Suppression of Glucose Absorption by Some Fractions Extracted from *Gymnema sylvestre* Leaves

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ABSTRACT. Extracts containing gymnemic acids, which were extracted from the leaves of *Gymnema sylvestre* (GS) as nine fractions, were evaluated for their effects on a high K^+-induced contraction of guinea-pig ileal longitudinal muscles, on glucose transport mediated by the difference of glucose-evoked transmural potential difference (ΔPD) in the inverted intestine of guinea-pig and rat, and on blood glucose in rat. Among nine fractions obtained by high performance liquid chromatography from the extract, f-2 and f-4 strongly suppressed the high K^+-induced contraction of the ileal muscle, f-3 and f-5 did so moderately, and f-8 and f-9 did so weakly, whereas the other fractions did not affect it. The degree of suppression of high K^+-induced contraction by f-2 at 74% was almost the same as that of f-4 at 67%, at concentrations of 0.1 mg/m*l*. The suppressed contraction by f-2 or f-4 was recovered by adding 5.5 mM pyruvate. The ΔPD increased by 5.5 mM glucose in the inverted intestines of guinea-pig and rat were equally suppressed by 0.1 mg/m*l* of f-2 or f-4 to 40%. In a rat sucrose tolerance test, f-2 and f-4 suppressed the elevation of blood glucose level. Both f-2 and f-4 suppressed the contraction of guinea-pig ileal longitudinal muscle, interfered with the increase in ΔPD induced by glucose in the inverted intestines of guinea-pig and rat, and inhibited the elevation of blood glucose level. In conclusion, it is suggested that some of the extracts containing gymnemic acids from GS leaves suppress the elevation of blood glucose level by inhibiting glucose uptake in the intestine. — KEY WORDS: blood glucose, gymnema extract, intestine, muscle contraction, potential difference.

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Gymnema sylvestre (GS) leaves, of the Asclepiadaceae species, have been known to be effective on diseases including diabetes, rheumatic arthritis and gout in India and South-east Asia. With regard to the physiological effects of this plant, suppression of sweetness and prevention of dental decay are also well known [9]. Furthermore, crude components separated from GS leaves are reported to suppress absorption of glucose by the glucose-evoked transmural potential difference (ΔPD) using the inverted rat intestinal sac, and to suppress blood glucose elevation in sucrose tolerance tests on rats [21]. The pharmacological effects of gymnemic acids with chemical structures already indentified [10] have not yet been revealed. As the first step to clarify the pharmacological activity of each gymnemic acid extracted from GS leaves, we examined the pharmacological effects of the extracts containing gymnemic acids (f-1 to f-9) which were obtained by high-performance liquid chromatography.

In this study, we evaluated whether extracts containing gymnemic acids extracted from GS leaves as nine fractions, a yellowish powder, have a suppressive effect on the high K^+-induced contraction in the guinea-pig ileal longitudinal muscle, on the glucose transport system exhibiting ΔPD using the inverted intestinal sac, and on the increment of blood glucose in the oral sucrose tolerance test on rats. These results show the possibility that some extracts containing gymnemic acids have an inhibitory effect on the glucose transport system in the intestine.

MATERIALS AND METHODS

Method of extraction and refinement from Gymnema sylvestre leaves: Gymnema sylvestre (GS), growing naturally in South-east Asia, was extracted by a method for ordinary glycosides. GS leaves were dried and crushed, and then treated with citric acid. The treated leaves were extracted with 75% ethanol, and then the extract was evaporated. The dried extract was mixed with *n*-butanol and water (2:1), 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 as nine fractions by high performance liquid chromatography (HPLC) (Fig.1). The yield of the extracts from the dried leaves of GS was 0.6 g/kg.

