Cardiovascular Pharmacology of *N,N'*-Bis-[α-(2-pyridyl)ethyl]-ethylenediamine Dimaleate

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N,N'-Bis-[α-(2-pyridyl)ethyl]-ethylenediamine dimaleate (JB 5058), a substituted polymethylene diamine with hypotensive action, was noted to have alpha adrenergic blocking activity, as demonstrated in the cat superior cervical ganglion nictitating membrane preparation. It did not produce a centrally mediated hypotensive response in the dog cross-circulation preparation. JB 5058 produced only a transient decrease in cardiac output while increasing femoral blood flow. The hypotensive activity of JB 5058 appears to be caused by a decrease in peripheral vascular resistance primarily due to alpha adrenergic blockade. It also appears to produce a modest antagonism of angiotensin II on vascular smooth muscle.

HALLIDAY ET AL. (1) have reported that *N,N'*-bis-[α-(2-pyridyl)ethyl]ethylenediamine dimaleate (JB 5058) was the most active hypotensive compound in a series of substituted polymethylene diamines. The compound produced marked hypotensive effects of long duration in anesthetized rats and dogs, and preliminary data suggested that the compound blocked alpha adrenergic receptors. This present study was undertaken to define more clearly the effects of JB-5058[1] on the cardiovascular system and to elucidate more fully the mechanisms of action of this compound.

METHODS

Dog Cross-Circulation Preparation.—The central and peripheral hypotensive activity of JB 5058 was investigated in the dog cross-circulation preparation, as described by Bickerton and Buckley (2). Mongrel dogs were anesthetized by an intravenous injection of 35 mg./Kg. of pentobarbital sodium and the vertebral venous sinuses and vertebral arteries occluded between C-2 and C-3 or C-3 and C-4 utilizing a 21-gauge stainless steel wire. Circulation was established between the left common carotid artery of the anesthetized donor dog, and the two common carotid arteries of the recipient, and the two jugular veins of the recipient, and the left jugular vein of the donor. Circulatory leakage between the head and trunk of the recipient was determined periodically utilizing the I^{131} method. Blood pressure was recorded from a femoral artery of each dog *via* a Statham transducer onto a Grass polygraph. Only those experiments in which no leakage was observed were utilized for this study. JB 5058, 35 mg./Kg., was administered into the arterial inflow to the recipient's head in three such preparations.

Cat Nictitating Membrane.—The cat superior cervical ganglion-nictitating membrane preparation was utilized to investigate the effects of JB 5058 on ganglionic transmission. Cats of either sex were anesthetized with pentobarbital sodium, 35 mg./Kg., by intraperitoneal injection. The head was rigidly secured and a nictitating membrane connected to an isotonic lever system which recorded on a kymograph. Responses of the membrane to submaximal electrical stimulation of the pre- and postganglionic fibers and to exogenous epinephrine, 10 mcg., i.v., were recorded prior to and following the administration of JB 5058, 35 mg./Kg., i.v.

Cardiac Output.—The effects of JB 5058 on cardiac output were investigated in anesthetized dogs using the method described by Olmsted (3). Dogs, 12 to 15 Kg., were anesthetized with pentobarbital sodium, 35 mg./Kg., i.v. The dogs were placed on intermittent positive-pressure respiration utilizing a Mine Safety Appliance respirator and the left thoracic wall opened at the fourth intercostal space using electrocautery. The lungs were retracted to expose the pericardium, which was then incised, retracted, and fastened to the chest wall with wound clips. The ascending aorta was carefully stripped of surrounding tissue and a 14-mm. i.d. probe placed around the vessel. Negative pressure was created in the thoracic cavity by overinflation of the lungs while the chest was tightly closed with sutures. Artificial respiration was then discontinued and the animal permitted to breathe voluntarily. Cardiac output (ascending aortic blood flow) was recorded *via* an electromagnetic flowmeter (Medicon FM-6) and femoral blood pressure *via* a Statham transducer onto a Grass polygraph. After femoral blood pressure and cardiac output had stabilized, JB 5058 was administered intravenously. In two of the experiments, the effects of epinephrine, 1 to 2 mcg./Kg., i.v., on cardiac output, prior to and following JB 5058, also were determined.

Peripheral Blood Flow.—The effects of the compounds on renal and hind-limb blood flow and coronary perfusion pressure were investigated in anesthetized dogs. Dogs were anesthetized with pentobarbital sodium, 35 mg./Kg., i.v. Renal and femoral blood flows were determined utilizing a Medicon electromagnetic flowmeter in which a

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[1] Kindly supplied by Lakeside Laboratories, Milwaukee, Wis.

