Antinociceptive Effect of *Gosha-jinki-gan*, a *Kampo* Medicine, in Streptozotocin-Induced Diabetic Mice

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ABSTRACT—We evaluated the antinociceptive effect of *Gosha-jinki-gan*, a *Kampo* medicine including processed *Aconiti* tuber, and its mechanism in streptozotocin-induced diabetic mice. *Gosha-jinki-gan* (0.1–1.0 g/kg, p.o.) showed a more potent antinociceptive effect in diabetic mice than in non-diabetic mice. The antinociceptive effect of *Gosha-jinki-gan* (0.3 g/kg, p.o.) in diabetic mice was inhibited by administration of either anti-dynorphin antiserum (5 μg, i.t.) or nor-binaltorphimine (10 mg/kg, s.c.), a κ-opioid antagonist. The antinociceptive activity of *Gosha-jinki-gan* (0.3, 1.0 g/kg, p.o.) was decreased by excluding processed *Aconiti* tuber. Furthermore, the antinociceptive effect of processed *Aconiti* tuber (0.03, 0.1 g/kg, p.o.) was also shown to be enhanced in diabetic mice. These results suggest that the increased antinociceptive effect of *Gosha-jinki-gan* in diabetic mice is partly derived from the action of processed *Aconiti* tuber and that it is based on stimulation of spinal κ-opioid receptors via dynorphin release. *Gosha-jinki-gan* was considered useful for treating painful diabetic neuropathy.

Keywords: *Gosha-jinki-gan*, Antinociception, Diabetes, Processed *Aconiti* tuber, Dynorphin

Neuropathy accompanied by anomalies in pain perception is the most frequent diabetic complication. Since pain related to diabetic neuropathy is difficult to control by nonsteroidal anti-inflammatory drugs, such pain is treated with tricyclic antidepressants (1–3), anticonvulsants (4) or antiarrhythmic drugs (5–7). However, treatment with these drugs does not always provide satisfactory results.

Gosha-jinki-gan is a *Kampo* medicine composed of 10 crude drugs including processed *Aconiti* tuber, and it has been used since ancient times to treat melosalgia, low back pain and numbness. In recent years, some clinical studies suggested that *Gosha-jinki-gan* is especially useful for improving subjective symptoms in diabetic patients (8, 9). Basic investigations also suggested that *Gosha-jinki-gan* improves diabetic neuropathy. Shoji et al. (10) demonstrated that *Gosha-jinki-gan* improved glucose tolerance in diabetic rats and inhibited aldose reductase activity. Nishizawa et al. (11) reported that *Gosha-jinki-gan* prevented the deterioration of sciatic nerve conduction velocity in diabetic rats. In addition, we suggested that *Gosha-jinki-gan* improves peripheral circulation in diabetic rats based on increased nitric oxide production (12, 13). However, there have not been any reports regarding pharmacological evaluation of the effect of *Gosha-jinki-gan* on increased pain perception in diabetics.

It was reported that the nociceptive threshold with respect to mechanical stimuli are selectively decreased in spontaneously or drug-induced diabetic animals (14–16). Furthermore, various analgesics administered to diabetic animals show changes in pharmacological activities corresponding to the therapeutic effects on patients with painful diabetic neuropathy (17, 18). Therefore, diabetic animals are thought to be an appropriate model for predicting the pharmacological effects of analgesics in clinical use as well as to elucidate the action mechanisms involved. In the present study, using streptozotocin-induced diabetic mice, we evaluated the antinociceptive effect of *Gosha-jinki-gan* and its mechanism.

MATERIALS AND METHODS

Animals and induction of diabetes

Male ddY mice (Japan SLC, Hamamatsu), weighing about 25 g at the beginning of the experiments, were em-

ployed for the studies. Animals were allowed free access to solid food and water in an animal room that was maintained at 22±2℃ with 12-hr light/dark cycles. Diabetes was induced by an injection of streptozotocin (STZ; 150 mg/kg, i.v.) dissolved in 33.3 mM citrate buffer solution at pH 4.5. Age-matched control mice were injected with vehicle alone. The experiments were conducted 2 weeks after injection of vehicle or STZ. Mice with blood glucose levels above 400 mg/dl were considered diabetic.

Determination of nociceptive threshold

Changes in nociceptive threshold were determined by the tail-pressure test using an analgesimeter (Ugo Basile, Milan, Italy). In brief, the tail of the mouse was pressed 1−1.5 cm from the root using a plastic plate (1.5-mm-thick and 8-mm-wide) at a loading rate of 16 g/sec. The weight (g) when the mouse withdrew its tail or struggled was considered the nociceptive threshold.