Measurement of muscle tension: Male Hartley guinea-pigs (Funabashi Farm, Funabashi) weighing 350 to 450g were killed by a blow to the head and exsanguinated. After exsanguination, the abdomen was opened, and the ileum was removed. The lower part of the ileum was placed in an incubation medium, and food residues were carefully removed with physiological salt solution (PSS). PSS employed was a modified Tyrode solution of the following composition (mM): NaCl, 136.8; KCl, 5.4; $CaCl_2$, 2.5; $MgCl_2$, 1.0; $NaHCO_3$, 11.9 and glucose, 5.5. The PSS was aerated with 95%O_2, 5%CO_2 gas mixture at 37°C and pH 7.2. Then, the ileal longitudinal muscle preparation, about 1.5 cm long and 0.5 cm wide, was prepared by the method

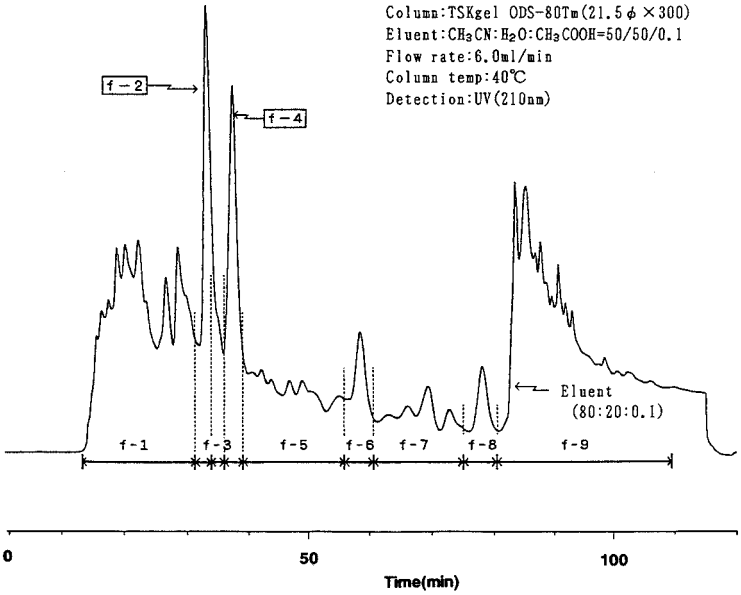

Fig. 1. HPLC characteristics of nine fractions (f-1 to f-9) of *Gymnema sylvestre* extracts.

described by Paton & Aboo Zar [11]. One end of the strip was bound to a glass holder with a silk thread, and the other end was connected by the thread to a strain gauge transducer (TB-611T, Nihon Kohden) to monitor the mechanical activity. The muscle was suspended in an organ bath containing 15 m*l* of PSS. The muscle tension was recorded isometrically. Muscle strips were loaded with a resting tension of 1.0 g.

Measurment of glucose-evoked transmural potential difference (ΔPD): Male guinea-pigs (350–450 g) and rats of either sex (Wistar strain, 300–350 g; Imamichi Institute for Animal Reproduction, Ibaraki) were killed by a blow to the head and exsanguinated. After exsanguination, the abdomen was opened, and the ileum was removed. The intestine (approximately 4 cm length) was suspended in PSS and was cleaned of all fat and connective tissue. Then the segment of intestine was everted in PSS, and the serosal or mucosal surface of the everted segment was washed gently with a modified Kreb's solution (NaCl, 27.4 mM; mannitol, 100 mM; Tris-HCl, 24 mM; KCl, 5.4 mM; $CaCl_2$, 2.5 mM and $MgCl_2$, 1.0 mM) which was adjusted to pH 7.2 by adding 1N HCl. One end of the segment was tied with a silk thread, and the other end was tied over a polyethylene tube (diameter, 6 mm) of about 7 cm length. A small volume, about 2 m*l*, of the Kreb's solution was injected through the tube into the sac of intestine, which we refer to as the serosal fluid. Then the inverted preparation was suspended in an organ bath containing 30 m*l* of the Kreb's solution. The fluid surrounding the sac refer to as the mucosal fluid, and was continuously gassed (95%O_2, 5%CO_2) and maintained at 36 ± 1°C and pH 7.2. The ΔPD was monitored continuously using KCl-agar bridges connected through silver-silver chloride electrodes to a high-sensitivity amplifier (AVH-9, Nihon Kohden), and was recorded on a recorder (R-52, Rika Denki). The KCl-agar bridges were immersed in the mucosal and serosal solutions for reading the potential. In some experiments, NaCl (27.4 mM) was substituted with equimolar choline. Applications of glucose, extracts containing gymnemic acids and other drugs, which were previously solved in an appropriate concentration, were added to the mucosal solution.

