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Antinociceptive effect of the novel compound OT-7100 in a diabetic neuropathy model

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Abstract

We previously reported that OT-7100 (5-*n*-butyl-7-(3,4,5-trimethoxybenzoylamino)pyrazolo[1,5-α]pyrimidine) had antinociceptive potency in various animal models. To further characterize this compound, the present study examined the effects of OT-7100 on mechanical hyperalgesia and motor nerve conduction velocity in streptozotocin-induced diabetic rats. OT-7100 significantly increased the nociceptive threshold in the diabetic rat in a dose-dependent manner. Gabapentin (anticonvulsant agent) and insulin strongly increased the nociceptive threshold but gabapentin increased it above normal levels. An aldose reductase inhibitor slightly increased the nociceptive threshold at a high dose. We also measured glucose levels and motor nerve conduction velocity in OT-7100-treated rats. Insulin decreased glucose levels but OT-7100 had no effect on glucose levels or on motor nerve conduction velocity. These results suggest that OT-7100 alleviates hyperalgesia in a diabetic neuropathy model in a different manner from gabapentin or aldose reductase inhibitor and may be a new treatment for the pain associated with peripheral nerve injury.

Keywords: OT-7100; Pain; Diabetic neuropathy; Streptozotocin

1. Introduction

Neuropathic pain is generally considered to be one of the most common and troublesome complications afflicting diabetic patients, and painful diabetic neuropathy is one of the more frequently encountered neuropathic pain syndromes (Clark and Lee, 1995; Vinik et al., 1992). In humans, painful diabetic neuropathy is associated with burning, tactile hypersensitivity (Bays and Pfeifer, 1988) and is treatable with antidepressants, anticonvulsants, capsaicin, nonsteroidal anti-inflammatory drugs, opioids and an aldose reductase inhibitor (Courteix et al., 1993; Hotta et al., 1996). However, such analgesic relief is often inadequate, potentially toxic, or is associated with dependence liability (Arner and Meyerson, 1988).

The streptozotocin-induced diabetic rat has been widely used as a model of insulin-dependent diabetes mellitus, and a number of anomalies in pain perception have been demonstrated in this model (Hounsom and Tomlinson, 1997). Mechanical hyperalgesia and thermal allodynia have also been observed following streptozotocin treatment (Wuarin-Bierman et al., 1987). A single dose of streptozotocin leads to the development of abnormal pain syndrome in rats similar to that seen in patients with painful diabetic neuropathy (Calcutt et al., 1996). A number of agents that are effective in clinical studies have been shown to alleviate mechanical hyperalgesia in this model (Courteix et al., 1994). For example, gabapentin (anticonvulsant agent) was reported to block static and dynamic allodynia (Field et al., 1999). This drug and other classical drugs generally have side effects affecting the central nervous system or stomach. In Japan, aldose reductase inhibitor is widely used. It inactivates the polyol pathway and has a beneficial effect on nerve function (Kikkawa et al., 1983). However, this drug has weak potential for diabetic complications. Thus, new therapies need to be developed for the treatment of painful neuropathy. We have been searching for new potent analgesic agents with no ulcerogenic effect that can be used for the treatment of peripheral neuropathic pain. We screened our compound library for an agent which had an analgesic effect in acute hyperalgesia models (yeast-induced pain model and substance P-induced pain model) without causing inhibition of prostaglandin E2 biosynthesis by cyclooxygenase, which is known to cause gastric side effects. We found OT-7100 (5-*n*-butyl-7-(3,4,5-tri-

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methoxybenzoylamino)pyrazolo[1,5-α]pyrimidine) to be a novel analgesic compound and tested it in various pain models. A previous report on OT-7100 focused primarily on its activity in models of acute and persistent pain induced by peripheral nerve injury without affecting prostaglandin formation. Also, this compound did not affect behavior in pharmacological studies and showed no toxicity in toxicological studies (Yasuda et al., 1999). Therefore, it is expected that OT-7100 will be an antinociceptive agent without side effects. The objectives of the current investigation were to further characterize the effects of OT-7100 in the streptozotocin-induced diabetic neuropathy model by studying the time course of effect following oral dosing, determining nerve function and comparing it with other agents that have been clinically used for the treatment of painful neuropathy, such as an anticonvulsant, an aldose reductase inhibitor, and a pancreatic hormone.

