

# Suppression of Glucose Absorption by Extracts from the Leaves of *Gymnema inodorum*

Kazumasa SHIMIZU, Mie OZEKI, Katsunori TANAKA, Kayo ITOH, Shinjiro NAKAJYO, Norimoto URAKAWA, and Mikito ATSUCHI<sup>1)</sup>

Division of Veterinary Pharmacology, Nippon Veterinary and Animal Science University, Musashino-shi, Tokyo 180 and <sup>1)</sup>Technical Research Laboratory, Kowa Chemical Industries Co., Ltd., Ohta-ku, Tokyo 144, Japan

(Received 21 February 1997/Accepted 7 May 1997)

**ABSTRACT.** *Gymnema sylvestre* (GS) is one of the Asclepiad strains that grows in South-east Asia. Their therapeutic effects for treating diabetes mellitus, rheumatic arthritis and gout have been well known for a long time. However, the problem is that GS suppresses sweetness and tastes bitter. For this study, we chose *Gymnema inodorum* (GI) instead of GS, since it has an advantage that it does not suppress sweetness nor is it bitter in taste. In this paper, effects of glucose availability of some saponin fractions (F-I to F-IV) extracted from GI leaves, which were obtained by high-performance liquid chromatography were studied on a high K<sup>+</sup>-induced contraction of guinea-pig intestinal smooth muscle, O<sub>2</sub> consumption on guinea-pig ileum, glucose-evoked transmural potential difference (APD) of guinea-pig everted intestine and blood glucose level in glucose tolerance tests on rats. The extracts of GI leaves suppressed the intestinal smooth muscle contraction, decreased the O<sub>2</sub> consumption, inhibited the glucose evoked-transmural potential, and prevented the blood glucose level. Our studies suggest that the component of GI inhibits the increase in the blood glucose level by interfering with the intestinal glucose absorption process. — **KEY WORDS:** glucose tolerance test, *Gymnema inodorum* leaf, muscle contraction, oxygen consumption, transmural potential.

J. Vet. Med. Sci. 59(9): 753–757, 1997

Gymnemic acids extracted from the leaves of *Gymnema sylvestre* (GS) is one of triterpene saponins [14] and consists of some compounds [7]. On the pharmacological action, crude components separated from the leaves of GS inhibits glucose absorption from the intestinal tract and suppresses the increase in glucose level in oral glucose tolerance tests in rat [16, 17]. Recently, we examined the pharmacological effects of the extracts containing gymnemic acids from GS leaves which were obtained by high-performance liquid chromatography (HPLC). It showed that some of the extracts from it suppress the elevation of blood glucose level by inhibiting glucose uptake in the intestine [12]. *Gymnema inodorum* (GI) belonging to the same group as GS, is also well known to have therapeutic effects similar to those of GS in treating certain diseases such as diabetes mellitus, rheumatic arthritis and gout. The difference between GI and GS is that GI does not have some of the GS effects which include suppression of sweetness and bitter in taste [3, 6].

In this study, we separated four different fractions from the saponin extract of GI leaves by HPLC and evaluated the effects of the fractions on glucose utilization by experiments with a high K<sup>+</sup>-induced contraction on guinea-pig intestinal smooth muscle, O<sub>2</sub> consumption on guinea-pig ileum, a glucose-evoked transmural potential difference (APD) on guinea-pig inverted intestine. And blood glucose levels were examined after glucose tolerance tests which were conducted on the rats.

## MATERIALS AND METHODS

**Extraction and fractionation from the leaves of *Gymnema inodorum* (GI):** GI leaves were dried and crushed, and then treated with citric acid solution (pH 2.5). The treated leaves

were extracted with 75% ethanol, and then the extract was evaporated. The dried extract was mixed with *n*-butanol and water (1:1), then the layer of *n*-butanol was evaporated under vacuum. The residue was washed with petroleum ether to remove fatty components, then extracted with methanol. After filtration, the methanol solution was concentrated under vacuum. The concentrated extract in methanol was separated by HPLC (Column; TSKgel ODS-80Tm (4.6 mm I.D. × 25 cm), eluent; CH<sub>3</sub>CN/H<sub>2</sub>O/AcOH = 40/60/0.1, flow rate; 0.8 ml/min, column temperature; 40°C detection; UV (210 nm)). Four fractions, F-I, F-II, F-III and F-IV, obtained with the chromatography were used in the following experiments (Fig. 1). The yield of each fraction from the dried leaves of GI was 4.60, 6.75, 1.11 and 0.33 g/kg, respectively.