TABLE I.—EFFECT OF JB 5058, 35 mg./Kg., ON DOG CROSS-CIRCULATION PREPARATION

Expt. No.	Animal	Route	Original Blood Pressure, mm. Hg	Max. Change, %	Duration, Min.
1	Donor		210/120	−47/46	80+
	Recipient	IA-R[a]	150/100	+33/25	7
2	Donor		200/125	−40/44	60+
	Recipient	IA-R	115/ 70	+26/28	20
3	Donor		160/100	−38/35	420+
	Recipient	IA-R	150/ 90	+84/94	5

[a] IA-R = Intra-arterially *via* the carotid inflow to the recipient's head.

suitable probe was placed around the respective artery. Vascular resistance was calculated using

$$\frac{\text{Mean Blood Pressure (mm.Hg)}}{\text{Flow (ml./min.)}} = \text{Vascular Resistance Units}$$

The left descending coronary arteries were perfused in the following manner. A carotid artery was cannulated and flow established between the carotid artery and the anterior descending branch of the left coronary artery. Flow was held constant utilizing a Sigmamotor pump and perfusion pressure recorded *via* a T-tube on the outflow side of the pump with a Statham transducer. Heparin, 1000 units/Kg., i.v., was administered to prevent clotting. In each experiment, femoral blood pressure and either renal blood flow, hind limb blood flow, or coronary perfusion pressure were recorded on a model 5 Grass polygraph.

Pithed Rat.—Rats were anesthetized with urethan, 1.2 Gm./Kg., i.p., and placed on positive-pressure respiration. A rod (2.2 mm. in diameter and 25 cm. long) was inserted obliquely into and through the eye socket and passed down the length of the spinal canal. The vagi were cut and a carotid artery cannulated for blood pressure recording. Blood pressure responses to 2 mcg. of epinephrine HCl and synthetic angiotensin II,[2] 1 mcg., were obtained prior to and after the administration of JB 5058, 10 mg./Kg., i.v.

Rabbit Aortic Spirals.—The method described by Furchgott and Bhadrakoni (4) was employed for this study. Albino rabbits, 2 to 4 Kg., were sacrificed by cervical dislocation. A segment of the descending aorta between the arch and the diaphragm was removed, pulled over polyethylene tubing (PE 360), and rotated against a scalpel blade so that spiral strips approximately 0.5 cm. in width were cut. A strip then was suspended in a magnus bath containing Tyrode's solution maintained at 38° and oxygenated with 95% O_2 and 5% CO_2. Contractions were recorded onto a Grass polygraph *via* a Grass force displacement transducer adjusted to apply 5-Gm. tension to the strip. Responses induced by epinephrine, 0.1 mcg./ml., of bathing solution or angiotensin II, 0.1 mcg./ml., were obtained prior to and after the addition of JB 5058 in doses ranging from 25 to 800 mcg./ml.

Denervated Dog Hind Limb.—Mongrel dogs were anesthetized with pentobarbital sodium, 35 mg./Kg., i.v., the right femoral artery and vein isolated, and the right sciatic and femoral nerves cut, as described by Clonninger and Green (5). A wire tourniquet was placed beneath the exposed vessels and tightened around the limb utilizing a Schiffrin wire tightener. Carotid blood was perfused into the cannulated femoral artery of the isolated limb by means of a constant output pump (Sigmamotor pump, model TM-10) and perfusion pressure recorded on a Grass polygraph *via* a force displacement transducer. Arterial blood pressure was recorded from the left femoral artery. Responses to epinephrine HCl, 5 mcg., and angiotensin II, 0.5 mcg./Kg., injected into the arterial inflow to the limb were recorded prior to and after administration of JB 5058, 35 mg./Kg., i.v.

[2] Kindly supplied by Ciba Pharmaceutical Products, Inc., Summit, N. J.

RESULTS

Dog Cross-Circulation Preparation.—The effects of JB 5058, administered *via* the arterial inflow to the recipient's head, on the blood pressure of the recipient and donor animals are summarized in Table I. JB 5058 produced marked hypotensive effects in the donor animal only, and the donor's blood pressure remained depressed throughout the remainder of the experiment. The compound produced pressor responses of relatively short duration in the recipient's trunk. It would appear that these effects in the recipient animal were of a reflex nature.

Cat Nictitating Membrane.—JB 5058, 35 mg./Kg., i.v., reduced the response of the nictitating membrane to stimulation of both pre- and postganglionic fibers of the superior cervical sympathetic nerve approximately 60% in three cats. Response of the membrane to exogenous epinephrine was completely abolished; in one experiment, four times the control dose of epinephrine failed to stimulate the nictitating membrane.

Cardiac Output.—JB 5058, 35 mg./Kg., i.v., produced a transient (1 to 2 minutes) decrease in cardiac output ranging from 10 to 43% in three of four dogs. Cardiac output then returned to predrug levels and remained constant for the duration of the experiments (2 to 3 hours). The compound did not alter the increase in cardiac output produced by epinephrine in two additional experiments.

