Evaluation of Certain Hypotensive Agents V

Substituted Polymethylenediamines

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The hypotensive activity of a series of substituted alkylenediamines was investigated in anesthetized rats and dogs. *N,N'*-Bis-[α-(2-pyridyl)ethyl]-ethylenediamine dimaleate (JB 5058) produced marked hypotensive effects of long duration in both species of animals. JB 5058 reversed the pressor response to epinephrine, 2 mcg./Kg., and decreased the pressor response to levarterenol, 2 mcg./Kg.; angiotensin II, 1 mcg./Kg.; and bilateral carotid occlusion. The oral administration of the compound to normotensive animals produced a mild transient depression of systolic blood pressure; however, 40 mg./Kg. administered orally to renal hypertensive dogs produced a marked decrease in systolic blood pressure which persisted from 34 to 57 hours. The data suggest that the primary mechanism of the hypotensive activity of JB 5058 is competitive blockade of the α adrenergic receptors.

SEVERAL ETHYLENEDIAMINE derivatives have been reported to produce hypotensive activity in experimental animals. Tonks (1) reported that a series of compounds structurally related to mepyramine, *N,N*-dimethyl-*N'*-(4-methoxybenzyl) - *N'* - (2 - pyridyl) - ethylenediamine, produced mild transient hypotension. The pressor response to epinephrine in the anesthetized dog and cat was potentiated by all of the compounds in this series except the *N*-ethyl-*N*-(2-naphthyl)-*N',N'*-dimethyl derivative which exhibited adrenolytic activity. Graham and Tonks (2) reported that 2 mg./Kg. of this compound reversed the pressor response to epinephrine and blocked the pressor response of levarterenol in the cat.

This report is concerned principally with the evaluation of the hypotensive activity of several substituted polymethylenediamines[1] in rats and dogs and the oral hypotensive activity in unanesthetized hypertensive dogs of the most promising compound of this series, JB 5058, *N,N'* - bis - [α - (2 - pyridyl)ethyl] - ethylenediamine dimaleate.

EXPERIMENTAL

Hypotensive Activity in Normotensive Rats.—The experimental compounds were screened for hypotensive activity in normotensive Wistar rats, anesthetized with 1.2 Gm./Kg., i.p., urethan as described by Bickerton, *et al.* (3). All compounds were administered in aqueous solution *via* a femoral vein in graded doses from 1–60 mg./Kg.; each animal received only a single dose of one of the compounds. In some instances, due to low water solubility or insufficient compound, the higher dosage range could not be administered.

Hypotensive Activity in Normotensive Dogs.—Certain compounds were further evaluated in the anesthetized normotensive dog. Mongrel dogs were anesthetized with pentobarbital sodium, 35 mg./Kg., i.v., and blood pressure recorded from a cannulated femoral artery *via* a Statham transducer onto a Grass polygraph. The effects of the experimental compounds on the pressor activity of the following were also recorded: epinephrine, 2 mcg./Kg.; levarterenol, 2 mcg./Kg.; bilateral carotid occlusion, 15 seconds; and synthetic angiotensin II, 1 mcg./Kg. All compounds were administered *via* the femoral vein.

Oral Hypotensive Activity in Unanesthetized Normotensive and Hypertensive Dogs.—Renal hypertension was produced in dogs utilizing the method described by Grollman (4). The blood pressures of the normotensive and hypertensive dogs were determined by the method of Prioli and Winbury (5). The dogs were restrained in a harness which limited movement but did not fully support the animals. The tail was shaved and a microphone of an Infraton unit secured to the tail beneath the coccygeal artery so that the pulse wave could be observed on the screen of an oscilloscope. A digital cuff (Beckman Instruments) was placed proximal to the microphone and the pressure increased in the cuff until pulse waves were no longer visible. The pressure was then reduced slowly until the pulse waves reappeared and the systolic pressure in mm. Hg recorded. JB 5058 was administered orally in capsule form to dogs fasted