Drugs

Gosha-jinki-gan is composed of 10 crude drugs in fixed proportions: *Rehmanniae* radix (5.0 g), *Achyranthis* radix (3.0 g), *Corni* fructus (3.0 g), *Moutan* cortex (3.0 g), *Alismatis* rhizoma (3.0 g), *Dioscoreae* rhizoma (3.0 g), *Plantaginis* semen (3.0 g), *Hoelen* (3.0 g), processed *Aconiti* tuber (1.0 g) and *Cinnamomi* cortex (1.0 g). The drug was prepared as a spray-dried powder from a hot-water extract (yield 16.1%) and obtained from Tsumura (Tokyo). In addition, TJ-3022 (Tsumura) was used as processed *Aconiti* tuber. "*Gosha-jinki-gan* minus processed *Aconiti* tuber" (incomplete formulation of *Gosha-jinki-gan* that excluded processed *Aconiti* tuber) was provided by Mr. Masanori Taki (Kampo and Pharmacognosy Laboratories, Tsumura). Other test drugs used in this study included morphine (Takeda Chemical Industries, Osaka); diclofenac (Sigma Chemical, St. Louis, MO, USA); desipramine amitriptyline and lidocaine (Wako Pure Chemical Industries, Osaka). Lidocaine dissolved in saline was injected i.v., while all the other test drugs were administered orally after suspending or dissolving in distilled water (10 ml/kg). Norbinaltorphimine (Research Biochemicals International (RBI), Natick, MA, USA) dissolved in saline was injected s.c. 2 hr before *Gosha-jinki-gan* administration. According to the method described by Hylden and Wilcox (19), anti-dynorphin A (1−13) antiserum (Peninsula Laboratories, Belmont, CA, USA) was injected intrathecally (i.t.) immediately after *Gosha-jinki-gan* administration. Five microliters of anti-human IgG antibody (RBI) was used as the control of anti-dynorphin A (1−13) antiserum. Naltorindole (Sigma), yohimbine (Sigma) and methysergide (RBI) were injected s.c. 15 min after *Gosha-jinki-gan* administration. Sulpiride (Sigma) was injected i.p. immediately after *Gosha-jinki-gan* administration.

Statistical analyses

The results are expressed as means±S.E.M. Significance of differences was determined by one way analysis of variance (ANOVA) followed by Student's *t*-test or Dunnett's *t*-test. In all cases, $P<0.05$ was considered significant.

RESULTS

Antinociceptive effects of Gosha-jinki-gan and processed Aconiti tuber in non-diabetic and diabetic mice

The nociceptive threshold in diabetic mice (71.3±0.2 g, N=142) was significantly ($P<0.01$, Student's *t*-test) decreased compared with that in non-diabetic mice (106±0.7 g, N=136). *Gosha-jinki-gan* (0.1−1.0 g/kg, p.o.) and processed *Aconiti* tuber (0.03, 0.1 g/kg, p.o.) showed dose-dependent antinociceptive effects in both non-diabetic and diabetic mice (Figs. 1 and 2). The antinociceptive activities of both *Gosha-jinki-gan* (0.1−1.0 g/kg) and processed *Aconiti* tuber (0.03, 0.1 g/kg) were significantly more potent in diabetic mice than in non-diabetic mice. The antinociceptive activity of processed *Aconiti* tuber was increased 1.7−1.9 times in diabetic mice compared with that in non-diabetic mice. *Gosha-jinki-gan* showed a more marked increase (2.5−5.0 times) in diabetic mice than in non-diabetic mice. The antinociceptive activity of *Gosha-jinki-gan* minus processed *Aconiti* tuber (0.3, 1.0 g/kg, p.o.) was approximately 60% of that of *Gosha-jinki-gan* (0.3, 1.0 g/kg, p.o.) (Table 1).

Antinociceptive effects of several other drugs in non-diabetic and diabetic mice

Antinociceptive activities of morphine (8, 16 mg/kg, p.o.) and diclofenac (20, 100 mg/kg, p.o.), a typical nonsteroidal anti-inflammatory drug, was decreased under diabetic conditions. Antinociceptive effects of two tricyclic antidepressants, desipramine (200 mg/kg, p.o.) and amitriptyline (200 mg/kg, p.o.), were not influenced by diabetes. Lidocaine (1 mg/kg, i.v.), an antiarrhythmic drug, showed more potent antinociceptive activity in diabetic mice than in non-diabetic mice (Table 1).

