Evaluation and Mechanism of Action of Two Hypotensive Substituted Diamines

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Two substituted diamines, *N,N'*-bis(α-2-pyridylethyl)-1,2-propanediamine difumarate (EX 4962) and *N,N'*-bis(α-2-pyridylethyl)-2,3-butanediamine difumarate (EX 10,074), produced marked hypotensive effects of long duration when administered intravenously in anesthetized rats and dogs. They abolished the pressor response to bilateral carotid occlusion, slightly reduced the pressor response to injected catecholamines, and abolished the pressor response to amphetamine sulfate. Both of the compounds decreased the response of the cat nictitating membrane to both pre- and postganglionic stimulation and to injected epinephrine and increased blood flow in the perfused denervated hindlimb of the dog following intra-arterial administration. These results suggested that the compounds possessed some adrenergic blocking properties and that their hypotensive activity possibly was due to the blockade of the activity of endogenously released catecholamines on α-adrenergic receptors.

THE ADRENERGIC blocking properties of certain substituted amines have been reported by a number of investigators. In 1953, Tonk (1) studied the pharmacology of a series of ethylenediamines that were structurally related to mepyramine. He reported that the *N*-ethyl-*N*-2-naphthyl-*N*-dimethyl derivatives exhibited antiepinephrine activity. Graham and Tonk (2) investigated a number of compounds structurally related to Antergan and reported that these compounds, *N*-benzyl (or ethyl)-*N*-1 (or 2) naphthyl-*N*:*N*-dimethyl (or diethyl) ethylenediamines, possessed antihistaminic and antiepinephrine properties. Halliday *et al.* (3) evaluated the hypotensive activity of several substituted polymethylene diamines in rats and dogs. The most promising compound of this series, JB 5058, *N,N'* - bis - [α - (2 - pyridyl)ethyl] - ethylenediamine dimaleate, produced marked hypotensive effects of long duration in both species of animals. JB 5058 reversed the pressor response to epinephrine and decreased pressor responses to norepinephrine, angiotensin II, and bilateral carotid occlusion. The data suggested that the primary mechanism of the hypotensive activity of JB 5058 was competitive blockade of the α-adrenergic receptors.

This report is concerned mainly with the hypotensive activity of EX 4962, *N,N'*-bis (α-2-pyridylethyl) - 1,2 - propanediamine difumarate[1] and EX 10,074, *N,N'*-bis (α-2-pyridylethyl)-2,3-butanediamine difumarate,[1] both structurally related to JB 5058 and both of which exhibited marked adrenergic blocking properties. (See Fig. 1.)

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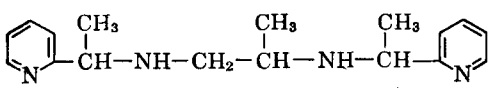
[1] Kindly supplied by Lakeside Laboratories, Inc., Milwaukee, Wis.

EXPERIMENTAL

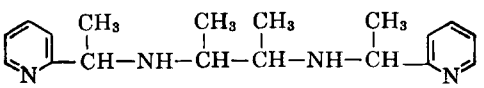
Hypotensive Activity in Normotensive Rats.—EX 4962 and EX 10,074 were screened for hypotensive activity in normotensive Wistar rats anesthetized with 1.2 Gm./Kg. i.p. of urethan, as described by Bickerton *et al.* (4). Blood pressure was recorded from a cannulated carotid artery. Both of the compounds were administered in an aqueous solution *via* a femoral vein in doses ranging from 1 to 40 mg./Kg., and the dose producing a 50% decrease in blood pressure was determined and administered to six additional animals. Each animal received only a single dose of one of the compounds.

Hypotensive Activity in Normotensive Dogs.—EX 4962 and EX 10,074 were studied further in the anesthetized dog. Mongrel dogs were anesthetized with sodium pentobarbital, 35 mg./Kg. i.v., and the blood pressure recorded from a cannulated



·2 HCCOOH ‖ HOOCHC

N,N'-Bis (α-2-pyridylethyl)-1,2-propanediamine difumarate



·2 HCCOOH ‖ HOOCHC

N,N'-Bis (α-2-pyridylethyl)-2,3-butanediamine difumarate

Fig. 1.—EX 4962 (top) and EX 10,074 (bottom).

