The Mechanism of Blood Pressure Depression by Ellagic Acid (34303)

UMESH C. BHARGAVA[1] AND BERTIS A. WESTFALL

Department of Pharmacology, University of Missouri, School of Medicine, Columbia, Missouri 65201

Ellagic acid has been reported to reduce blood pressure in several species of animals (1–3). Botti and Ratnoff (2) suggested the fall in blood pressure might be related to activation of the Hageman factor, since ellagic acid primarily decreases the clotting time by activation of the Hageman factor (4, 5) and this in turn causes vasodilation as determined by an increase in blood flow in the hind limb of the dog (6). The purpose of the present study was to elucidate further the possible mechanism of blood pressure depression by determining whether histamine might play a role in this fall in blood pressure following ellagic acid administration.

Materials and Methods. Wistar male rats (200–280 g) from Harlan Industries and Simonsen Laboratories were maintained in a rather constant environment and fed Purina chow and water *ad libitum*. Ellagic acid was extracted from black walnut (3). Fresh aqueous solutions were prepared in NaOH solution and adjusted to pH 10 ± 0.5 with HCl and kept frozen during the day until time for the experiment.

Rats were anesthetized with 50 mg/kg of pentobarbital sodium (ip). Blood pressure was measured through the left carotid artery using a Statham pressure transducer attached to a polygraph recorder (Grass). Drugs were injected through the left femoral vein.

Data are presented as the mean ± SE. The following drugs were injected: reserpine (CIBA Pharmaceutical Co.); diphenhydramine hydrochloride (Parke, Davis and Co.); atropine sulfate (Eli Lilly and Co.); propranolol (Ayerst Lab.); dextran (McGraw Lab.); 48/80 (Burroughs Wellcome and Co.); acetyl choline chloride (Merck & Co.); histamine phosphate (Eli Lilly and Co.); norepinephrine bitartrate (Winthrop Lab.; epinephrine hydrochloride (Parke, Davis and Co.). All doses were expressed in the form of the salts.

Results. In a typical experiment when 5 mg/kg of ellagic acid were injected (iv) into the rat, blood pressure dropped after a latency of 20–30 sec and the maximum fall (−75 ± 2.8 mm Hg) was observed at approximately 1 min after ellagic acid injection. Five min following an injection the blood pressure approached a steady level but was somewhat less than the control level (−17.3 ± 4.83 mm Hg).

Effect of various treatment on blood responses. Two types of experiments were conducted:

I. Animals were pretreated with one of the blocking agents (reserpine, diphenhydramine, atropine, propranolol, dextran, or 48/80) and after appropriate time ellagic acid (5 mg/kg, iv) was injected. All drugs used except atropine and propranolol significantly altered the blood pressure response by ellagic acid, e.g., the maximum blood pressure drop after 1 min and the blood pressure depression when measured between 5–10 min (see Table I).

II. In this experiment, one of the following drugs was injected before and after ellagic acid treatment (5 mg/kg, iv): acetylcholine, histamine, norepinephrine, and epinephrine. The central end of the cut left vagus was stimulated before and after treatment with ellagic acid. None of the above drug responses was altered by ellagic acid. The response from central vagus stimulation was not changed significantly after ellagic acid treatment (see Table II).

Effect of a second dose of ellagic acid. In one group of rats, ellagic acid was injected (5 mg/kg, iv) and then a second dose of the

[1] Present address: Diamond Laboratories, Inc., P. O. Box 863, Des Moines, Iowa 50304.

TABLE I. Effect of Ellagic Acid (5 mg/kg, iv) on Blood Pressure in Rats after Various Pretreatments.[a]

Treatment	Dose (mg/kg)	Control BP	Δ In BP at 0–1 min (mm Hg)	Δ In BP at 0–1 min (%)	Δ In BP at 5–10 min (mm Hg)	Δ In BP at 5–10 min (%)
Control	0	102.2 ± 2.48	−65.3 ± 2.85	−63.9 ± 2.78	−17.3 ± 4.85	−16.9 ± 4.74
Reserpine	4	81.3 ± 4.69	−39 ± 4.52	−48 ± 5.56	−1.8 ± 3.85	−2.2 ± 4.73
Diphenhydramine	2.5	84.7 ± 6.65	−45.8 ± 4.43	−54 ± 5.23	+4.3 ± 3.60	+5.1 ± 4.25
Atropine	1	103 ± 5.20	−61.7 ± 3.63	−59.9 ± 3.52	−21.7 ± 4.19	−21.1 ± 4.07
Propranolol	0.5	110.6 ± 6.96	−64 ± 4.82	−57.9 ± 4.36	−18 ± 5.18	−16.3 ± 4.68
Dextran	20	78.3 ± 9.77	−43 ± 8.02	−54.9 ±10.24	+7.3 ± 5.08	+9.3 ± 6.49
48/80	0.1	88.8 ± 6.43	−49 ± 6.15	−58.5 ± 7.33	+0.77± 5.74	+0.92± 6.85

[a] Ellagic acid was injected 10 min after each drug treatment with the exceptions of: reserpine, 24 hr; dextran and 48/80, 0.5 hr. Each response is the average of six animals.

same compound at the same level (5 mg/kg) was administered either 7 min, 1, 2, or 3 hr after the first injection. It was observed that there was virtually no effect on blood pressure when a second dose of ellagic acid was injected at approximately 7 min after the first injection. But when a second dose was injected 1, 2, or 3 hr after the first injection, an increasing response respectively was observed (see Fig. 1).