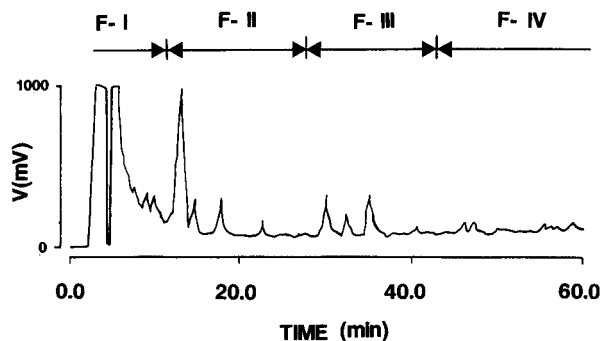


Fig. 1. HPLC elution patterns of crude saponin mixture (F-I, F-II, F-III and F-IV) extracted from the leaves of *Gymnema inodorum*. Column; TSKgel ODS-80Tm (4.6 mm I.D. × 25 cm), eluent; CH<sub>3</sub>CN/H<sub>2</sub>O/CH<sub>3</sub>COOH = 40/60/0.1, flow rate; 0.8 ml/min, column temperature; 40°C, detection; UV (210 nm). Ordinate: Voltage (mv), Abscissa: Retention time (min).

**Measurement of muscle tension:** Hartley male guinea-pigs, weighing between 350 and 450 g (Funabashi Farm, Funabashi), were killed by a blow on the head and bled to death. The lower part of the ileum was removed and placed in modified Tyrode solution with the following compositions (mM): NaCl, 136.8; KCl, 5.4; CaCl<sub>2</sub>, 2.5; MgCl<sub>2</sub>, 1.0; NaHCO<sub>3</sub>, 11.9 and glucose, 5.5. The solution was bubbled continuously with a gas mixture containing 95% O<sub>2</sub> and 5% CO<sub>2</sub>. The bath was maintained at 37°C, with the solution kept at pH 7.2. The ileal longitudinal muscle strips prepared using the ordinary technique were approximately 10 and 5 mm in length and width, respectively. One end of the muscle strip was bound to a glass holder with a silk thread, and the other end was connected to a force transducer (TB-611, Nihon Kohden) with a thread. The muscle contractions were isometrically drawn on a recorder (RJG-4004, Nihon Kohden). A resting tension of 1 g was applied to each strip.

**Rate of oxygen consumption:** Preparations being 20 mm in length and 5 mm in width were prepared from isolated guinea-pig ileal longitudinal muscle with the foresaid method. The preparation was incubated in the Tyrode solution for approximately 60 min until the muscle became stabilized. An oxygen consumption was measured by a similar method as described earlier [13] using a Clark-type polarograph electrode connected with a biological oxygen monitor (model 53, YSI).

**Measurement of transmural potential difference by glucose transport:** The excised ileum was cut 4 cm in length, and then adipose and connective tissues were removed. The ileum was inverted in modified Krebs solution (pH 7.2) in a petri plate. This solution contained: 27.4 mM NaCl; 100 mM mannitol; 24.0 mM Tris HCl; 5.4 mM KCl and 1.0 mM CaCl<sub>2</sub>. After carefully cleaning both the mucosal and serosal sides, one end of the inverted ileum was tied to create an ileal sac. The other end was then connected to a polyethylene tube being approximately 7 cm in length. The sac was placed in a Magnus tube containing 30 ml of the Krebs solution. The serosal lumen was also filled with the Krebs solution. Two agarose bridge electrodes were placed on both the serosal and mucosal sides. Both electrodes were connected to a DC bioamplifier (AVH-9, Nihon Kohden), and a potential difference ( $\Delta$ PD) between internal and external lumen was recorded on the recorder (R-52, Rika Denki). The Krebs solution was continuously bubbled with a mixed gas of 95% O<sub>2</sub> and 5% CO<sub>2</sub> in the temperature kept at  $36 \pm 1^\circ\text{C}$ .

