PHARMACOLOGICAL STUDIES ON CHINESE CINNAMON. IV. EFFECT OF CINNAMALDEHYDE ON THE ISOLATED HEART OF GUINEA PIGS AND ITS CATECHOLAMINE RELEASING EFFECT FROM THE ADRENAL GLAND OF DOGS

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Cinnamaldehyde increased contractile force and beating rate of the isolated perfused heart of guinea pigs. These effects were antagonized by propranolol and virtually abolished by reserpinization. Cinnamaldehyde released catecholamines from the isolated adrenal gland of dogs, whereas cinnamyl alcohol and cinnamic acid were devoid of such effect. Benzaldehyde also released catecholamines in the same preparation but its potency was lower than that of cinnamaldehyde. Cinnamaldehyde released catecholamines from the adrenals of pentobarbital-anesthetized and splanchnicotomized dogs by its close *i.a.* application to the organ, which was confirmed by measuring catecholamine contents in adrenal venous blood. Blood pressure in the same *in situ* preparation was raised by *i.a.* application of cinnamaldehyde and this pressor response was antagonized by phentolamine but not by combination of hexamethonium and atropine. When adrenal venous blood was taken out from the circulation immediately after an *i.a.* application of cinnamaldehyde, the pressor response was not observed in most cases. In conclusion, cinnamaldehyde released catecholamines from the adrenal gland of dogs through a direct action (independent of cholinergic receptor sites) on the organ, cinnamaldehyde was presumed to elicit positive ino- and chronotropic actions on the heart of guinea pigs by releasing endogenous catecholamines, and the aldehyde group in cinnamaldehyde molecule played a role in its catecholamine releasing effect from the organs.

Keywords—benzaldehyde; cardiostimulative effect; catecholamine release; Chinese cinnamon; cinnamaldehyde; dog adrenal gland *in situ*; isolated dog adrenal gland; isolated guinea pig heart; pressor effect

Chinese cinnamon, Japanese apricot seed, and Ephedra are important crude drugs in Chinese medicine and include cinnamaldehyde, amygdalin, and ephedrine as the main component, respectively. Amygdalin easily undergoes enzymatic hydrolysis to generate benzaldehyde. Many prescriptions which are used for common cold with cough and for asthma in Chinese medicine include combination of these 2 or 3 crude drugs.

Ephedrine is one of the sympathomimetic amines and the therapeutic effects of Ephedra can partly be explained from the sympathomimetic properties of ephedrine. Previously, we reported that cinnamaldehyde exerted positive ino- and chronotropic actions on isolated atria and isolated

perfused hearts of guinea pigs and that neither cinnamyl alcohol nor cinnamic acid showed these actions.¹⁾ It was reported that acetaldehyde exerted sympathomimetic actions such as sinus tachycardia and arterial hypertension in dogs by direct application of acetaldehyde to the sinus node through its nutrient artery and that acetic acid as well as ethyl alcohol lacked these actions.²⁾ These facts suggest similarity between the pharmacological effects of cinnamaldehyde and acetaldehyde. Moreover, acetaldehyde was capable of releasing catecholamines from the adrenal gland of cats³⁾ and cows⁴⁻⁶⁾ and ethyl alcohol was devoid of this effect in cats.⁷⁾ The present investigation was undertaken to study the sympathomimetic effects of cin-

namaldehyde and benzaldehyde on guinea pig hearts, dog blood pressure and adrenal glands.

EXPERIMENTAL

Method — 1) *Experiment on Isolated Perfused Heart of Guinea Pigs* — Male guinea pigs weighing 300–400 g were killed by a blow on the head. The heart was quickly removed and the coronary vessels were perfused with Krebs-Henseleit solution according to the Langendorff's technic. The composition of this solution was as follows: (g/l) NaCl 6.9, KCl 0.35, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.29, CaCl_2 0.28, KH_2PO_4 0.16, NaHCO_3 2.1, glucose 2.0. The solution was bubbled with a mixture of 95% O_2 and 5% CO_2 and maintained at 35°. The perfusing pressure was 55–60 cm H_2O and the flow rate was 5–8 ml/min. Contractions of the heart were recorded on an ink writing oscillograph *via* a force displacement transducer (Nihon Kohden, SB-1T). Drugs were dissolved in saline and the drug solutions were administered in a constant volume of 0.1 ml and a constant speed of 20 sec through a rubber tube connected to the cardiac cannula.

