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PHARMACOLOGICAL STUDIES ON CHINESE CINNAMON. V. CATECHOLAMINE RELEASING EFFECT OF CINNAMALDEHYDE IN DOGS

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Effect of *i.v.* and *i.d.* cinnamaldehyde on plasma catecholamine concentration along with intestinal absorption of the drug was studied in anesthetized dogs. Cinnamaldehyde increased plasma catecholamine concentration, the effect produced through *i.d.* route (50–100 mg/kg) being dose-dependent and more lasting compared with that through *i.v.* route (20 mg/kg). In the case of 200 mg/kg of *i.d.* cinnamaldehyde, an exceeding increase in this parameter was obtained during the later period of time course. Intestinal absorption of cinnamaldehyde *i.d.* administered, which was investigated through measurement of cinnamaldehyde concentration in the portal venous blood and in blood of the postcava, occurred very early and was long-lasting. Increase in plasma catecholamine concentration produced by *i.v.* cinnamaldehyde disappeared after blood circulation through the adrenal glands was stopped, and was not influenced with pretreatment of hexamethonium plus atropine. Almost all the increased portion of plasma catecholamines by *i.v.* or *i.d.* cinnamaldehyde was epinephrine. It was concluded that cinnamaldehyde, entering the circulatory system, reaches the adrenals and releases catecholamines from the organ through a mechanism(s) independent of affecting the cholinergic system. *i.v.* DMPP which was used as a referential drug also increased plasma catecholamine concentration.

Keywords —adrenal gland; adrenal gland ligation; catecholamine release; Chinese cinnamon; cinnamaldehyde; DMPP; ganglion blocking; intestinal absorption; intraduodenal administration

INTRODUCTION

In the preceding paper,[1] we reported that cinnamaldehyde released catecholamines from the isolated dog adrenal and also from the adrenals *in situ* of anesthetized dogs by local *i.a.* application. The purpose of the present report is to study catecholamine releasing effect of cinnamaldehyde applied *i.v.* and intraduodenally (*i.d.*) along with intestinal absorption of the drug in dogs, since Chinese cinnamon which contains cinnamaldehyde as a main component has frequently been used in Chinese medicine. Dimethylphenylpiperazinium, a nicotinic agonist, was used as a reference drug.

EXPERIMENTAL

Method

1) Catecholamine Releasing Effect of Cinnamaldehyde and Dimethylphenylpiperazinium (i.v. Administration)—Mongrel dogs of either sex weighing 7.5–17.5 kg were anesthetized with 35 mg/kg of sodium pentobarbital *i.v.* or *i.p.* After intubation of the trachea, the right common carotid artery and right cephalic vein were cannulated for recording of blood pressure and injection of drugs and the additional anesthetic when necessary, respectively. Another cannula filled with heparin solution (50 U/ml) was inserted in the left femoral vein with the tip at the xiphisternal level in the postcava for blood collection. About 1 h after completion of the surgical operation, when basal blood pressure showed a constant level, 20 mg/kg of cinnamaldehyde was *i.v.* administered. Blood was sampled 8 min before, and 2, 10 or 20, and 30 min after the drug administration for determina-

tion of blood catecholamine concentration. The 30-min value thus measured served as a basal catecholamine concentration at the succeeding experimental series of drug administration, since it had returned to the basal level. One experimental series was mostly composed of 3 blood samplings, *i.e.*, sampling before, and 2 and 20 min after the drug administration and 3 to 4 experimental series were successfully conducted. In the case of *i.v.* administration of 30 μg/kg of dimethylphenylpiperazinium (DMPP), blood was sampled 9.5 min before, and 0.5, 10, and 20 min after the drug administration. The 20-min value of catecholamine concentration also served as a basal value of the succeeding experimental series. Using a syringe which contained 0.4 ml of 5% sodium metabisulfite solution, 20 ml of blood were taken for 30 s, accompanied with an infusion of the same volume of Ringer solution. When necessary, ligation of the adrenals and treatment with hexamethonium plus atropine were performed. Blood pressure and heart rate triggered from the puls wave were recorded by means of a pressure transducer (Nihon Kohden, MPU-0.5) and a heart rate meter (San-ei Sokki, 2140), respectively.

