whole blood, rather than to plasma subsequently reconstituted as whole blood. Whenever oleate enters whole human blood *in vitro,* red cells and plasma compete for binding oleate; that fraction of oleate bound by plasma is rendered hemolytically inert whereas that fraction bound by erythrocyte constitutes the hemolytically active moiety.

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Pharmacologic Studies of a New Antihypertensive Compound N-(o-Methoxyphenyl)-N'-(3-methoxypropyl)-piperazine Phosphate (HT1479) (24872)

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In screening of compounds for antihypertensive activity certain members of a series of piperazines showed significant power to produce a fall of blood pressure, both in normotensive and hypertensive animals. Further testing of this group (all synthesized by Sommers *et al.* of Organic Chemistry Dept., Abbott Labs) yielded one (HT1479) which possessed satisfactory antihypertensive characteristics, and promised to excell in one important phase, that of relatively prolonged duration of action in anesthetized animals.

Methods. All injections were given intravenously unless otherwise specified. Blood pressure was recorded from carotid artery of cats, dogs and monkeys anesthetized with phenobarbital-pentobarbital mixture, and recorded with ink-writing mercury manometer. Blood pressure was also recorded from femoral artery of trained unanesthetized normotensive dogs. Cats were used for studies on carotid reflexes, effect of anoxia and nictitating membrane activity. Carotid sinus occlusion was achieved by 2 pneumatic cuffs which applied pressure to the carotids *in situ,* with minimum of trauma or traction. Anoxia was produced by connecting tracheal cannula to spirometer containing 100% nitrogen. Nictitating membrane experiments employed direct mechanical recording of membrane movements. The preganglionic portion of the superior cervical nerve was stimulated by square wave or 60 cycle impulses. Standardized doses of epinephrine were given before and at intervals after administration of piperazine compound. Duration and degree of inhibition of pressor response was used as semi-quantitative measure of relative potencies. Parasympatholytic action was determined in anesthetized cats by comparing depressor response to standardized doses of acetylcholine (1 μg/kg) before and after administration of drug. Antihistaminic potency of compounds was evaluated in anesthetized cats by their influence on depressor response to standardized dose of histamine (0.25 μg). Renal hypertensive rats, prepared by Grollman method (1) were used to study hypotensive effect of the compounds. Blood pressure was measured in these unanesthetized rats by photoelectric method of Kersten *et al.*(2). Direct effect on heart was studied in the Langendorff heart preparation, perfused at pressure of 40-50 cm with Ringer-Locke solution. The drugs

were added to the perfusing solution. Heart contractions were recorded with ink-writing level, and coronary outflow was registered continuously by modified Stephenson volume recorded(3). Acute toxicity was determined in mice. LD_{50} and standard errors were calculated by log-probit method of Miller and Tainter(4). Chronic toxicity studies were carried out in rats and dogs.

Results. Anesthetized dogs and cats. HT1479 in dose of 0.5 mg/kg produced a lowering of blood pressure of 55-70 mm Hg. At the end of 6 hours, blood pressure was still 30 mm below usual normal level after this period. At 250 μg/kg there was a fall of 40-50 mm with return to normal in about ½ hour in dogs and cats. 250 μg/kg administered intraduodenally produced a decline beginning in 7 minutes and progressing gradually to 30 mm below normal. This level was sustained over 30 minutes before slowly returning to normal. In the above dose range, the heart rate slowed, but seldom as much as 10%. In 28 animals many other tests in the range from 25-250 μg/kg produced results comparable to those already mentioned. In the pithed, vagotomized cat, doses of 75 and 100 μg/kg intravenously caused prolonged drops of blood pressure and a slight inhibition of epinephrine response. One mg/kg produced complete reversal of 5 and 10 μg of epinephrine. In both species bilateral occlusion of renal blood flow prolonged depressor effect up to 100%. When occlusion was terminated the pressure rose above control level. In anesthetized bilaterally nephrectomized monkeys depressor effects were similar to those osberved in cats or dogs with occlusion of renal artery except that duration of effect was much prolonged but magnitude of initial response was about the same.

Langendorff heart preparation. The effects of HT1479 and one of its homologues, HT507, on the isolated heart were studied in 22 cat hearts, 10 monkey hearts and 2 rabbit hearts. When mixed with Ringer-Locke solution in concentrations from 0.25 to 10 μg/ml and perfused through coronary circulation, they produced no consistent effect except a slight decrease or increase in coronary flow. There were no significant alterations in heart rate or amplitude of contractions.

