Cardiovascular Activity of Mecamylamine, Pempidine, and Several Pempidine Analogs

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The cardiovascular activities of mecamylamine, pempidine, and several pempidine analogs were investigated. The active compounds produced a significant drop in blood pressure and cardiac output in anesthetized normotensive dogs, markedly inhibited superior cervical ganglionic transmission in the cat, and produced a slight transient decrease in vascular resistance when administered intraarterially into the denervated perfused hind limb of the dog. Pempidine and *N*-amino-*N*-[β-(2,2,6,6-tetramethylpiperidino)ethyl]guanidine sulfate (EX 4510) exhibited quantitatively and qualitatively similar hypotensive responses when administered orally to unanesthetized renal hypertensive dogs. The hypotensive activities of hexamethonium, pentolinium, chlorisondamine, mecamylamine, and pempidine were evaluated in the prostigminized rat. Prostigmine antagonized the hypotensive response of only hexamethonium, suggesting that hexamethonium alone produces a competitive type of antagonism at the autonomic ganglia.

THE HYPOTENSIVE activities of pempidine (1,2,2,6,6-pentamethylpiperidine) and the pempidine analogs, *N*-amino-*N*-[β-(2,2,6,6-tetramethylpiperidino)ethyl]guanidine sulfate (EX 4510), 1,1-bis-[β-(2,2,6,6-tetramethylpiperidino)-ethyl] hydrazine trihydrochloride (EX 4513), 1 - (2 - hydrazinoethyl) - 2,2,6,6 - tetramethylpiperidine dihydrochloride (EX 4531), and 4-ethoxycarbonylhydrazino - 2,2,6,6 - tetramethylpiperidine dihydrochloride (EX 4601), in anesthetized normotensive rats and dogs were reported previously by Buckley *et al.* (1). The preliminary data suggested that the active compounds, like pempidine, are ganglionic blocking agents. This report is concerned with the evaluation of certain cardiovascular effects of mecamylamine, pempidine, and the previously mentioned pempidine analogs along with an investigation of the mechanism of ganglionic blockade produced by quaternary, tertiary, and secondary amines.

EXPERIMENTAL

Ganglionic Blockade in the Cat Nictitating Membrane Preparation.—The compounds were evaluated for ganglionic blocking activity in the cat nictitating membrane–superior cervical ganglion preparation. Cats of both sexes were anesthetized with a pentobarbital–urethan mixture (1.0 ml./Kg. i.p. of 30 mg. pentobarbital and 250 mg. urethan per milliliter). The blood pressure was obtained by direct cannulation of a femoral artery and recorded on a Grass polygraph. Fresh aqueous solutions of the compounds were administered intravenously *via* a femoral vein. The nictitating membrane contractions were measured with a force-displacement transducer (Grass FT03). The pre- and postganglionic fibers were stimulated submaximally, using a Grass stimulator (model SD5), 60-cycle frequency with a duration of 10 sec.

Cardiac Output in Dogs.—The electromagnetic flowmeter (Medicon FM-6) described by Olmsted (2) was utilized to determine cardiac outputs in anesthetized normotensive dogs. Mongrel dogs were anesthetized with sodium pentobarbital (35 mg./Kg. i.v.) and the blood pressure recorded from a femoral artery and cardiac output *via* a 12- or 14-mm. probe placed around the ascending aorta. Fresh aqueous solutions of the compounds were administered into an isolated saphenous vein and the physiological responses recorded on a polygraph.

Hypotensive Activity in Renal Hypertensive Dogs.—Renal hypertensive dogs were prepared according to the method of Grollman (3). Blood pressure determinations were obtained utilizing the method of Prioli and Winbury (4). Control blood pressure and heart rate levels were determined daily, at eight 1-hr. intervals, for a period of 1 week. Two hypertensive dogs were utilized in this study, one being subjected to a cross-over study. Both animals were fasted at least 12 hr. prior to the oral administration of the drug.