2) Catecholamine Releasing Effect of Cinnamaldehyde (i.d. Administration)——Mongrel dogs of either sex weighing 9—17.5 kg were fasted with free access to water for 18 h and were subjected to the same surgical operation as in 1. Then, the abdomen was opened and a cannula for injecting a test solution was distally introduced in the duodenum about 5 cm away from the pylorus with maintenance of passage of gastric contents. About 1 h after completion of the surgical operation, blood was sampled (20 ml, for 1 min) 30 and 15 min before *i.d.* administration of drugs. Again, blood was sampled at 1, 5, 10, 20, 30, 45, 60, 90, and 120 min in the case of 200 mg/kg of cinnamaldehyde, and at 20, 60, 120, 180, 240, 300, and 360 min in the case of 50 and 100 mg/kg of it, thereafter. The same volume of Ringer solution was simultaneously infused at each sampling.

3) Intestinal Absorption of Cinnamaldehyde in Dogs——Mongrel dogs of either sex weighing 7—11.5 kg were used. Fasting and the surgical operation were the same as in 2. The animal preparation having a cannula filled with 0.7 ml of heparin solution (50 U/ml) which was inserted in the splenic vein with the tip in the portal vein toward the liver instead of a cannula placed in left femoral vein in 2 was also provided. About 1 h after the surgical operation, cinnamaldehyde was administered. Blood was sampled (10 ml, for 30 s) from the portal vein or the postcava at 1, 2, 5, 10, 20, 30, 45, 60, 90, and 120 min (200 mg/kg, *i.d.*) or 0.5, 1, 2, 5, and 10 min (10 and 20 mg/kg, *i.v.*) after the drug administration. The same volume of Ringer solution was infused at each sampling.

4) Ligation of the Adrenals——After completion of the surgical operation in 1, the abdomen was opened. The bilateral adrenals were freed from the surrounding tissues and a thread was placed around the organ in order to stop the adrenal circulation. Under temporal closure of the opened abdomen with forceps, the drug administration and succeeding blood collection were made. Then, the adrenals were ligated and the same series of the experimental procedure were repeated with an intramuscular injection of 5 mg/kg of corticosterone or 20 mg/kg of hydrocortisone suspended in 2% CMC solution. Validity of adrenal ligation was confirmed through observation of no bleeding from the organ immediately after its dissection.

5) Ganglion Blocking——Monitoring blood pressure, 5 mg/kg of hexamethonium was *i.v.* administered as an initial dose. Then, 10% as much as dose as the initial one was consecutively added until no further decrease in blood pressure was observed. Next, an initial dose (1 mg/kg) of atropine and, if necessary, succeeding doses (0.1 mg/kg each) of it were consecutively *i.v.* administered again till no further decrease in blood pressure was observed.

6) Assay of Catecholamines——Extraction of catecholamines from the samples and determination of their content were carried out with a slight modification according to the methods of

Shellenberger and Gordon[2] and Anton and Sayre,[3] respectively. Results were expressed as total catecholamines or epinephrine and norepinephrine.

7) Determination of Cinnamaldehyde Concentration in Blood—Ten ml of blood sample and 5 ml of benzene were placed in an ice-cold 50-ml glass-stoppered centrifuge tube and cinnamaldehyde was extracted in the benzene under mechanical shaking for 10 min. Recovery ratio of the drug from blood was 95.6±1.4% ($N=4$). Concentration of cinnamaldehyde was determined by means of gas chromatography using *l*-menthol (Wako) as an internal standard. A gas chromatograph equipped with a hydrogen flame ionization detector (Hitachi 163) and a stainless column 1 m long and 3 mm internal diameter was used. The column was packed with 60/80 mesh Uniport B coated with 10% SE-30. A flow rate of carrier gas (N_2) and hydrogen was 20 and 35 ml/min, respectively. The column temperature was 110°C and the injection part was kept at 160°C.