2. Materials and methods

All experiments were conducted in accordance with the guidelines on ethical standards for investigations of experimental pain in animals defined by the International Association for the Study of Pain (IASP; Zimmermann, 1983). In particular, the duration of the experiments was as short as possible and the number of animals was kept to a minimum.

2.1. Animals

Male Sprague–Dawley rats (Charles River Japan, Yokohama, Japan) weighing 200–250 g were used. On arrival at the laboratory, the rats were allowed to acclimate for 3 weeks in groups of 5 rats per cage under a 12-h light/dark cycle with free access to food and water.

2.2. Induction of diabetes and measurement of blood glucose level

The rats were rendered diabetic by i.v. injection through a tail vein of 50 mg/kg streptozotocin (Sigma, St. Louis, USA) dissolved in 0.01 M citrate buffer. Three weeks later, the presence of diabetes was confirmed by measurement of tail vein blood glucose levels with the Wako glucose test (Wako Chemical, Osaka, Japan). Blood samples were obtained from the tail by pinprick, and only rats with a final blood glucose level >400 mg/dl were included in this study. After final drug administration, we collected and measured blood samples in the same way.

2.3. Drug administration

OT-7100 (Otsuka Pharmaceutical Factory, Tokushima, 10, 30, and 100 mg/kg), gabapentin (Neurontin, Park-Davis, USA, 30 and 100 mg/kg) and aldose reductase inhibitor (epalrestat, Ono Pharmaceutical, Osaka, 30 and 100 mg/kg) were suspended in 5% acacia and administered orally once daily for 21 days. Insulin (Novo Nordisk Pharma, Tokyo, 30 U/kg) was administered subcutaneously once daily for 21 days.

2.4. The Randall–Selitto mechanical hyperalgesia test

The antinociceptive effect was determined by measuring the foot-withdrawal threshold elicited by pressure on the left hind paw (Randall and Selitto, 1957), using the Ugo Basile analgesimeter (Unicom, Chiba, Japan). Mechanical hyperalgesia was chosen as the endopoint in the diabetic rats based on preliminary studies in our laboratory, which indicated that this measure was more reliable than the tactile allodynia measure. This instrument generates a linearly increasing mechanical force applied by a dome-shaped plastic tip placed on the dorsal surface of the rat's hind paw. The force was applied until the rat withdrew the paw. We measured the nociceptive threshold 20 days after streptozotocin injection and chose animals whose nociceptive threshold was lower than 30 mm Hg. During the 3 weeks' administration, this measurement was carried out once a week.

2.5. Measurement of motor nerve conduction velocity

Motor nerve conduction velocity was measured in the most rapidly conducting fibers of the rat tail nerve supplying the segmental muscle according to the method previously described (Miyoshi and Goto, 1973). The rats were kept on a heated pad in a room maintained at 25 °C to preserve a constant rectal temperature. After intraperitoneal injection of sodium pentbarbital (30–40 mg/kg per

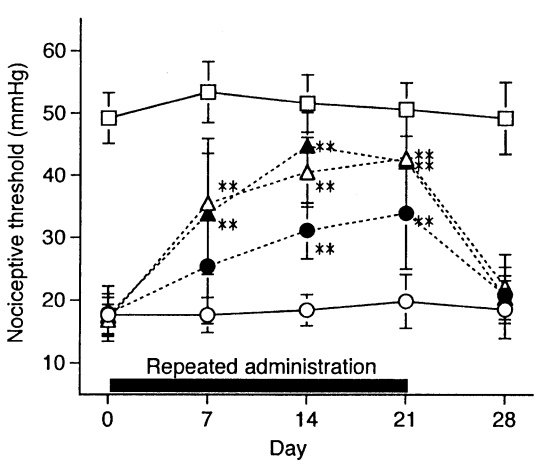

Fig. 1. Time course of the effects of p.o. administration of OT-7100 on mechanical nociceptive threshold in streptozotocin-induced diabetic rats (□: nondiabetic rats; ○: diabetic control; ●: OT-7100, 10 mg/kg; △: OT-7100, 30 mg/kg; ▲: OT-7100, 100 mg/kg). Data are expressed as means ± S.D.; $n = 10$ rats per group. $^{**}P < 0.01$ versus diabetic control, Dunnett's multiple comparison test.