Effects of anti-dynorphin A (1−13) antiserum and various antagonists on Gosha-jinki-gan−induced antinociception in diabetic mice

The increased antinociceptive activity of *Gosha-jinki-gan* (0.3 g/kg, p.o.) in diabetic mice was not influenced by naltorindole (0.1 mg/kg, s.c.), a δ-opioid antagonist. However, approximately 60% of the antinociceptive

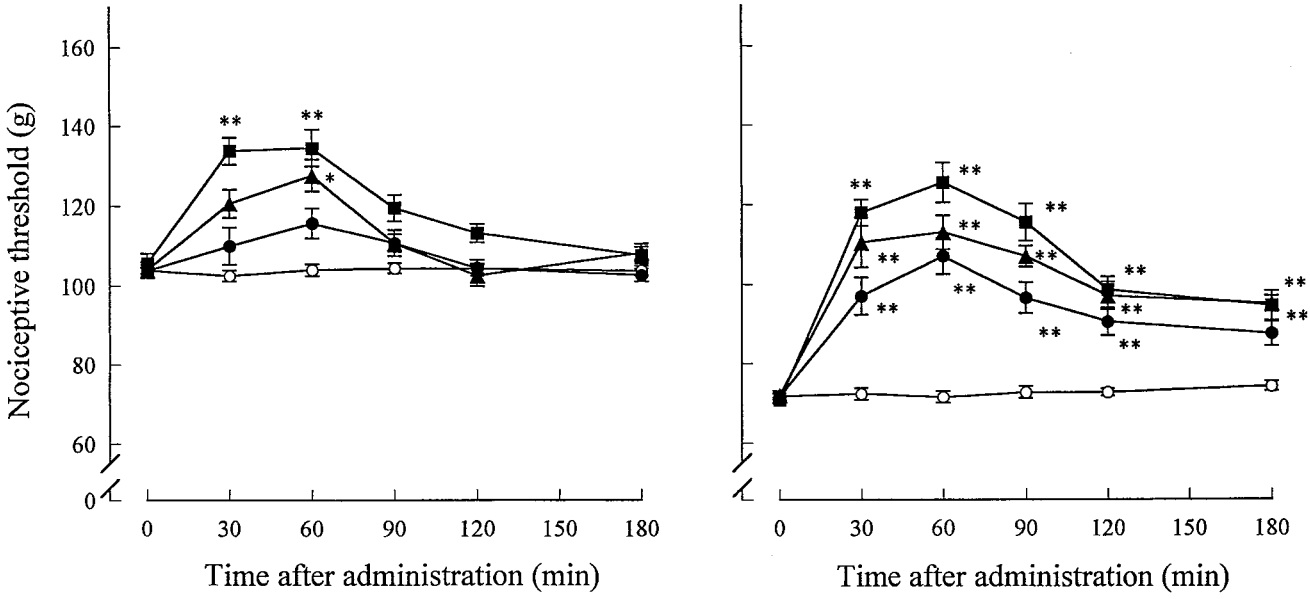

Fig. 1. Time course of the effect of *Gosha-jinki-gan* on nociceptive threshold in non-diabetic (left panel) and diabetic (right panel) mice. ○: distilled water (10 ml/kg, p.o.), ●: *Gosha-jinki-gan* (0.1 g/kg, p.o.), ▲: *Gosha-jinki-gan* (0.3 g/kg, p.o.), ■: *Gosha-jinki-gan* (1.0 g/kg, p.o.). Each point and vertical bar represents the mean±S.E.M. of 8 to 10 animals. *P<0.05, **P<0.01, compared with the value of distilled water-treated animals (Dunnett's *t*-test).