Statistics

All data were statistically analyzed using Student's *t*-test and expressed as mean ±s.e.

Drugs

Drugs used were as follows; Atropine sulfate (Wako), cinnamaldehyde (Wako), corticosterone (Sigma), DMPP (1,1-dimethyl-4-phenylpiperazinium iodide, Aldrich), *dl*-norepinephrine (Sankyo, for the pharmacological test), *l*-norepinephrine bitartrate (Sigma, for norepinephrine determination), hexamethonium bromide (Yamanouchi), hydrocortisone 21-acetate (Wako), and *l*-epinephrine bitartrate (Sigma). Doses of drugs refer to those of the salt in the case where drugs were described as a salt form.

RESULTS

1) Catecholamine Releasing Effect of Cinnamaldehyde and Dimethyphenylpiperazinium (i.v. Administration)

Results are summarized in Table I. Total catecholamine concentration approx. doubled 2 min after administration of cinnamaldehyde, reaching a maximal level, and returned to the basal level in approx. 20 min. As shown in Fig. 1, magnitude of increase in 2 min was almost equal through 4 series repeated. Almost all the increased portion of catecholamines was epinephrine. No constant response of blood pressure was observed. On the other hand, DMPP produced a marked increase in total catecholamine concentration in 30 s, which disappeared as early as 1.5 min thereafter. The effect of DMPP was reproducible through 4 consecutive series as in the case of cinnamaldehyde. Increase in epinephrine concentration was markedly greater than that in norepinephrine concentration, both being significantly elevated. Blood pressure markedly increased with DMPP. CMC solution which served as control showed no effect in this experiment.

Influence of ganglion blocking on the catecholamine releasing effect of both drugs is shown in Fig. 2. The effect of DMPP was markedly antagonized with hexamethonium plus atropine but that of cinnamaldehyde was not. Under treatment with the ganglion blocking agents, increase in blood pressure by DMPP substantially disappeared, whereas responses of blood pressure to cinnamaldehyde tended to increase regardless of a fashion of the responses before the treatment.

Influence of the adrenals on catecholamine releasing effect of both drugs is shown in Table II. The effect of both drugs disappeared or markedly decreased after ligation of the adrenals. Basal catecholamine concentration after ligation of the adrenals fell to the level approx. a half that before ligation (Total catecholamine concentration 4±1 ng/ml ($N=4$)). On the contrary, pressor response to 1 μg/kg of norepinephrine (base) *i.v.* showed no practical change before and after the ligation (30±3 mmHg → 25±4 mmHg ($N=5$)).

2) Catecholamine Releasing Effect of Cinnamaldehyde (i.d. Administration)

As shown in Table III, cinanmaldehyde increased catecholamine concentration in a dose-dependent way. At 50 mg/kg, the 20-min value

was approx. twice that of the basal level, reaching a maximal level and the catecholamine concentration seemed still to remain at an increased level in 1 h. At 100 mg/kg, the 20-min value was almost the same as that at 50 mg/kg and the increased level seemed still to be maintained even

TABLE I. *Catecholamine Releasing Effect of Cinnamaldehyde and DMPP*

Drug		Basal(22)[a]	2(21)	10(7)	20(17)	30(14)	min
Cinnamaldehyde	Epi	3.4±0.9[b]	9.8±1.7[c]	12.4±7.0	4.6±1.2	5.6±1.4	
20 mg/kg, *i.v.*	Nor	3.6±0.7	5.6±1.7	7.6±3.6	2.4±0.4	2.7±1.0	
	Total	6.9±1.3	15.5±2.8[d]	20.0±10.5	6.9±1.4	8.3±1.5	
		Basal(41)	0.5(31)	2(9)	10(41)	20(21)	min
DMPP	Epi	3.6±1.0	63.5±18.4[c]	3.6±1.8	2.9±0.6	3.1±0.6	
30 μg/kg *i.v.*	Nor	3.2±0.4	12.2±2.8[c]	3.0±1.1	3.1±0.7	2.6±0.5	
	Total	6.8±1.1	75.7±19.6[c]	6.5±2.9	6.0±0.9	5.7±0.8	

a) number of animal, b) plasma concentration (ng/ml), c) $p < 0.01$ (against the basal value), d) $p < 0.05$ (against the basal value).