Hypotensive Activity in Prostigminized Rats.—Normotensive Wistar rats were prepared for direct blood pressure measurements from a carotid artery as previously described (1). After the blood pressure stabilized, the test rats were given an intravenous dose of prostigmine (0.1 mg./Kg.). After allowing the blood pressure to stabilize again, each prostigminized rat received an intravenous dose of a ganglionic blocking compound. The hypotensive responses in the prostigminized rats were compared to the effects produced in untreated anesthetized rats.

Isolated Perfused Guinea Pig Heart.—The method of Langendorff (5) was utilized to determine the effects of the experimental compounds on coronary blood flow and myocardial contractions. Any changes in the caliber of the coronary vessels were reflected in the rate of drainage of the perfusion fluid. Changes in the amplitude of cardiac contractions reflected changes in the force of myocardial

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TABLE I.—EFFECTS OF THE INTRAVENOUS ADMINISTRATION OF CERTAIN HYPOTENSIVE COMPOUNDS ON THE CAT NICTITATING MEMBRANE PREPARATION

Compd.	Dose, mg./Kg.	Predrug Mean Blood Pressure, mm. Hg	Drop in Blood Pressure, %	Response of Nictitating Membrane to Preganglionic Stimulation		Ganglionic Blockade, %
				Predrug, mm.	Postdrug, mm.	
Pempidine	2.5	90	50	16	4	75
Pempidine	2.5	90	44	20	8	60
EX 4510	2.5	150	67	16	7	56
EX 4510	2.5	130	54	12	4	67
EX 4513	10	120	38	26	17	35
EX 4513	10	130	19	23	15	35
EX 4531	10	150	60	21	14	33
EX 4531	10	130	50	32	15	53
EX 4568	10	160	44	35	5	86
EX 4568	10	135	33	23	10	57
EX 4601	20	150	43	29	13	56
EX 4601	20	140	46	26	14	46

TABLE II.—EFFECTS OF THE INTRAVENOUS ADMINISTRATION OF MECAMYLAMINE, PEMPIDINE, AND EX 4510 (5 mg./Kg.) ON THE CARDIAC OUTPUT OF ANESTHETIZED NORMOTENSIVE DOGS

Compd.	Predrug Mean Blood Pressure, mm. Hg	Postdrug Mean Blood Pressure, mm. Hg	Predrug Cardiac Output, ml./min.	Postdrug Cardiac Output, ml./min.	Drop in Cardiac Output, %
Pempidine	90	70	1144	676	50
Pempidine	110	65	860	650	24
Mecamylamine	130	100	1092	728	33
Mecamylamine	120	80	960	720	24
EX 4510	125	80	775	450	43
EX 4510	140	70	1300	728	44

contractions. Guinea pigs were sacrificed by cervical dislocation and the heart removed from the thoracic cavity. The aorta was cannulated and immediately perfused with oxygenated Locke's solution (38°). A thread was attached to the apex of the heart with a pin hook and connected through a system of pulleys to an isotonic writing lever. Heart contractions were recorded on a slowly moving smoked kymograph. All drug doses were administered in volumes of 0.5 ml. and were injected into the perfusion fluid just prior to the entry of the fluid into the heart. The milligram dosage per 0.5 ml. of perfusion fluid was based on the effective dose of the compound in rats and dogs, the concentration of the compound being estimated from the average plasma volume of the guinea pig.

Denervated Perfused Hind Limb of the Dog.—The method described by Clonninger and Green (6) was utilized. Mongrel dogs were anesthetized with sodium pentobarbital (35 mg./Kg. i.v.) and the femoral artery isolated and cannulated for the recording of blood pressure. The contralateral limb was denervated by severing the femoral and sciatic nerves. The femoral artery in the denervated limb was isolated and the distal portion cannulated with polyethylene tubing. Sodium heparin (1000 U.S.P. units/Kg. i.v.) was administered to prevent coagulation. A circulatory circuit was established between the cannulated femoral artery and a carotid artery, using polyethylene tubing. Similarly, venous return was shunted to the ipsilateral jugular vein. The limb was vascularly isolated from the body by use of a stainless steel wire tourniquet. A Sigmamotor pump was interposed within the carotid-femoral circuit to maintain a constant perfusion pressure. The initial perfusion pressure was adjusted to the approximate level of the systemic blood pressure. A T-tube was placed between the pump outflow and the perfused limb and the pressure monitored *via* a Statham pressure transducer. Any changes in this pressure reading reflected either vasodilation or vasoconstriction. The test compounds were injected into the arterial inflow to the leg and changes in perfusion pressure recorded.