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

TABLE I.—EVALUATION OF CERTAIN SUBSTITUTED POLYMETHYLENEDIAMINES ON THE BLOOD PRESSURE OF ANESTHETIZED NORMOTENSIVE RATS

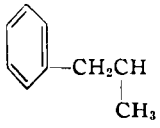
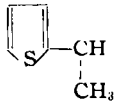
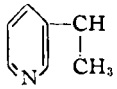
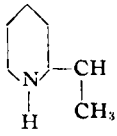
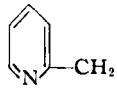
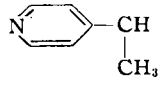
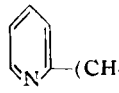
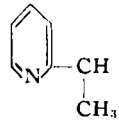
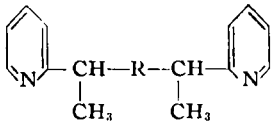
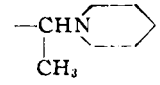
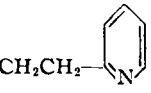
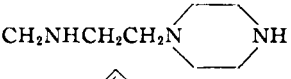
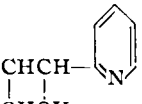
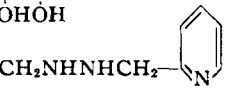
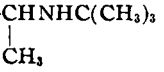
Compd.	R	Salt	Animals, No.	Dose, mg./Kg.	% Drop, b.p. $\bar{x} \pm$ S.D.	Duration, min. $\bar{x}$ ● S.D.
		Group I R—HN—CH_2—CH_2—NH—R				
EX 2837		Dimaleate	6	20	0	0
EX 3144		Dimaleate	15	40	0	0
EX 3460		4 HCl	6	60	0	0
EX 4420		Tetramaleate	6	20	0	0
EX 4467		Dimaleate	6	10	51 ± 4	190 to 490
EX 4468		Dimaleate	8	60	0	0
EX 4947		Dimaleate	6	60	42 ± 8	63 ± 20
JB 5058		Dimaleate	10	10	48 ± 10	150 to 840
		Group II 				
EX 3571	—$N(CH_3)(CH_2)_2N(CH_3)$	4 HCl	6	10	48 ± 5	140 to 360
EX 4525	—$NH(CH_2)_5NH$	Dimaleate	13	20	0	0
EX 10006	—$NH(CH_2)_3NH$	4 HCl	11	10	47 ± 6	270 to 490
		Group III 				
EX 4914	—$CH(CH_3)N(CH_3)_2$	CH_3Br	6	30	0	0
EX 4937		Fumarate	8	80	0	0

TABLE I.—(*Continued*)

Compd.	R	Salt	Animals, No.	Dose, mg./Kg.	% Drop, b.p. x ± S.D.	Duration, min. x ± S.D.
EX 4938		2 HCl	6	20	0	0
EX 4949	—CH_2NHCH_3	Maleate	7	60	0	0
EX 4950		4 HCl	6	60	40	45
EX 4955		2 HCl	5	60	0	0
EX 4959		Diacetate	6	40	0	0
EX 4963		Maleate	7	40	0	0
	Group IV					
EX 3132	$(CH_2)_3N(CH_3)_2$	Trimaleate	17	50	0	0
EX 3500	$(CH_2)_2N(C_2H_5)_2$	Trimaleate	6	50	0	0

12 to 18 hours and the systolic blood pressure obtained periodically.

RESULTS

Hypotensive Activity in Rats.—The hypotensive activity of the experimental compounds in anesthetized rats is summarized in Table I. Insertion of a needle into the rat femoral vein, or the physical characteristics of the solution injected, often produce transient hypotensive responses. To eliminate these possible artifacts, blood pressure recordings were measured 10 minutes after administration of the compound. This procedure, by necessity, also evaluated drug effects of less than 10 minutes duration as inactive. The compounds were divided into four structural groups, and doses listed in this table are either the HD_{50} (hypotensive dose$_{50}$, that dose which was estimated to produce approximately a 50% reduction in mean arterial pressure) or the highest dose tested. Three com-

TABLE II.—EFFECTS OF EXPERIMENTAL COMPOUNDS ON THE BLOOD PRESSURE AND SEVERAL VASCULAR RESPONSES IN THE ANESTHETIZED DOG

Compd.	Dose, mg./Kg.	Original Blood Pressure, mm. Hg	% Fall, b.p.	Duration, min.	% Control Responses BCO	EPI	L-ART	ANG
EX 2837	20	170	70	3	75	90	110	120
EX 3144	20	92	0	...	60	75	100	100
EX 3460	20	170	0	...	100	110	86	100
EX 4467	20	120	50	80+[c]	62	Reversal	...	160
EX 4468	25	140	0	...	100	100	...	100
EX 4525	20	150	0	...	100	170	160	130
EX 4914	20	150	33	1.5	90	80	86	100
EX 4937	40	140	28	1.0	63	180	112	120
EX 4938	40	136	+58	11	100	100	91	71
EX 4947	60	160	31	80+[a]	60	Reversal	40	67
EX 4949	20	145	+20	7	120	100	100	120
EX 4950	20	138	86	138	15	150	140	160
EX 4955	20	130	0	...	100	100	100	100
EX 4959	20	130	0	...	100	100	100	100
EX 4961	20	124	0	...	75	140	140	100
EX 4963	20	135	26	5	230	120	135	120
EX 10006	25	132	62	168	25	78	108	113
JB 5058	10	150	33	60	84	Reversal	...	...
...	20	96	40	80+[b]	50	Reversal	60	69
...	25	120	50	660	25	Reversal	...	...
...	40	140	49	1200+	28	Reversal	32	77