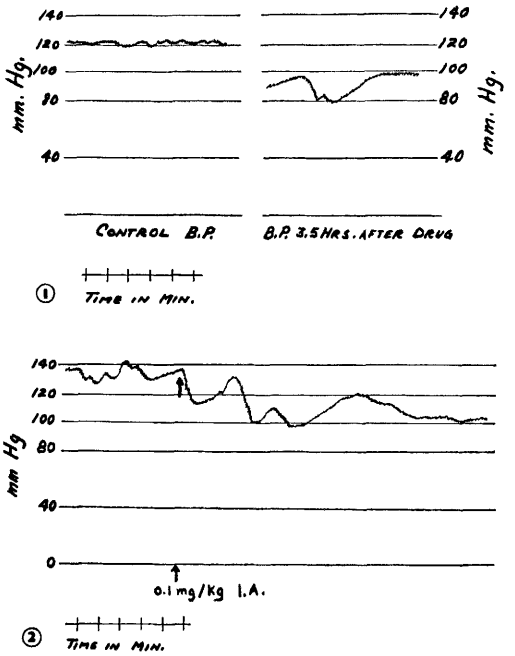


FIG. 1. Effect of HT1479 on blood pressure of an unanesthetized normotensive dog given 5 mg/kg orally.

FIG. 2. Effect of HT1479 on blood pressure (femoral artery) of an unanesthetized normotensive dog after 0.1 mg/kg given intra-arterially into femoral artery.

Coronary Flow in the Dog. In 5 experiments using a Morawitz cannula(5) the coronary flow of anesthetized dogs was directly measured. Intravenous (femoral) infusion of 0.1 to 1 mg/kg of HT1479 caused a slight increase in coronary flow. When 10 mg (approximately 1 mg/kg) was injected directly into the vena cava there was a slight decrease in flow. In either case the change was less than 15%.

Unanesthetized normotensive dogs. A single dose of 5 mg/kg orally produced lowering of blood pressure of 16 mm Hg when determined after 3 hours in one dog and of 20 mm after 3.5 hours in another dog (Fig. 1). A dose of 0.1 mg/kg into femoral artery produced immediate lowering of 25 mm in the artery of this extremity (Fig. 2). Electrocardiograms recorded following intravenous dose of 1 mg/kg showed no significant abnormalities. A compensatory increase in heart rate occurred from a control of 90 beats/minute to 120 at 2

TABLE I. Effects on the Anesthetized Animal.

Compound No.	Dose/kg I.V. (mg)	Effect on blood pressure mm Hg	Duration	Epinephrine effect	Nictitating membrane response	Serotonin effect
			Anesthetized cat			
HT1479	.1	-55	Returning to -20 sustained	60% inhibited	50% inhibited	Normal
	1	-70	80 min.	Reversed	80% "	40% inhibited
507	.25	-40	60 "	Normal	Normal	
			Spinal cat			
HT1479	.075	-35	60 min.	Slightly inhibited		
507	.25	-55	Sustained			
			Anesthetized dog			
HT1479	.1	-20	Sustained	Absent		
507	.5	-45	60 min.	Normal		
			Anesthetized monkey			
HT1479	.05	-50	Returning to -20 sustained	Normal		
507	.5	-45	Sustained	"		Normal

minutes and 105 at 5 minutes. This change is probably attributable to fall in systemic blood pressure when the drug reaches general circulation.

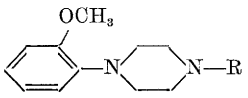
Peripheral blood flow in the dog. Measurement of peripheral arterial blood pressure with Nolf triple-manometer preparation likewise indicated that intra-arterial injection of 25 μg/kg of HT1479 had a direct vasodilator effect. The dilator action was usually less than subsequent systemic response when the drug entered the general circulation. This confirms the observation in the unanesthetized dog.

Unanesthetized hypertensive rats. In a group of 6 chronic hypertensive rats with average blood pressure of 160 mm Hg (range 152-168) HT1479 in single dose of 0.5 mg orally (equivalent to approximately 2.5 mg/kg) produced a marked hypotensive effect. All animals showed significant decrease in blood pressure of about 25-30 mm, returning gradually to pretreatment levels in about 3 hours.

