Investigation of the Cardiovascular Response of the Dog to 1-Phenyl-2-hydrazinopropane

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Results of a further attempt to determine a basis for the qualitative difference in blood pressure response of the human and the dog to 1-phenyl-2-hydrazinopropane are reported. Elimination of arterial pressure level as a factor influencing the circulatory response of the dog to this compound removes the last possibility for a circumstantial basis for the difference. Mechanism studies indicate that the canine pressor response is a neurotropic, sympathetic effect mediated, for the most part, as a direct action on myoneural junction receptor sites with a lesser component of indirect action on these sites related to MAO inhibition or the peripheral release of catecholamines from blood vessel walls. Evidence presented by certain clinical investigators points to either a ganglionic or an adrenergic block as responsible for the human antihypertensive effect.

WHILE 1-phenyl-2-hydrazinopropane[1] has been reported to produce marked orthostatic hypotension in humans with elevated blood pressure, it would appear to elicit only an inconstant and relatively weak depressor response in the supine hypertensive (1, 2). Certain clinical studies have indicated that this hydrazine analog of amphetamine exhibits only a slight hypotensor activity in normotensive individuals. These findings are in sharp disagreement with the pressor activity demonstrated in both pentobarbital-anesthetized and conscious dogs by Groves (3) and in the present work with conscious dogs in the orthostatic position.

The goal of this and a previous investigation was the establishment of a basis for the apparent species difference in the responses of the human and the dog to 1-phenyl-2-hydrazinopropane. Realization of this goal was attempted first through the stepwise elimination of species differences pertaining to the conditions surrounding the administration of the drug on the one hand, and the measurement of blood pressures on the other. A second attempt at goal achievement involved a study of the mechanism of the hypertensive response of the dog to this compound. Information provided in the latter study together with that relating to the mechanism of the hypotensive response of the human to this drug might provide the explanation for a true species difference in response.

EXPERIMENTAL

Methods.—Since the work of Groves served to eliminate route of administration, anesthesia and recording position of the individual as bases for the difference in the response of the human and the dog to 1-phenyl-2-hydrazinopropane, the first concern of this work was to examine the influence of blood pressure level on the canine response to this compound. Accordingly, Page's method was employed for the production of chronic renal hypertension in dogs (4). Work with 1-phenyl-2-hydrazinopropane was begun only after significant sustained elevation in blood pressure was observed, usually about 4 to 5 weeks after the encapsulation of the kidneys.

Blood pressure recordings were obtained in these renal hypertensive animals both in the conscious and pentobarbital-anesthetized state by means of a femoral artery puncture. In conscious dogs the puncture area was previously anesthetized with 2% ethyl chloride. A continuous flow of a 0.02% heparin solution in saline (5 ml./hour) from the infusion pump prevented clotting in the artery. The infusion pump was connected to a Sanborn recorder through a transducer and amplifier. Changes in respiratory rate and depth were recorded by means of a bellows-type pneumograph which was connected through another transducer and another amplifier to the same recorder.

All injections of this compound were made in the femoral vein through an 18-gauge indwelling needle which was connected by rubber tubing to a saline-filled buret. The injection volume was flushed in each time at the same rate with the same saline volume.

Results.—Prior to the actual testing of the compound in hypertensive dogs, work was carried out in normotensive animals, both conscious and pentobarbital-anesthetized (see Table I). The data obtained were qualitatively similar to but quantitatively different from comparable results appearing in the literature. The cardiac rate decrease generally took place in normotensive dogs at the beginning of blood pressure elevation rather than at or after the peak response was reached. In a few instances, a cardiac rate increase rather than a decrease followed administration of the compound, and when this occurred the pressor response was more marked than when a decrease in rate resulted. Work with atropinized dogs indicated that the cardiac rate decrease usually seen in normotensive dogs is reflex-ordered.

The peak blood pressure response in both con-

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[1] Supplied by Lakeside Laboratories.

TABLE I.—BLOOD PRESSURE EFFECTS OF 1-PHENYL-2-HYDRAZINOPROPANE IN THE NORMOTENSIVE DOG

Dog No.	Dose, i.v., mg./Kg.	No. Expt.	Av. Mean Pressure	Av. Rise Mean Pressure
		Anesthetized		
1	0.25	3	162	52
	0.50	3	161	66
	0.75	3	159	68
	1.0	2	152	96
2	0.50	2	147	92
	0.75	2	143	104
	1.0	2	160	119
		Conscious		
1	1.0	4	167	117
2	1.0	4	164	116
		Anesthetized-Phentolamine Pretreated		
1	1.0	3	135	7

scious and anesthetized normotensive dogs was attained, in every case, within $^1/_2$ to $2^1/_2$ minutes subsequent to administration of the compound. The respiratory rate was usually increased when initially low and decreased when initially high. In most instances, the depth of respiration was decreased when blood pressure reached peak response, but the effect was of short duration only.

