EFFECT OF CONCAVALIN A ON THE ASPIRIN CONCENTRATION AND DISTRIBUTION IN THE BRAIN AND PLASMA OF RATS

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(Received December 27, 1985)

The effects of concanavalin A (Con A) on plasma and brain concentration of aspirin (Asp) were investigated in rats. When Asp was administered in rats treated with Con A, the plasma and brain concentration of Asp and its metabolites (salicylic acid (SA), salicyluric acid (SU) and gentisic acid (GA)) were increased. Distribution of Asp and its metabolites into the hippocampus, striatum and hypothalamus were increased by treatment with Con A.

Asp esterase activities in the small intestinal mucosa, liver and brain were increased by pretreatment with Con A, but these enzyme activities were decreased by a high dose of Con A. The inhibitory effect of Asp, SA, SU and GA on the writhing induced by acetic acid in Con A-pretreated mice was stronger than that in the control. A lowering of rectal temperature, after the administration of Asp, was stronger in Con A-treated rats than in control rats.

These results suggested that Con A facilitated the absorption of Asp and enhanced the transfer of SA into the brain, and increased Asp esterase activities in various tissues.

Keywords — concanavalin A; aspirin; salicylic acid; aspirin esterase; plasma concentration; blood; brain; antipyretic action; analgesic action

INTRODUCTION

Concanavalin A (Con A), a carbohydrate-binding protein,¹⁾ is isolated from Jack beans, *Canavalia ensiformis*. Con A accelerated the degradation of the intestinal brush border membrane, resulting in cell lysis and exfoliation, and induces diarrhea.²⁾ Con A has also been used as a carrier of chemotherapeutic agents,³⁾ since Con A agglutinates the transformed cells at much lower concentrations than their normal counterparts. Recently, Nishihata *et al.* reported that Con A enhances then rectal permeability of compounds which are normally poorly absorbed, such as cefoxitin and phenol red.⁴⁾ However, there have been no reports concerning the effect of Con A on drug disposition in animals.

We previously reported that Con A promotes the absorption of 5-fluorouracil in the rat small intestine⁵⁾ and accelerates the acetylation of *p*-aminobenzoic acid in the intestinal mucosa and the liver in rats.⁶⁾

In the present study, we examined the effect of Con A on the absorption of aspirin (Asp) in the small intestine, *in situ* and *in vitro*, and on the salicylic acid (SA) distribution in the brain after oral administration of Asp. Asp esterase activities were investigated in the various tissues of rat treated with Con A, *in vitro* and *in vivo*. Moreover, we examined the effect of Con A on

the analgesic and antipyretic action of Asp.

MATERIALS AND METHODS

Materials — JP X grade of Asp and SA, reagent grade of gentisic acid (GA) (Nakarai Chemical Ltd.) and salicyluric acid (SU), prepared as described in the literature,⁷⁾ were recrystallized and used. All other chemicals and solvents were of analytical reagent grade. Water deionized by the Milli-Q water system (Millipore Corporation) was used in all experiments.

Animals — Male rats (Funabashi Farm Co.), weighing 180—200 g, and male ddY mice, weighing 20 g were used. Rats were fed a commercial diet and were fasted for 20 h prior to experiments, but allowed water *ad libitum*. Con A in water and in saline solution were administered *p.o.* (5 ml/kg) and *i.p.* (0.5 ml/kg), respectively, once a day for 5 d in rats. The ddY mice were also subjected to exactly the same conditions as the rats.

Procedure for Plasma Samples — At 15, 30, 60, 120 and 180 min after the oral administration of Asp (100 mg/kg) in 0.3% sodium carboxymethyl cellulose (CMC-Na), rats were killed by decapitation. Blood samples collected were immediately added to chilled small tube containing heparinized saline solution. Plasma was then separated by centrifugation at 3000 rpm

for 10 min at about 2 °C.

One ml of saline solution containing 25 mg/kg of SA was injected into the femoral vein of rats under ether anesthesia and 0.5 ml of the plasma sample was obtained as in the case of Asp treatment.