Dog Cross-Circulation Preparation.—The cross-circulation technique, described by Bickerton and Buckley (7), was utilized to determine any possible centrally mediated hypotensive responses. The donor and recipient animals were anesthetized with sodium pentobarbital (35 mg./Kg. i.v.). The circulatory system of the recipient was occluded at the level of C-3 and C-4, producing a vascularly isolated neurally intact head. The blood pressures were recorded *via* a Statham pressure transducer (No. P23AC), utilizing a Grass polygraph (model 5). A common circulation was established between the head of the recipient dog and the general circulation of the donor dog using polyethylene tubing. The test compounds were administered *via* the carotid inflow to the recipient's head, and the effects on recipient and donor femoral blood pressure were recorded.

Vagotomized Pithed Cat Preparation.—Cats were anesthetized with sodium pentobarbital (35 mg./Kg. i.p.). The trachea was cannulated and the animal placed on artificial respiration. Both vagi were cut and a pithing rod inserted between the atlanto-occipital articulation and forced down the length of the spinal column. Blood pressure was recorded from a femoral artery, and the experi-

mental compounds were administered *via* a femoral vein.

Isolated Dog Mesenteric Arterial Strip Preparation.—The method described by Waugh (8) was utilized to determine the effects of the experimental compounds on the vasoconstricting activity of norepinephrine. A 2-cm. length of an intramesenteric branch of the superior mesenteric artery of a dog was cannulated with a 23-gauge stainless steel T-cannula and perfused with oxygenated Tyrode's solution (38°) delivered at a constant rate by means of a Sigmamotor pump. A Statham pressure transducer was attached to the side arm of the T-cannula, and the perfusion pressure was recorded on a Grass polygraph. The vasoconstriction produced by norepinephrine alone and following perfusion with the test compounds was compared by determining alterations in perfusion pressure.

TABLE III.—EFFECTS OF PEMPIDINE AND EX 4510 (5 mg./Kg.) *per os* ON THE BLOOD PRESSURE OF ANESTHETIZED RENAL HYPERTENSIVE DOGS

Controls	Time, hr.	Systolic Pressure, mm.Hg	Heart Rate, min.
Dog *1*, 10.2 Kg., F			
Systolic Blood pressure: 180 ± 9.5[c] mm. Hg	Predrug EX 4510	175	105
Heart Rate: 110 ± 11.0 beats/min.	0.50	155	180
	1.50	130[a]	135
	3.00	130	125
	12.50	140	130
	23.50	155	140
	24.50	175	125
Dog *2*, 12.1 Kg., M			
Systolic blood pressure: 194 ± 4.9 mm. Hg	Predrug Pempidine	195	115
Heart rate: 127 ± 10.0 beats/min.	0.50	180	175
	1.00	130[a]	160
	3.00	145	135
	11.50	130	140
	25.00	185	125
	Predrug[b] EX 4510	200	130
	0.50	175	205
	1.75	145[a]	145
	3.75	155	125
	10.75	155	115
	21.75	190	120

[a] Maximal change. [b] Animal permitted a 3-day drug-free period between dosages. [c] Deviations of the means are expressed as ± standard deviation.

RESULTS

Ganglionic Blockade in the Cat Nictitating Membrane Preparation.—The ganglionic blocking action of the compounds studied in the current investigation are summarized in Table I. All the experimental compounds were effective in inhibiting transmission across the superior cervical ganglion. No significant changes were noted in postganglionic fiber transmission.

Cardiac Output in Dogs.—The effect of the experimental compounds on the cardiac output in normotensive anesthesized dogs is summarized in Table II. All three compounds tested produced a significant decrease in cardiac output, suggesting that this is a major mechanism in the production of the hypotensive response.

