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CARDIOVASCULAR ACTIONS OF GUANCYDINE IN NORMOTENSIVE AND HYPERTENSIVE ANIMALS[1]

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ABSTRACT

CUMMINGS, J. R., A. N. WELTER, J. L. GRACE, JR. AND L. M. LIPCHUCK: Cardiovascular actions of guancydine in normotensive and hypertensive animals. J. Pharmacol. Exp. Therap. **161**: 88–97, 1968. In conscious normotensive rats, oral administrations of guancydine caused a dose-related hypotension and bradycardia. Vasopressor responses to angiotensin, renin and phenethylamine were blocked. Responses to epinephrine and norepinephrine were reduced to a lesser degree and responses to vasopressin and $BaCl_2$ were unaltered. Repeated oral doses of guancydine given to metacorticoid hypertensive rats caused a reduction in both mean blood pressure and cardiac hypertrophy. Guancydine, 8 to 15 mg/kg p.o., produced hypotension in hypertensive (but not normotensive) dogs and had no effect on heart rate. Larger amounts (30 mg/kg) caused hypotension and tachycardia in all types of dogs. This dosage also increased cardiac output in conscious dogs. Vasodilation of the cranial mesenteric and renal vascular beds of anesthetized dogs developed after i.p. injections. Renal vascular resistance also decreased in one conscious dog after p.o. administration. Propranolol partially blocked the hypotensive effects of guancydine. Chronic treatment with guancydine, 80 mg/kg/day, reduced norepinephrine concentrations in the hearts of rats whereas a lesser hypotensive dose (20 mg/kg/day) and an acute dose (100–200 mg/kg) appeared to be without effect.

In searching for novel antihypertensive agents, several structurally related cyanoguanidines were found which reduced the mean blood pressure of conscious rats and blocked vasopressor responses to angiotensin without altering responses to epinephrine. One of the most active of these compounds was guancydine (1-cyano-3-*tert.*-amylguanidine), $CH_3—CH_2—C(CH_3)_2—NH—C(=NCN)—NH_2$. It is a white crystalline compound, m.p. 156–157°C, which is soluble in alcohol but only sparingly soluble in water, alkali and acid. Syntheses for the series of related cyanoguanidines have been described recently by Gadekar *et al.* (1968).

The purpose of this report is to consider various pharmacologic actions of guancydine in conscious and anesthetized normotensive and hypertensive animals.

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METHODS. *Rats.* Blood pressures of conscious male albino rats weighing 200 to 300 g were recorded. Normotensive and hypertensive animals were of the Wistar and Sherman strains, respectively. The rats were fastened to boards in a supine position by means of canvas vests and limb ties. The femoral area was anesthetized by s.c. infiltration of lidocaine before insertion of catheters into the iliac artery and vein. Hypertension was induced by s.c. injections of 0.2 ml of a 5% aqueous suspension of desoxycorticosterone acetate 5 days a week for 3 weeks, during which the rats drank isotonic saline *ad libitum*. In the metacorticoid testing period (the 12 days after the last desoxycorticosterone acetate injection), they drank tap water. Both normotensive and hypertensive rats were given guancydine in a 2% starch suspension by gavage. Control animals received only the 2% starch. The volume administered was 0.5 ml.

Dogs. Hypertension of renal origin was produced in dogs by the method of Goldblatt *et al.* (1934). Neurogenic hypertension was induced by the technique of Wakerlin *et al.* (1954). New dogs entering our colony which maintained an elevated

mean blood pressure of >140 mm Hg for at least 4 months were classed as spontaneous hypertensive animals. The hypertensive and normotensive dogs were trained to lie in a recumbent position, and, after their hind limbs had been secured with straps, a 26-gauge thin wall needle attached to a pressure transducer was advanced percutaneously into their femoral arteries.

In all experiments using rats and dogs, blood pressure was measured using a P23Db Statham pressure transducer-polygraph system. Mean blood pressure was determined by integrating the pulse curves using either Sanborn Polyviso or Offner Dynograph averaging circuits.