**Glucose tolerance test by oral administration of glucose in rat:** Male Sprague-Dawley strain rats, weighing between 300 and 350 g (Nihon SLC, Hamamatsu), were used after 16 hr of fasting. An appropriate volume of 10% glucose solution was administered to the control group using a gastric tube in 1 g/kg body weight. The treated group was administered with the same volume of glucose solution which was mixed with each fraction of GI leaves. Blood samples were drawn from the tail veins and treated by the *o*-toluidine method. Blood glucose levels were measured before administration of glucose, and at 15, 30, 60 and 120

min after the administration of glucose, by automatic glucose analyzer (GA-1120, Kyoto Daiichi-Kagaku).

**Statistical analyses:** Results are shown in mean  $\pm$  S.E.M. Statistical analyses were performed by either analysis of variance followed by Dunnett's multicomparison test (glucose tolerance) or by means of the unpaired Student's *t*-test (others).  $P < 0.05$  or less was considered as indicative of significance.

## RESULTS

**Effect of the extracts on high K<sup>+</sup>-induced contraction in the ileal longitudinal muscle:** The effects of GI extracts were examined on a contraction evoked by 65.4 mM KCl (H-65K<sup>+</sup>) added hyperosmotically in the ileal longitudinal muscle. The components used in this experiment were broken down into the following four fractions, F-I, F-II, F-III and F-IV, which were separated by HPLC (Fig. 1). A sustained contraction induced by H-65K<sup>+</sup> was gradually suppressed by application of 0.1 mg/ml of F-I or F-II, and the suppressed contraction by F-II was maintained at a steady level for a certain period, but that by F-I gradually recovered. F-III (0.1 mg/ml) also suppressed H-65K<sup>+</sup>-induced contraction, which continued to suppress for the longest period, even as long as 60 min after the application. F-IV (0.1 mg/ml) showed a minimum decrease in the H-65K<sup>+</sup>-induced sustained contraction (Fig. 2). The degree of H-65K<sup>+</sup>-induced contraction at 60 min after the application of F-I, F-II or F-III was expressed as  $70.2 \pm 2.9\%$  ( $n=6$ ),  $81.5 \pm 3.1\%$  ( $n=6$ ) or  $64.5 \pm 2.2\%$  ( $n=6$ ) of that in the absence of any fraction, respectively (Fig. 3).

It is well-known that the sustained contraction induced by H-65K<sup>+</sup> solution in guinea-pig intestine is dependent on the external glucose in the presence of Na<sup>+</sup>. Pyruvate, substrate for oxidative metabolism, is as effective as glucose on the contraction even in the absence of Na<sup>+</sup> [10]. When the muscle strip was relaxed by each fraction, addition of 5.5 mM pyruvate restored the contraction to approximately 90% of that by H-65K<sup>+</sup> (data not shown).

**Changes in the rate of oxygen consumption:** In the ileal longitudinal muscle, the rate of basic oxygen consumption measured for 15 min was  $0.42 \pm 0.04 \mu\text{mol/g/min}$  ( $n=6$ ). The rate of oxygen consumption increased about 2.5 times by the addition of H-65K<sup>+</sup> solution, which was  $1.03 \pm 0.06 \mu\text{mol/g/min}$  ( $n=6$ ) at 15 min. When F-I, F-II or F-III with H-65K<sup>+</sup> solution was applied to the muscle, the rate of oxygen consumption was significantly reduced to  $0.82 \pm 0.04$  ( $n=4$ ),  $0.79 \pm 0.05$  ( $n=4$ ) or  $0.72 \pm 0.05 \mu\text{mol/g/min}$  ( $n=6$ ), respectively.