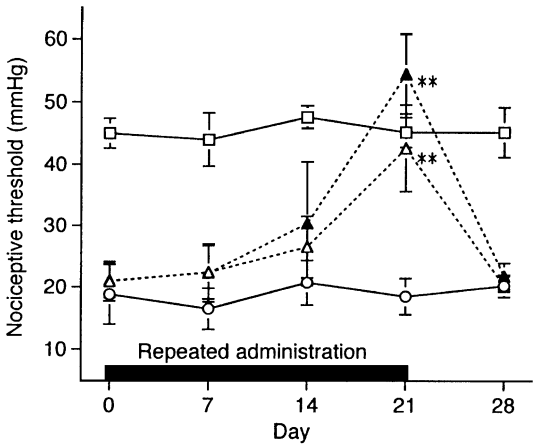

Fig. 2. Time course of the effects of p.o. administration of gabapentin on mechanical nociceptive threshold in streptozotocin-induced diabetic rats (□: nondiabetic rats; ○: diabetic control; △: gabapentin, 30 mg/kg; ▲: gabapentin, 100 mg/kg). Data are expressed as means ± S.D.; $n = 8$ rats per group. $^{**}P < 0.01$ versus diabetic control, Dunnett's multiple comparison test.

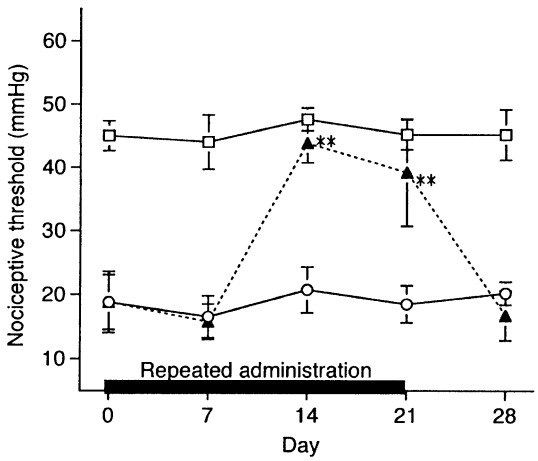

Fig. 4. Time course of the effects of s.c. administration of insulin on mechanical nociceptive threshold in streptozotocin-induced diabetic rats (□: nondiabetic rats; ○: diabetic control; ▲: insulin, 30 U/kg). Data are expressed as means ± S.D.; $n = 8$ rats per group. $^{**}P < 0.01$ versus diabetic control, Dunnett's multiple comparison test.

body weight), motor nerve conduction velocity was determined using a Neuropak 2 instrument (Nihon-Koden, Osaka, Japan) at 0 and 3 weeks following the treatment.

2.6. Drugs

OT-7100 (5-*n*-butyl-7-(3,4,5-trimethoxybenzoylamino)pyrazolo[1,5-α]pyrimidine) was synthesized at Otsuka Pharmaceutical Factory. Streptozotocin was purchased from Sigma. Insulin was purchased from Novo Nordisk Pharma. Gabapentin was extracted from Neurontin (Park-Davis). Aldose reductase inhibitor was extracted from epalrestat preparation (Kinedak, Ono Pharmaceuticals).

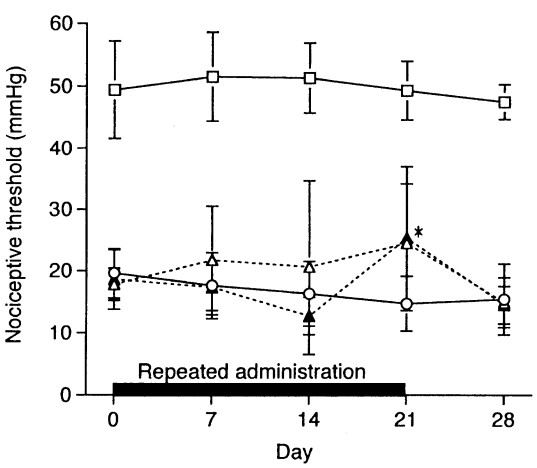

Fig. 3. Time course of the effects of p.o. administration of aldose reductase inhibitor on mechanical nociceptive threshold in streptozotocin-induced diabetic rats (□: nondiabetic rats; ○: diabetic control; △: aldose reductase inhibitor, 30 mg/kg; ▲: aldose reductase inhibitor, 100 mg/kg). Data are expressed as means ± S.D.; $n = 8$–9 rats per group. $^{*}P < 0.05$ versus diabetic control, Dunnett's multiple comparison test.