TABLE I.—EFFECTS OF EX 4962 AND EX 10,074 ON THE BLOOD PRESSURE AND VASCULAR RESPONSES IN NORMOTENSIVE ANESTHETIZED MONGREL DOGS

Compd.	Sex	Wt., Kg.	Dose, mg./Kg.	Original Blood Pressure, mm. Hg	Decrease in Blood Pressure, %	Duration, min.	% of Control Responses			
							BCO[a]	Epi[b]	NEpi[c]	Ang[d]
EX 4962	M	9.4	2.5	130	40	100+	0	87	91	116
EX 4962	F	7.7	5.0	116	51	120+	0	87	90	138
EX 4962	M	9.4	5.0	126	57	240+	0	92	100	71
EX 10,074	F	9.5	5.0	164	26	100+	0	80	90	87
EX 10,074	F	9.2	10.0	100	36	240+	0	85	74	90
EX 10,074	M	7.2	10.0	124	39	120+	0	96	83	103

[a] BCO, bilateral carotid occlusion. [b] Epi, epinephrine, 1 mcg./Kg. [c] NEpi, norepinephrine, 1 mcg./Kg. [d] Ang, angiotensin II, 1 mcg./Kg.

TABLE II.—EFFECTS OF EX 4962 AND EX 10,074 ON THE PRESSOR RESPONSE TO AMPHETAMINE SULFATE, 0.5 mg./Kg. i.v., IN ANESTHETIZED DOGS

Compd.	Sex	Wt., Kg.	Dose, mg./Kg.	Original Blood Pressure, mm. Hg	Blood Pressure after Amphetamine, mm. Hg	Blood Pressure after Exptl. Compd., mm. Hg	Duration, min.
EX 4962	F	5.2	5	114	190	100	10
EX 4962	F	11.2	5	146	220	138	8
EX 10,074	M	9.0	10	156	224	126	12
EX 10,074	F	9.4	10	158	270	128	25

TABLE III.—EFFECTS OF EX 4962 AND EX 10,074 ON THE CAT NICTITATING MEMBRANE PREPARATION

Compd.	Sex	Wt., Kg.	Dose, mg./Kg.	Original Blood Pressure, mm. Hg	Decrease in Blood Pressure, %	Duration, min.	Control Epi[a] Response, %		% of Control N.M. Response		Duration, min.
							B.P.	N.M.[b]	Pre-G[c]	Post-G[d]	
EX 4962	F	2.0	5.0	120	37	150+	100	50	29	45	150+
EX 4962	F	2.4	10.0	165	36	150+	75	40	80	57	150+
EX 10,074	F	2.7	2.5	190	21	75+	50	0	50	63	75+
EX 10,074	F	2.5	5.0	175	31	100+	108	85	53	82	100+
EX 10,074	M	1.5	10.0	120	25	170+	...	..	0	0	170+

[a] Epi, epinephrine, 1 mcg./Kg. [b] N.M., nictitating membrane. [c] Pre-G, preganglionic nerve fibers. [d] Post-G, postganglionic nerve fibers.

TABLE IV.—EFFECTS OF EX 4962 AND EX 10,074 ON THE CARDIAC OUTPUT OF ANESTHETIZED DOGS

Compd.	Sex	Wt., Kg.	Dose, mg./Kg.	Original Blood Pressure, mm. Hg	Decrease in Blood Pressure, %	Duration, min.	Predrug Cardiac Output, ml./min.	% of Predrug Cardiac Output	Duration, min.
EX 4962	F	15.3	5	110/65	18/23	150+	630	135	2
EX 4962	F	14.1	5	110/55	18/9	150+	675	120	2
EX 4962	F	13.6	5	135/55	25/33	150+	1456	117	3
EX 10,074	M	11.6	5	110/50	31/20	100+	1665	68	100+
EX 10,074	M	13.8	10	130/65	23/7	150+	1485	61	150+

TABLE V.—EFFECTS OF EX 4962 AND EX 10,074 ON THE FEMORAL BLOOD FLOW OF ANESTHETIZED DOGS

Compd.	Sex	Wt., Kg.	Dose, mg./Kg.	Original Blood Pressure, mm. Hg	Decrease in Blood Pressure, %	Duration, min.	Predrug Femoral Flow, ml./min.	% of Predrug Femoral Flow	Duration, min.	Vascular Resistance Units[a]	
										Predrug	Postdrug
EX 4962	M	13.0	2.5	200/125	22/20	180+	12	150	150	12.50	6.38
EX 4962	F	8.6	2.5	185/110	29/31	150+	20	135	5	6.75	3.55
EX 4962	F	14.3	5.0	150/75	46/60	100+	22	145	22	4.54	1.50
EX 10,074	M	11.4	10.0	175/125	23/24	140+	14	285	95	10.14	2.82
EX 10,074	M	10.6	10.0	135/100	25/35	150+	30	153	130	3.73	1.78

[a] Vascular resistance units = $\frac{\text{mean blood pressure (mm. Hg)}}{\text{femoral flow (ml./min.)}}$

femoral artery *via* a mercury manometer onto a smoked kymograph. The effects of the experimental compounds on the pressor activity of the following challengers were: epinephrine, 1 mcg./Kg.; norepinephrine, 1 mcg./Kg.; 15 sec. bilateral carotid occulsion; and synthetic angiotensin, 1 mcg./Kg. In several experiments, amphetamine sulfate, 0.5 mg./Kg., was used also as a cardiovascular challenge. All of the compounds were administered *via* a femoral vein.