Procedure with Brain Samples — At 15, 30, 60, 120 and 180 min after the oral administration of Asp (100 mg/kg) in 0.3% CMC-Na suspension, rats were killed by decapitation. The brain was removed and immediately washed with ice-cold isotonic sodium phosphate buffer (pH 6.8). For the assay of drugs, the whole brain was used. In the assay of various brain regions, the brain was dissected into 7 regions: cerebellum, medulla oblongata, hypothalamus, striatum, midbrain, hippocampus and cortex. In the case of intravenous administration of SA, the brain samples were obtained in the manner described above. The whole brain or dissected portions were homogenized with 4 ml of water, and centrifuged at 4000 rpm (2 °C). The pH of the homogenate was adjusted to 2.5 by the addition of 0.1 N HCl. After extraction with chloroform-ether (1:1) twice, the extracts were dried by evaporation.

High-Performance Liquid Chromatography (HPLC) — The HPLC apparatus consisted of a Toyo Soda Model HLC-803D solvent delivery system, a Toyo Soda Model UV-8II, Absorption Detector monitoring at 220 nm and a Hitachi Model 833 Chromatoprocessor. Separation was performed on a reversed-phase "ODS-120A" (particle size 5 μ m Toyo Soda, Japan) column (25 cm $\times$ 4.6 mm, i.d.). A sample of 20 μ l was injected into the chromatograph. All separations were done at ambient temperature using 0.03% phosphoric acid solution-acetonitrile (7:3, v/v) as a mobile phase at a flow-rate of 1.2 ml/min, producing a pressure of 150 kg/cm². The retention times of GA, SU, Asp, benzoic acid (internal standard) and SA were 4.4, 5.6, 7.8, 9.2 and 11.1 min, respectively. The solvent was ultrasonicated before use.

Absorption Experiments by the in Situ Recirculating Method — We used the modified method of Yasuhara *et al.*⁸⁾ Thirty ml of Asp solution (pH 6.8 isotonic sodium phosphate buffer) were recirculated through the small intestine at the rate of 5 ml/min. The section from the proximal end of the duodenum to the ileocecal junction was examined. The initial concen-

tration of the Asp solution was 2 mM. We sampled 1 ml of solution at 0, 15, 30, 45, and 60 min, but there was a lag time of 3 min.

Uptake of Asp by the Brain — Brain slices were incubated in isotonic sodium phosphate buffer (pH 6.8) containing Asp (5 mg/ml) for 15 min at 37 °C, homogenized in 4 volumes of water, and centrifuged. The supernatant fluid was assayed.

Enzyme Assays — The activities of Asp esterase in the small intestine, liver, kidney, brain and plasma were determined by the method of Morgan⁹⁾ with a slight modification. The small intestinal mucosa, liver, kidney, brain and brain regions were homogenized in 8, 10, 5, 10 and 5 volumes of 50 mM Tris-HCl buffer (pH 7.5) respectively, using a Potter Elvehjem type homogenizer. The homogenate and the blood were centrifuged at 10000 $\times g$ for 30 min. To assay the enzyme activity, 1 ml of the supernatant fraction of these tissue homogenates, and plasma were mixed with 1 ml of 50 mM Tris-HCl buffer (pH 7.5) containing Asp. The reaction was stopped by the addition of 0.5 ml of 1 N HCl and 5 ml of chloroform, after incubation for 30 min at 37 °C. The mixture was extracted twice with 5 ml of chloroform. The absorbance of SA in chloroform was measured at 303 nm. Protein content was determined by the method of Lowry *et al.*¹⁰⁾

Analgesic Effects on Writhing in Mice — Analgesic activity was evaluated by the method of Koster¹¹⁾ with a slight modification. For each experimental group, 8–10 mice were used. The drugs (Asp, SA, SU and GA, 150 mg/kg) in 0.3% acacia solution were orally administered 10 or 30 min before the acetic acid injection. The number of writhings was counted during a period of 10 to 20 min after the intraperitoneal administration of 0.1 ml/10 g of acetic acid and the inhibition percentage was calculated. For the control, 0.1 ml/10 g of 0.3% acacia solution was orally administered.