**Effect of four fractions on  $\Delta$ PD in the inverted ileal sac:**  $\Delta$ PD in the inverted ileal sac in the Krebs solution without glucose was increased to 3–4 mV by adding 5.5 mM glucose to the Krebs solution, which was maintained at a certain degree. The increased  $\Delta$ PD returned to the base line by changing the Krebs solution to the solution without glucose. FI (0.1 mg/ml) which was applied at 10 min after the glucose treatment promptly decreased the  $\Delta$ PD increased with the

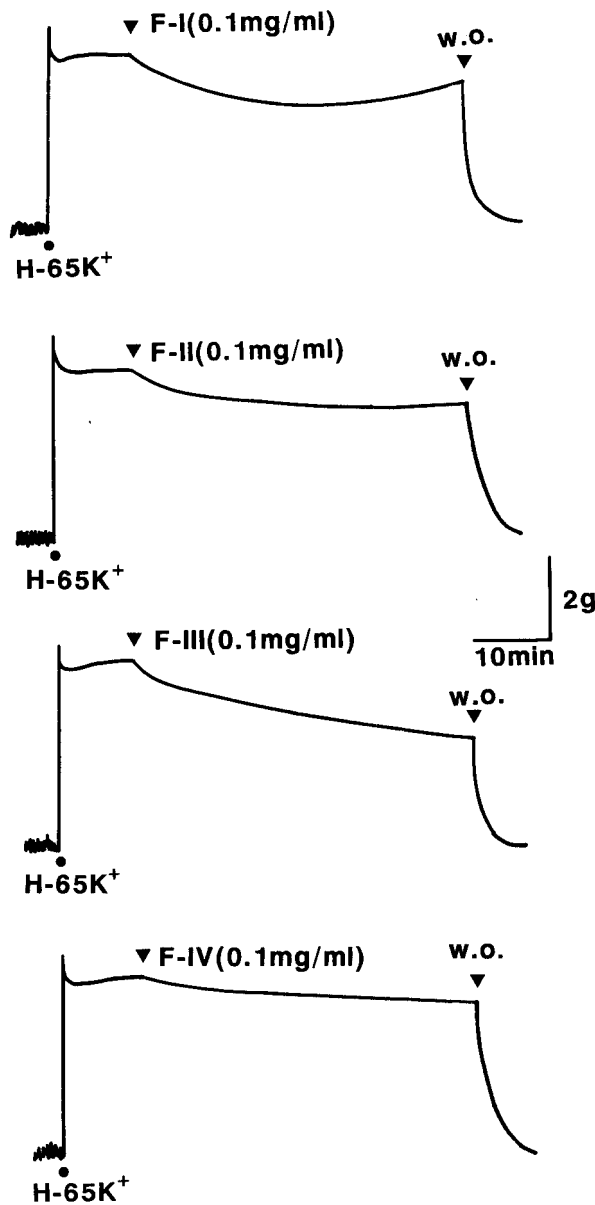


Fig. 2. Effects of F-I, F-II, F-III and F-IV on high K<sup>+</sup>-induced contraction in guinea-pig ileal longitudinal muscle. Typical traces demonstrating the effect of each fraction (0.1 mg/ml) on a hyperosmotically added 65 mM KCl (H-65K<sup>+</sup>)-induced contraction. W.O. is a abbreviation of "wash out".

glucose treatment. F-II (0.1 mg/ml) and F-III (0.1 mg/ml) promptly decreased  $\Delta$ PD which further decreased than FI did. A residual  $\Delta$ PD after the treatment with F-I, F-II or F-III returned to the base line level by removing glucose from the Krebs solution (Fig. 4). The maximum increase in  $\Delta$ PD induced by 5.5 mM glucose was referenced as 100%, and  $\Delta$ PD changes by adding F-I, F-II and F-III were expressed as  $84.0 \pm 4.0\%$  (n=4),  $70.0 \pm 2.0\%$  (n=4) and  $70.0 \pm 3.0\%$  (n=6), respectively (Fig. 5).