Regional blood flows in 18 dogs under chloralosane anesthesia were measured extracorporeally by means of a square-wave electromagnetic flowmeter. In the renal blood flow studies during the time the left renal artery was being cannulated, the involved kidney was perfused with blood from the common carotid artery. Throughout this maneuver, blood flow to the kidney was interrupted for only a few seconds. Renal blood flow was also measured in three dogs anesthetized with pentobarbital and in one conscious dog by a miniature EMP-4081 blood flow transducer (Electromagnetic Probe Co.) connected to a Carolina Medical Electronics two-channel Flowmeter. In the chronic preparation, the probe was placed around the left renal artery under aseptic conditions and the wound was repaired. After 8 days were allowed for recovery, recordings were made periodically throughout a 2-week interval. Splanchnic blood flow was estimated by cannulating the cranial mesenteric artery with an extracorporeal blood flow transducer. Blood flows through innervated and denervated hindlimbs of dogs were recorded simultaneously by connecting extracorporeal flow-meters between the lower abdominal aorta and the femoral arteries. In these experiments, one hindlimb of an animal was denervated by severing the sciatic and femoral nerves and stripping the adventitia from the femoral artery. Ten days elapsed between the time of surgery and the measurement of blood flow to allow for degeneration.

The procedure for chronic measurements of pulmonary hemodynamic events in nonthoracotomized, conscious dogs has been reported (Welter and Cummings, 1966). Under fluoroscopic visualization, the transseptal puncture technique was used to advance a catheter into either the left atrium or the pulmonary vein. Catheters were also placed in the pulmonary artery, pulmonary arterial wedge and descending thoracic aorta. Several days later, the conscious dogs were put in a canvas sling and their pressures were recorded by P23Db Statham transducers.

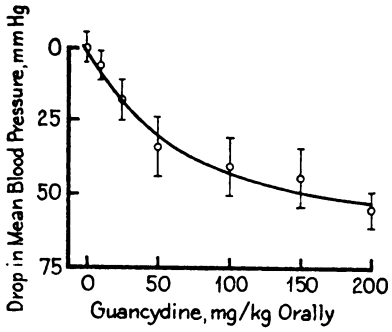

FIG. 1. Effect of graded doses of guancydine on the mean blood pressure of conscious rats 2 hr after p.o. dosing. Data are averages ± S.D. Animals per dose: 32 rats, 0 and 100 mg/kg; 20 rats, 10 mg/kg; 15 rats, 50 mg/kg; 6 rats, 25, 150 and 200 mg/kg.

Cardiac output was determined in conscious and anesthetized dogs by the indocyanine green dye-dilution technique (Fox and Wood, 1957). A Walton strain gauge arch sutured to the right ventricle of anesthetized dogs was used to record the contractile force of the heart.

Studies were performed on the storage and liberation of catecholamines in mice and rats. In mice, H^3-norepinephrine was injected i.v.; guancydine or starch was given 17 hr later and, after another 7 hr, the hearts were removed and placed in cellulose bags. The radioactivity was determined by an oxygen flask combustion method (Kelly *et al.*, 1961). The norepinephrine in the tissues of rats was measured according to the procedure of Maynert and Klingman (1962).

The following drugs were used: epinephrine HCl, *l*-norepinephrine, angiotensin amide (Hypertensin), vasopressin (Pitressin), renin (Nutritional Biochemicals Corp.), barium chloride, dimethylphenylpiperazinium iodide (DMPP), dichloroisoproterenol, propranolol (Inderal[8]), acetylcholine chloride and histamine diphosphate. Where applicable, all doses refer to the salts of these agents.

The statistical significance of the data was determined by Student's *t* test. The procedure described by Box (1954) was used for contour representation of certain three-factor systems.

RESULTS. EFFECTS IN CONSCIOUS RATS. *Dose response.* Figure 1 summarizes the effect of guancydine on the mean blood pressure of conscious rats 2 hr after p.o. doses of 0 to 200 mg/kg. Along with a progressive fall in blood

[8] Kindly supplied by Dr. D. A. Buyske, Ayerst Laboratories, Montreal, Canada.