Renal and Hind-Limb Blood Flow and Coronary Perfusion Pressure.—Effects of JB 5058, 35 mg./Kg., i.v., on renal blood flow and vascular resistance are summarized in Table II. Renal vascular resistance was reduced approximately 35%, even though only a slight change in actual renal blood flow occurred. JB 5058, 35 mg./Kg. administered intravenously reduced vascular resistance in the hind limb and produced an increase in blood flow in three experiments (Table III). JB 5058, 35 mg./Kg., i.v., reduced coronary perfusion pressure 28% in two dogs (Table IV). Perfusion pressure returned to predrug levels approximately 30 minutes following administration of the compound, even

TABLE II.—EFFECT OF JB 5058, 35 mg./Kg., I.V., ON RENAL VASCULAR RESISTANCE

Time, Min.	Control Responses,[a] % Mean Blood Pressure	Renal Blood Flow	Vascular Resistance Units
Control	100	100	100
5	59 ± 11	83 ± 30	74 ± 17
15	62 ± 12	102 ± 24	73 ± 8
30	70 ± 7	105 ± 17	74 ± 6
60	70 ± 6	113 ± 18	66 ± 9
120	62 ± 5	109 ± 16	65 ± 8

[a] $\bar{X}$ ± S. E. $N = 3$.

TABLE III.—EFFECT OF JB 5058, 35 mg./Kg., I.V., ON VASCULAR RESISTANCE IN HIND LEG OF THE DOG

Time, Min.	Control Responses,[a] % Mean Blood Pressure	Femoral Blood Flow	Vascular Resistance Units
Control	100	100	100
5	58 ± 9	155 ± 23	43 ± 8
15	68 ± 3	156 ± 8	47 ± 3
30	69 ± 1	140 ± 13	50 ± 6
60	67 ± 5	123 ± 13	59 + 6
120	75 ± 3	140 ± 13	52 ± 4

[a] $\bar{X}$ ± S. E. $N = 3$.

TABLE IV.—EFFECT OF JB 5058, 35 mg./Kg., I.V., ON CORONARY PERFUSION PRESSURE IN THE DOG

Expt. No.	Time, Min.	Mean Blood Pressure, mm. Hg	Coronary[a] Perfusion Pressure, mm. Hg
1	Control	120	125
	5	50	90
	15	60	100
	30	60	120
2	Control	140	175
	5	70	125
	15	80	150
	30	85	170

[a] A Sigmamotor pump was used to perfuse the anterior descending coronary artery.

TABLE V.—EFFECT OF JB 5058, 10 mg./Kg., I.V., ON PRESSOR RESPONSE TO EPINEPHRINE AND ANGIOTENSIN II IN THE PITHED RAT

Expt. No.	Control Response, mm. Hg Epi.	Ang.	Response after JB 5058, mm. Hg Epi.	Ang.
1	115	28	60	12
2	100	96	40	65
3	115	32	61	17
4	140	..	60	..

though arterial pressure was still depressed approximately 50%.

Pithed Rats.—The effects of JB 5058 on the epinephrine and angiotensin II pressor responses in the pithed rat are summarized in Table V. The compound depressed the pressor response to both epinephrine and angiotensin II 48 and 42%, respectively. Administration of the compound itself produced a mild transient pressor effect. Injections of normal saline solution, equal to the volume of drug solution injected, produced similar pressor responses. However, the saline solution did not alter the responses to epinephrine and angiotensin II.

Rabbit Aortic Strips.—JB 5058 blocked or diminished contractions of the rabbit aorta induced by epinephrine and angiotensin II. The inhibitory $dose_{50}$ for JB 5058 against epinephrine-induced contractions was approximately 70 mcg./ml. of bath, whereas the inhibitory $dose_{50}$ against angiotensin II-induced contractions was approximately 350 mcg./ml.

Dog Denervated Limb.—JB 5058, 35 mg./Kg., produced a mean decrease in perfusion pressure of 38% in three preparations. Hind-limb perfusion pressure returned to predrug levels within 20 minutes, even though the hypotensive effect was still at its maximum. The compound inhibited the vasoconstricting effects of epinephrine and angiotensin administered directly into the perfused femoral artery 75 and 23%, respectively.