[a] Blood pressure at termination of experiment was 104 mm. Hg. [b] Blood pressure at termination of experiment was 65 mm. Hg. [c] Blood pressure at termination of experiment was 80 mm. Hg.

pounds in Group I exhibited hypotensive activity: EX 4947 at 60 mg./Kg. and JB 5058 and EX 4467 at 10 mg./Kg. EX 4947 produced a mean drop of 42% with a mean duration (time to return to pre-drug level) of 63 minutes. EX 4467 produced a mean drop in blood pressure of 51% and a duration of action ranging from 190 to 430 minutes. JB 5058 was the longest acting compound of this series since 10 mg./Kg. produced a mean fall in blood pressure of 48% persisting from 150 to 840 minutes. Two of the three compounds in Group II produced marked hypotensive effects. EX 3571 and EX 10006 produced hypotensive responses in excess of 4 hours. Group III contained one compound which was moderately active. EX 4950 at 60 mg./Kg. produced a 40% fall in blood pressure with a duration of 45 minutes. Group IV contained no active hypotensive agents when tested in the rat.

Modification of the basic structure of JB 5058 from the (2-pyridyl) to the (3-pyridyl) or (4-pyridyl) as seen in compounds EX 3460 and EX 4468 apparently eliminated the hypotensive activity. The conversion of both α-(2-pyridyl)ethyl groups to picolyl groups (EX 4467) produced a slight decrease in the duration of action. EX 10006, the trimethylenediamine homolog of JB 5058, was highly effective, whereas EX 4525, the pentamethylenediamine homolog, was inactive as was the hydrazine, EX 4959, which has no alkylene chain.

The data obtained from this series of compounds in anesthetized rats suggest that the following structural moieties should be present for optimal hypotensive activity: (*a*) the compound should contain two 2-pyridylmethyl groups joined by an alkylenediamine chain, and (*b*) the distance between the alkylenediamine nitrogens has a bearing on the hypotensive activity since all the active compounds contained a 2 or 3 carbon chain at this site.

Hypotensive Activity in Normotensive Dogs.—The effects of the experimental compounds on the blood pressure of anesthetized dogs and on the various pressor responses in these animals are summarized in Table II. All of the compounds which were active in rats were also active in the anesthetized dog. EX 4950 produced only moderate effects in anesthetized rats; however, 20 mg./Kg., i.v., produced a mean drop in blood pressure of 86% and a duration of action of 138 minutes. EX 3571 was not investigated in dogs because of the limited quantity of the compound available. EX 4947, JB 5058 (Fig. 1), and EX 4467 reversed the pressor response of exogenous epinephrine. EX 4947 and JB 5058 also depressed the levarterenol and angiotensin II pressor responses, and both compounds markedly depressed the pressor effect produced by bilateral carotid occlusion. JB 5058 did not alter the pressor response to central vagal stimulation but did produce atropine-like effects on the cardiac response to peripheral vagal stimulation.

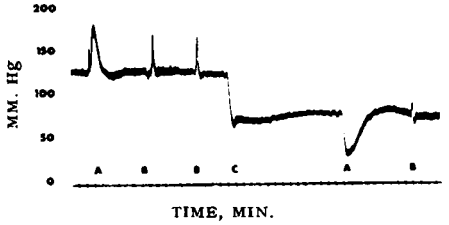


Fig. 1.—The effects of 40 mg./Kg., i.v., JB 5058 on epinephrine, 2 mcg./Kg., i.v., and bilateral carotid occlusion, 15 seconds, pressor responses. Key: A, epinephrine; B, carotid occlusion; C, JB 5058.

Oral Hypotensive Activity in Unanesthetized Normotensive and Hypertensive Dogs.—Single doses of JB 5058 were administered to two hypertensive dogs and one normotensive dog. Forty milligrams/Kg., orally, reduced the systolic pressure of the first hypertensive dog from 200 to 110 mm. Hg and was effective for approximately 34 hours (Fig. 2). This same dose lowered the systolic pressure of the

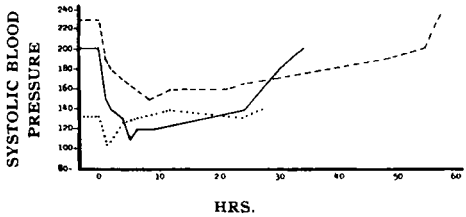


Fig. 2.—The effects of 40 mg./Kg. JB 5058, per os, on the blood pressure of unanesthetized normotensive and renal hypertensive dogs. Key: ---, hypertensive; ——, hypertensive; ..., normotensive.