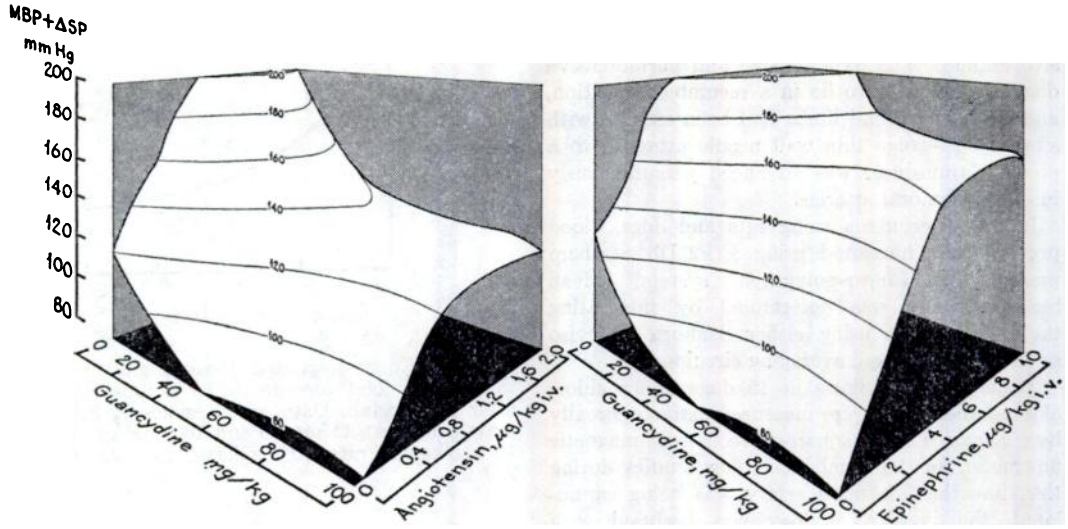

FIG. 2. Contour representation of the effect on blood pressure of injections of agonists (angiotensin and epinephrine) 2 hr after p.o. administrations of an antagonist (guancydine). MBP + ΔSP, mean blood pressure of conscious rats just before agonist administration plus the maximal rise in femoral systolic pressure caused by the agonist. The MBP of rats receiving neither antagonist nor agonist averaged 120 mm Hg.

pressure, increasing dosages caused a bradycrotic response in this species. For example, the heart rates (±S.D.) of rats 2 hr after 0, 50 and 200 mg/kg were 450 ± 20, 360 ± 20 and 340 ± 15 beats/min, respectively. The duration of the hypotensive response produced by a single p.o. dose of 100 mg/kg of guancydine was more than 8 hr but less than 24 hr.

Effect on vasopressor responses. Two hours after p.o. administrations of 50 mg/kg of guancydine to rats, the vasopressor response to angiotensin, 0.25 μg/kg i.v., was blocked, whereas the response to epinephrine, 2 μg/kg i.v., was essentially unaltered. This observation prompted a study on the effect of varying doses of guancydine (0, 10 and 100 mg/kg) on the pressor responses to graded amounts of angiotensin (0.08, 0.4 and 2 μg/kg) and epinephrine (0.4, 2 and 10 μg/kg). A total of 48 rats, 16 in each group, was used in a randomized incomplete block experiment. The responses to angiotensin and epinephrine were measured 2 hr after the administration of guancydine or 2% starch. The changes in blood pressure in relation to varying doses of two agonists and an antagonist are shown in a three-dimensional response-surface graph (fig. 2). Blood pressure represents the mean blood pressure (MBP) just prior to injecting the agonist plus the maximal rise in femoral systolic pressure (ΔSP) produced by the agonist, a relatively constant value under control conditions (Clark, 1935; Schaper *et al.*, 1963). It may be noted that the MBP + ΔSP, for example, of rats treated with 100 mg/kg of guancydine was 100 mm Hg immediately after the injection of 0.4 μg/kg of angiotensin. Statistical analysis of the data revealed that a dose of 10 mg/kg of guancydine caused a significant reduction ($P < .01$) in the vasopressor responses to the low and medium doses of angiotensin. A dose of 100 mg/kg caused a further reduction in the responses which was significantly greater than that achieved with 10 mg/kg ($P < .01$). The responses to epinephrine were also reduced, but to a lesser degree than those to angiotensin ($P < .05$). The high dose of angiotensin and especially epinephrine tended to override the blocking action of guancydine.