Sucrose tolerance test 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% sucrose solution of 1 g/kg body weight was administered to the control rat group using a gastric tube. The treated group was then administered with the same volume of sucrose solution which was mixed with each fraction of the GS leaves. Blood glucose levels were measured before feeding, and, at 15, 30, 60 and 120 min after feeding. Then, blood samples were drawn from the tail veins and analyzed by an automatic glucose analyzer (GA-1120, Kyoto Daiichi Kagaku).

Drugs: The drugs used in the experiments were pyruvic acid (Wako Pure Chemical Industries, Osaka) and phloridzin (Sigma Chemical Co., St. Louis, MO, U.S.A.).

Statistical analyses: Results of the experiments are expressed as the mean ± S. E. M. Statistical analysis was performed by Student's *t* test or analysis of variance (ANOVA). A P value of less than 0.05 was considered to be significant.

RESULTS

Effects of extracts containing gymnemic acids extracted from Gymnema sylvestre leaves on high K^+-induced contraction in the ileal longitudinal muscle: Extracts containing gymnemic acids extracted from *Gymnema*

sylvestre (GS) leaves as a yellowish powder were prepared with high performance liquid chromatography (HPLC) and then broken down into nine fractions as shown in Fig. 1. These fractions were then examined for their effects upon high K^+-induced contraction of the ileal longitudinal muscles of the guinea-pig. It was found that hyperosmotically added 65 mM KCl (H-65K^+) induced a transient contraction followed by a sustained contraction. Specimens of all nine fractions (f-1 to f-9) were prepared by diluting in a solution of ethanol at 0.1 mg/m*l*, and these specimens were applied to the muscle. Fractions f-2, f-3, f-4 and f-5 suppressed the H-65K^+-induced contraction by 74.3 ± 0.4%, 42.2 ± 2.2%, 67.8 ± 2.9% and 34.6 ± 2.0%, respectively. While fractions f-8 and f-9 suppressed the contraction by 17.1 ± 2.2% and 11.7 ± 0.6%, respectively. Fractions f-1, f-6 and f-7 did not affect the contraction. In summary, f-2 and f-4 strongly (Fig. 2-A), f-3 and f-5 moderately, and f-8 and f-9 weakly suppressed the H-65K^+-induced contraction in the ileal longitudinal muscle, whereas, f-1, f-6 and f-7 did not affect it (Fig. 2-B). Fraction f-4 having a strong relaxing activity in the ileal longitudinal muscle, transiently enhanced the H-65K^+-induced contraction preceding the suppressed contraction in most cases (Fig. 2-A).

Effect of pyruvate on the muscle relaxation induced by f-2 or f-4: The actions of f-2 and f-4, which significantly suppressed high K^+-induced contraction in the ileal longitudinal muscle, were assessed. An application of 5.5 mM pyruvate, which is directly utilized as an energy substrate without mediating a Na^+-glucose co-transport system, recovered the relaxation induced by f-2 in the H-65K^+-induced contraction. Pyruvate also recovered the f-4-induced suppression but more weakly than the f-2-induced one (Fig. 3-A, B).

Changes in glucose evoked-potential difference (ΔPD) in the inverted ileal sac: Glucose was applied to the bath after the ΔPD of the inverted ileal sac of the guinea-pig or rat was stabilized in a glucose-removal solution. An application of 5.5 mM glucose increased ΔPD by 3 to 4 mV in both models. The ΔPD returned to the original level when the glucose-added solution was replaced by the modified Kreb's solution without glucose.

Change in ΔPD by adding glucose and Na^+: The ΔPD in the inverted ileal sac of the guinea-pig or rat increased in a dose-dependent manner when glucose was added to the Kreb's solution without glucose in the bath at various concentrations (0.1, 0.5, 1.0, 2.5 and 5.5 mM). The maximum increase in ΔPD induced by 5.5 mM glucose was referenced as 100%, and increases in ΔPD induced by 0.1, 0.5, 1.0 and 2.5 mM glucose resulted in increases of 5, 20, 30 and 60% of the maximum, respectively (Fig. 4-A). An increase in ΔPD of the inverted guinea-pig or rat ileal sac was also seen when NaCl was added at various concentrations (5, 10, 20 and 40 mM) to a NaCl-free medium with glucose (5.5 mM). The maximum increase in ΔPD induced by 40 mM NaCl was referenced as 100% and ΔPD changes by adding 5, 10 or 20 mM NaCl were 20, 45 and 70% of the maximum, respectively (Fig. 4-B).