Measurement of Body Temperature in Rats — Six rats were orally given 150 mg/kg of Asp. The control group was given 5 ml/kg of 0.3% accasia solution. The rectal temperature was measured by a thermometer with a thermister element (Sibaura Electronics, MGA-III) every 30 or 60 min for 330 min at a room temperature of 23 °C.

FIG. 1. Time Concentration Curves of Aspirin and Its Metabolites in Plasma after Oral Administration of 100 mg/kg of Aspirin to Rats

—●—, control; —○—, simultaneous administration of aspirin and concanavalin A (500 µg/kg); —■—, administration of aspirin to rats pretreated with concanavalin A (5 d, p.o., 3 mg/kg); —□—, administration of aspirin to rats pretreated with concanavalin A (5 d, i.p., 3 mg/kg). (A) aspirin, (B) salicylic acid, (C) gentisic acid, (D) salicyluric acid. Each result represents the mean $\pm$ S.E. of 6 rats.

FIG. 2. Time Concentration Curves of Aspirin and Its Metabolites in Brain after Oral Administration of 100 mg/kg of Aspirin to Rats

—●—, control; —○—, simultaneous administration of aspirin and concanavalin A (500 µg/kg); —■—, administration of aspirin to rats pretreated with concanavalin A (5 d, p.o., 3 mg/kg); —□—, administration of aspirin to rats pretreated with concanavalin A (5 d, i.p., 3 mg/kg). (A) aspirin, (B) salicylic acid, (C) gentisic acid, (D) salicyluric acid. Each result represents the mean $\pm$ S.E. of 6 rats.

RESULTS

Plasma Concentration of Asp

Figure 1 shows the plasma concentrations of Asp and its metabolites (SA, SU and GA) during a period of 180 min after the oral administration of Asp (100 mg/kg) in rats. The plasma concentration of Asp increased in Con A-pretreated rats. The plasma concentrations of metabolites, SA and SU, slightly increased.

Since the concentration of Asp in the plasma increased after the oral administration of Asp in Con A-pretreated rats, the absorption of Asp in the gut lumen was examined by the *in situ* recirculating method. As shown in Table I, the percentage of Asp remaining in the lumen decreased and the appearance of SA was increased by the pre-treatment with Con A.

Brain Concentration of Asp

The concentrations of Asp in the brain after the oral administration of Asp (100 mg/kg) were examined in Con A-treated rats. As shown in Fig. 2, Asp concentrations in the brain after the oral administration of Asp decreased in rats pre-

treated intraperitoneally with Con A. The SA concentrations increased in Con A-pretreated (i.p. and p.o.) rats, and the concentrations of SU and GA in the brain slightly increased in rats treated with intraperitoneal administration of Con A and with simultaneous administration of Con A and Asp.

Since SA concentration increased in the brains of rats pretreated with Con A after the oral administration of Asp, the Asp concentration and its metabolites in the various brain regions were measured at 30 min after the oral administration of Asp. As shown in Table II, the SA concentration in the brain regions increased in rats which were given oral or intraperitoneal administration of Con A.

Uptake of Asp into the Brain

SA concentration in the brain was investigated after SA (25 mg/kg) was intravenously administered in rats treated with Con A. As shown in Table III, SA concentration increased in the brain of the rats pretreated intraperitoneally with Con A (3 mg/kg) for 5 d. The uptake of

TABLE I. *The Effect of Concanavalin A on Small Intestine Absorption of Aspirin and Appearance of Salicylic Acid during the in Situ Recirculation of Aspirin in Rats*