Cat Nictitating Membrane Preparation.—Cats were anesthetized with sodium pentobarbital, 35 mg./Kg. i.p. Blood pressure was recorded on a Grass polygraph from a cannulated femoral artery *via* a Statham transducer, and contractions of the nictitating membrane were recorded using a force displacement transducer (FT03). Both pre- and postganglionic fibers of the superior cervical ganglion innervating the nictitating membrane were isolated, and electrical stimulation was performed using a Grass square wave stimulator and platinum electrodes. Control membrane contractions to a submaximal stimulation of both pre- and postganglionic nerve fibers and to an intravenous injection of 1 mcg./Kg. of epinephrine hydrochloride were recorded. EX 4962 and EX 10,074 were administered intravenously *via* a femoral vein, and the pre- and postganglionic nerve stimulations and the epinephrine dosage were repeated.

Cardiac Output and Femoral Blood Flow.—Mongrel dogs were anesthetized and prepared for the recording of arterial blood pressure, as previously described. Cardiac output was measured by means of an electromagnetic flowmeter (Medicon FM-6), as described by Olmsted (5). A 12- or 14-mm. electromagnetic flo-probe was placed around the ascending aorta of a dog maintained by artificial respiration. Following probe placement, the chest was closed, and respiration was unaided during the remainder of the experiment. Femoral flow was similarly recorded by placing a 2-mm. probe around an isolated femoral artery. All measurements were recorded on a Grass polygraph. The experimental compounds were administered intravenously.

Denervated Hindlimb of the Dog.—The effects of EX 4962 and EX 10,074 on peripheral vasculature were studied on the denervated perfused hindlimb of the dog. Mongrel dogs were anesthetized with sodium pentobarbital, 35 mg./Kg. i.v., and a femoral artery was isolated for the recording of blood pressure. The contralateral limb was denervated by severing the femoral and sciatic nerve trunks and vascularly isolated by clamping the muscles with stainless steel wire and a Schiffrin wire tightener. The femoral artery of the denervated leg was cannulated, and the limb was perfused with blood from the reservoir maintained at 37°. Reservoir volume was maintained by blood from a carotid artery. A Sigmamotor pump was utilized to perfuse the leg at a constant rate of flow. Perfusion pressure was measured between the pump and isolated limb by a Statham transducer. The use of the reservoir in this experiment eliminated the influence of cardiac output on the perfusion pressure. Thus, changes in perfusion pressure could be related to changes in the vascular resistance. In some experiments, a donor dog was used instead of a blood reservoir, and perfusion flow instead of perfusion pressure was measured using a Shipley-Wilson flowmeter. EX 4962 and EX 10,074 were administered intra-arterially into the leg; physiological measurements were recorded on a Grass polygraph.

RESULTS

Hypotensive Activity in Normotensive Rats.—EX 10,074 and EX 4962 produced prolonged hypotensive effects in all doses tested. EX 10,074, 20 mg./Kg. i.v., produced a mean decrease in blood pressure of 29.5 ± 3.9% (S.D.) (N = 6), with a duration of action of over 3.5 hr. EX 4962, 10 mg./Kg. i.v., produced a mean decrease in blood pressure of 48 ± 4.29% (S.D.) (N = 6), which did not return to predrug levels after 3.5 hr.

Hypotensive Activity in Normotensive Dogs.—The results are summarized in Tables I and II. EX 4962 and EX 10,074 exhibited significant hypotensive activity of relatively long duration in all doses tested. Both compounds completely blocked the pressor response to bilateral carotid occlusion and slightly decreased the pressor responses to epinephrine and norepinephrine. They produced variable effects on the pressor response to angiotensin II. When the compounds were administered at the height of the amphetamine pressor response, EX 4962, 5 mg./Kg. i.v., and EX 10,074, 10 mg./Kg. i.v., abolished the pressor response of amphetamine sulfate, 0.5 mg./Kg. i.v., and the blood pressure was depressed to subnormal levels for short duration of 10 to 25 min. Subsequent doses of amphetamine failed to produce significant pressor response. The hypotensive activity of EX 10,074 was not affected by pretreatment of the animals with dichloroisoproterenol or atropine sulfate.