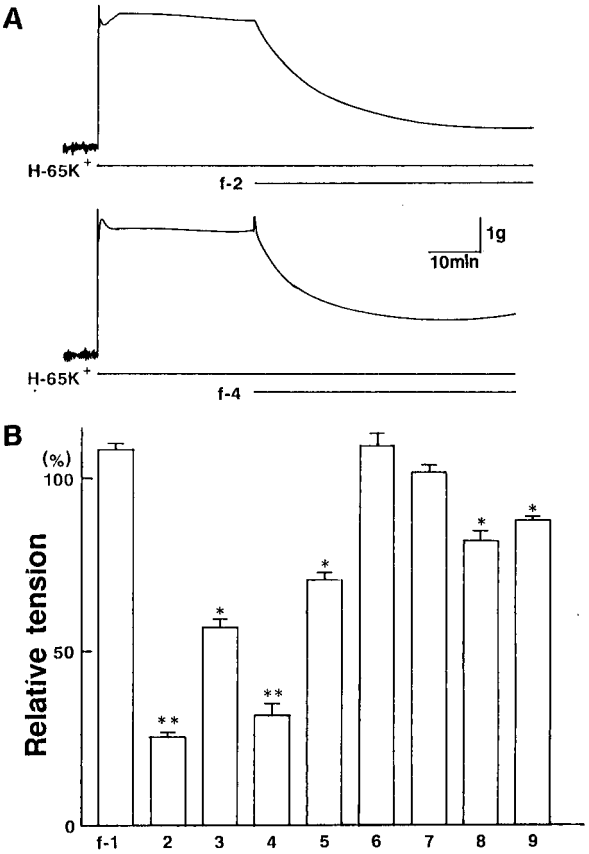

Fig. 2. Inhibitory effects of extracts containing gymnemic acids extracted from *Gymnema sylvestre* leaves on a high K^+-induced contraction in guinea-pig ileal longitudinal muscle. A: Typical traces demonstrating the inhibitory effect of f-2 or f-4 (0.1 mg/m*l*) on a hyperosmotically added 65 mM KCl (H-65K^+)-induced contraction. B: As shown in A, effects of the nine fractions (0.1 mg/m*l*) obtained by HPLC on the H-65K^+-induced contraction. A steady level of H-65K^+-induced contraction was taken as 100%. Values of mean (± S. E. M.) of 6 experiments are given. * and **: Statistically significant differences from the untreated group at $P<0.05$, $P<0.01$, respectively.

Effect of phloridzin on change in ΔPD: After ΔPD was increased by applying 5.5 mM of glucose, phloridzin of varying concentrations from 10^{-6} M to 10^{-4} M was applied. The result showed that the ΔPD increased by glucose in the inverted guinea-pig or rat ileal sac decreased in a dose-dependent manner (Fig. 5).

Effects of f-2 and f-4: ΔPD was increased by applying 5.5 mM of glucose and then f-2 or f-4 was applied. The increased ΔPD in the inverted guinea-pig or rat ileal sac was decreased by adding 0.1 mg/m*l* of f-2. The same degree of ΔPD change was observed with the same dose of f-4 (Fig. 6).

Effects of f-2 and f-4 on sucrose tolerance test in rat: One g/kg body weight of sucrose was given orally to rats in the control group. Then, the blood glucose level was measured at 15, 30, 60 or 120 min after the administration.