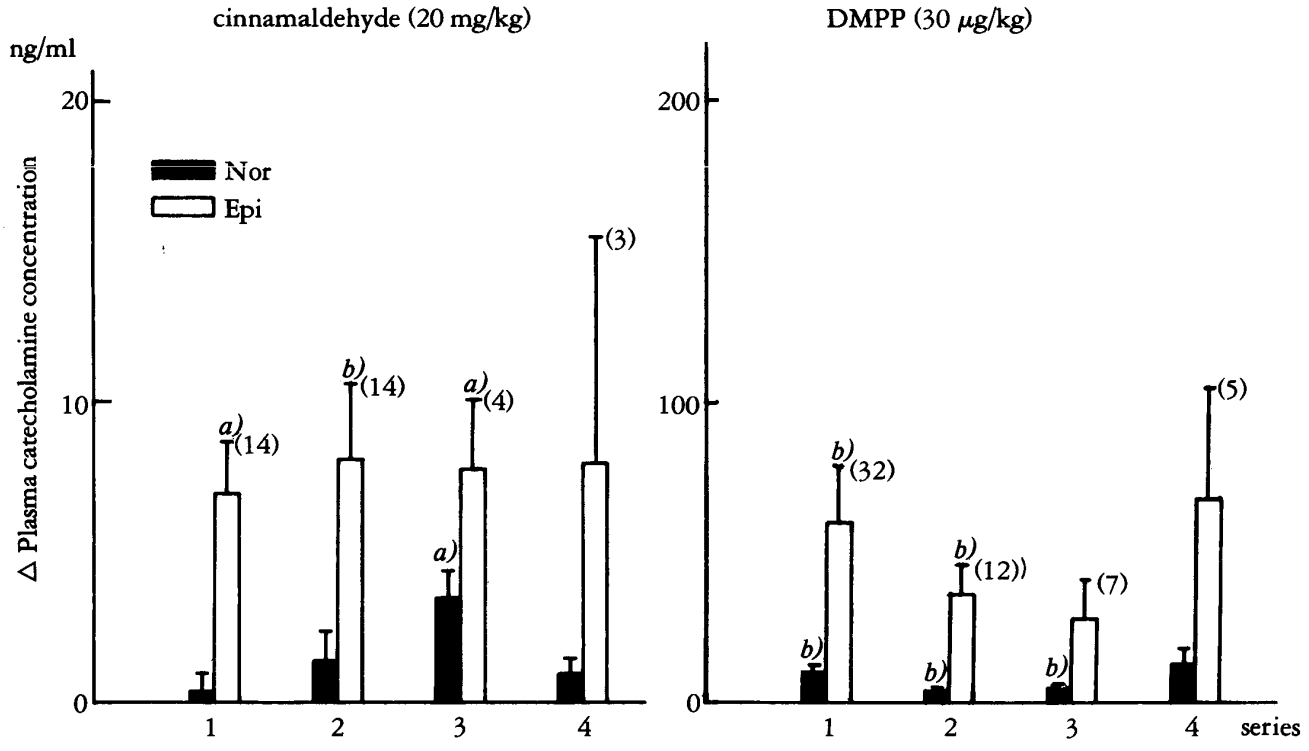

FIG. 1. *Catecholamine Releasing Effect of Repeated i.v. Administration of Cinnamaldehyde and DMPP*
Numbers in parentheses indicate the number of animals.
a) $p < 0.05$ (against the basal level), b) $p < 0.01$ (against the basal level).
Values at each series represent the 2-min value (cinnamaldehyde) and the 30-s value (DMPP).
Note different scale of the ordinate of the two drugs.

in 3 h. Further, 200 mg/kg of cinnamaldehyde increased catecholamine concentration in 20 min and produced a marked increase in 2 h with the magnitude of approx. 14 times as much as the basal level. In the early period of time course, cinnamaldehyde at doses used here showed an