Table 1 lists the responses to seven vasopressor agents in groups of rats receiving either starch or guancydine by gavage. In addition to angiotensin, the vasopressor actions of renin and phenethylamine were markedly reduced by guancydine. With the doses selected, the responses to norepinephrine and epinephrine, although less affected, were also significantly lower ($P < .05$) in the rats which received

guancydine as compared with the starch-treated control animals. The hypertensive effects of vasopressin and barium chloride appeared unaltered by the hypotensive agent.

Repeated administrations to hypertensive rats. A series of metacorticoid hypertensive rats, six in each group, received guancydine, 20 or 80 mg/kg, or starch by gavage daily for 5 days. A second identical series received the same treatment over a 12-day period. As shown in figure 3, both the increased blood pressure and the unilateral left ventricular hypertrophy typically associated with metacorticoid hypertension (Benitz *et al.*, 1961) were affected by guancydine. At the .05 probability level, the mean blood pressures and ventricular ratios of rats given 20 and 80 mg/kg/day of guancydine were lower than those of the starch-tested rats. The change in hemodynamics appeared to precede the change in cardiac morphology. As previously reported (Cummings and Stokey, 1963), the mean blood pressure and ventricular ratio of normotensive untreated rats of the same species, sex and weight averaged 127 mm Hg and 3.4, respectively.

EFFECTS IN CONSCIOUS DOGS. *Normotensive and hypertensive.* The effect of guancydine, 7.5 and 15 mg/kg, on the mean blood pressure of conscious normotensive and hypertensive dogs over a 6-hr period is shown in table 2. The compound and the lactose control were given p.o. in cellulose acetate phthalate-coated, soft shell capsules. According to a randomized block design, all animals were given capsules of both lactose and the two doses of guancydine at 2-week intervals. With the doses employed, guancydine had no activity in normotensive dogs but produced hypotension in renal, neurogenic and spontaneous hypertensive dogs. Heart rate was not significantly altered in any of the animals ($P > .05$).

Repeated administrations to hypertensive dogs. The effects of repeated daily doses of guancydine, 30 mg/kg, over a 5-day period on the blood pressures of four conscious renal hypertensive dogs are shown in figure 4. In these experiments, the drug was packed in hard shell capsules. The figure illustrates the marked hypotensive action of the guancydine administered p.o. There was no evidence of tolerance or of an accumulative drug effect. With this larger dose, tachycardia was encountered. The average heart rates (±S.D.) before and 4 hr after guancydine were 80 ± 14 and 162 ± 19 beats/min, respectively.

Other hemodynamic actions. Cardiac output, stroke volume, mean blood pressure, total peripheral resistance and pulmonary arteriolar and venous resistances were measured in con-

TABLE 1

Responses to various vasopressor agents after p.o. administration of guancydine to conscious normotensive rats[a]

Vasopressor Agent	Dose	Rise in Femoral Systolic Blood Pressure	
		After starch[b]	After guancydine[c]
	μg/kg i.v.	*mm Hg ± S.D.*	
Epinephrine	2	46 ± 4	34 ± 4
Norepinephrine	2	47 ± 5	32 ± 6
Angiotensin	0.4	42 ± 6	12 ± 4
Renin	1000	53 ± 4	17 ± 5
Phenethylamine	1000	46 ± 7	10 ± 6
Barium chloride	1000	43 ± 7	44 ± 5
Vasopressin	50	54 ± 5	55 ± 6

[a] One group of 20 rats received starch; another group of 20 received guancydine. Both groups were given all seven vasopressor agents in identical sequence. Doses of renin and vasopressin were estimated on the basis that 1 U of renin was equal to 100 μg and 1 U of vasopressin was equal to 500 μg.

[b] Measurements were taken 2 hr after administration of 2% starch.

[c] Measurements were taken 2 hr after administration of 100 mg/kg of guancydine.