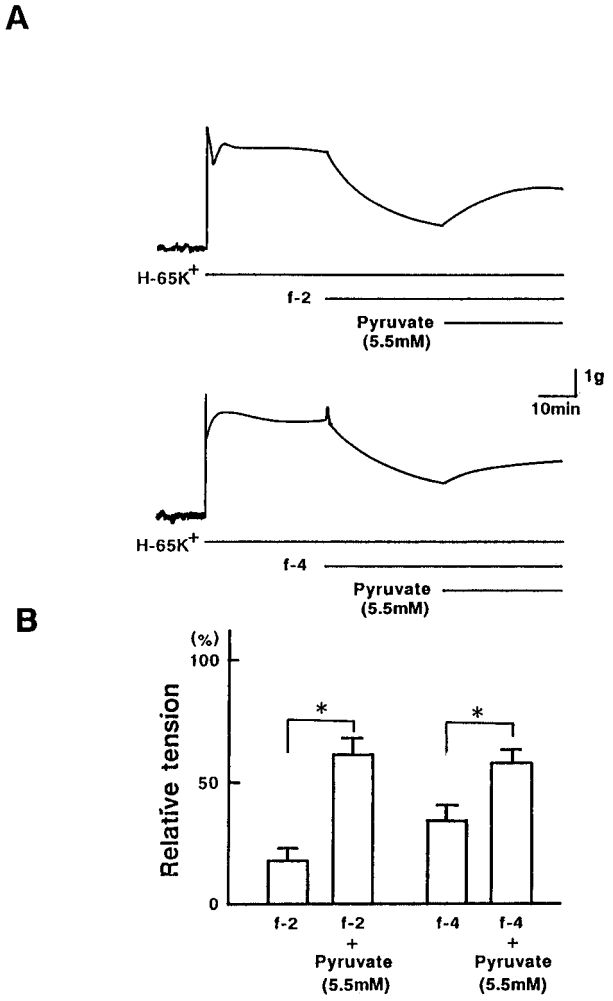

Fig. 3. Effect of pyruvate on the smooth muscle relaxation induced by f-2 and f-4. A: Effects of Pyruvate on the relaxation induced by f-2 (0.1 mg/m*l*) (upper trace) and f-4 (0.1 mg/m*l*) (lower trace) fractions on the H-65K$^+$-induced contraction. B: Summary of the results in A. A steady level of H-65K$^+$-induced contraction was taken as 100%. Values represent means ± S. E. M., n=4–6 experiments. *: Significantly different from control at $P<0.05$.

It was found that the blood glucose level increased at 15 min and peaked at 30 min, then declined afterwards. An administered sucrose solution with 6 mg/kg of f-2 did not show any significant differences in the blood glucose levels compared with those of the control group, but an administration of 30 mg/kg of f-2 showed a significant suppression of the glucose levels at 15 and 30 min (Fig. 7-A). An application of 10 mg/kg f-4 also significantly suppressed the increased glucose level at 15 min, and 50 mg/kg f-4 further suppressed the increased glucose level induced by the oral sucrose loading (Fig. 7-B).