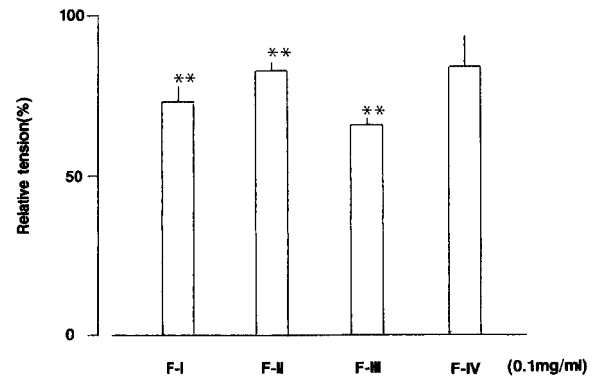


Fig. 3. Inhibitory effects of each fraction (0.1 mg/ml) on the H-65K<sup>+</sup>-induced contraction. A steady level of H-65K<sup>+</sup>-induced contraction just before an addition of the fraction was taken as 100%. Values were obtained 60 min after adding the each fraction. Values of mean ( $\pm$  S. E. M.) of 6 experiments are given. \*\*: Statistically significant difference from the control with  $P < 0.01$ .

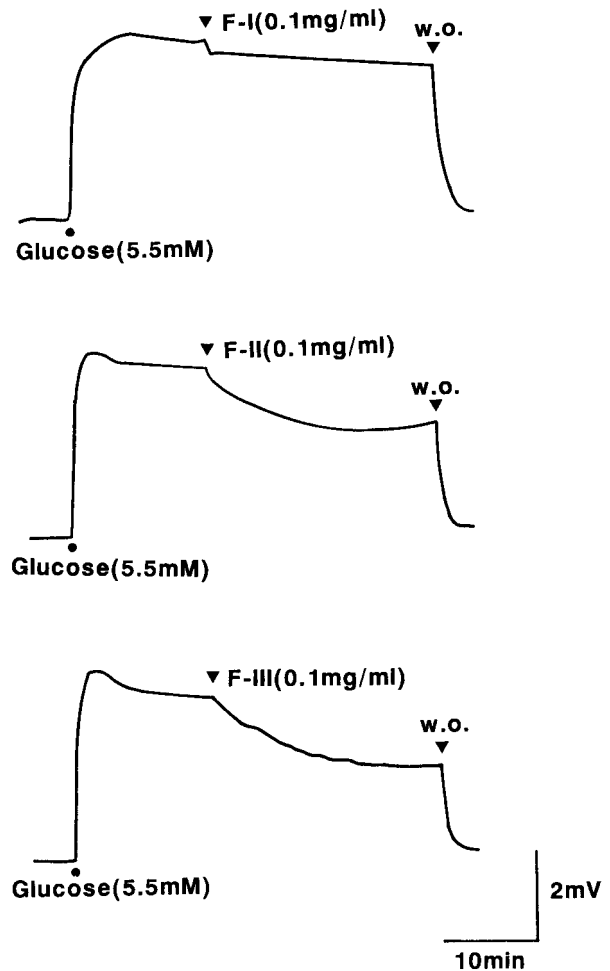


Fig. 4. Typical traces for demonstrating the effect of F-I, F-II or F-III on glucose-evoked transmembrane potential difference (APD). After the  $\Delta$ PD of inverted intestine of guinea-pig was stabilized in the medium adding 5.5 mM glucose, each fraction (0.1 mg/ml) was added. W.O. is a abbreviation of "wash out".