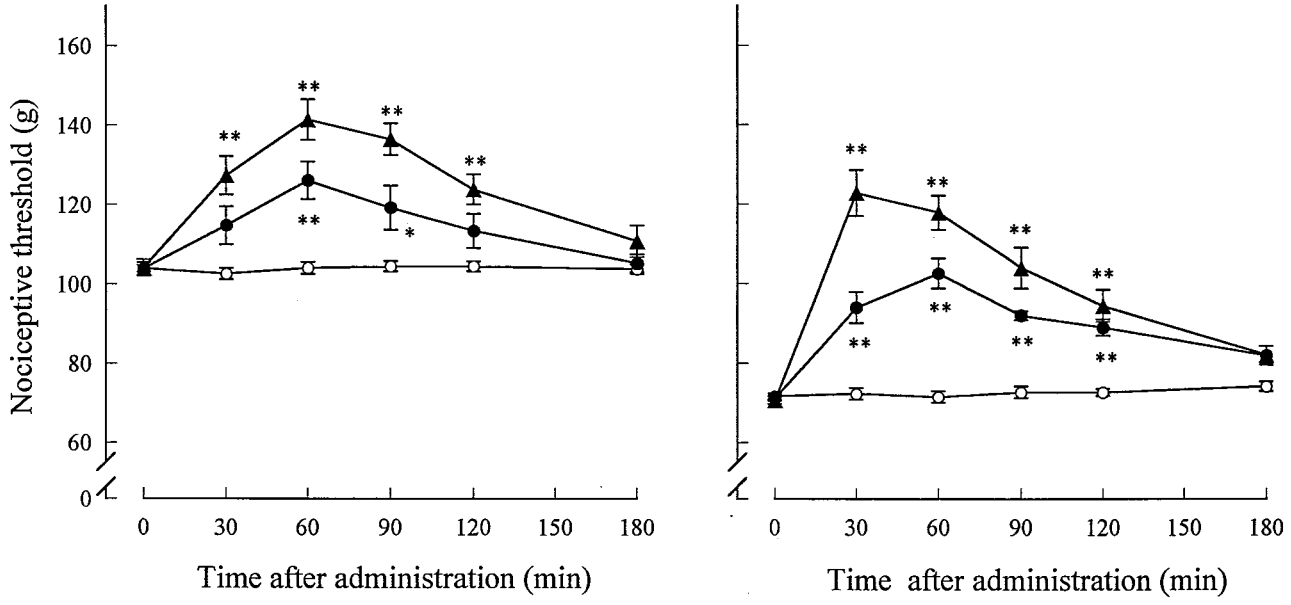

Fig. 2. Time course of the effect of processed *Aconiti* tuber on the nociceptive threshold for pressure stimulation in non-diabetic (left panel) and diabetic (right panel) mice. ○: distilled water (10 ml/kg, p.o.), ●: processed *Aconiti* tuber (0.03 g/kg, p.o.), ▲: processed *Aconiti* tuber (0.1 g/kg, p.o.). Each point and vertical bar represents the mean±S.E.M. of 9 to 10 animals. *P<0.05, **P<0.01, compared with the value of distilled water-treated animals (Dunnett's *t*-test).

activity of *Gosha-jinki-gan* was inhibited by administration of either nor-binaltorphimine (10 mg/kg, s.c.), a κ-opioid antagonist, or anti-dynorphin A (1−13) antiserum (5 μg, i.t.). Sulpiride (3, 10 mg/kg, i.p.), a dopamine D_2-antagonist, decreased the antinociceptive activity of *Gosha-jinki-gan* by approximately 50%. The anti-

Table 1. Antinociceptive effects of *Gosha-jinki-gan*, processed *Aconiti* tuber, *Gosha-jinki-gan* minus processed *Aconiti* tuber and several other drugs in non-diabetic and diabetic mice

	Dose	AUC	
		Non-diabetes	Diabetes
Distilled water		−0.2±3.2	5.3±3.0
	(g/kg)		
Gosha-jinki-gan	0.1	13.1±6.4	64.8±8.2$^{**\#\#}$
	0.3	24.4±4.7	87.5±7.0$^{**\#\#}$
	1	41.9±6.9*	106.3±5.2$^{**\#\#}$
Processed *Aconiti* tuber	0.03	42.3±8.7*	78.3±3.7$^{**\#\#}$
	0.1	75.5±9.3**	127.0±10.5$^{**\#\#}$
Gosha-jinki-gan	0.3		49.3±4.7*††
minus Processed *Aconiti* tuber	1		67.8±3.1**††
	(mg/kg)		
Morphine	8	105.6±7.6**	44.4±4.1$^{\#\#}$
	16	157.1±16.8**	51.1±4.0$^{**\#\#}$
Diclofenac	20	51.4±9.4*	22.6±4.0$^{\#\#}$
	100	83.2±10.6**	32.2±5.1$^{\#\#}$
Desipramine	200	54.7±9.9*	62.7±8.0**
Amitriptyline	200	62.4±14.8**	68.5±5.3**
Lidocane	1	17.5±6.4	68.0±3.4$^{**\#\#}$