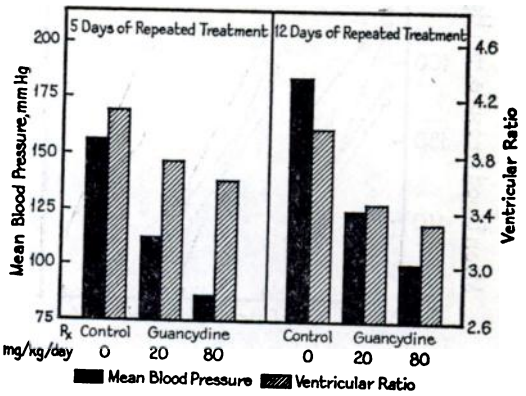

FIG. 3. Antihypertensive effects of guancydine in metacorticoid hypertensive rats. Ventricular ratio is calculated as weight of left ventricle and septum/weight of right ventricle.

TABLE 2

Effect of guancydine administered p.o. in conscious normotensive and hypertensive dogs[a]

Type o Dog	Dose	Change from Control Mean Blood Pressure					
		Lactose			Guancydine		
		2 hr	4 hr	6 hr	2 hr	4 hr	6 hr
	mg/kg	*mm Hg*			*mm Hg*		
Normotensive	7.5	5	7	8	−1	3	0
	15	4	1	1	−7	7	3
Hypertensive Renal	7.5	−2	1	4	−16[b]	−10	−7
	15	−4	−2	6	−41[b]	−32[b]	1
Neurogenic	7.5	11	11	20	−5	−5	−9
	15	−8	15	18	−13	−20[c]	−22[c]
Spontaneous	7.5	2	3	6	3	8	10
	15	2	3	2	−17[b]	−21[b]	3

[a] Data are average changes from initial control, three dogs per type. Each animal received capsules of both lactose and guancydine.

[b] Significant at the .01 probability level.

[c] Significant at the .05 probability level.

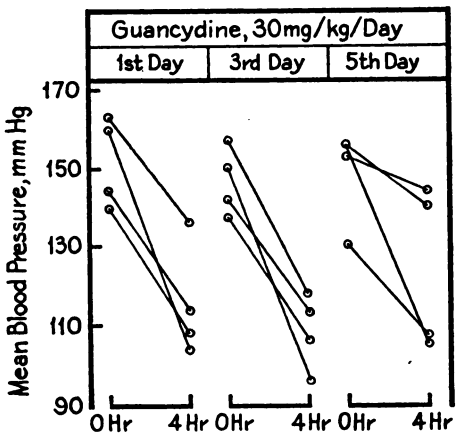

FIG. 4. Hypotensive effects of daily p.o. administrations of guancydine to four conscious renal hypertensive dogs. Hypertension was induced 6 to 7 months prior to administration of drug.

scious, nonthoracotomized dogs (table 3). Starch and doses of 20 and 30 mg/kg of guancydine were administered in randomized order at 5-day intervals. Two of the dogs received starch and 30 mg/kg of drug; one received starch and 20 mg/kg of drug; one received only 30 mg/kg of drug. The original plan of giving each dog both starch and the two doses of guancydine was not possible for technical reasons, *e.g.*, a cerebral vascular accident and clotted indwelling catheters. These preliminary data indicate that p.o. doses of 20 to 30 mg/kg of guancydine increased cardiac output and decreased peripheral resistance. Pulmonary arteriolar resistance appeared to increase after starch and guancydine treatment.

EFFECT ON BLOOD FLOW. A summary of the effects on 14 cardiovascular parameters after the i.p. administration of guancydine, 10 mg/kg, to anesthetized dogs is shown in table 4. For comparative purposes, the results after starch treatment throughout an identical time period are included. In the statistical analysis, each animal served as its own control. Of the measurements, only mean blood pressure, cranial mesenteric blood flow and splanchnic and renal vascular resistances were significantly altered (*i.e.*, reduced) 5, 20 or 35 min after an i.p. administration of guancydine ($P < .05$). All of the regional blood flows were measured extracorporeally.