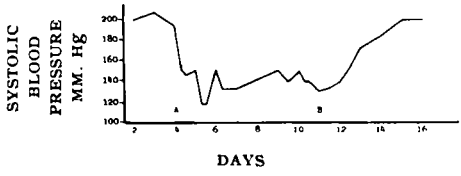


Fig. 3.—The effects of 20 mg./Kg. JB 5058, per os, daily in hypertensive dog No. 1. Key: A, initiation of JB 5058 administration; B, termination of treatment.

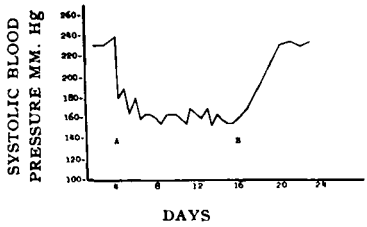


Fig. 4.—The effects of 20 mg./Kg. JB 5058, per os, daily in hypertensive dog. No. 2. Key: A, initiation of JB 5058 administration; B, termination of treatment.

second hypertensive dog from 230 to 150 mm. Hg within 8 hours with pressure returning to pretreatment levels in approximately 57 hours. The oral administration of 40 mg./Kg. of this compound to a normotensive dog produced a transient decrease in systolic blood pressure of 30 mm. Hg. The only observable effect of the compound in these animals was a relaxation of the nictitating membrane. JB 5058 was also administered once a day, 20 mg./Kg., per os, to two hypertensive dogs for periods of 7 and 12 days, respectively. The systolic pressure of the first dog decreased from 200 to 120 mm. Hg by the second day (Fig. 3). The blood pressure taken periodically each day

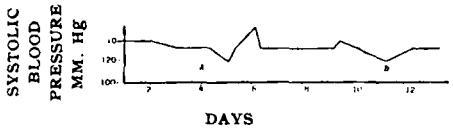


Fig. 5.—The effects of 20 mg./Kg. JB 5058, per os, daily in a normotensive dog. Key: A, initiation of JB 5058 administration; B, termination of treatment.

during the 7-day period varied from a low of 120 mm. Hg to a high of 150 mm. Hg. Treatment was then stopped, and the arterial pressure gradually returned to predrug levels in 4 days. The systolic blood pressure of the second hypertensive dog dropped from 240 to 155 mm. Hg over the 12-day period, returning to predrug levels 5 days after withdrawal of the compound (Fig. 4). JB 5058, 20 mg./Kg., administered orally each day to a normotensive dog did not significantly lower the systolic blood pressure of the animal over a 7-day period (Fig. 5).

DISCUSSION

JB 5058, *N,N'*-bis-[α-(2-pyridyl)ethyl]-ethylenediamine was the most active compound in a series of substituted polymethylenediamines. This compound produced marked hypotensive effects of long duration in anesthetized rats and dogs, reversed the pressor response to moderate doses of epinephrine, and decreased the pressor response to levarterenol. The reversal of epinephrine pressor response could be overcome by administering relatively large doses of epinephrine. The pressor response to bilateral carotid occlusion was decreased but not abolished, and the pressor response to single injections of angiotensin II was decreased approximately 25%. Pretreatment of anesthetized dogs with tripelannamine (sufficient to abolish the depressor response of 2.5 mcg./Kg. of histamine) and dichloroisoproterenol (up to 10 mg./Kg.) did not alter the hypotensive effect of this compound. These preliminary data suggest that JB 5058 blocks α adrenergic receptors (6) and that this action is most likely the primary mechanism of its hypotensive activity.

SUMMARY

Twenty-one substituted polymethylenediamines were investigated for their hypotensive activity. JB 5058, *N,N'*-bis-[α-(2-pyridyl)ethyl]-ethylenediamine dimaleate; EX 4467, *N,N'*-bis-[2-pyridylmethyl]-ethylenediamine dimaleate; EX 3571, *N,N'*-bis-[α-(2-pyridyl)ethyl]-*N,N'*-dimethylethylenediamine tetrahydrochloride; and EX 10006, *N,N'*-bis-[α-(2-pyridyl)ethyl]-trimethylenediamine tetrahydrochloride were the most active hypotensive compounds in the current series.

JB 5058 is a long-acting, orally effective hypotensive compound whose primary mechanism of action appears to be a blockade of α adrenergic receptors.

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