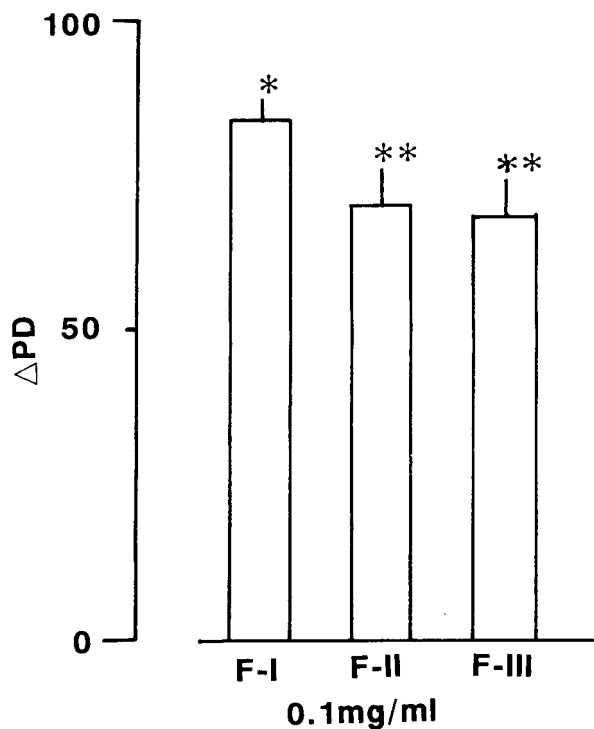


Fig. 5. Changes in  $\Delta$ PD by adding F-I, F-II and F-III. The magnitude of  $\Delta$ PD induced by 5.5 mM glucose just before an addition of each fraction was taken as 100%. Each column indicates the mean  $\pm$  S. E. M. of 4–6 experiments. \* and \*\*: Statistically significant difference from the control with  $P < 0.05$  and  $0.01$ , respectively.

*Effect of extracts on glucose tolerance test in rat:* When 1 g/kg of glucose in solution was administered to rats, the blood glucose level increased at 15 min and peaked at 30 min, and dropped thereafter. The administration of a glucose solution containing F-I (360 mg/kg) demonstrated almost the same values of the control at 15, 30, 60 and 120 min. That is, F-I did not show any effect on the change of blood glucose level. The administration of a glucose solution containing F-II (300 mg/kg) demonstrated an approximate 10% decrease compared to the control at 15 min. In contrast, the F-III (60 mg/kg) group showed a blood glucose level of approximately 60% of control at 15 min, and 40% at 30 min. Both of them were statistically significant from the control. F-IV (120 mg/kg) did not show any significant change in blood glucose level compared to the control (Fig. 6).

## DISCUSSION

In this study, we extracted some saponin fractions from GI leaves by modifying the extraction technique employed for GS [7]. As the first step to clarify the pharmacological activity of some fractions extracted from GI leaves, we examined the pharmacological effects of the extracts (F-I, F-II, F-III and F-IV) which were obtained by HPLC. Each of these fraction was then used in experiments on the smooth

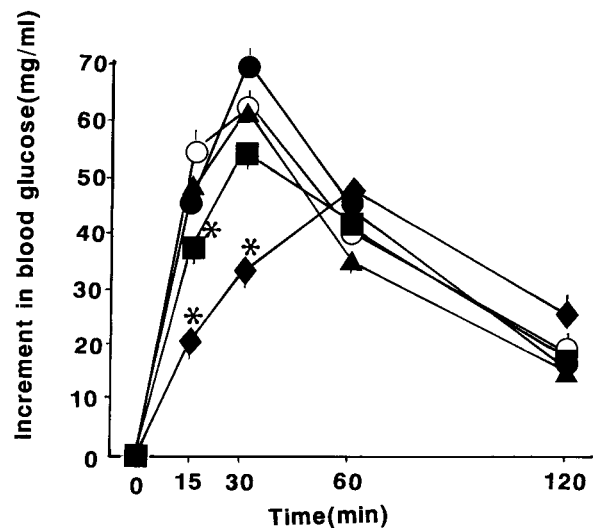


Fig. 6. Effects of each fraction on glucose tolerance test in rat. Rat was orally given 1 g/kg body weight of glucose as 10% solution. , control; , F-I (360 mg/kg); , F-II (300 mg/kg); , F-III (60 mg/kg); , F-IV (120 mg/kg). Glucose solution without (control) or with F-I, F-II, F-III or F-IV was orally given at time 0. Increment in blood glucose was measured at 15, 30, 60 and 120 min. Values represent means  $\pm$  S.E.M., in 5 animals. \*  $P < 0.05$ : the treated groups are compared with the untreated one.

muscle contraction,  $O_2$  consumption and  $\Delta$ PD in the inverted ileal sac of guinea-pig, and on blood glucose level in glucose tolerance test in rats. The purpose of these experiments was to evaluate the effects of GI on glucose utilization.