Renal blood flow was also measured in four other dogs anesthetized with pentobarbital by means of a noncannulating electromagnetic flow probe. Under these conditions, guancydine (10 mg/kg i.p.) reduced systemic blood pressure without significant alteration of renal blood flow. In addition, weekly measurements of renal blood were made before and after the p.o. administration of 30 mg/kg of guancydine to a conscious dog with a chronically implanted electromagnetic probe. As illustrated in figure 5, guancydine caused tachycardia, a reduction in blood pressure and an apparent increase in renal blood flow. These parameters seemed to be unaffected by the administration of a starch capsule.

AUTONOMIC EFFECTS. The effects of i.p. injections of guancydine, 10 mg/kg, and 2% starch on vasomotor responses to acetylcholine, histamine, epinephrine, phenethylamine, DMPP, angiotensin and centrifugal and centripetal vagal stimulation in anesthetized dogs are summarized in table 5. Large variations were encountered within both groups during the control and experimental periods, and no significant action of guancydine on vasoactive stimuli was demonstrated. Vasomotor, inotropic and chronotropic responses to cardiac nerve stimulation (5 V, 30 cps, 5-msec pulse width, 15 sec in duration) and isoproterenol (1 μg/kg i.v.) were recorded in dogs under chloralosane

TABLE 3

Effect of guancydine on cardiac and pulmonary measurements in conscious normotensive dogs[a]

Capsule	Dose	N	Cardiovascular Parameter				
			Cardiac output	Stroke volume	Total PR	Pulmonary AR	Pulmonary VR
	mg/kg		*ml/kg/min*	*ml/kg/beat*			
Starch		3					
0 hr			145 ± 4	1.2 ± 0.2	65 ± 10	3.0 ± 0.9	0.5 ± 0.1
1–2 hr			136 ± 13	1.0 ± 0.1	60 ± 8	3.2 ± 0.2	0.6 ± 0.2
2–4 hr			132 ± 9	1.0 ± 0.1	61 ± 8	3.7 ± 0.6	1.3 ± 0.7
Guancydine	20	1					
0 hr			140	1.3	51	3.7	0.2
1–2 hr			144	0.9	52	5.2	0.1
2–4 hr			165	1.0	27	6.8	0.4
Guancydine	30	3					
0 hr			144 ± 32	1.0 ± 0.3	61 ± 6	4.2 ± 1.0	0.4 ± 0.1
1–2 hr			151 ± 43	0.9 ± 0.1	44 ± 10	4.7 ± 1.6	0.7 ± 0.3
2–4 hr			146 ± 41	0.9 ± 0.1	52 ± 6	4.8 ± 1.4	0.8 ± 0.5

[a] Data are means ± S.D. *N*, number of dogs per treatment. Total PR, total peripheral resistance, calculated as mean blood pressure/cardiac output; pulmonary AR, pulmonary arterial resistance, calculated as (pulmonary arterial pressure − pulmonary arterial wedge pressure)/cardiac output; pulmonary VR, pulmonary venous resistance, calculated as (pulmonary arterial wedge pressure − left auricular pressure)/cardiac output. In determining the resistance values, cardiac output was given as liters per minute and blood pressure as millimeters of mercury.

TABLE 4

Hemodynamic effects of guancydine administered i.p. in anesthetized dogs[a]

Cardiovascular Parameter	Average Change from Initial Control Values					
	2% starch (10 ml i.p.)			Guancydine (10 mg/kg i.p.)		
	5 min	20 min	35 min	5 min	20 min	35 min
Mean blood pressure (mm Hg)	−1	−6	−9	−13[b]	−27[b]	−29[b]
Heart rate (beats/min)	2	2	−3	−5	5	−5
Cardiac output (liters/min)	0.1	0	−0.2	0.1	−0.2	−0.2
Stroke volume (ml/beat)	0.7	0.1	0.8	1.2	−1.3	−1.2
Total peripheral resistance	−2.0	−1.6	−.23	−11.1	−9.2	−12.0
Central venous pressure (mm Hg)	−0.3	0.1	−0.4	0.2	0.1	−0.2
Innervated hindlimb flow (ml/min)	−13.7	−14.3	−10.3	−1.3	−8.3	−9.0
Innervated limb resistance	1.2	1.6	0.4	−0.3	3.1	1.6
Denervated hindlimb flow (ml/min)	0.3	−6.3	−6.7	0.6	−15.0	−12.0
Denervated limb resistance	1.7	0.2	0	−7.0	0.3	−0.1
Cranial mesenteric flow (ml/min)	−10.0	−18.3	−21.9	3.0	7.7[c]	6.7[c]
Splanchnic resistance	0.1	0.2	0.1	−0.3	−0.6[b]	−0.5[b]
Renal arterial flow (ml/min)	1.2	−0.8	−6.0	4.0	−13.0	−15.5
Renal resistance	0	0	0	−0.4[b]	−0.4[b]	−0.4[c]