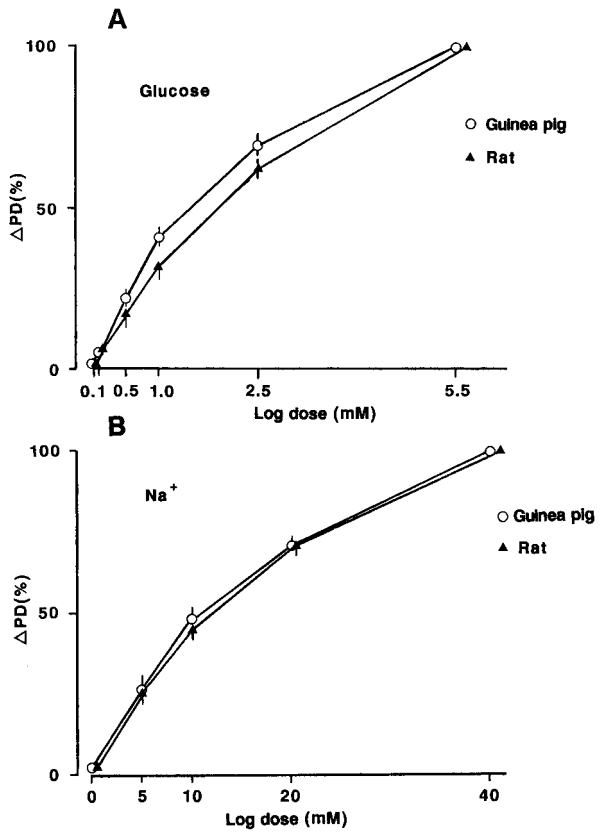

Fig. 4. Changes in glucose-evoked transmural potential difference (ΔPD) by adding glucose (A) and Na$^+$ (B). After the ΔPD of the inverted intestines of guinea-pig (○) and rat (▲) were stabilized in a glucose removal medium with 27.4 mM Na$^+$ or Na$^+$ removal medium with 5.5 mM glucose, glucose (0.1–5.5 mM) or Na$^+$ (5–40 mM) was added. One hundred% represents the magnitude of ΔPD induced by 5.5 mM glucose or 40 mM Na$^+$. Each point indicates the mean ± S. E. M. of 4–6 experiments.

DISCUSSION

Crude components extracted from the leaves of *Gymnema sylvestre* (GS) is one of triterpene saponins [19] and consists of some compounds [5, 10]. The components are also known to have physiological effects, including suppression of sweetness by a reversible effect on the sweet taste receptors [5, 9]. Pharmacological effects of crude components separated from the leaves of GS have been reported as an inhibitory effect on glucose absorption in the rat intestine and suppression of blood glucose elevation in the oral glucose tolerance test in rats [21]. Although, the chemical structures of gymnemic acids have been identified already [10], pharmacological studies of the respective substances have not yet been reported. In this study, as the first step to clarify the pharmacological effects of each gymnemic acid, we examined the effects of extracts containing gymnemic acids (f-1 to f-9) which were obtained by HPLC.

The extraction from the leaves of GS in this study was

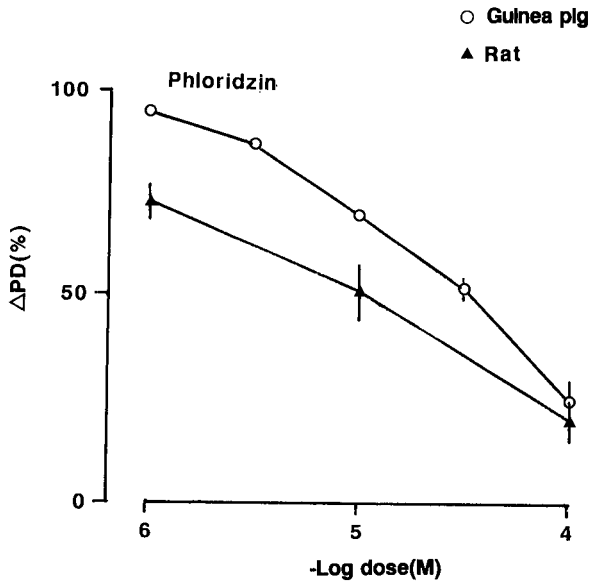

Fig. 5. Effects of phloridzin on the glucose-evoked ΔPD in the inverted intestine of guinea-pig (○) and rat (▲). After the glucose-evoked ΔPD was increased and stabilized by applying 5.5 mM of glucose, phloridzin of varying concentrations from 10^{-6} to 10^{-4} M was applied. ΔPD measured at 25 min after 5.5 mM glucose application was taken as control (100%). Each point indicates the mean ± S. E. M. of 4–6 experiments.

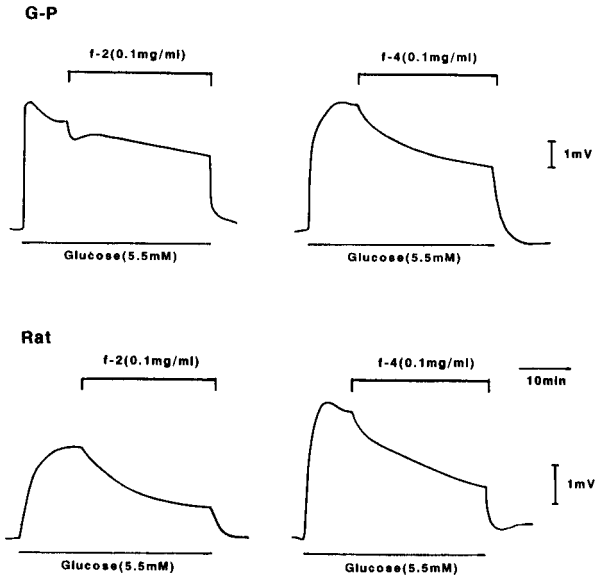

Fig. 6. Effects of f-2 and f-4 on the glucose evoked ΔPD induced by 5.5 mM glucose in inverted intestine of guinea-pig or rat.