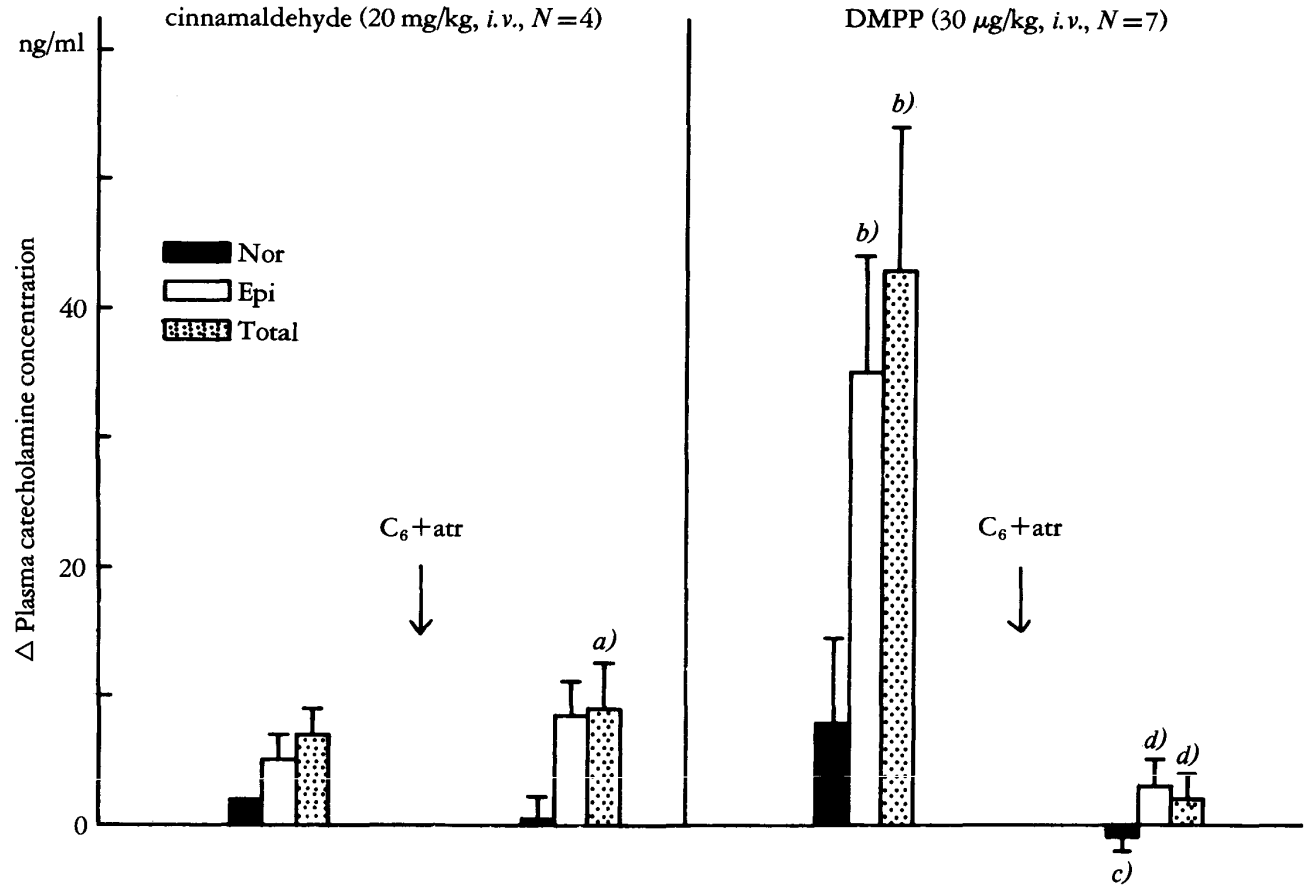

FIG. 2. *Influence of Hexamethonium plus Atropine (C_6 +atr) on Catecholamine Releasing Effect of Cinnamaldehyde and DMPP*
a) $p< 0.05$ (against the basal level), b) $p< 0.01$ (against the basal level), c) $p< 0.05$ (against the level before C_6 +atr), d) $p< 0.01$ (against the level before C_6 +atr).