DISCUSSION

JB 5058 has been reported to produce hypotensive effects of long duration in anesthetized rats and dogs, to reverse the pressor response to epinephrine, and to decrease the pressor response to *l*-norepinephrine, angiotensin II, and bilateral carotid occlusion in anesthetized dogs (1). In the present study, the compound decreased the response of the nictitating membrane in the cat to both pre- and postganglionic stimulation by the same magnitude, thus indicating no interference with the impulse transmission across the ganglion. The response of the nictitating membrane to injected epinephrine was abolished, further substantiating the previously observed alpha adrenergic blocking activity of JB 5058. The compound appeared to produce its hypotensive effects through purely peripheral mechanisms when investigated in the dog cross-circulation preparation. Administration of the compound into the arterial inflow to the isolated head of the recipient failed to produce a centrally induced depressor response.

Page and Bumpus (6) have reported that other adrenolytic agents have relatively little effect on the pressor response to angiotensin II. JB 5058 appears to inhibit angiotensin II directly, independent of its alpha adrenergic blocking activity and mild vascular smooth muscle depressing action. JB 5058 inhibited the constricting effects of angiotensin II *in vitro* (rabbit aortic spirals) and *in vivo* (pithed rat and perfused denervated dog hind limb). In the denervated hind limb, the compound produced only transient direct relaxation of arterial smooth muscle, whereas the inhibitory effects of angiotensin II could be demonstrated long after this brief dilatory effect of JB 5058.

JB 5058 produced only a transient decrease in cardiac output, an indication that the hypotensive activity of the compound was due to the marked decrease in peripheral resistance. Blood flow studies suggest that this decreased resistance was, at least in part, due to increased blood flow to skeletal muscle and cutaneous tissue. The renal blood flow remained approximately unchanged, even though systemic blood pressure was greatly reduced. In conclusion, the hypotensive activity of JB 5058 results in a decrease in peripheral vascular resistance, primarily due to alpha adrenergic blockade. The compound also appears to produce a

mild inhibitory effect on the direct vasoconstrictor activity of angiotensin II on vascular smooth muscle.

SUMMARY

No centrally induced hypotensive effect could be demonstrated with JB 5058 in the dog cross-circulation preparation.

Alpha adrenergic blocking activity in the absence of ganglionic blockage was demonstrated with JB 5058 in the cat superior cervical ganglion nictitating membrane preparation.

Cardiac output, except for a brief (1–2 minutes) reduction, was not altered by the administration of JB 5058.

Following the administration of JB 5058, femoral blood flow was increased, coronary vascular resistance was transiently decreased, and renal blood flow was unchanged.

A modest antagonism of angiotensin II was observed with JB 5058 both *in vivo* and *in vitro*.

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Effect of the Site of Release on the Absorption of Trimeprazine-S^{35} and Penicillin G in Dogs

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A radiofrequency generator described in a previous paper was applied to release trimeprazine-S^{35} and potassium penicillin G at selected sites in the gastrointestinal tract of dogs. Plasma and urinary excretion data indicate that trimeprazine-S^{35} is readily and efficiently absorbed when released in the stomach and the upper and middle small intestine. Potassium penicillin G in its biologically active form is more available for absorption when released just beyond the pylorus than when released in either the stomach or more than 3 ft. beyond the pylorus.

THE EFFECT of drug release site on the rates and efficiency of drug absorption has been a subject of great interest to pharmaceutical investigators. A technique developed in our laboratories using a radiofrequency generator for opening specially designed capsules at selected sites in the gastrointestinal tract offers a useful tool for studying the effect in intact animals under normal physiologic conditions. Useful information thus can be gathered for the design and evaluation of oral dosage forms. This report describes the application of this technique to a study of the effect of release site on the absorption of a phenothiazine antipruritic, trimeprazine, and an antibiotic, potassium penicillin G.

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EXPERIMENTAL

The design and the filling of the capsule and the release of drugs from the capsule by induction heating from a radiofrequency generator have been described in detail in a previous report (1).

Adult mongrel dogs were fasted (18–20 hours) prior to the oral administration of a capsule. Induction heating (approximately 5 minutes) was applied either 15–30 minutes after capsule administration to release the drug in the stomach or after sufficient time had been allowed for the capsule to reach certain sites in the intestine, determined by a marked thread attached to the capsule or by fluoroscopy (1). Usually the dog had been fasted for 21–25 hours prior to drug release. Urine was collected up to 24 hours following drug release by catheterizing the dogs and for longer periods by housing the animals in a metabolic cage. Blood samples were drawn from the jugular vein.

Absorption of Trimeprazine-S^{35}.—Each capsule was filled with 0.3–0.4 ml. of an aqueous solution of the drug to provide the dose of 2.5 mg. of trimeprazine-S^{35} tartrate/Kg. body weight. To help provide adequate urine collection, each dog was given 20 ml./Kg. of water 1 hour before drug release. Blood was drawn prior to induction heating and at 0.5, 1, 2, 3, 6, 9, 12, and 24 hours thereafter. Urine was collected for the periods: 0–0.5, 0.5–1,