2.7. Data analysis and statistics

Data are expressed as means ± S.D. The time course of the effects of the various treatments was analyzed statistically by means of an analysis of variance followed by Dunnett's test. The significance level was $P < 0.05$.

3. Results

3.1. Effects of OT-7100 or other drugs on nociceptive threshold in diabetic rats

Three weeks after the induction of diabetes, the nociceptive threshold was significantly decreased in the diabetic control group (nondiabetic group: 49.2 ± 4.1 mm Hg; diabetic control group: 17.6 ± 3.4 mm Hg). Injection of citrate buffer did not influence the nociceptive threshold. OT-7100 significantly increased the nociceptive threshold from day 7 at doses of 30 and 100 mg/kg (OT-7100 30 mg/kg group: 35.5 ± 10.4 mm Hg; OT-7100 100 mg/kg group: 33.8 ± 9.7 mm Hg) and from day 14 at a dose of 10 mg/kg (OT-7100 10 mg/kg group: 31.1 ± 4.5 mm Hg; Fig. 1). Gabapentin strongly increased the nociceptive threshold from day 21 at doses of 30 and 100 mg/kg

Table 1
Effect of OT-7100 on motor nerve conduction velocity in diabetic rats

Treatment	Before	After 21 days (m/s)
Nondiabetic rats	44.1 ± 1.5	48.3 ± 2.4
Diabetic control rats	41.3 ± 1.5[a]	44.2 ± 1.5[a]
OT-7100 100 mg/kg	41.1 ± 1.7[a]	44.4 ± 0.9[a]

Each value represents the mean ± S.D.; $n = 10$.
[a] $P < 0.05$ vs. nondiabetic rats, Dunnett's test.

(gabapentin 30 mg/kg group: 42.6 ± 7.0 mm Hg; gabapentin 100 mg/kg group: 54.5 ± 6.3 mm Hg; Fig. 2). Aldose reductase inhibitor slightly increased the nociceptive threshold on day 21 at a dose of 100 mg/kg (aldose reductase inhibitor 100 mg/kg group: 25.3 ± 11.7 mm Hg; Fig. 3). Insulin (pancreatic hormone) significantly increased the nociceptive threshold from day 14 (insulin 30 U/kg group: 43.8 ± 3.1 mm Hg; Fig. 4).

3.2. Effects of OT-7100 on motor nerve conduction velocity in diabetic rats

We measured motor nerve conduction velocity before OT-7100 administration and after 3 weeks of OT-7100 administration. Before administration, motor nerve conduction velocity decreased in diabetic rats (nondiabetic group: 44.1 ± 1.5 m/s; diabetic control group: 41.3 ± 1.5 m/s; OT-7100 100 mg/kg group: 41.1 ± 1.7 m/s). Three weeks of repeated OT-7100 administration had no effect to motor nerve conduction velocity in diabetic rats (nondiabetic group: 48.3 ± 2.4 m/s; diabetic control group: 44.2 ± 1.5 m/s; OT-7100 100 mg/kg group: 44.4 ± 0.9 m/s; Table 1).

3.3. Effects of OT-7100 or other drugs on glucose levels in diabetic rats

Blood was collected from a tail vein 3 h after drug administration. OT-7100, gabapentin and aldose reductase inhibitor had no effect on glucose levels in diabetic rats. Insulin strongly decreased glucose levels to normal (diabetic control group: 492 ± 196 mg/dl; insulin 30 U/kg group: 158 ± 71 mg/dl; Table 2).