	Time (min)	Aspirin (remained %)	Salicylic acid (appearance) ^{a)}
Control	15	96.8 ± 1.5	25.1 ± 2.9
	30	90.2 ± 2.0	39.7 ± 5.6
	45	80.5 ± 3.1	54.9 ± 8.3
	60	69.5 ± 5.8	64.3 ± 5.9
Simultaneous administration ^{b)}	15	83.9 ± 7.6 ^{e)}	28.1 ± 4.7
	30	80.1 ± 5.0 ^{e)}	41.3 ± 3.2
	45	75.2 ± 8.2	69.1 ± 3.2
	60	67.7 ± 2.8	88.6 ± 5.1 ^{e)}
Oral administration ^{c)}	15	93.5 ± 3.5 ^{e)}	32.0 ± 3.6
	30	84.9 ± 3.5 ^{e)}	46.5 ± 5.3
	45	69.5 ± 8.3	75.4 ± 6.8
	60	62.5 ± 7.9	107.1 ± 17.1 ^{e)}
Intraperitoneal administration ^{d)}	15	84.1 ± 9.9	34.4 ± 4.7
	30	77.6 ± 6.7 ^{e)}	61.0 ± 9.7 ^{e)}
	45	66.6 ± 8.7 ^{e)}	87.6 ± 11.3 ^{e)}
	60	60.8 ± 2.2 ^{e)}	108.2 ± 12.9 ^{e)}

30 ml of aspirin (initial concentration of 2 mM) in rat small intestine.

a) The concentration of salicylic acid in the lumen expressed as the ratio of initial concentration salicylic acid in lag time at 3 min.

b) Simultaneous administration of concanavalin A (100 µg/ml).

c) Administration of aspirin to rat pretreated with concanavalin A (5 d, p.o., 3 mg/kg).

d) Administration of aspirin to rat pretreated with concanavalin A (5 d, i.p., 3 mg/kg).

Each result represents the mean ± S.E. of 5 rats.

e) Significantly different from the control ($p < 0.10$).

TABLE II. Concentration of Aspirin and its Major Metabolites in Hippocampus, Striatum and Hypothalamus of Concanavalin A-Treated Rats

Segment		Aspirin (ng/g wet tissue)	Salicylic acid (μ g/g wet tissue)	Salicyluric acid (ng/g wet tissue)	Gentisic acid (μ g/g wet tissue)
Hippocampus	Control	—	3.39 $\pm$ 0.58	—	1.71 $\pm$ 0.11
	Simultaneous administration ^{a)}	—	3.13 $\pm$ 0.33	—	1.64 $\pm$ 0.34
	Intraperitoneal administration ^{b)}	—	3.27 $\pm$ 0.63	—	2.39 $\pm$ 0.45 ^{d)}
	Oral administration ^{c)}	—	5.74 $\pm$ 0.24 ^{e)}	—	2.53 $\pm$ 0.15 ^{e)}
Striatum	Control	47.4 $\pm$ 10.6	1.99 $\pm$ 0.42	72.1 $\pm$ 5.3	0.22 $\pm$ 0.04
	Simultaneous administration ^{a)}	68.0 $\pm$ 2.4 ^{e)}	2.06 $\pm$ 0.19	86.8 $\pm$ 6.2 ^{d)}	0.22 $\pm$ 0.04
	Intraperitoneal administration ^{b)}	—	3.07 $\pm$ 0.14 ^{e)}	257.2 $\pm$ 54.2 ^{e)}	0.67 $\pm$ 0.18 ^{e)}
	Oral administration ^{c)}	—	3.35 $\pm$ 0.40 ^{e)}	321.5 $\pm$ 61.3 ^{e)}	0.80 $\pm$ 0.16 ^{e)}
Hypothalamus	Control	—	1.41 $\pm$ 0.26	126.2 $\pm$ 8.5	0.13 $\pm$ 0.01
	Simultaneous administration ^{a)}	—	1.43 $\pm$ 0.10	139.8 $\pm$ 17.9	0.13 $\pm$ 0.01
	Intraperitoneal administration ^{b)}	—	1.43 $\pm$ 0.52	141.2 $\pm$ 12.6	0.13 $\pm$ 0.02
	Oral administration ^{c)}	—	2.10 $\pm$ 0.26 ^{e)}	127.8 $\pm$ 11.2	0.16 $\pm$ 0.01 ^{d)}

Concentration of aspirin, salicylic acid, salicyluric acid and gentisic acid at 30 min after oral administration of aspirin (100 mg/kg).

a) Simultaneous administration of concanavalin A (500 μ g/kg).

b) Administration of aspirin to rat pretreated with concanavalin A (5 d, i.p., 3 mg/kg).

c) Administration of aspirin to rat pretreated with concanavalin A (5 d, p.o., 3 mg/kg).