[a] Renal, cranial mesenteric and innervated-denervated limb flows were recorded extracorporeally in 18 dogs under chloralosane anesthesia. Cardiac output and central venous pressure were measured in five dogs anesthetized with pentobarbital.

[b] Significant at the .01 probability level.

[c] Significant at the .05 probability level.

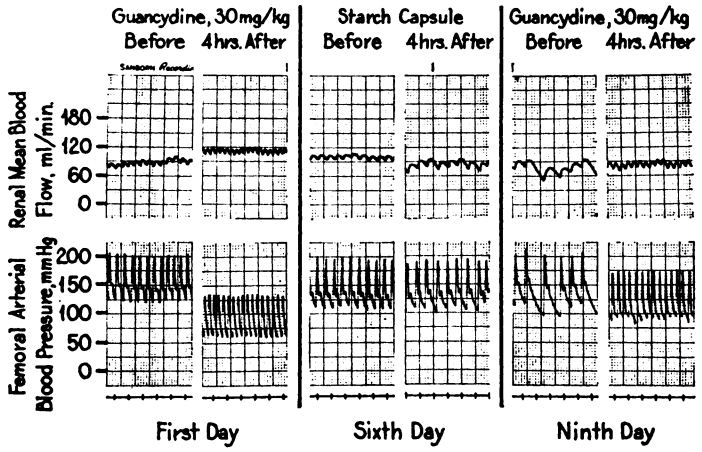

FIG. 5. Effect of an oral dose of guancydine on renal mean blood flow and femoral blood pressure in a conscious dog. An electromagnetic probe was implanted around the animal's left renal artery 8 days prior to the initial recording.

TABLE 5

Effect of guancydine on responses to autonomic agents and vagal stimulation in anesthetized dogs[a]

Vasomotor Agent or Stimulus and i.v. Dose	2% starch (10 ml i.p.)			Guancydine (10 mg/kg i.p.)		
	0 min	30 min	90 min	0 min	30 min	90 min
	mm Hg			*mm Hg*		
Acetylcholine (2 μg/kg)	−54 ± 19	−35 ± 10	−38 ± 10	−52 ± 31	−34 ± 11	−48 ± 8
Histamine (10 μg/kg)	−59 ± 12	−45 ± 8	−47 ± 5	−52 ± 18	−55 ± 24	−63 ± 7
Epinephrine (2 μg/kg)	118 ± 37	99 ± 36	56 ± 28	120 ± 27	78 ± 16	83 ± 40
Phenethylamine (50μg/kg)	78 ± 16	47 ± 17	43 ± 14	80 ± 23	52 ± 15	43 ± 8
Angiotensin (0.25 μg/kg)	64 ± 28	53 ± 23	44 ± 10	71 ± 30	75 ± 40	63 ± 18
DMPP (10 μg/kg)	127 ± 45	141 ± 43	136 ± 44	134 ± 44	134 ± 40	155 ± 37
Peripheral vagus (6 V, 30 cps for 3 sec)	−79 ± 10	−73 ± 9	−70 ± 16	−78 ± 26	−71 ± 7	−72 ± 15
Central vagus (14 V, 30 cps for 10 sec)	80 ± 32	76 ± 30	95 ± 43	93 ± 23	110 ± 34	115 ± 34

[a] Under chloralosane anesthesia, six dogs received starch and five received guancydine. Decreases in femoral diastolic pressure (−) or increases in femoral systolic pressure given as the mean ± the standard deviation.