Miscellaneous effects. Blood pressure responses in anesthetized cats to intravenous injections of l-arterenol. acetylcholine and histamine were not modified by doses of this compound which reverses the response to epinephrine. Neither were typical responses of blood pressure to electrical stimulation to the central end of sciatic or caudal end of vagus nerves changed. The pressor effect of serotonin was reduced by about 40%. Doses of 0.5-1 mg/kg produced a 75% inhibition of the nictitating membrane response to superior cervical nerve stimulation. Carotid sinus reflex was 50-60% decreased and the response to anoxia reversed. In the expected therapeutic range (1-5 mg/kg) the compounds showed no potentiation of barbiturate sleep in mice. The same was true to 50 mg/kg orally. Table I shows activity of HT1479 and HT507 upon blood pressure and certain other functions. Tachyphylaxis does not occur in anesthetized cats or dogs.

Acute toxicity. The LD_{50} (Table II) of HT1479 was determined in mice, at least 10/level and analyzed by graphic method of Miller and Tainter(4). The symptomatology was entirely that of a depressant, with ataxia, depression and coma. Respiratory depression, slowed heart rate and terminal cyanosis were sequentially seen. At no time were convulsions observed. The depressant effects were seen only when the dose approached the lethal range. In dogs, HT1479 at doses up to 100 mg/kg subcutaneously produced no deaths, but doses of 120 mg/kg and above were lethal. A normal monkey was given 10 mg/kg of HT1479 by stomach tube. Only minimal behavioral effects were noted. There was a brief period of possible ataxia. The animal appeared tranquilized, he was drowsy, but

TABLE II. LD_{50} Was Determined in Mice, at Least 10/Level, by Graphic Method of Miller and Tainter(4).



Compound No.	R	LD_{50} in mice, mg/kg I.V.	I.P.	Oral
HT1479	$-(CH_2)_3-OCH_3$	47 ± 3	146 ± 3.8	350 ± 18.7
507	–H	44 ± 1.8	108 ± 2.9	148 ± 7.8

was readily alerted. Eating habits were normal. One week later the same animal was given 30 mg/kg. There was no significant increase in observable symptoms except perhaps a greater degree of docility.

Chronic toxicity. Of a greater number of animals placed on test, at the end of 12 weeks, 2 dogs and 8 rats were sacriced for histopathologic examination. The dogs had received, respectively, 15 and 30 mg/kg orally, 6 days a week for 12 weeks. The following organs were examined histologically: brain, bone marrow, heart, lungs, spleen, liver, kidneys, adrenals, gonads, pancreas, stomach, lymph nodes, small and large intestine. Microscopic examination of tissues did not reveal morphologic alterations attributable to drug action. Two dogs on HT1479 at 15 mg/kg and 1 dog at 30 mg/kg orally received the drug 6 days a week for 20 months. Fifty rats on 20 and 50 mg/kg orally for 10 months showed no symptoms attributable to the drug. All animals maintained good health and normal weight gains. In dogs, complete hematological tests, urinalysis, liver BSP function test and blood NPN showed no significant effects attributable to the drug.

Summary. In a series of piperazines, N-(o-Methoxyphenyl) - N'-(3-methoxypropyl)-piperazine (HT1479) had potent hypotensive activity. Its homologue HT507 possesses similar hypotensive actions, but appears to be less potent and less adrenolytic. In nephrectomized animals duration of effect of HT1479 was much prolonged. This was also true in pithed vagotomized animals. The mechanism of hypotensive effect of this drug has not yet been fully established. While central effects of HT1479 may play a part in its hypotensive action, occurrence of a drop of blood pressure in pithed cat as well as experiments with triple manometer technic indicates that this is not the only mode of action. Paralysis of ganglia appears unlikely in view of the failure of HT1479 to block the effect of peripheral vagal stimulation of the heart. Peripheral adrenolytic properties of the drug may well play a role in its hypotensive action though they are too weak in low dosage to explain the total hypotensive potency. Other peripheral dilator actions could be involved. The effects upon heart rate have been inconsistent and the role of this organ in the overall effect cannot yet be fully appraised. HT1479 is now on clinical trial and will be reported later.

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