The antinociceptive activity was measured by the tail-pressure test every 0.5 hr after oral administration of the drugs (except for i.v. injection of lidocane) for 3 hr and expressed as the area under the time-response curve (AUC) obtained by plotting the increase in nociceptive threshold (g) on the ordinate and the time interval (hr) on the abscissa. Each value represents the mean±S.E.M. of 8 to 14 animals. $^{*}P<0.05$, $^{**}P<0.01$, compared with the value of distilled water-treated animals (Dunnett's *t*-test). $^{\#\#}P<0.01$, $^{\dagger\dagger}P<0.01$, compared with the value of non-diabetic and *Gosha-jinki-gan*−treated animals, respectively (Student's *t*-test).

nociceptive effect of *Gosha-jinki-gan* was also inhibited by administration of yohimbine (3, 10 mg/kg, s.c.), a noradrenaline α_2-antagonist, or methysergide (5, 20 mg/kg, s.c.), a serotonin antagonist. However, the higher doses of yohimbine and methysergide inhibited the antinociceptive activity of *Gosha-jinki-gan* by 49% and 51%, respectively (Table 2).

DISCUSSION

The major finding of the experiment reported here was that *Gosha-jinki-gan* has an antinociceptive effect that increases under diabetic conditions. The antinociceptive effect of *Gosha-jinki-gan* was considered at least partially responsible for improving subjective symptoms in diabetic patients.

The results of this study suggest that few drugs are suitable for the treatment of pain in diabetic patients. The decreased antinociceptive activity of morphine in diabetic mice may be attributed to hypo-responsiveness to supraspinal μ_1-opioid receptor-mediated antinociception (20). The result obtained with diclofenac in diabetic mice was thought to be supported by the lack of an antinociceptive effect by aspirin, a nonsteroidal anti-inflammatory drug, in STZ-induced diabetic rats (17). It was speculated that tricyclic antidepressants were widely used to treat patients with painful diabetic neuropathy (1−3) because the antinociceptive activities of these drugs were not decreased under diabetic conditions. However, the antinociceptive activity of lidocaine was increased in diabetic mice in contrast with that of morphine or diclofenac. It was also demonstrated that lidocaine shows a more potent antinociceptive effect in diabetic rats than in non-diabetic rats (17). Kastrup et al. (6) demonstrated evidence of an activated endogenous opioid system after i.v. lidocaine administration. In addition, we suggested that an antinociceptive mechanism via δ_1-opioid receptor was involved in the increased antinociception of mexiletine in diabetic mice (18). The molecular structure of mexiletine is similar to that of lidocaine. Selective increase in opioid analgesia associated with supraspinal δ_1-opioid receptors in diabetic mice (21) may explain the

Table 2. Effects of anti-dynorphin A (1–13) antiserum and various antagonists on *Gosha-jinki-gan*–induced antinociception in diabetic mice

		AUC
Gosha-jinki-gan (0.3 g/kg, p.o.) +		
Nor-binaltorphimine	–	79.8±6.6
10 mg/kg s.c.	+	31.0±4.2**
Naltorindole	–	98.9±7.8
0.1 mg/kg s.c.	+	97.5±12.4
Anti-dynorphin	–	67.5±2.2
5 μg i.t.	+	26.0±2.6**
Sulpiride	–	65.8±5.9
3 mg/kg i.p.	+	33.5±5.5**
10 mg/kg i.p.	+	34.1±3.8**
Yohimbine	–	70.8±5.2
3 mg/kg s.c.	+	41.9±3.0**
10 mg/kg s.c	+	36.1±2.9**
Methysergide	–	60.7±4.7
5 mg/kg s.c.	+	41.6±3.8**
20 mg/kg s.c.	+	29.5±4.4**

The antinociceptive activity was measured by the tail-pressure test every 0.5 hr after administration of *Gosha-jinki-gan* (0.3 g/kg, p.o.) for 3 hr and expressed as the area under the time-response curve (AUC). Each value represents the mean±S.E.M. of 7 to 9 animals. **$P<0.01$, compared with the corresponding value (Student's *t*-test).

increased antinociceptive effect of lidocaine in diabetic mice. *Gosha-jinki-gan* was shown to be one of the few drugs whose antinociceptive activities are increased in a diabetic condition similar to those of lidocaine and mexiletine.