Each result represents the mean $\pm$ S.E. of 5 rats.

d) Significantly different from the control ($p < 0.10$).

e) Significantly different from the control ($p < 0.05$).

Asp into the brain *in vitro*, was also examined. As shown in Table IV, the uptake of Asp and the appearance of SA were enhanced by Con A, except in rats pretreated orally with Con A.

Asp Esterase Activities in the Various Tissues

Hydrolytic activities of Asp in the brain, small intestinal mucosa, liver and kidney of rats were measured to elucidate the relationship between blood and tissue concentrations and enzyme activities in these tissues. As shown in Fig. 3, Asp esterase activities in the brain were increased significantly by incubation with Asp and Con A (10, 100 μ g/ml), but were decreased at 1 mg/ml of Con A. Similarly, Asp esterase activities increased in rats administered orally or intraperitoneally with Con A, except in rats pretreated intraperitoneally with Con A (20 mg/kg).

The relationship between Asp esterase activity and Asp concentration in the brain after the administration of Asp was investigated. The Asp esterase activity was measured in various brain regions under the following conditions; 1) Con A (100 μ g/ml) and Asp were simultaneously incubated with the supernatant fraction of the homogenate of the various brain regions. 2) Asp was incubated with a supernatant fraction of the various brain regions of the rats administered orally or intraperitoneally with Con A (3 mg/kg) for 5 d. As shown in Table V, Asp esterase activities in some brain regions were increased significantly by Con A, especially, the activities in striatum and hypothalamus.

We investigated the effects of Con A on Asp esterase activities in the small intestinal mucosa, liver and kidney. Asp esterase activities increased

TABLE III. Concentration of Salicylic Acid in Brain of Concanavalin A-Treated Rats

	Brain ($\mu\text{g/g}$ wet tissue)
Control	6.33 ± 0.78
Simultaneous administration ^{a)}	6.11 ± 0.49
Intraperitoneal administration ^{b)}	10.53 ± 1.00^d
Oral administration ^{c)}	6.98 ± 0.57

Concentration of free salicylic acid in the brain after 30 min intravenous administration of salicylic acid (25 mg/kg, 1 ml).

a) Simultaneous administration (i.v.) of concanavalin A (100 $\mu\text{g/ml}$, 1 ml).

b) Administration of salicylic acid (i.v.) to rat pretreated with concanavalin A (5 d, i.p., 3 mg/kg).

c) Administration of salicylic acid (i.v.) to rat pretreated with concanavalin A (5 d, p.o., 3 mg/kg).

Each result represents the mean $\pm$ S.E. of 9 rats.

d) Significantly different from the control ($p < 0.05$).

TABLE IV. Effect of Concanavalin A on Uptake of Aspirin and Appearance of Salicylic Acid in Rat Brain

	Drug concentration ($\mu\text{g/g}$ wet tissue)	
	Aspirin	Salicylic acid
Control	380.6 ± 13.7	97.6 ± 7.5
Simultaneous administration ^{a)}	516.6 ± 14.1^d	115.5 ± 5.9^d
Oral administration ^{b)}	385.8 ± 13.8	113.2 ± 6.7
Intraperitoneal administration ^{c)}	457.9 ± 30.2^d	101.9 ± 6.6

Concentration of aspirin and salicylic acid after 15 min incubation of slices of the brain in the aspirin solution (5 mg/ml) at 37 °C.

a) Slices of the brain incubated in the aspirin and concanavalin A (100 $\mu\text{g/ml}$) solution.

b) Slices of the brain (concanavalin A-pretreated, 5 d, p.o., 3 mg/kg) incubated in the aspirin solution.

c) Slices of the brain (concanavalin A-pretreated, 5 d, i.p., 3 mg/kg) incubated in the aspirin solution.