Table 2
Effects of OT-7100, gabapentin, aldose reductase inhibitor, and insulin on blood glucose level on day 21

	Doses	Blood glucose (mg/dl)
Diabetic control rats	–	492 ± 196
Diabetic rats + OT-7100		
	10 mg/kg	612 ± 113
	30 mg/kg	606 ± 129
	100 mg/kg	580 ± 85.0
Diabetic rats + gabapentin		
	30 mg/kg	626 ± 256
	100 mg/kg	594 ± 46.0
Diabetic rats + aldose reductase inhibitor		
	30 mg/kg	518 ± 85.2
	100 mg/kg	588 ± 130
Diabetic rats + insulin		
	30 U/kg	158 ± 71.0[a]
Nondiabetic rats	–	120 ± 8.2[a]

Each value represents the mean ± S.D.; $n = 8$.
[a] $P < 0.01$ vs. nondiabetic rats, Dunnett's test.

4. Discussion

This study examined the antinociceptive potency of OT-7100 in rats with streptozotocin-induced diabetic neuropathy. We confirmed that streptozotocin-treated rats showed mechanical hyperalgesia, as previously reported (Ahlgen and Levine, 1993). This hyperalgesia was evident within 14 days of streptozotocin injection and lasted for at least 10 weeks. In this diabetic rat model, the mechanical hyperalgesia induced had a similar time course and magnitude to that seen after partial sciatic nerve ligation, which serves as a model of peripheral neuropathic pain (Seltzer et al., 1990; Bennett and Xie, 1988).

We examined the effects of OT-7100 on mechanical hyperalgesia in diabetic rats and compared the activity with that of other drugs that are clinically used for the same condition. OT-7100 significantly increased the nociceptive threshold in rats with streptozotocin-induced diabetic neuropathy in a dose-dependent manner. OT-7100 had no effect on the glucose levels in diabetic rats. In a previous study, this compound restored the nociceptive threshold in a chronic constriction injury model (Yasuda et al., 1999). This compound was reported to have a specific effect on neuropathic hyperalgesia without affecting the normal nociceptive threshold, and the antinociceptive effect of OT-7100 decreased when administration stopped, which showed that this effect was reversible. We used a receptor binding assay to study the mechanism of OT-7100. The receptors related to pain and nerve function (adrenergic, serotonergic, opioids, dopaminergic, Ca^{2+} channels) were investigated, but this compound had no binding affinity for these receptors (data not shown). So, we presently do not know the mechanism of action of OT-7100. This compound has a pyrazolopyrimidine structure that is similar to adenosine ligands (Jacobson and van Rhee, 1997) and adenosine is an inhibitory neuromodulator that have shown antinociceptive effect in animal model of neuropathic pain (Lee and Yaksh, 1996). We predict that the site of action of OT-7100 might be an adenosine-related enzyme or receptor.

We tested the effects of other agents that are used clinically for the treatment of diabetic neuropathy in this diabetic model. Classical anticonvulsant drugs have a long history of use in the treatment of chronic pain (McQuay et al., 1995). Gabapentin is a new anticonvulsant that is effective for alleviating neuropathic pain in animal models as well as in humans (Rosner et al., 1996; Hunter et al., 1997). This compound has been reported to show antinociceptive efficacy after 4 weeks of treatment with few side effects (Backonja et al., 1998). In the present study, gabapentin increased the nociceptive threshold above the normal level in rats with streptozotocin-induced diabetic neuropathy after 3 weeks' treatment with 100 mg/kg. We examined the effect of gabapentin on the nociceptive threshold in the chronic construction injury model. Gabapentin increased the nociceptive threshold in both the

injured paw and the uninjured paw in this model (data not shown). These results suggest that gabapentin has not only an antinociceptive effect but also the ability to induced mechanical hypoalgesia, by increasing the nociceptive threshold above the normal level. OT-7100 administration did not increase the nociceptive threshold above the normal level and did not affect the nociceptive threshold in the uninjured paw in the chronic construction injury model (Yasuda et al., 1999). These results suggest that OT-7100 specifically suppresses hyperalgesia induced by diabetic neuropathy and has little likelihood of causing side effects related to hypoalgesia.

Aldose reductase inhibitor is clinically used for diabetic complications, especially numbness and pain induced by diabetic neuropathy (Hotta et al., 1996). In this study, this compound slightly increased the nociceptive threshold in diabetic rats. Aldose reductase inhibitor inhibits the transformation of glucose to sorbitol, inactivating the polyol pathway, and also improves nerve conduction velocity (Judzewitsch et al., 1983). In animal models, there are no reports that aldose reductase inhibitor improves pain caused by hyperglycemia. These results suggest that aldose reductase inhibitor has a weak potency to improve pain in diabetic rats.