A pressor activity, corresponding in extent and duration to that evidenced in normotensive dogs, was exhibited by 1-phenyl-2-hydrazinopropane in hypertensive dogs (see Table II). Elevation of blood pressure usually began immediately after administration of the compound to either normotensive or hypertensive dogs. In most instances a decrease in cardiac rate, beginning with blood pressure rise and continuing until return of pressure to preinjection levels, occurred in both animals. Peak pressures were reached in hypertensive dogs within the same time interval as in normotensive dogs. Respiratory changes were similar in both animals, the rate being increased if previously low and decreased if previously high, and depth being decreased fleetingly during the zenith of the blood pressure response.

Tolerance to the compound was reflected in the tachyphylaxis associated with pressor responses to repeated doses in a given test run. Also a distinct sedating effect, related in magnitude to dose, accompanied other manifestations of activity. The latter effect had a duration similar to that of the blood pressure response. Salivation occurred intermittently, and tachycardia and mydriasis were occasionally encountered following the administration of 1-phenyl-2-hydrazinopropane. Ataxia was noted in hypertensive dogs after two injections of 2.0 mg./Kg. Cardiac arrhythmias usually became more marked as the dosage level was increased. Soft stools and emesis were observed after repeated administration of the compound.

With the elimination in the portion of the work of the influence of pre-existing blood pressure as a factor in the response to 1-phenyl-2-hydrazinopropane in the dog, the last of the circumstantial bases for an apparent species difference in the response of the human and the dog to this compound was removed.

Since the mode of action involved in the pressor response to 1-phenyl-2-hydrazinopropane in the dog is not generally agreed upon by investigators, it was considered advisable to pursue this area further in an effort to uncover a mechanistic basis for the compound's species response difference. If there is a definitive difference in the pharmacology of this compound at the action level in the human and in the dog, as indicated by this and comparable clinical studies, this would, of course, account for the response difference in the two species. A number of dogs were used in this portion of the investigation, but in a given series, to avoid animal variation, the same dog was employed repeatedly.

Results of experiments by Eltherington and Horita in intact dogs demonstrate the ability of dibenzyline to block completely the pressor response to 1-phenyl-2-hydrazinopropane (5). These findings support the likelihood of a neurotropic, rather than a musculotropic, activity in the dog's pressor response to this compound. That this neurotropic activity is not concerned with a block of parasympathetic pathways is supported by other findings of these investigators in intact dogs. Previous administration of the compound to such animals was shown to cause no significant changes in the pressure responses elicited by acetylcholine chloride (10 mcg./Kg.).

The possibility that the neurotropic activity is an active proposition involving sympathetic pathways, either the ganglia or the myoneural junction, was contemplated. The appraisal of this possibility comprises the latter portion of this investigation. The postulation of ganglionic stimulation as the mechanism of action of 1-phenyl-2-hydrazinopropane was examined with the use of the ganglionic blocking agent hexamethonium. The latter was administered by intravenous injection (2 mg./Kg.) to anesthetized normotensive and conscious hypertensive dogs prior to the administration of 1 and 2 mg./Kg. doses of 1-phenyl-2-hydrazinopropane by the same route. Hexamethonium did not exert any observable effect on the pressor response of either animal to 1-phenyl-2-hydrazinopropane. Actually, the reverse was true, *i.e.*, following the

TABLE II.—BLOOD PRESSURE EFFECTS OF 1-PHENYL-2-HYDRAZINOPROPANE IN THE HYPERTENSIVE DOG

Dog No.	Dose, i.v., mg./Kg.	No. Expt.	Av. Mean Pressure	Av. Rise Mean Pressure
		Anesthetized		
3	0.1	4	174	45
	0.25	7	183	96
	0.50	4	186	114
	0.75	2	169	140
	1.0	4	172	155
4	0.25	2	177	54
	0.50	2	179	98
	0.75	3	180	103
	1.0	3	178	115
		Conscious		
3	0.1	2	197	30
	0.25	3	198	51
	0.50	5	200	77
	0.75	7	201	85
	1.0	3	186	108
4	0.50	3	198	68
	0.75	3	174	70
	1.0	3	174	68

former, the pressor response to the latter appeared enhanced. The effectiveness of the hexamethonium block was confirmed by the subsequent injection of 1-dimethyl-4-phenylpiperazine iodide (DMPP).