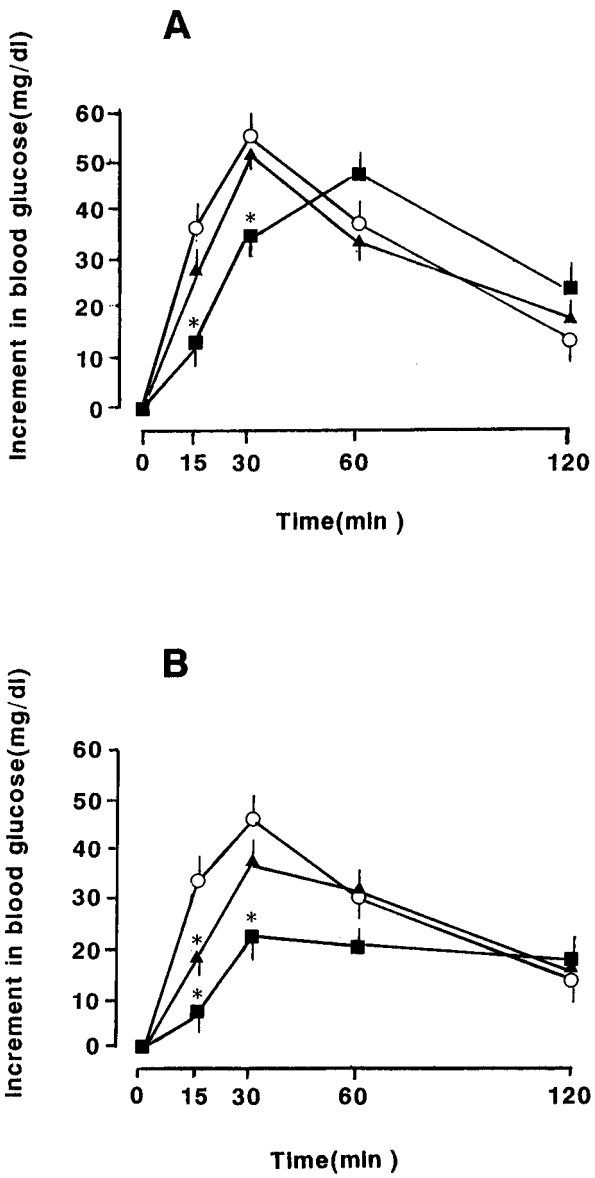

Fig. 7. Effects of f-2 and f-4 on sucrose tolerance test. The control group of rat (○) was orally given 1 g/kg body weight of sucrose, in which the increment in blood glucose was measured at 15, 30, 60 or 120 min. A: F-2 fraction at doses of 6 mg/kg (▲) and 30 mg/kg (■) sucrose solution. B: F-4 fraction at doses of 10 mg/kg (▲) and 50 mg/kg (■) sucrose solution. Values represent means ± S. E. M., in five animals. ※ $P<0.05$: F-2-and f-4-treated groups are compared with the untreated one.

based on the extraction method for ordinary glycosides. HPLC revealed nine fractions which were a yellowish powder. Among the nine fractions of GS, the f-2 and f-4 fractions had significant suppressive effects on H-65K$^+$-induced contraction of the guinea-pig ileal longitudinal muscle. We have already reported that a sustained contraction induced by high K$^+$ in the intestinal smooth muscle is suppressed by an application of metabolic inhibitors, hypoxia, and the removal of glucose [6–8, 12, 16]. Furthermore, the high K$^+$-induced contraction was suppressed by the removal of Na$^+$ from the extracellular fluid, and the suppression of high K$^+$-induced contraction was recovered by an application of pyruvate which is directly utilized as energy substrate. Therefore, the

inhibitory effect of the muscle contraction is involved with the inhibition of glucose absorption by Na^+ depletion [16, 17, 20]. Recently, we examined the simultaneous measurements of reduced pyridine nucleotide (PNred) or oxidized flavin protein (FPox) fluorescense and contractile force, and f-2 and f-4 reduced the increase of PNred fluorescence and contractile force induced by high K^+. Reduced muscle contraction induced by f-2 or f-4 was restored by 5.5 mM pyruvate (unpublished data). Therefore, the relaxing effect of guinea-pig ileal muscle with f-2 or f-4 was considered to be mediated by inhibition of glucose utilization.