2) *Experiment on Isolated Perfused Adrenal Gland of Dogs* — Mongrel dogs of either sex weighing 8–18 kg were anesthetized with 30–40 mg/kg of *i.p.* or *i.v.* sodium pentobarbital. The left adrenal gland was removed and perfused with phosphate buffered Locke's solution *via* the adrenolumbar vein in a retrograde fashion as described by Robinson.⁸⁾ The composition of this solution was as follows: (g/l) NaCl 9.0, KCl 0.42, CaCl_2 0.24, Na_2HPO_4 0.31, NaH_2PO_4 0.12, glucose 1.8. The solution in reservoirs was bubbled with O_2 and maintained at 30°. The perfusing pressure was 55–60 cm H_2O and the flow rate was 0.5–2.5 ml/min. The effluent from the gland was collected in ice-cold test tubes every 5 min and 2.4 N perchloric acid solution containing EDTA- Na_2 (3 mg/ml) and $\text{Na}_2\text{S}_2\text{O}_5$ (6 mg/ml) was added to each collected sample in a volume ratio of 1:5. These prepared solutions were stored at –15° and their catecholamine contents were determined within 3 days. Each drug was dissolved in the buffer solution. The drug solution was introduced to the

adrenal cannula for a period of 2 min by changing flow from a reservoir of the perfusing solution to that from a reservoir of the test solution and simultaneously a collecting tube was renewed.

3) *Experiment on Adrenal Gland of Anesthetized Dogs* — Mongrel dogs of either sex weighing about 10 kg were anesthetized as mentioned above. After an intubation of the trachea, abdomen was opened and the right renal artery and vein were ligated near the kidney. A cannula for an *i.a.* injection of drugs was inserted in the superior mesenteric artery with its tip in the abdominal aorta. A thread was loosely placed around the aorta just above the left renal artery. The bilateral greater splanchnic nerves were dissected and cut under the diaphragm. Blood pressure was recorded from the right carotid artery on an ink writing oscillograph by means of a pressure transducer (Nihon Kohden, MPU-0.5). Drugs were *i.a.* administered in a constant volume of 3 ml including a flushing saline and a constant speed of 30 sec with a simultaneous occlusion of the aorta by pulling the thread upward or were *i.v.* injected through the left femoral vein. In the case of injection of cinnamaldehyde, it was emulsified in 0.5% carboxymethylcellulose (CMC) solution. At the end of the experiments, an ink solution was *i.a.* injected to confirm perfusion of the organ with the drug solutions. In these preparations, the left adrenal gland was recognized to receive much more portions of the ink solution compared to the right adrenal gland. In another series of the experiments, adrenal venous blood was collected for catecholamine determination. Following the operative procedures mentioned above, dogs were heparinized (300 μ /kg, then 100 μ /kg every 1 hr), the left adrenolumbar vein was ligated near the postcava, and a polyethylene T-tube was interposed between the adrenolumbar vein and the ipsilateral cephalic vein. Venous outflow from the left adrenal gland was collected through the T-tube drainage in centrifuge tubes successively at intervals of 2–5 min (1–4 ml aliquot) before and after a drug injection. These tubes contained 0.2 ml of 30 mg/ml of $\text{Na}_2\text{S}_2\text{O}_5$ solution and were chilled with ice in advance. During a period of blood collection, Ringer solution was *i.v.* infused at a rate of 1 ml/min.

Plasma was separated from the blood sample by centrifugation and was added to an equal volume of 0.8 N perchloric acid solution containing EDTA- Na_2 (1 mg/ml). After centrifugation of this plasma sample in a refrigerated centrifuge (14000 rpm, 15 min), the supernatant fractions were stored at -15° for catecholamine assay within 1 week.

4) *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 Gordon⁹⁾ and Anton and Sayre.¹⁰⁾ Results were expressed as total catecholamines or as norepinephrine and epinephrine.

Drugs

Drugs used were as follows—acetaldehyde (Wako), acetylcholine chloride (Daiichi), atropine sulfate (Wako), benzaldehyde (Wako), cinnamaldehyde (Wako), cinnamic acid (Daiichi Kagaku), cinnamyl alcohol (Wako), ephedrine hydrochloride (Fuji), *l*-epinephrine (Sankyo, for the pharmacological tests), *l*-epinephrine bitartrate (Sigma, for epinephrine determination), hexamethonium bromide (Yamanouchi), histamine dihydrochloride (Wako), *dl*-norepinephrine (Sankyo, for the pharmacological tests), *l*-norepinephrine bitartrate (Wako, for norepinephrine determination), phenolamine mesylate (CIBA), propranolol hydrochloride (Sumitomo), reserpine (Daiichi), and tyramine hydrochloride (Wako). Doses of only catecholamines and reserpine are expressed in terms of the free base in the text.