Each result represents the mean $\pm$ S.E. of 7 samples.

d) Significantly different from the control ($p < 0.05$).

in the intestinal mucosa of the rats treated intraperitoneally with Con A (Fig. 4).

Asp esterase activities in the liver also increased, except for the case where Con A and Asp were incubated together (Fig. 5).

Asp esterase activities in the kidney were stimulated by the simultaneous incubation with Asp and Con A, but no changes were seen in the rats pretreated orally or intraperitoneally with Con A (3 mg/kg) for 5 d (Fig. 6).

Table VI shows the results of Asp esterase activities in the plasma. Except for the case of oral administration of Con A, there were no significant change in the esterase activities in the plasma when compared to the activity in the control. The esterase activity in plasma from rats administered Con A orally was significantly higher than that in the control.

Inhibitory Effects in Acetic Acid-Induced Writhing

Since it was recognized that the Con A facilitated the transport of Asp and SA into the brain, the effects of Con A on analgesic and antipyretic actions were examined in mice. The drugs (Asp, SA, SU and GA, 150 mg/kg) were administered orally at 10 or 30 min before the intraperitoneal administration of 0.7% acetic acid (10 ml/kg). The number of writhings were recorded in each mouse during a period of 10 to 20 min after the acetic acid injection. All drugs showed inhibitory effects on the writhing syndrome at 10 min after the administration of acetic acid in normal mice but exhibited no effect at 30 min after the administration (Fig. 7). In mice treated with Con A, all drugs showed significant inhibition even at 30 min after the administration of acetic acid.

FIG. 3. Effect of Concanavalin A on Aspirin Esterase Activity in Brain Homogenates of Rats

(A) incubation of brain homogenate with concanavalin A and aspirin. (B) incubation of homogenate from rats treated with concanavalin A (5 d, p.o.) and aspirin, (C) incubation of homogenate from rats treated with concanavalin A (5 d, i.p.) and aspirin.

--●--, control; —●—, 10 μ g/ml; —■—, 100 μ g/ml; —▲—, 1 mg/ml; —○—, —●—, 500 μ g/kg; —□—, —■—, 3 mg/kg; —△—, —▲—, 20 mg/kg.

Each result represents the mean $\pm$ S. E. of 4 to 6 rats.

The Effect of Asp on The Normal Rectal Temperature in Con A Treated Rats

Asp significantly reduced the rectal temperature of rats administered orally or intraperitoneally with Con A (Fig. 8). However, no significant lowering of the normal rectal temperature

was observed after the simultaneous administration of Asp and Con A.

DISCUSSION

It has been reported that Con A binds a protein receptor, a nicotinic acetylcholine

TABLE V. Effect of Concanavalin A on Aspirin Esterase Activity in Brain of Rats

Segment	Esterase activity ($10^{-6} \cdot \text{nmol/min}/\mu\text{g protein}$)			
	Control	Simultaneous administration ^{a)}	Intraperitoneal administration ^{b)}	Oral administration ^{c)}
Cortex	2.38 ± 0.13	3.15 ± 0.31^d	3.33 ± 0.51^e	2.66 ± 0.32
Midbrain	3.15 ± 0.31	3.57 ± 0.21	4.04 ± 0.24^d	3.93 ± 0.18^d
Cerebellum	3.58 ± 0.09	4.18 ± 0.19^d	3.99 ± 0.38^d	4.27 ± 0.25^d
Medulla oblongata	4.28 ± 0.79	4.24 ± 0.23	4.15 ± 0.30	4.41 ± 0.68
Hippocampus	4.92 ± 1.04	4.75 ± 0.57	5.18 ± 0.46	5.24 ± 1.46
Striatum	2.30 ± 0.25	2.20 ± 0.29	3.78 ± 0.50^d	4.98 ± 0.89^d
Hypothalamus	2.24 ± 0.40	4.56 ± 0.61^d	4.41 ± 0.52^d	4.91 ± 0.17^d

a) Incubation of brain homogenate with concanavalin A (100 μ g/ml) and aspirin.

b) Incubation of brain homogenate from rats treated with concanavalin A (5 d, i.p., 3 mg/kg) and aspirin.

c) Incubation of brain homogenate from rats treated with concanavalin A (5 d, p.o., 3 mg/kg) and aspirin.