The findings that the antinociceptive activity of *Gosha-jinki-gan* was not influenced by naltorindole but was inhibited by nor-binaltorphimine suggest that part of the antinociceptive effect of *Gosha-jinki-gan* is mediated by κ-opioid receptors, unlike that of lidocaine. U-50488H, a κ-opioid agonist, administered s.c. or i.t. but not i.c.v. to diabetic mice, showed more potent antinociceptive activity than that in non-diabetic mice (Y. Suzuki et al., unpublished observations). Hyper-responsiveness to spinal κ-opioid receptor-mediated antinociception in diabetic mice may explain the increased antinociceptive activities of *Gosha-jinki-gan* under diabetic conditions. Since effects similar to the administration of nor-binaltorphimine were obtained by the i.t. administration of anti-dynorphin antiserum, *Gosha-jinki-gan* is considered to indirectly stimulate intraspinal κ-opioid receptors via the release of dynorphin, which is an endogenous κ-opioid ligand.

Takeshige et al. (22, 23) demonstrated that stimulation of spinal κ-opioid receptors by dynorphin induced an increase in dopaminergic transmissions in the hypothalamic arcuate nucleus (HARN) and subsequently showed an antinociceptive effect via activation of the descending pain inhibitory system transmitted by both noradrenaline and serotonin. Therefore, it was speculated that sulpiride decreased the antinociceptive effect of *Gosha-jinki-gan* resulting from stimulation of spinal κ-opioid receptors by blocking dopaminergic transmissions in HARN. Similarly, the decrease in the antinociceptive activity of *Gosha-jinki-gan* due to inhibition of the descending pain inhibitory system by the administration of either yohimbine or methysergide suggests that part of the antinociceptive effect of *Gosha-jinki-gan* is based on activation of the descending pain inhibitory system.

The antinociceptive activity of *Gosha-jinki-gan* was decreased by excluding processed *Aconiti* tuber. The enhancement of the antinociceptive effect of processed *Aconiti* tuber in diabetic mice also suggests that processed *Aconiti* tuber plays an important role in the antinociceptive effect of *Gosha-jinki-gan*. We have demonstrated in an experiment using non-diabetic mice the possibility that the antinociceptive effect of processed *Aconiti* tuber is derived from activation of the descending pain inhibitory system based on stimulation of intraspinal non-μ-type opioid receptors (24). Therefore, the action of *Gosha-jinki-gan* in inducing the release of intraspinal dynorphin is considered ascribable to the activity of processed *Aconiti* tuber.

In this study, nor-binaltorphimine, anti-dynorphin antiserum or sulpiride was administered at a dose that mostly obliterated the antinociceptive activity of processed *Aconiti* tuber (0.3 g/kg, p.o.) (Y. Omiya et al., unpublished observations). Doses of 0.1 mg/kg of naltorindole, 3 mg/kg of yohimbine and 5 mg/kg of methysergide were used as the sufficient ones to inhibit the effects of δ-opioid, α_2-noradrenergic and 5-HT receptors, respectively (25–27). However, about 40% of the antinociceptive activity of *Gosha-jinki-gan* remained even after the administration of nor-binaltorphimine or anti-dynorphin antiserum. Also, about 50% of the antinociceptive activity was observed after the treatment with the higher doses of sulpiride, yohimbine, or methysergide, which are considered to suppress the descending pain inhibitory system, and about 60% of the activity remained after exclusion of processed *Aconiti* tuber. From these observations, processed *Aconiti* tuber accounts for approximately half (40–60%) of the antinociceptive effect of *Gosha-jinki-gan*. *Gosha-jinki-gan* showed more marked enhancement of the antinociceptive activity in diabetic mice than processed *Aconiti* tuber. This difference in the enhancement of the antinociceptive

effect between *Gosha-jinki-gan* and processed *Aconiti* tuber suggests that there is a mechanism involved in the enhancement of the antinociceptive activity by *Gosha-jinki-gan* other than the hyper-responsiveness to spinal κ-opioid receptor-mediated antinociception. Further evaluation of the antinociceptive mechanism of *Gosha-jinki-gan* not explained by processed *Aconiti* tuber is warranted.

In conclusion, *Gosha-jinki-gan* may be useful for treating painful diabetic neuropathy because its antinociceptive effect was increased in diabetic mice. It was suggested that the action of processed *Aconiti* tuber, which stimulates spinal κ-opioid receptors via dynorphin release and subsequently activates the descending pain inhibitory system, was responsible for approximately half (40–60%) of the antinociceptive effect of *Gosha-jinki-gan*.

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