Cat Nictitating Membrane Preparation.—EX 4962 and EX 10,074 decreased the response of the nictitating membrane to injected epinephrine and to both preganglionic and postganglionic electrical stimulation (Table III). A dose of 10 mg./Kg. i.v. of EX 10,074 in one animal completely inhibited the response of the nictitating membrane to electrical stimulation of both nerve trunks. Both EX 4962 and EX 10,074 appeared to be more effective in blocking the epinephrine response on the nictitating membrane than the epinephrine pressor response. The duration of the blocking activity of these compounds appeared to be as long as the duration of their activity on blood pressure.

Cardiac Output and Femoral Blood Flow.—The effects of EX 4962 and EX 10,074 on cardiac output differed significantly. EX 10,074 markedly reduced cardiac output in doses of 5 and 10 mg./Kg. i.v., with the duration of this activity lasting as long as the duration of hypotensive effects. Cardiac output was transiently increased immediately after the administration of EX 4962, then returned to predrug levels within 2 to 3 min. Thus, cardiac output was an important factor in the mechanism of hypotensive activity of EX 10,074 but not in that of EX 4962. Both of the compounds markedly increased femoral blood flow, indicating a decrease in vascular resistance in femoral beds following the administration of the drug (Tables IV and V).

Denervated Hindlimb of the Dog.—The results of the isolated denervated perfused hindlimb experiments confirmed the ability of EX 4962 and EX 10,074 to lower the peripheral vascular resistance

in the doses that reduced blood pressure. EX 4962, 5 mg./Kg., increased flow to the hindlimb 258 and 184%, respectively, in two dogs, with a duration of action of 30 min. EX 10,074 decreased perfusion pressure 58 and 61%, respectively, in two additional dogs, with a duration of action of 35 min. The results suggested that the primary mechanism of action of these compounds lies in their ability to reduce peripheral vascular resistance.

DISCUSSION

Two substituted diamines, EX 10,074 and EX 4962, produced marked hypotensive effects in both rats and dogs. While EX 10,074 was able to reduce cardiac output markedly, no such effect was produced by EX 4962. Both of the compounds were capable of markedly reducing peripheral vascular resistance, indicated by an increase in femoral blood flow or a decrease in perfusion pressure in denervated hindlimb preparations. Thus, while the resultant hypotension produced after the administration of EX 10,074 was the algebraic summation of decrease in cardiac output and a decrease in peripheral vascular resistance, the hypotension produced after the administration of EX 4962 appeared to be due mainly to a decrease in peripheral vascular resistance.

The decrease in peripheral resistance facilitated by these compounds could be due to their inherent ability to dilate blood vessels directly, to their capacity to inhibit the activity of endogenously released catecholamines on blood vessels, or to the combination of both of these effects.

Burn and Rand (6) postulated that amphetamine produces its pressor effect by the release of catecholamines in experimental animals, and this was confirmed further by Day and Rand (7). Both EX 4962 and EX 10,074 were able to abolish the pressor response of amphetamine in dogs, thus suggesting the ability of the compounds to antagonize the activity of endogenously released catecholamines. Also they decreased slightly the pressor responses of injected catecholamines in dogs and reduced responses of the nictitating membrane to electrical stimulation of pre- and postganglionic fibers and also to exogenous epinephrine.

The results of this investigation, which included the inhibition of pressor responses to bilateral carotid occlusion, a decrease in nictitating membrane responses, and a blockade of the activity of endogenously released catecholamines, suggest that the compounds EX 4962 and EX 10,074 possess some adrenergic blocking properties and decrease blood pressure by possibly blocking the activity of the endogenously released catecholamines on α adrenergic receptors (8).

SUMMARY

Two substituted diamines, *N,N'*-bis (α-2-pyridylethyl)-1,2-propanediamine difumarate (EX 4962) and *N,N'*-bis (α-2-pyridylethyl)-2,3-butanediamine difumarate (EX 10,074), were evaluated for their hypotensive activity. Both of the compounds produced marked hypotensive effects of long duration when administered intravenously in anesthetized rats and dogs. They abolished the pressor response to bilateral carotid occlusion, slightly reduced the pressor response to injected catecholamines, and abolished the pressor response to amphetamine sulfate. Both of the compounds decreased the response of the cat nictitating membrane to both pre- and postganglionic stimulation and to injected epinephrine. Peripheral vasodilatory activity of the compounds was indicated by an increase in blood flow in the perfused denervated hindlimb of the dog, following intra-arterial administration. These results suggested that the compounds possessed some adrenergic blocking properties and that their hypotensive activity was due possibly to the blockade of the activity of endogenously released catecholamines on α adrenergic receptors. In addition, the data also suggest the possibility that the compounds directly depress vascular smooth musculature.

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