Each result represents the mean $\pm$ S.E. of 4 rats.

d) Significantly different from the control ($p < 0.10$).

e) Significantly different from the control ($p < 0.05$).

FIG. 4. Effect of Concanavalin A on Aspirin Esterase Activity in Small Intestinal Mucosa Homogenates of Rats

(A) incubation of homogenate with concanavalin A and aspirin, (B) incubation of homogenate from rats treated with concanavalin A (5 d, p.o.) and aspirin, (C) incubation of homogenate from rats treated with concanavalin A (5 d, i.p.) and aspirin.

--●--, control; —●—, 10 $\mu\text{g/ml}$; —■—, 100 $\mu\text{g/ml}$; —▲—, 1 mg/ml; —○—, —●—, 500 $\mu\text{g/kg}$; —□—, —■—, 3 mg/kg; —△—, —▲—, 20 mg/kg.

Each result represents the mean $\pm$ S.E. of 4 to 6 rats.

FIG. 5. Effect of Concanavalin A on Aspirin Esterase Activity in Liver Homogenates of Rats

(A) incubation of homogenate with concanavalin A and aspirin, (B) incubation of homogenate from rats treated with concanavalin A (5 d, i.p.) and aspirin, (C) incubation of homogenate from rats treated with concanavalin A (5 d, p.o.) and aspirin.

--●--, control; —●—, 10 $\mu\text{g/ml}$; —■—, 100 $\mu\text{g/ml}$; —▲—, 1 mg/ml; —○—, —●—, 500 $\mu\text{g/kg}$; —□—, —■—, 3 mg/kg; —△—, —▲—, 20 mg/kg.

Each result represents the mean $\pm$ S.E. of 4 to 6 rats.

FIG. 6. Effect of Concanavalin A on Aspirin Esterase Activity in Kidney Homogenates of Rats

—●—, control; —○—, incubation of homogenate with concanavalin A (100 μ g/ml) and aspirin, —■—, incubation of homogenate from rats treated with concanavalin A (5 d, p.o., 3 mg/kg) and aspirin, —□—, incubation of homogenate from rats treated with concanavalin A (5 d, i.p., 3 mg/kg) and aspirin.

Each result represents the mean $\pm$ S.E. of 4 to 6 rats.

receptor,¹²⁾ but the effect of Con A on the central nervous system and drug disposition is not clear. The present investigation revealed the effect of Con A on the absorption of Asp, the analgesic and antipyretic activities of Asp in mice and rats, the transfer of its metabolite, SA, into the brain and Asp esterase activities in plasma and other tissues. The plasma and brain concentra-

tions of SA after the oral administration of Asp or the intravenous administration of SA in Con A-treated rats were increased compared with that of the control. The absorption of Asp from the small intestine by *in situ* recirculation system also increased in rats treated with Con A. The brain uptake of Asp *in vitro* experiments increased by the treatment of Con A.

When the brain of rats was dissected into 7 regions after the oral administration of Asp and the concentration of Asp in each brain region was examined, high concentrations of SA were observed in the hippocampus, striatum and hypothalamus. These results suggested that Con A may enhance the absorption of Asp in the small intestine and the transfer of SA into the brain.

Asp esterase activity in the whole brain and in most of the regions of the brain of Con A-treated rats was higher than that of the control. The highest enhancement of activity was observed in the hypothalamus. Asp esterase activities of small intestine and liver in Con A-treated rats were enhanced, but no significant changes were seen in the kidney. Accordingly, Con A may increase the activity of Asp esterase.