Hypotensive Activity in Renal Hypertensive Dogs.—The effects of pempidine and EX 4510 on the blood pressure of unanesthetized renal hypertensive dogs are summarized in Table III. Both compounds produced similar quantitative hypotensive responses. Both compounds were rapidly absorbed, the onset of drug action occurring within 15 min. and the maximal hypotensive response occurring within 1 to 1.5 hr. Tachycardia was evident with both compounds; however, this increase in heart rate persisted for only 2 to 3 hr., whereas the hypotensive activity was evident in excess of 12 hr.

Hypotensive Activity in Prostigminized Rats.—The effects of the six ganglionic blocking agents on prostigminized rats are summarized in Table IV. The hypotensive response to hexamethonium in the control rats was almost completely blocked by prostigmine, which was not the case with pentolinium and chlorisondamine. This suggests a difference in the mechanism of action of hexamethonium and the two other potent ganglionic blockers, pentolinium and chlorisondamine. The hypotensive activities of mecamylamine, pempidine, and EX 4510 were antagonized partially by prostigmine.

Isolated Perfused Guinea Pig Heart.—None of the experimental compounds significantly altered the rate of coronary flow; however, the effects on myocardial contractility varied. Pempidine and EX 4601 produced a slight increase; EX 4510 and EX 4531 produced a slight decrease in the amplitude of myocardial contractions.

Denervated Perfused Hind Limb of the Dog.—Mecamylamine, pempidine, and EX 4510, in doses of 5 mg./Kg. intraarterially, to the denervated hind limb produced slight transient decreases in perfusion pressure, with pempidine being the most effective and EX 4510 the least effective.

TABLE IV.—EFFECTS OF PROSTIGMINE METHYLSULFATE ON THE HYPOTENSIVE RESPONSES PRODUCED IN ANESTHETIZED NORMOTENSIVE RATS BY QUATERNARY, TERTIARY, AND SECONDARY AMINES

	Controls		Prostigminized[a]		
Compd.	Dose, mg./Kg. i.v.	Drop in Mean Blood Pressure,[b] mm. Hg $\bar{x} \pm$ S. D.	Dose, mg./Kg. i.v.	Drop in Mean Blood Pressure,[b] mm. Hg $\bar{x} \pm$ S. D.	P^c
Hexamethonium	5.0	37.9 ± 8.2	5.0	2.03 ± 2.05	<0.001
Chlorisondamine	2.5	37.8 ± 15.1	2.5	42.4 ± 14.5	>0.95
Pentolinium	5.0	29.7 ± 12.5	5.0	29.8 ± 7.4	>0.95
Pempidine	5.0	35.1 ± 10.5	5.0	25.7 ± 8.7	<0.05
EX 4510	5.0	42.5 ± 7.8	5.0	30.8 ± 7.3	<0.01
Mecamylamine	5.0	36.7 ± 7.5	5.0	24.5 ± 4.7	<0.01

[a] Dose = 0.1 mg./Kg. i.v. [b] $N = 8$. [c] = Significance determined by subjecting data to Student t test.

Dog Cross-Circulation Preparation.—Administration of the experimental compounds *via* the carotid inflow to the neurally intact vascularly isolated recipient's head in dog cross-circulation studies, in doses ranging from 5 to 10 mg./Kg. (calculated on the donor's weight), produced hypotensive effects in the donor animal only. The data indicated that none of the compounds produced centrally mediated hypotensive activity.

Vagotomized Pithed Cat Preparation.—The experimental compounds in doses of 5 to 40 mg./Kg. i.v. failed to alter the blood pressure of pithed vagotomized cats.

Isolated Dog Mesenteric Arterial Strip Preparation.—Pempidine, mecamylamine, and EX 4510, in doses of 0.1 mg., did not affect the vasoconstrictive activity of *l*-norepinephrine.