RESULT

1) *Effect of Cinnamaldehyde and Benzaldehyde on the Isolated Perfused Heart of Guinea Pigs*

Cinnamaldehyde 250 μg increased contractile force and heart rate after a transient and slight decrease in both parameters. The same dose of cinnamaldehyde produced a tachyphylaxis 20 min after the first application. Both positive responses decreased by approximately 40%. Acetaldehyde 250 μg , norepinephrine 0.2 μg , and histamine 0.4 μg increased contractile force and rate. Benzaldehyde 250 to 500 μg produced no positive responses. Pretreatment with 1 μg propranolol 10

min before the second application significantly antagonized positive responses to cinnamaldehyde. The actions of acetaldehyde and norepinephrine were also reduced by this pretreatment but those of histamine were not modified. Reserpine 2.5 mg/kg, injected *i.p.* 24 and 48 hr before sacrifice, abolished positive ino- and chronotropic responses to cinnamaldehyde 250 μg , acetaldehyde 250 μg , and tyramine 100 μg . Responses to norepinephrine and histamine were not modified. Typical inotropic actions are shown in Fig. 1 and summarized data are given in Table I.

2) *Catecholamine Releasing Effect of Cinnamaldehyde and Benzaldehyde from the Isolated Perfused Adrenal Gland of Dogs*

Spontaneous secretory rate of catecholamines was between 0.21 and 4.48 $\mu\text{g}/5$ min. Cinnamaldehyde 4.0×10^{-4} to 1.2×10^{-3} g/ml increased catecholamine secretion with the maximal effect at the first or second aliquot, and thereafter the secretion returned to the original level. Although the cardiostimulative actions of cinnamaldehyde decreased at its second application, the catecholamine releasing action of cinnamaldehyde did not decrease up to the third trial. Acetaldehyde and benzaldehyde showed a similar effect to that of cinnamaldehyde, potency of the latter being lower. The increased portion of catecholamine amounts released by 1.2×10^{-3} g/ml of cinnamaldehyde, benzaldehyde, and acetaldehyde during the first 10-min period were approximately 13, 4, and 17 μg , respectively. In contrast, cinnamyl alcohol and cinnamic acid (neutralized with NaOH) did not show such action in a dose of 1.2×10^{-3} g/ml. Acetylcholine (ACh) 1×10^{-5} g/ml markedly released catecholamines but ephedrine 1×10^{-3} g/ml showed no action. Typical time courses of catecholamine releasing action of drugs are given in Fig. 2 and summarized results are shown in Table II.

3) *Catecholamine Releasing Effect of Cinnamaldehyde from the Adrenal Gland of Anesthetized Dogs*

Twelve dogs were used in this experiment. CMC control solution exerted no action on blood pressure. Cinnamaldehyde 10–50 mg injected *i.a.*

FIG. 1. Inotropic Effect of Cinnamaldehyde (CA) and Acetaldehyde (AA) on the Isolated Perfused Heart of Guinea Pigs

A: Propranolol treatment, B: Reserpine treatment.

FIG. 2. Time Course of Catecholamine Releasing Effect of Cinnamaldehyde (CA) and Acetaldehyde (AA) from the Isolated Perfused Adrenal Gland of Dogs

Ach: acetylcholine. CA and AA were tested successively at 40-min intervals on the same gland.

FIG. 3 Effect of Cinnamaldehyde (CA) on Blood Pressure of an Anesthetized Dog

Epi: epinephrine, CMC: 0.5% carboxymethylcellulose solution, Ach: acetylcholine, hexamethonium: 3 mg/kg *i.v.*, atropine: 1 mg/kg *i.v.*, phentolamine: 2 mg/kg *i.v.*, dog: ♂ 8 kg.

(per animal) raised blood pressure, preceded by a transient fall, whereas these doses injected *i.v.* either caused a fall in blood pressure or did not affect it. Ach 30–100 µg injected *i.a.* also raised blood pressure and these doses were enough to lower it when given by *i.v.* route. The onset of pressor responses evoked by cinnamaldehyde was slower and the duration of action was prolonged over that of Ach. Pretreatment with phentolamine 2 mg/kg *i.v.* blocked pressor responses evoked by cinnamaldehyde, Ach, and norepinephrine (2 µg/kg, *i.v.*). Pretreatment with hexamethonium 3 mg/kg *i.v.* blocked the pressor response by Ach and when the block was not complete, the combined use of hexamethonium and atropine (1 mg/kg, *i.v.*) blocked the Ach-induced pressor

response. However, the pressor response by cinnamaldehyde was not blocked by the pretreatment with these drugs (Fig. 3).

