergic system plays an important role in nociceptive transmission occurring in the dorsal horn of the spinal cord (Basbaum and Fields, 1984). α_2-adrenoreceptors are present not only in laminae I and II, but throughout the entire gray matter of the spinal cord (Sullivan et al., 1987). Furthermore, studies of analysis of mRNA of muscarinic receptors have revealed the existence of five muscarinic receptor subtypes in the spinal cord (Wei et al., 1994). Autoradiographic studies have shown the existence of muscarinic M_1 receptors in dorsal horn of spinal cord where it may be localized on the terminals of the primary afferents (Villiger and Faull, 1985; Wamsley et al., 1984). Immunocytochemical localization of choline acetyltransferase has demonstrated the existence of intrinsic spinal cholinergic neurons in laminae III–V, and laminae II and III of the spinal cord (Borges and Iversen, 1986; Ribeiro-da-Silva and Cuello, 1990). The signals caused in mechanoreceptors in response to von Frey filaments seem to be conveyed into the inner layers of laminae II (IIi), III, and IV in the spinal cord via Aβ-fibers (Millan, 1999). These findings suggest that the distributions of α_2-adrenoreceptors and muscarinic receptors in laminae III and IV are related to the termination of Aβ-afferents into the spinal dorsal horn.

The electrophysiological study revealed ectopic discharges and higher spontaneous activity predominantly in Aβ- and Aδ-fiber afferents in diabetic rats (Khan et al., 2002). Thus, the myelinated primary afferent fibers may play an important role in the development of diabetic neuropathic pain. We have previously reported that spinal muscarinic receptors are involved in the mechanism of the intrathecal clonidine-induced increase of mechanical withdrawal threshold, but not that of thermal threshold in naïve mice (Honda et al., 2002). Taken together, these results suggest that intrathecal clonidine may inhibit diabetic mechanical allodynia through inhibition of the input from myelinated primary afferents via spinal muscarinic M_1 receptors.

Klimscha et al. (1997) reported that intrathecal clonidine caused release of acetylcholine from the spinal cord in sheep. Recently, Abelson and Höglund (2004) have reported that intrathecal α_2-adrenergic receptor agonists (including clonidine) and antagonists caused the release of acetylcholine via nicotinic receptors in rat spinal cord. But, yohimbine decreased the acetylcholine release via nicotinic receptors. The findings indicate that stimulation of spinal α_2-adrenergic receptors enables activation of the muscarinic receptors through the release of acetylcholine in the spinal cord. In addition, acetylcholine and muscarine activated the GABA receptors via the release of γ-aminobutyric acid (GABA) in the rat spinal cord (Baba et al., 1998; Li et al., 2002). Chen and Pan (2003) reported that activation of the $GABA_B$ receptor was involved in the antinociceptive effect of intrathecal muscarine in rats. These findings suggest the possibility that the activation of muscarinic M_1 receptors may inhibit the transmission of mechanical information through the activation of inhibitory inter-neurons such as GABAergic neurons in the spinal dorsal horn. On the other hand, it has been reported that spinal muscarinic receptors play a critical role in the analgesic effect of intrathecal clonidine in a rat model of neuropathic pain of spinal nerve injury through interaction with nitric oxide (Xu et al., 2000).

In conclusion, the present study suggests that intrathecally administered clonidine inhibits mechanical allodynia via activation of spinal α_2-adrenoreceptors and muscarinic M_1 receptors in diabetic mice. Thus, administration of α_2-adrenoreceptor agonists and muscarinic M_1 receptor agonists may become important alternatives in the treatment of diabetic painful neuropathy.

Acknowledgements

This work was supported in part by a Grant-in-Aid from The Ministry of Education, Culture, Sports, Science and Technology of Japan (#1457210), and by a Research Grant (#HSP 11006) from the Japan Health Sciences Foundation, and a grant from the Advanced Materials Institute and the Central Research Institute of Fukuoka University.

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