Insulin is the most common therapy for diabetes mellitus. In this study, insulin normalized blood glucose levels and significantly increased the nociceptive threshold in diabetic rats. Blood glucose level is the most important factor regarding diabetic complications (UKPDS, 1998). In this study, we found that control of blood glucose levels prevented the progression of painful diabetic neuropathy.

Changes in motor nerve conduction velocity are an indication of peripheral nerve disorder. In diabetic patients, motor nerve conduction velocity is decreased by hyperglycemia (Halar et al., 1982). It is known that motor nerve conduction velocity is decreased in streptozotocin-induced diabetic neuropathy (Van Dam et al., 1999). Aldose reductase inhibitor increases motor nerve conduction velocity in diabetic rats by inhibition of sorbitol accumulation (Judzewitsch et al., 1983). In this study, streptozotocin injection decreased on motor nerve conduction velocity. OT-7100 orally administered had no effects on motor nerve conduction velocity despite increasing the nociceptive threshold. These results suggest that OT-7100 improves the nociceptive threshold without affecting motor nerve conduction velocity, and that the mechanism of OT-7100 is different from that of aldose reductase inhibitor.

To summarize, we reported the effects of OT-7100 on nociceptive threshold, glucose levels and motor nerve conduction velocity in streptozotocin-induced diabetic rats. This compound significantly increased the nociceptive threshold in diabetic rats without affecting glucose levels and motor nerve conduction velocity. These results suggest that OT-7100 alleviates hyperalgesia in rats with streptozotocin-induced diabetic neuropathy in a different manner from gabapentin, aldose reductase inhibitor or insulin, and that it may be a new treatment for the pain associated with peripheral nerve injury.

References

Ahlgen, S.C., Levine, J.D., 1993. Mechanical hyperalgesia in streptozotocin-diabetic rats. Neuroscience 52, 1049–1055.

Arner, S., Meyerson, B.A., 1988. Lack of analgesic effect of opioids on neuropathic and idiopathic forms of pain. Pain 33, 11–23.

Backonja, M., Beydoun, A., Edwards, K.R., Schwartz, S.L., Fonseca, V., Hes, M., LaMoreaux, L., Garofalo, E., 1998. Gabapentin for the symptomatic treatment of painful neuropathy in patients with diabetes mellitus. JAMA 280, 1831–1836.

Bays, H.E., Pfeifer, M.A., 1988. Peripheral diabetic neuropathy. Med. Clin. North Am. 72, 1439–1464.

Bennett, G.J., Xie, Y.K., 1988. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 33, 87–107.

Calcutt, N.A., Jorge, M.C., Yaksh, T.L., Chaplan, S.R., 1996. Tactile allodynia and formalin hyperalgesia in streptozotocin-diabetic rats: effects of insulin, aldose reductase inhibition and lidocaine. Pain 68, 293–299.

Clark, C.M., Lee, D.A., 1995. Prevention and treatment of the complications of diabetes mellitus. N. Engl. J. Med. 332, 1210–1217.

Courteix, C., Eschalier, A., Lavarenne, J., 1993. Streptozocin-induced diabetic rats: behavioural evidence for a model of chronic pain. Pain 53, 81–88.

Courteix, C., Bardin, M., Chantelauze, C., Lavarenne, J., Eschalier, A., 1994. Study of the sensitivity of the diabetes-induced pain model in rats to a range of analgesics. Pain 57, 153–160.

Field, M.J., McCleary, S., Hughes, J., Singh, L., 1999. Gabapentin and pregabalin, but not morphine and amitriptyline, block both static and dynamic components of mechanical allodynia induced by streptozotocin in the rat. Pain 80, 391–398.

Halar, E.M., Graf, R.J., Halter, J.B., Brozovich, F.V., Soine, T.L., 1982. Diabetic neuropathy: a clinical, laboratory and electrodiagnostic study. Arch. Phys. Med. Rehabil. 63, 298–303.

Hotta, N., Sakamoto, N., Shigeta, Y., Kikkawa, R., Goto, Y., 1996. Clinical investigation of epalrestat, an aldose reductase inhibitor, on diabetic neuropathy in Japan: multicenter study. Diabetic Neuropathy Study Group in Japan. J. Diabetes Complications 10, 168–172.