The sustained contraction of the ileal longitudinal muscle induced by high  $K^+$  solution was suppressed with F-I, F-II and F-III. There was very little change with F-IV. It is known that high  $K^+$  solutions increased the rate of oxygen consumption in the intestinal smooth muscles in dose-dependent manner [9]. In this experiment, the rate of oxygen consumption of the ileal longitudinal muscle was increased 2.5 times by the H-65K $^+$  solution. The addition of F-I, F-II or F-III with H-65K $^+$  solution suppressed the increase of the rate of oxygen consumption. We have already demonstrated that high  $K^+$ -induced contraction of the ileal smooth muscle was inhibited by several metabolic inhibitors, hypoxia, and the removal of glucose from external medium [4, 5, 8, 10]. Therefore, the relaxing effect of smooth muscle with F-I, F-II or F-III which showed a close correlation with the decrease in the oxygen consumption was considered to be mediated by inhibition of aerobic metabolism.

The inhibitory effects of glucose absorption from the intestinal tract have been evaluated by measuring the  $\Delta$ PD [17]. The  $\Delta$ PD level serves as a good indicator of pharmacological effects on the glucose transport system, since  $\Delta$ PD results from a transmembranous electric potential difference produced by a  $Na^+$  shift when glucose is absorbed by a  $Na^+$ -dependent co-transport system [1, 2]. Our recent studies showed that administration of glucose (0.1 to 5.5

mM) in the inverted intestine of rat or guinea-pig proportionally increased  $\Delta$ PD at a given  $\text{Na}^+$  concentration (27.4 mM) [12]. Moreover,  $\Delta$ PD was also increased by  $\text{Na}^+$  (10 to 40 mM) to  $\text{Na}^+$  removal medium containing at a given glucose concentration (5.5 mM) in both the intestine [12]. On the other hand, the high  $\text{K}^+$ -induced contraction of the intestinal smooth muscle was inhibited by a removal of  $\text{Na}^+$  from medium, and the inhibition was recovered by an addition of pyruvate [10, 11, 15]. It is well known that pyruvate enters directly into the cytoplasm through the membrane without the  $\text{Na}^+$ -dependent co-transport system. From these findings, both the decrease of  $\Delta$ PD and the suppression on muscle contraction seem to be due to the same inhibitory action on glucose utilization by  $\text{Na}^+$ -co-transport. In this study, the increased  $\Delta$ PD with a glucose supply as well as the high  $\text{K}^+$ -induced contraction was suppressed by some of the fractions such as F-I, F-II and F-III and recovered by the application of pyruvate. These results suggest a possibility that some components of GI have an inhibitory effect on the  $\text{Na}^+$ -glucose co-transport system.

It has been suggested that crude components extracted from GS suppress the increase in blood glucose level in the oral glucose tolerance tests in rats [16]. This suppressing effect on the glucose level has been assumed to be result of the inhibition of glucose uptake incorporated with  $\text{Na}^+$  entry processes [12, 16, 17]. In this study, F-III significantly suppressed the blood glucose increase at 15 and 30 min in glucose tolerance test. F-II mildly suppressed the blood glucose increase at 15 min. However, F-I and F-IV did not show any significant effects. These results suggest that GI, especially F-III, suppress the blood glucose increase by inhibiting glucose absorption from the intestinal tract.

In conclusion, some components of GI leave which do not suppress sweetness and are not bitter in taste have suppressive effects on the intestinal smooth muscle contraction,  $\text{O}_2$  consumption and  $\Delta$ PD. The effects are probably caused by the inhibition of the  $\text{Na}^+$ -glucose co-transport system. Furthermore, the components inhibit blood glucose increase by interfering with glucose absorption from the intestinal tract in rat.