The measurement of change in ΔPD evoked by glucose is essential to evaluate the pharmacological effect on the active transport system, because the change of ΔPD indicates a Na^+ shift in co-transport when glucose is absorbed in the intestine by Na^+ dependent active transport regulated by glucose carriers [3, 4]. As the effects of the extracts containing gymnemic acids from GS leaves on smooth muscle contraction were assessed on the guinea-pig ileum in our study, we added the measurement of ΔPD in the guinea-pig inverted intestine to that of the rat. Both the inverted intestines showed a similar result in the increment of ΔPD when various concentrations of glucose were added. Moreover, the inhibitory effects of f-2 and f-4 were almost the same in both the intestines. That is, there was no species difference in the ΔPD experiments. On the other hand, ΔPD was also increased by Na^+ (10 to 40 mM) to the same degree as a Na^+-free medium containing glucose. Phloridzin, a phenol glycoside, is widely used as a competitive inhibitor on Na^+-glucose co-transport [1, 2], and we also demonstrated that ΔPD increased by glucose was suppressed dose-dependently by applications of phloridzin (10^{-6} to 10^{-4} M) in the inverted intestines of the guinea-pig and rat. These findings suggest that f-2 and f-4 have the same effect as phloridzin in suppressing Na^+-glucose co-transport. A H-65K^+-induced contraction suppressed by removal of extracellular Na^+ or extracellular glucose was recovered by adding Na^+ or glucose, respectively [17], and in our study the H-65K^+-induced contraction suppressed by f-2 or f-4 was recovered by adding pyruvate which does not require Na^+ during its absorption and is directly utilized as an energy substrate. These results suggest that the extracts containing gymnemic acids from GS leaves inhibit the smooth muscle contraction through suppression of glucose utilization in co-transport with Na^+ in the same way as they inhibit the ΔPD evoked by glucose.

Moreover, f-2 and f-4 showed suppression of blood glucose elevation at 15 and 30 min. These results suggest that the effects of f-2 and f-4, which suppress the increment of blood glucose level, are caused by inhibition of glucose uptake in the intestines. Crude components separated from GS leaves are reported to have raised serum insulin levels recorded during oral glucose tolerance tests in diabetic rats and rabbits [13–15]. In this study, the effects of the extracts containing gymnemic acids from GS leaves on serum insulin levels were not examined, and the relationships between blood glucose levels and insulin level changes still remains unclear.

Yoshioka [21] has reported that crude components separated from GS leaves inhibit the increment of ΔPD by glucose and elevation of blood glucose levels in the rat. The results of the effects of the extracts containing gymnemic acids, a yellowish powder, presented in the present paper are consistent with his results obtained from crude components separated from GS leaves. After these experiments, a crystal in f-2 was identified as (3β, 4α, 16β, 21β, 22α)-21-tigloxy-16, 22, 23, 28-tetrahydroxyolean-12-en-3-yl-β-D-glucopyranosiduronic acid. Another crystal in f-4 was identified as (3β, 4α, 16β, 21β, 22α)-21-(2-methylbutyroxy)-16, 22, 23, 28-tetrahydroxyolean -12-en-3-yl-β-D-glucopyranosiduronic acid [18].

In summary, f-2 and f-4 which are the major extracts containing gymnemic acids from GS leaves, suppressed both the high K^+-induced contraction of guinea-pig ileal longitudinal muscles and the ΔPD increase in the inverted intestines. It is suggested that this suppressive effect is a result of the inhibition of glucose uptake incorporated with Na^+ entry processes. The extracts may suppress the increment of blood glucose by inhibiting glucose uptake in the intestines.

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