Discussion. The data indicate that the blood pressure fall caused by ellagic acid is not like the effect of acetylcholine, since neither acetylcholine nor atropine blocked the effect of ellagic acid or vice versa. Furthermore ellagic acid does not seem to cause vasodilation by stimulating β receptors since propranolol did not block its effect. It appears that the blood pressure depression produced by ellagic acid is due to histamine release, since the blood pressure dropped only after a latency of 20–30 sec, which is the unique characteristic of histamine liberators (7). Diphenhydramine, dextran, or compound 48/80 all slightly antagonize the blood pressure response by ellagic acid which further supports the above statement. Dextran and compound 48/80 were injected to reduce the histamine content in intact animals. In our laboratory it has been observed that ellagic acid prevented the blood pressure fall produced by compound 48/80, dextran, or polymyxin B sulfate which are potent histamine releasers in the rat (8). It is already established that the blood pressure fall caused by histamine releasers (compound 48/80) is due to histamine release (9, 10). Therefore, in order to test whether ellagic acid decreases blood pressure by the mecha-

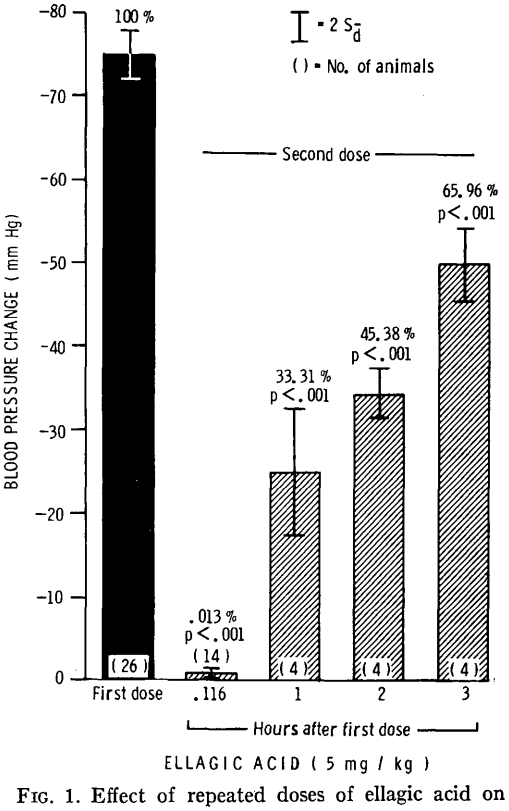

FIG. 1. Effect of repeated doses of ellagic acid on blood pressure in rats; each bar was obtained from the mean ± SE of the animals indicated in bracket; the black bar represents the effect of the first dose of ellagic acid, and the striped bars represent the effect of a second dose of ellagic acid in rats which were pretreated with ellagic acid. Significance (p value) was calculated by comparing the mean following one dose with the mean following a second dose.

TABLE II. Effect of Various Drugs before and after Ellagic Acid Treatment (5 mg/kg, iv).

			Before ellagic acid		After ellagic acid			
			Control		Same group[a]		Different group[b]	
Treatment	No. of rats	Dose (mμ/kg)	Control BP (mm Hg)	BP (mm Hg; %)	Control BP (mm Hg)	BP (mm Hg; %)	Control BP (mm Hg)	BP (mm Hg; %)
Acetylcholine	6	5.0	109.7 ± 6.47	−69 ± 4.55	95 ± 10.56	−55.3 ± 6.79	99.7 ± 2.50	−61.7 ± 1.44
Histamine	6	5.0	118.3 ± 4.38	−34.5 ± 8.56	95.5 ± 4.36	−27.0 ± 6.83	102.2 ± 2.67	−28.0 ± 4.64
Norepinephrine	6	2.5	108.7 ± 9.14	+50.2 ± 6.68	92.5 ± 5.07	+39.8 ± 5.55	98.8 ± 3.1	+56.8 ± 2.14
Epinephrine	6	2.5	100.3 ± 4.05	+58 ± 3.18	90 ± 7.09	+55.3 ± 7.04	98 ± 2.53	+61.7 ± 2.49
Vagal Stim.	7	—	111.3 ± 3.04	+28.8 ± 4.39	101 ± 4.86	+15.8 ± 5.92	—	—

[a] Same group of rats as controls.
[b] Different group of rats than controls.

nism of histamine release, animals were pretreated with ellagic acid and the effect of a second dose was determined. Indeed, ellagic acid blocked the effect of the second dose as it did with other histamine releasers. Furthermore, injection of histamine liberators or histamine increases the T wave of the EKG, which was also observed when ellagic acid was injected (3). Therefore, the results obtained suggest strongly that the blood pressure fall due to ellagic acid is due to histamine release. Data do not explain why reserpine pretreatment diminished the response of ellagic acid.

Summary. The mechanism of the blood pressure fall produced by ellagic acid was evaluated. Pretreatment of rats with diphenhydramine hydrochloride, compound 48/80, or dextran significantly reduced the blood pressure fall caused by ellagic acid. These results suggest that histamine is involved in producing the blood pressure fall following ellagic acid administration. The latency period (20–30 sec) before the blood pressure fall, and the blockade effect of ellagic acid on the effect of a second dose of ellagic acid suggest the release of histamine by this compound. Effects of acetylcholine, norepinephrine, epinephrine, and histamine (on blood pressure) administered intravenously were not significantly changed after the rats received ellagic acid.

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