To ascertain catecholamine releasing effect of cinnamaldehyde from the adrenal gland, adrenal venous blood was collected and plasma catecholamines were measured. The resting amounts of plasma catecholamines were 4.0–248.1 ng/min and they were not increased with an injection of CMC solution, but increased with 10–50 mg of cinnamaldehyde or 30–50 µg of Ach. Cinnamaldehyde and Ach showed a maximal catecholamine releasing effect in the first sampling period. Increases in blood pressure which were observed at cinnamaldehyde and Ach injections were not seen in most cases during the

FIG. 4. Time Course of Catecholamine Releasing Effect of Cinnamaldehyde (CA) from the Adrenal Gland of Anesthetized Dogs
 CMC: carboxymethylcellulose, Ach: acetylcholine. Dog: ♂ 9.5 kg (♂ 11.5 kg for CMC). Open column: epinephrine, closed column: norepinephrine. Drugs were i.a. administered.

period of blood sampling. Typical time courses of catecholamine releasing effect by cinnamaldehyde and Ach are illustrated in Fig. 4 and summarized results are shown in Table III.

DISCUSSION

Cinnamaldehyde exerted positive ino- and chronotropic actions on the isolated perfused heart of guinea pigs. The cardiostimulatory effects of cinnamaldehyde were reduced by pretreatment with propranolol and virtually abolished by reserpine. These results suggest that cinnamaldehyde acts on the heart by releasing endogenous catecholamines. Acetaldehyde also showed an effect similar to that of cinnamaldehyde. Since acetaldehyde can release catecholamines from the adrenal medulla of cats³⁾ and cows,⁴⁻⁶⁾ a similar

effect was anticipated with cinnamaldehyde. Certainly cinnamaldehyde released catecholamines from the adrenal gland of dogs both in isolated and in *in situ* preparations. On the contrary, cinnamyl alcohol and cinnamic acid did not show such effect in the isolated preparation. Benzaldehyde released catecholamines from the same preparation. From these findings, it was concluded that the aldehyde group in cinnamaldehyde molecule played a role in its catecholamine releasing effect from the adrenal gland of dogs and this conclusion was also the case with acetaldehyde.

The mode of the action of cinnamaldehyde on the dog adrenal gland was studied with a guidance of blood pressure responses. Catecholamines released by cinnamaldehyde and Ach from the organ were considered to be responsible for their

TABLE I. *Effect of Cinnamaldehyde (CA) and Acetaldehyde (AA) on the Isolated Perfused Heart of Guinea Pigs*

Drug	Dose (μ g)	No. of heart	1st response		2nd response	
			Increase in amplitude (%)	Increase in heart rate (beats/min)	Increase in amplitude (%) ^{a)}	Increase in heart rate (beats/min)
CA	250	5	102 $\pm$ 38 ^{b)}	32 $\pm$ 6	63 $\pm$ 7	18 $\pm$ 6
AA	250	5	51 $\pm$ 15	19 $\pm$ 5	101 $\pm$ 16	23 $\pm$ 8
Norepinephrine	0.2	6	114 $\pm$ 36	25 $\pm$ 10	125 $\pm$ 9	29 $\pm$ 9
Histamine	0.4	3	93 $\pm$ 21	11 $\pm$ 5	91 $\pm$ 7	14 $\pm$ 5
Drug	Dose (μ g)	No. of heart	1st response		2nd response (after propranolol) ^{e)}	
			Increase in amplitude (%)	Increase in heart rate (beats/min)	Increase in amplitude (%) ^{a)}	Increase in heart rate (beats/min)
CA	250	5	116 $\pm$ 33	42 $\pm$ 8	17 $\pm$ 4 ^{f)}	2 $\pm$ 4 ^{e)}
AA	250	3	66 $\pm$ 25	16 $\pm$ 2	35 $\pm$ 10	4 $\pm$ 2
Norepinephrine	0.2	4	165 $\pm$ 70	14 $\pm$ 7	31 $\pm$ 10 ^{f)}	2 $\pm$ 3 ^{e)}
Histamine	0.4	3	102 $\pm$ 34	27 $\pm$ 11	79 $\pm$ 6	22 $\pm$ 10
Drug	Dose (μ g)	No. of heart	1st response (reserpine pretreated) ^{d)}			
			Increase in amplitude (%)	Increase in heart rate (beats/min)	Increase in amplitude (%) ^{a)}	Increase in heart rate (beats/min)
CA	250	3	15 $\pm$ 8	-4 $\pm$ 5		
AA	250	3	1 $\pm$ 3	0 $\pm$ 0		
Norepinephrine	0.2	3	295 $\pm$ 21	16 $\pm$ 4		
Histamine	0.4	3	141 $\pm$ 23	0 $\pm$ 0		
Tyramine	100	3	11 $\pm$ 2	4 $\pm$ 4		