Hounsom, L., Tomlinson, D.R., 1997. Dose neuropathy develop in animal models? Clin. Neurosci. 4, 380–389.

Hunter, J.C., Gogas, K.R., Hedley, L.R., Jacobson, L.O., Kassotakis, L., Thompson, J., Fontana, D.J., 1997. The effect of novel anti-epileptic drugs in rat experimental models of acute and chronic pain. Eur. J. Pharmacol. 324, 153–160.

Jacobson, K.A., van Rhee, A.M., 1997. Development of selective purinoceptor agonists and antagonists. In: Jacobson, K.A., Jarvis, M.F. (Eds.), Purinergic Approaches in Experimental Therapeutics. Wiley-Liss, NY, pp. 101–128.

Judzewitsch, R.G., Jaspan, J.B., Polonsky, K.S., Weinberg, C.R., Halter, J.B., Halar, E., Pfeifer, M.A., Vukadinovic, C., Bernstein, L., Schneider, M., Liang, K.Y., Gabbay, K.H., Rubenstein, A.H., Porte Jr., D., 1983. Aldose reductase inhibition improves nerve conduction velocity in diabetic patients. N. Engl. J. Med. 308, 119–125.

Kikkawa, R., Hatanaka, I., Yasuda, H., Kobayashi, N., Shigeta, Y., Terashima, H., Morimura, T., Tsuboshima, M., 1983. Effect of a new aldose reductase inhibitor, (E)-3-carboxymethyl-5-[(2*E*)-methyl-3-phenylpropenylidene]rhodanine (ONO-2235) on peripheral nerve disorders in streptozotocin-diabetic rats. Diabetologia 24, 290–292.

Lee, Y.W., Yaksh, T.L., 1996. Pharmacology of the spinal adenosine receptor which mediates the antiallodynic action of intrathecal adenosine agonists. J. Pharmacol. Exp. Ther. 277, 1642–1648.

McQuay, H.J., Tramer, M., Nye, B.A., Carroll, D., Wiffen, P.J., Moore, R.A., 1995. A systematic review of antidepressants in neuropathic pain. Br. Med. J. 311, 1047–1052.

Miyoshi, T., Goto, I., 1973. Serial in vivo determinations of nerve conduction velocity in rat tails: physiological and pathological changes. Electroencephalogr. Clin. Neurophysiol. 35, 125–131.

Randall, L.O., Selitto, J.J., 1957. A method of measurement of analgesic activity on inflamed tissue. Arch. Int. Pharmacodyn. Ther. 111, 409–419.

Rosner, H., Rubin, L., Kestenbaum, A., 1996. Gabapentin adjunctive therapy in neuropathic pain states. Clin. J. Pain 12, 56–58.

Seltzer, Z., Dubner, R., Shir, Y., 1990. A novel behavioural model of neuropathic pain disorders produced in rats by partial sciatic nerve injury. Pain 43, 205–218.

UKPDS, 1998. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. Lancet 352, 854–865.

Van Dam, P.S., Van Asbeck, B.S., Bravenboer, B., Van Oirschot, J.F., Marx, J.J., Gispen, W.H., 1999. Nerve conduction and antioxidant levels in experimentally diabetic rats: effects of streptozotocin dose and diabetes duration. Metabolism 48, 442–447.

Vinik, A.I., Holland, M.T., LeBeau, J.M., Liuzzi, F.J., Stansberry, K.B., Colen, L.B., 1992. Diabetic neuropathies. Diabetes Care 15, 1926–1975.

Wuarin-Bierman, L., Zahnd, G.R., Kaufmann, F., Burcklen, L., Adler, J., 1987. Hyperalgesia in spontaneous and experimental animal models of diabetic neuropathy. Diabetologia 30, 653–658.

Yasuda, T., Iwamoto, T., Ohara, M., Sato, S., Kohri, H., Noguchi, K., Senba, E., 1999. The novel analgesic compound OT-7100 (5-*n*-butyl-7-(3,4,5-trimethoxybenzoylamino) pyrazolo[1,5-α]pyrimidine) attenuates mechanical nociceptive responses in animal models of acute and peripheral neuropathic hyperalgesia. Jpn. J. Pharmacol. 79, 65–73.

Zimmermann, M., 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16, 109–110.