TABLE II. *Influence of Adrenal Ligation on the Catecholamine Releasing Effect of Cinnamaldehyde and DMPP*

Drug		Before adrenal ligation	After adrenal ligation
Cinnamaldehyde (N=2)	Epi	39,[a] 13	1, 0
20 mg/kg, *i.v.*	Nor	−1, 0	−3, −3
	Total	38, 13	−2, −3
DMPP (N=5)	Epi	110.0±38.9	6.0±5.1
30 μg/kg, *i.v.*	Nor	30.0±15.2	2.2±2.0
	Total	140.0±53.3	8.2±7.1

a) Increase in plasma concentration (ng/ml).

increase in catecholamine concentration 2—3 times as much as that of the basal level which well corresponded to an increase 2 min after 20 mg/kg of *i.v.* cinnamaldehyde. The increased portion of catecholamines was almost all epinephrine and no significant increase in norepinephrine concentration was observed. CMC solution produced no effect on catecholamine concentration when determined in the same schedule of blood sampling as that at 200 mg/kg of cinnamaldehyde.

Blood pressure decreased 5 min after administration of 100 mg/kg of cinnamaldehyde, show-

TABLE III. *Catecholamine Releasing Effect of i.d. Administered Cinnamaldehyde*

Time	CMC ($N=3$)			50 mg/kg ($N=3$)			100 mg/kg ($N=5$)			200 mg/kg ($N=10$)		
min	Epi	Nor	Total	Epi	Nor	Total	Epi	Nor	Total	Epi	Nor	Total
Basal	1.9±0.8[a]	5.4±1.7	7.3±1.5	2.8±0.3	5.1±1.0	7.9±0.7	3.5±0.6	3.6±0.2	7.2±0.6	2.5±0.4	4.4±0.4	6.9±0.3
1										4.9±1.6	2.9±0.6	7.8±1.1
5	2.4±0.8	4.0±1.5	6.5±1.6							7.3±2.9	2.9±0.6	10.2±2.7
10	2.4±0.7	4.1±1.9	6.5±1.5							11.7±5.4	2.3±0.6	14.0±5.1
20	2.7±0.5	3.6±1.6	6.3±1.4	9.7±2.2[b]	6.6±2.6	16.3±3.6	8.8±1.6[b]	4.7±0.9	13.5±2.0[b]	19.0±9.1	3.0±0.6	22.0±8.8
30	4.0±1.1	2.8±1.9	6.8±0.9							13.5±3.5[c]	2.1±0.4	15.6±3.3[b]
45	4.2±2.0	3.5±2.0	8.0±0.4							15.6±4.5[c]	3.1±0.6	18.6±4.6[b]
60	4.0±0.9	2.9±1.8	6.8±0.9	6.2±2.2	6.4±1.9	12.6±2.7	7.2±2.2	3.0±0.8	10.2±1.6	23.7±7.2[c]	3.6±1.3	27.3±7.6[b]
90	4.8±1.3	2.1±1.7	6.9±0.4							35.5±12.2[b]	6.0±2.6	41.5±12.6[b]
120	3.0±1.3	3.1±1.7	4.9±2.0	3.3±0.5	5.7±1.3	8.9±1.1	7.7±2.1	5.0±0.8	12.7±2.8	76.4±28.7[b]	20.5±7.1	96.1±31.6[b]
180				3.9±1.5	5.0±1.1	8.9±1.3	9.8±3.6	5.2±1.3	15.0±4.5			
240				2.3±1.3	4.1±1.4	6.4±0.6	4.6±1.9	4.5±0.8	9.1±1.9			
300				2.8±1.4	4.4±1.4	7.3±0.1	4.7±0.9	3.0±0.6	7.8±1.0			
360				2.4±1.0	3.6±0.6	6.1±0.4	3.2±0.9	3.8±0.4	7.0±0.9			

a) plasma concentration (ng/ml), b) $p<0.05$ (against the basal value), c) $p<0.01$ (against the basal value).