DISCUSSION

A comparison of the hypotensive activity of pempidine and several of its analogs in the rat and dog, previously reported by Buckley *et al.* (1), to that in the cat shows evidence that the cat is more sensitive to ganglionic blockade produced by mecamylamine and pempidine, since the effective dose in the cat was only 50% of the rat or dog dose. In addition, EX 4513, which was relatively inactive in the rat and dog, showed a significant potency in the cat; and the compound exhibited a duration of action comparable to the other long-acting compounds.

The effects of the experimental compounds on the cat nictitating membrane preparation suggested that the main mechanism of action of the compounds is ganglionic blockade. All of the compounds markedly inhibited the response of the nictitating membrane to preganglionic response without interfering with the response to postganglionic stimulation.

Mecamylamine, pempidine, and EX 4510 produced a significant decrease in cardiac output of anesthetized normotensive dogs. This hemodynamic effect appears to be a major factor responsible for the hypotensive effect produced by these ganglionic blocking agents. The mechanism(s) involved in the production of the decreased cardiac output is controversial. Zimmerman (9) states that ganglionic blocking agents reduce cardiac output primarily by blocking tonic sympathetic activity to the heart. His view is challenged by Trapold (10) and Rowe (11), who conclude that ganglionic blocking agents reduce cardiac output primarily by reducing venous return due to peripheral pooling of blood.

The vagotomized pithed cat preparation demonstrated that, aside from ganglionic blockade, no apparent peripheral component was involved in the production of the hypotensive response to pempidine and several of its analogs. Although the perfused isolated guinea pig heart preparation indicated variable inotropic effects and the perfused denervated hind limb preparation indicated a slight vasodilatory action, none of these actions were evident in the vagotomized pithed cat preparation. The transient vasodilatory response observed in the perfused denervated hind limb preparation could be attributed to the heavy concentration of drug reaching the site upon intraarterial injection; however, once the drug became distributed throughout the general circulation, the direct vasodilatory action was no longer evident.

The comparison of the responses to hexamethonium, pentolinium, chlorisondamine, mecamylamine, pempidine, and EX 4510 in control and prostigminized rats suggests that hexamethonium alone acts principally by competitive antagonism of acetylcholine at the ganglionic site. The hypotensive response to hexamethonium was blocked in the prostigminized rats, which was not so in the case of pentolinium and chlorisondamine. Since the hypotensive response to pempidine, EX 4510, and mecamylamine was partially antagonized by prostigmine, this may indicate that these ganglionic blocking agents have a dual mechanism of action, one of which is competitive antagonism of acetylcholine.

SUMMARY

1. The hypotensive activities of mecamylamine, pempidine, *N*-amino-*N*-[β-(2,2,6,6-tetramethylpiperidino)ethyl]guanidine sulfate (EX 4510), 1,1-bis-[β-(2,2,6,6-tetramethylpiperidino)ethyl] hydrazine trihydrochloride (EX 4513), and 1-(2-hydrazinoethyl)-2,2,6,6-tetramethylpiperidine dihydrochloride (EX 4531) in the cat were compared to the activities in the rat and dog. The results showed that the cat was more sensitive to these ganglionic blocking agents.

2. All the compounds were effective in inhibiting superior cervical ganglionic transmission. No changes were noted in postganglionic fiber transmission.

3. Pempidine and the several analogs investigated showed variable inotropic effects on the perfused isolated guinea pig heart. The compounds did not significantly alter the rate of coronary flow.

4. Pempidine and the several analogs investigated were devoid of any centrally mediated hypotensive effects in the dog cross-circulation preparation.

5. Pempidine and the several analogs investigated failed to produce hypotensive effects in the vagotomized pithed cat.

6. Mecamylamine, pempidine, and EX 4510 produced a significant decrease in the cardiac output of anesthetized normotensive dogs.

7. Pempidine and EX 4510 exhibited similar hypotensive responses in the unanesthetized renal hypertensive dog. The hypotensive responses were accompanied by marked tachycardia.

8. Prostigmine blocked the hypotensive response to hexamethonium in the rat; the response to pentolinium and chlorisondamine was not blocked. Prostigmine partially blocked the hypotensive responses to mecamylamine, pempidine, and EX 4510.

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