## REFERENCES

- Barry, R. J. C., Dikstein, S., Matthew, J., Smyth, D. H., and Wright, E. M. 1964. Electrical potentials associated with intestinal sugar transfer. *J. Physiol. (Lond.)* 171: 316–333.
- Debnam, E. S. and Levin, R. J. 1975. An experimental method of identifying and quantifying the active transfer electrogenic component from the diffusive component during sugar absorption measured *in vivo*. *J. Physiol. (Lond.)* 246: 181–196.
- Ikeuchi, H. 1990. Effects of gymnemic acid in a large dose on the plasma glucose concentrations of rats. *J. Yonago Med. Assoc.* 41: 414–431 (in Japanese).
- Ishida, Y., Takagi, K., and Urakawa, N. 1984. Tension maintenance, calcium content and energy production of the taenia of the guinea-pig caecum under hypoxia. *J. Physiol. (Lond.)* 347: 149–159.
- Kishimoto, T., Ozaki, H., Karaki, H., Urakawa, N., and Ishida, Y. 1982. The inhibitory effects of monensin on high  $\text{K}^+$ -induced contraction in guinea-pig taenia coli. *Eur. J. Pharmacol.* 84: 25–32.
- Kurihara, Y. 1969. Antisweet activity of gymnemic acid AI and its derivatives. *Life Sci.* 8: 537–543.
- Liu, H. M., Kiuchi, F., and Tsuda, Y. 1992. Isolation and structure elucidation on gymnemic acids, antisweet principles of *Gymnema sylvestre*. *Chem. Pharm. Bull.* 40: 1366–1375.
- Pfaffman, M., Urakawa, N., and Holland, W. C. 1965. Role of metabolism in  $\text{K}^+$ -induced tension changes in guinea-pig taenia coli. *Am. J. Physiol.* 280: 1203–1205.
- Saito, N., Sakai, Y., Ikeda, M., and Urakawa, N. 1968. Oxygen consumption during potassium induced contracture in guinea-pig taenia coli. *Jpn. J. Pharmacol.* 18: 321–331.
- Shimizu, K., Kaburagi, T., Nakajyo, S., and Urakawa, N. 1991a. Decrease in muscle tension and reduced pyridine nucleotides of the guinea-pig ileal longitudinal smooth muscle in high  $\text{K}^+$ ,  $\text{Na}^+$ -deficient solution. *Jpn. J. Pharmacol.* 56: 53–59.
- Shimizu, K., Kaneda, T., Nakagiri, Y., Arakawa, T., Nakajyo, S., and Urakawa, N. 1991b. Inhibitory mechanism of  $\text{Na}^+$  deficiency on high  $\text{K}^+$ -induced contraction in the smooth muscles of aorta and ileum of guinea-pig. *Bull. Nippon Vet. Anim. Sci. Univ.* 40: 5–14.
- Shimizu, K., Iino, A., Nakajima, J., Tanaka, K., Nakajyo, S., Urakawa, N., Atsuchi, M., Wada, T., and Yamashita, C. 1997. Suppression of glucose absorption by some fractions extracted from *Gymnema sylvestre* leaves. *J. Vet. Med. Sci.* 59: 245–251.
- Stephens, N. L., Kloege, E. A., and Wroegemann, K. 1975. Energy metabolism: Method in isolated smooth muscle and methods at cellular and subcellular level. pp. 555–591. In: *Methods in Pharmacology*, vol. 3, Smooth Muscle (Daniel, E. E. and Paton, D. M. eds.), Plenum Press, New York.
- Stocklin, W., Weiss, E., and Reichstein, T. 1967. Gymnemasäure, das antisaccharine Prinzip von *Gymnema sylvestre* R. Br. Isolierungen Identifizierungen. *Helv. Chim. Acta* 50: 474–490.
- Suzuki, T., Karaki, H., and Urakawa, N. 1980. Mechanism of inhibition of contraction by high  $\text{K}^+$ ,  $\text{Na}^+$ -deficient solution in smooth muscle of guinea-pig taenia coli. *Arch. Int. Pharmacodyn. Ther.* 248: 43–49.
- Yoshioka, S., Takeuchi, T., Imoto, T., Kasagi, T., and Hiji, Y. 1985. Inhibitory effects of gymnemic acid from the indian plant *Gymnema sylvestre* on rising blood glucose levels after sucrose administration. *Igaku-no-Ayumi* 135: 241–242 (in Japanese).
- Yoshioka, S. 1986. Inhibitory effect of gymnemic acid and an extract from the leaves of *Zizyphus jujuba* on glucose absorption in the rat small intestine. *J. Yonago Med. Assoc.* 37: 142–154 (in Japanese).