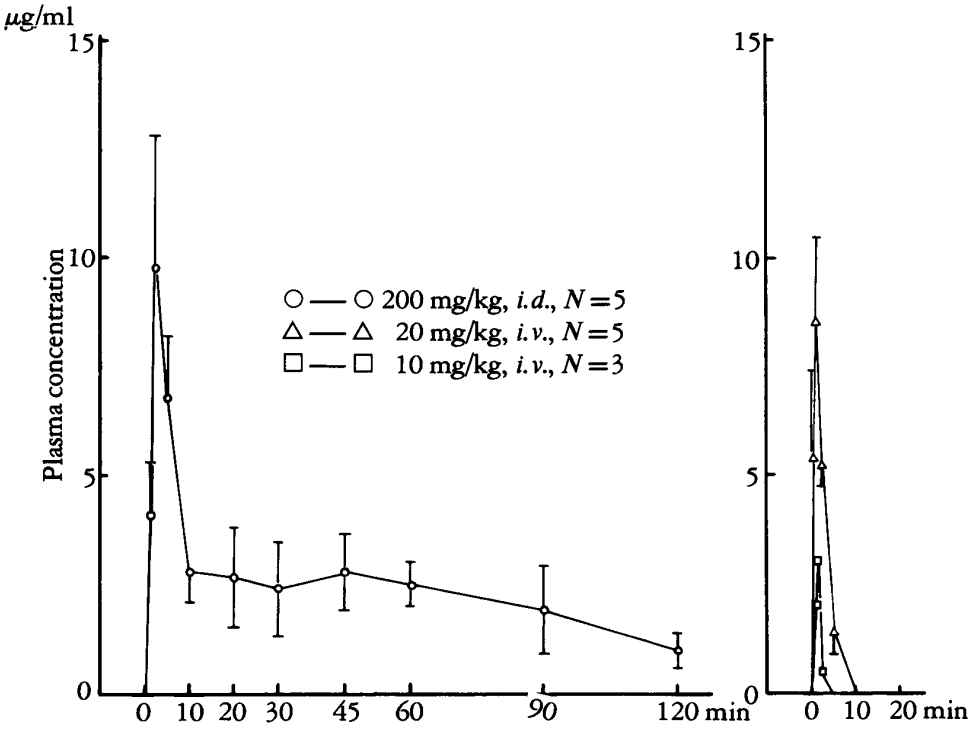

FIG. 3. *Plasma Concentration of Cinnamaldehyde in the Portal Venous Blood after i.d. and i.v. Administration of the Drug*

ing a maximal decrease of approx. 20 mmHg thereafter and returning to almost the basal level in 60 min. In a dose of 200 mg/kg, blood pressure gradually decreased with progression of time, the magnitude of which was 20 mmHg in 20 min, 30 mmHg in 90 min, and 50 mmHg in 120 min.

3) Intestinal Absorption of Cinnamaldehyde

As shown in Fig. 3, as early as 1 min after *i.d.* administration of 200 mg/kg, cinnamaldehyde was detected in the portal venous blood and the maximal concentration was reached in 2 min. Although declined thereafter, cinnamaldehyde was still detected in a concentration 1/10 as low as the maximal level in 2 h. Since no cinnamaldehyde was detected in the blood of the postcava through the experimental period, the amount of cinnamaldehyde in the portal blood is considered to reflect that of unchanged drug absorbed from the intestinal tract. *I.v.* administration of 20 mg/kg of cinnamaldehyde gave a maximal concentration in the portal venous blood in 1 min, with no detectable amount in 10 min. In the case of 10 mg/kg, *i.v.*, the magnitude of concentration and duration of the response were smaller and shorter compared with those at 20 mg/kg, respectively.