The effect of Con A on the writhing syndrome induced by acetic acid in mice was investigated. An inhibitory effect after the administration of drugs (Asp and its metabolites, SA, SU and GA) in normal mice was not seen at 10 min, but a significant inhibition, compared to that of the control, was observed in Con A-treated mice. A lowering of the normal rectal temperature by Asp was observed in rats treated orally and intravenously with Con A. It may be especially interesting to compare the antipyretic activity of Asp and the SA concentration in the hy-

TABLE VI. Effect of Concanavalin A on Aspirin Esterase Activity in Plasma of Rats

	Esterase activity (nmol/min/ μ g protein)		
	Aspirin		(10 mM)
Control	3.34	$\pm$	0.45
Simultaneous administration ^{a)}	3.55	$\pm$	0.14
Intraperitoneal administration ^{b)}	3.51	$\pm$	0.32
Oral administration ^{c)}	4.15	$\pm$	0.32 ^{d)}

a) Incubation of plasma with concanavalin A (100 μ g/ml) and aspirin.

b) Incubation of plasma from rats treated with concanavalin A (5 d, i.p., 3 mg/kg) and aspirin.

c) Incubation of plasma from rats treated with concanavalin A (5 d, p.o., 3 mg/kg) and aspirin.

Each result represents the mean $\pm$ S.E. of 5 rats.

d) Significantly different from the control ($p < 0.05$).

FIG. 7. Analgesic Effects of Aspirin, Salicylic Acid, Salicyluric Acid and Gentisic Acid on Stretching Induced by Acetic Acid in Mice

The drugs were administered orally 10 min or 30 min before the intraperitoneal injection (20 ml/kg) of 0.7% acetic acid solution. The number of writhes were counted in each mouse during a period of 10 to 20 min after the acetic acid injection.

(A) control, (B) simultaneous administration of the test drug and concanavalin A (500 μ g/kg), (C) pretreated with concanavalin A (5 d, i.p., 3 mg/kg), (D) pretreated with concanavalin A (5 d, p.o., 3 mg/kg).

□, control; ▨, aspirin; ▩, salicylic acid; ▤, salicyluric acid; ■, gentisic acid.

Each result represents the mean $\pm$ S.E. of 8 to 10 mice.

a) Differences are significantly from the control ($p < 0.10$).

pothalamus to determine whether or not Asp has a function in regulating the body temperature in Con A-treated rats.

Nishihata *et al.*⁴⁾ reported that Con A-enhanced rectal absorption of cefoxitin and phenol red may occur at the protein fraction site in the rectal epithelial membrane of rats and that the process of endocytosis may be partially involved in the absorption-enhancing mechanism of Con A. It is known that Con A enhances Ca^{2+} activated ATPase in the brain corpus of rats¹³⁾ and plasma membrane of small lymphocytes from mesenteric ganglia of pig. Dornand *et al.*¹⁴⁾ revealed that this enzyme was involved in Ca^{2+} transport through the membrane and that it was bound to a contractile protein involved in membrane deformability and permeability. More-

over, it is known that the radioiodinated *Ricinus lectin*¹⁵⁾ administered intraperitoneally, was distributed in rat tissues, and that lectins (wheat germ agglutinin and soybean agglutinin)¹⁶⁾ from the ventricular cerebrospinal fluid penetrated into brain parenchyma in mice. It has also been reported that ^{125}I -labeled Con A binds rat brain synaptosomes *in vitro*.¹⁷⁾ From these reports, Con A administered in rats appears to distribute into other tissues as well as into intestinal membrane, even though the amount is slight. The present experiment suggested that the enhancing action of Con A on the intestinal absorption of Asp and uptake into the brain was due to the alteration of the permeability of membrane by the interaction between Con A and cell surface carbohydrate moieties. However, it is not possible to elucidate

FIG. 8. Effect of Oral Administration of Aspirin (150 mg/kg) on Rectal Temperature of Rats

—●—, control; —○—, aspirin; —■—, simultaneous administration of aspirin and concanavalin A (500 μ g/kg); —□—, administration of aspirin to rat pretreated with concanavalin A (5 d, i.p., 3 mg/kg), —▲—, administration of aspirin to rat pretreated with concanavalin A (5 d, p.o., 3 mg/kg).

Each result represents the mean $\pm$ S.E. of 6 rats.

the enhancing action of Con A at this time without further investigations of this subject.

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