With ganglion stimulation thus ruled out of sympathetic involvement, a next step was to test the postulation of adrenergic receptor site stimulation. This was done by administering 25 mg./Kg. of phentolamine[2] (methanesulfonate) intravenously prior to the compound to block these receptor sites at the sympathetic myoneural junction. The administration of 1-phenyl-2-hydrazinopropane in amounts which, in the absence of phentolamine, had been effective in eliciting a marked elevation of blood pressure, failed to evoke a significant pressor response (see Table I). Confirmation of the adrenergic blockade was provided by a fall in blood pressure following intravenous administration of 4 mg. of epinephrine hydrochloride. The intravenous administration of RA-1226,[3] a compound which has been demonstrated to possess both neurotropic and musculotropic spasmogenic activity (6), was found effective in elevating canine blood pressure following doses of 1 to 25 mg./Kg. of phentolamine. This latter finding corroborates the discovery by Eltherington and Horita of the lack of a musculotropic spasmogenic component in the pharmacologic makeup of 1-phenyl-2-hydrazinopropane.

Since the previous work had thus limited the action site of the pressor response to 1-phenyl-2-hydrazinopropane in the dog to the sympathetic myoneural junction, a distinction had to be drawn among the three conceivable types of action at this point: direct stimulation of junctional receptor sites, indirect stimulation of these sites through peripheral release of catecholamines stored in the blood vessel walls (7) and monoamine oxidase (or similar enzyme) inhibition (8). It was thought that one or a combination of these types actually occurs. In an effort to determine which, 1-phenyl-2-hydrazinopropane was administered to normotensive dogs previously reserpinized (chemical sympathectomy) by the subcutaneous injection of 0.05 mg./Kg. for 5 to 7 consecutive days (see Table III). The pressor response to the compound in such animals was not so great by some 40% as in the nonreserpinized dogs, these results indicating that the major part of the compound's pressor activity is exerted by direct stimulation of adrenergic receptor sites, the remainder through either MAO inhibition or the peripheral release of catecholamines stored in blood vessel walls. Studies conducted in dogs pretreated with 15 mg./Kg. of guanethidine for periods of 24 to 48 hours prior to the administration of 1-phenyl-2-hydrazinopropane support the above results.

TABLE III.—BLOOD PRESSURE EFFECTS OF 1-PHENYL-2-HYDRAZINOPROPANE IN THE RESERPINIZED DOG

Dog No.	Dose, i.v., mg./Kg.	No. Expt.	Av. Mean Pressure	Av. Rise Mean Pressure
		Anesthetized		
1	0.25	2	118	30
	0.50	2	105	33
	0.75	3	127	43
	1.0	2	123	70
		Conscious		
2	1.0	4	146	72

DISCUSSION

The discovery in the first portion of this work that the arterial blood pressure level of the dog receiving 1-phenyl-2-hydrazinopropane does not materially affect his blood pressure response to this compound served to remove the last possibility for a circumstantial basis for the difference in its effect upon human and canine circulatory systems. There remained then the task of uncovering, from a mode of action study, a possible mechanistic basis for the species response difference.

The data of Eltherington and Horita demonstrating the ability of dibenzyline to block effectively the pressor response to this compound in the anesthetized dog point to the lack of a musculotropic component in its activity makeup. Support for the lack of musculotropic activity on the part of 1-phenyl-2-hydrazinopropane is provided by that portion of this study in which phentolamine (25 mg./Kg.) was shown capable of preventing the effect of this compound while RA-1226, an agent possessing both neurotropic and musculotropic activity, was only partially blocked by the same dose of adrenergic blocker. Eltherington and Horita also maintained that the neurotropic action of 1-phenyl-2-hydrazinopropane is not involved with the parasympathetic pathway, since previous administration of the compound caused no alteration in the blood pressure response to acetylcholine. In the current study, it was shown that the previous administration of blocking doses of hexamethonium does not alter the ability of the compound to elevate pressure. From this information, it would appear that stimulation along sympathetic pathways does not occur at the ganglia nor is it of central origin. Data of similar investigations with tetraethylammonium by Eltherington and Horita concur in these findings.