DISCUSSION

Cinnamaldehyde increased plasma catecholamine concentration through *i.v.* and *i.d.* route, the effect produced through the latter being dose-dependent and more lasting, *i.e.*, 2—3-h duration at doses of 50—100 mg/kg. In the case of 200 mg/kg, an exceeding increase was obtained during the later period of time course. In contrast, the effect of *i.v.* DMPP was very transient. In a perfusing experiment on the cat spleen, Brown and Gillespie reported that output of norepinephrine induced by electrical stimulation in saline perfused spleens is considerably greater than the corresponding outputs in the blood-perfused spleens.[4] Blakely *et al.* showed an existence of norepinephrine uptake process which required blood cells in an experiment *in vivo* on the cat spleen.[5] Zileli *et al.* found an increase of catecholamine release, especially epinephrine release, from the adrenals owing to decrease in blood pressure and blood volume in an experiment of bleeding shock in dogs.[6] Accordingly, an exceeding increase in catecholamine concentration observed during the later period of time after *i.d.* administration of 200 mg/kg of cinnamaldehyde may at least partly be explained from an aspect that dilution of blood took place at that time, which resulted in acceleration of catecholamine releasing effect of cinnamaldehyde although the dilution of blood *per se* (control experiment) showed no augmentation of catecholamine concentration. In addition, there exists a possibility that a marked decrease in blood pressure at that time may participate in the increase in catecholamine concentration. Intestinal absorption of cinnamaldehyde *i.d.* administered at a dose of 200 mg/kg occurred very early and was lasting. In contrast, its portal venous blood concentration was rapidly diminished beyond the detectable threshold in the case of *i.v.* administration. These findings may explain the reason why catecholamine releasing effect of *i.d.* cinnamaldehyde lasted longer than that of the *i.v.* administered drug.

The conclusion that origin of plasma catecholamines released by systemically administered cinnamaldehyde is mainly the adrenals can be derived from the following findings; 1) increase in plasma catecholamine concentration by *i.v.* cinnamaldehyde disappeared in the animal preparation whose adrenals were ligated. 2) increased portion of plasma catecholamines by *i.v.* or *i.d.* cinnamaldehyde was almost all epinephrine. 3) as reported earlier,[1] cinnamaldehyde released catecholamines from the isolated dog adrenal and from the adrenals *in situ* of anesthetized dogs by local *i.a.* application. The finding that catecholamine releasing effect of *i.v.* cinnamaldehyde was not influenced by pretreatment of hexamethonium and atropine indicates that the drug increased plasma catecholamine concentration through a mechanism(s) independent of increase in adrenergic nervous activity either originated in the central nervous system or

generated from peripheral events *via* reflex pathway at least under such an anesthetized condition. Except the later period of time after *i.d.* administration of 200 mg/kg of cinnamaldehyde where a marked decrease in blood pressure was noted, no relation between decrease in blood pressure and increase in plasma catecholamines released was recognized in the present experiment. From above findings, it is concluded that cinnamaldehyde, entering the circulatory system, reached the adrenals and released catecholamines from the organ through a mechanism(s) independent of affecting the cholinergic receptors. Blood catecholamine concentration measured here may express a highest one because of blood collection from a position in the postcava which is closely distal to the adrenals.

The following discussion is on a pharmacodynamic effect of plasma catecholamines released with cinnamaldehyde. As observed in the present study or a previous study,[7] *i.v.* cinnamaldehyde produced a hypotensive phase followed either by a presence or absence of hypertensive phase. Some discussions were made on mode of this hypotensive effect.[7] On the other hand, as illustrated in Fig. 3 in the preceding paper,[1] blood pressure response to *i.v.* cinnamaldehyde after treatment with hexamethonium plus atropine, not after treatment with atropine alone,[7] also was more vasopressive compared with that before the drug treatment in the present study. Nelson *et al.* reported an augmentation of pressor response to exogenous norepinephrine with hexamethonium treatment.[8] Moreover, *i.a.* cinnamaldehyde administered closely to the adrenals produced a vasopressive effect which was blocked by pretreatment of phentolamine.[1] From these findings, it is considered that *i.v.* cinnamaldehyde-induced hypertensive phase at least partly originated from plasma catecholamines released by the drug.

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