Spontaneous heart rate: 1st response 188 $\pm$ 2.4/min, 2nd response 186 $\pm$ 3.4/min, after propranolol 189 $\pm$ 4.8/min, and reserpine pretreated 223 $\pm$ 4.3/min.

a) compared to values at 1st responses, b) mean $\pm$ S.E., c) propranolol 1 μ g, d) reserpine 2.5 mg/kg i.p. 2 days, e) $p < 0.05$ and f) $p < 0.01$ compared to 2nd responses (Student's *t* test, in the groups ($N \geq 4$)).

pressor effect, since this effect was not observed in most cases when left adrenal venous blood was taken out from the circulation immediately after the drug injection. The left adrenal was exposed to *i.a.* drugs much more than the right adrenal. Ach-induced pressor response was inhibited by pretreatment with phentolamine or hexamethonium alone, and was completely blocked by combination of hexamethonium and atropine. This indicates the existence of nicotinic and muscarinic receptors in the adrenal medulla of dogs.¹¹⁻¹³⁾ Cinnamaldehyde-evoked pressor responses were blocked by phentolamine but not by combination of these 2 drugs. These results suggest that cinnamaldehyde releases catecholamines from the adrenal gland through a mechanism without participation of the nicotinic and muscarinic receptors. In this connection, acetaldehyde has been reported to release cate-

cholamines from the adrenal medulla not *via* the nicotinic receptor site and independently of Ca^{++} .^{3, 4, 6)}

It is premature to make discussion on relation between the present pharmacological effects of cinnamaldehyde as well as benzaldehyde and the therapeutic effects of Chinese cinnamon and Japanese apricot seed, since these substances were examined by means of an unpractical route. It seems, however, of interest that in many prescriptions in Chinese medicine, we can find combination of Chinese cinnamon, Japanese apricot seed, and Ephedra all of which main components have similar sympathomimetic actions.

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TABLE II. *Catecholamine Releasing Effect of Cinnamaldehyde (CA), Benzaldehyde (BA), and Acetaldehyde (AA) from the Isolated Perfused Adrenal Gland of Dogs*

Drug	Dose (g/ml)	No. of adrenal	Increase in catecholamine output ($\mu\text{g}/10\text{ min}$) ^{a)}
CA	1.0×10^{-4}	2	0.07 ^{b)} 1.38
	4.0×10^{-4}	4	5.62 ± 4.07
	1.2×10^{-3}	5	12.86 ± 2.79
AA	4.0×10^{-4}	1	4.52
	1.2×10^{-3}	5	16.54 ± 6.16
BA	4.0×10^{-4}	1	3.21
	1.2×10^{-3}	5	3.86 ± 0.85
Acetylcholine	1.0×10^{-5}	2	11.15 51.59
Cinnamyl alcohol	1.2×10^{-3}	2	-0.21 -1.10
Cinnamic acid	1.2×10^{-3}	2	0.07 -0.13
Ephedrine	1.0×10^{-5}	1	-0.69
	1.0×10^{-4}	2	0.78 0.98
	1.0×10^{-3}	2	-1.09 0.35

a) Increased portion from the resting level and total amounts of the first 2 aliquots.

b) Each value represents the original value or mean $\pm$ S.E.TABLE III. *Catecholamine Releasing Effect of Cinnamaldehyde (CA) from the Adrenal Gland of Anesthetized Dogs*

Drug	Dose (mg)	No. of adrenal	Increase in plasma catecholamines (%) ^{a)}
CMC ^{b)}	1 ml	5	8.4 ± 12.0 ^{c)}
CA	10	3	135.6 133.9 102.7
			2429.3 460.5 204.1
	50	5	866.5 ± 308.3
	0.03	3	440.6 413.5 21.1
Acetylcholine	0.05	2	510.1 1023.7

a) Increased portion from the resting level.

b) 0.5% carboxymethylcellulose solution.

c) Each value represents the original value or mean $\pm$ S.E.

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