The rise in blood pressure following 1 mg./Kg. doses of 1-phenyl-2-hydrazinopropane was completely blocked by phentolamine (1 and 25 mg./Kg.), partially blocked by guanethidine (15 mg./Kg.), and reserpine (0.05 mg./Kg. for 5 days). These results point to four action possibilities with respect to the compound's pressor effect in dogs. These possibilities include direct stimulation of effector cell receptor sites at the sympathetic myoneural junction, indirect stimulation of these sites through MAO inhibition, indirect stimulation through the peripheral release of catecholamines stored in the blood vessel walls and, finally, a combination of two or more of these.

The results in dogs pretreated with reserpine and guanethidine indicate that the major component of the pressor activity of this agent, some 60%, is exerted through direct stimulation of adrenergic receptors. The lesser activity component, the 40% blocked by reserpine and guanethidine, is achieved through either MAO (or other enzyme) inhibition or the peripheral release of catecholamines stored in the walls of the blood vessels. In the human, the compound may act like a ganglionic blocker (9) or an adrenergic blocker (10).

[2] Marketed as Regitine (methanesulfonate) by Ciba Pharmaceutical Co.

[3] 4-Methyl-2-aminopyridine, supplied by Lakeside Laboratories.

SUMMARY

The pressor response to 1-phenyl-2-hydrazinopropane is essentially the same in hypertensive as in normotensive dogs.

The cardiac rate decrease which accompanies the blood pressure elevation following the administration of this compound does not occur in atropinized dogs.

The pressor response to 1-phenyl-2-hydrazinopropane is completely blocked by phentolamine, indicating the absence of a musculotropic component and the involvement of the sympathetic pathway in the response.

The pressor response to this compound is not affected by the prior administration of hexamethonium, making unlikely the possibility of ganglion and central stimulation.

The diminution of the pressor response to 1-phenyl-2-hydrazinopropane in reserpinized dogs and those which had received guanethidine gives support to the combined mechanism at the myoneural junction of direct and indirect effector cell receptor site stimulation, the latter by MAO (or other enzyme) inhibition or by the peripheral release of catecholamines in the blood vessel walls.

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Phase Solubility Study of Solid Species Formed by Magnesium Aluminate from Aqueous Solutions Containing Sulfate Ions

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A phase solubility technique has been used to detect and establish the nature of several hydrated solid species formed by sulfated magnesium aluminate. The most stable form at lower magnesium level appears to correspond to a hydrate of 4MgO : Al_2O_3:SO_3. Equilibrium behavior of this substance is such as to obey the solubility product principle with regards to both magnesium and sulfate. Under other conditions species corresponding to MgO:Al_2O_3, 3MgO : Al_2O_3 : SO_3, and 8MgO:Al_2O_3 : SO_3 seem to be produced. The results demonstrate the great value of the phase solubility technique in studying complex inorganic systems.

SERIOUS INVESTIGATIONS of the physical states of precipitated hydrous oxides and mixed hydrous oxides have been severely limited by the instability of these inorganic systems and by the difficulty in establishing their true states and compositions without washing or drying. For this reason, despite wide pharmaceutical usage of many of these compounds as bases for antacids, no detailed physical-chemical studies on these systems have been published in recent years. The present report is concerned with the results of an investigation on a hydrous sulfated magnesium aluminate system which makes use of a phase solubility technique developed earlier for studies on formations of organic complexes. The data presented show that the $m\text{MgO}\cdot n\text{Al}_2\text{O}_3\cdot p\text{SO}_3\cdot x\text{H}_2\text{O}$ systems could exist in a number of different stoichiometric ratios.

Results of the present studies suggest that formations of distinct solid species having the following approximate compositions are favored by hydrated aluminum and magnesium oxides in contact with aqueous solutions containing sulfate ions: (*a*) $3\text{MgO}\cdot\text{Al}_2\text{O}_3\cdot\text{SO}_3\cdot x\text{H}_2\text{O}$, (*b*) $4\text{MgO}\cdot\text{Al}_2\text{O}_3\cdot\text{SO}_3\cdot y\text{H}_2\text{O}$, and (*c*) $8\text{MgO}\cdot\text{Al}_2\text{O}_3\cdot\text{SO}_3\cdot z\text{H}_2\text{O}$. The presence of sulfate in the structure appears to be necessary. These systems appear to possess distinct solubility products based on the concentrations of the three components.

These findings also attest to the value of phase solubility determinations in the detection and

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