anesthesia. Three of the animals were dosed with dichloroisoproterenol, 5 mg/kg i.v.; three with guancydine, 10 mg/kg i.p.; and three with 2% starch, 10 ml i.p. Dichloroisoproterenol blocked the responses to both stimuli, whereas guancydine and starch were without effect. In support of the findings obtained with the ganglionic stimulant, DMPP, data recorded in four anesthetized cats indicated that guancydine has no effect on the conduction of nerve impulses across sympathetic ganglia. In these latter experiments, contractions of the nictitating membrane evoked by preganglionic cranial cervical nerve stimulation (6 V, 10 cps, 1-msec pulse width, 3 min in duration) were similar before and after i.p. injections of guancydine, 10 mg/kg.

BETA ADRENERGIC STIMULATION. Under certain conditions, hypotensive doses of guancydine produced effects indicative of *beta* adrenergic stimulation, *e.g.*, increased cardiac output, heart rate and contractile force. In examining this concept, several experiments were performed to determine whether these actions could be inhibited by propranolol. Data obtained in anesthetized and conscious dogs given

TABLE 6

Influence of propranolol on the effects of guancydine in chloralosane-anesthetized dogs and conscious renal hypertensive dogs[a]

State of Dogs	Guancydine			Control		
	Dose	Mean blood pressure	Heart rate	Treatment	Mean blood pressure	Heart rate
	mg/kg	*mm Hg*	*beats/min*		*mmHg*	*beats/min*
Anesthetized	20			10% ethanol		
Prepropranolol (30 min after infusion)		90 ± 16	189 ± 23		149 ± 10	121 ± 19
Postpropranolol (45 min after 3 mg/kg i.v.)		105 ± 15	138 ± 20		140 ± 16	114 ± 16
Conscious	30			Starch capsule		
Prepropranolol (4 hr after p.o. dose)		110 ± 8	161 ± 25		153 ± 13	92 ± 31
Postpropranolol (20 min after 1 mg/kg i.v.)		140 ± 9	128 ± 21		176 ± 3	76 ± 27

[a] Data are averages ± S.D. Both guancydine and ethanol were infused at a rate of 0.6 ml/min for 30 min.

guancydine i.v. or p.o. are summarized in table 6. An equal number of animals received treatment with only the infusion vehicle or a starch capsule over an identical time period. After propranolol, there was a slight elevation in the blood pressures of the dogs dosed with guancydine and a slight fall in the blood pressure of the dogs dosed with ethanol. Although the standard deviations of the values recorded before and after propranolol overlapped in the two groups, all of the guancydine-treated dogs had an increase in mean blood pressure, whereas all of the alcohol-treated dogs had a decrease after *beta* adrenergic blockade. In both groups, heart rate was slowed after propranolol. The negative chronotropism, however, was statistically significant ($P < .05$) only in the drug-treated dogs. The conscious animals given guancydine had an increase in mean blood pressure and a decrease in heart rate after propranolol. However, similar but less pronounced changes were also noted in the starch-treated dogs. The altered responses in both groups after propranolol were statistically significant by paired *t* test analysis ($P < .05$). Myocardial contractile force was measured in two of the anesthetized dogs which received an infusion of guancydine or ethanol. A positive inotropic response was recorded during and after the administration of guancydine (maximum, 70% increase from control). Immediately after *beta* adrenergic blockade, the response was abolished. Little significance, however, is attached to these data since a positive inotropic response was produced by infusing ethanol (maximum, 50% increase from control) which was blocked by propranolol.

CATECHOLAMINES.[4] Single doses of guancydine did not appear to affect the storage of tritiated norepinephrine in the myocardium of mice. With eight mice serving as controls and eight receiving 100 to 200 mg/kg of guancydine i.p., the drug caused a 19% average reduction in the counts of H^3-norepinephrine per minute per heart ($P > .05$). In contrast, the i.p. administration of 2.5 mg/kg of reserpine to another group of eight mice caused an 82% average reduction from control ($P < .05$).

During the course of the previously described metacorticoid hypertension experiment, ptosis was noted in several of the rats which received 80 mg/kg/day p.o. of guancydine. As a result, the hearts of five rats from each group were analyzed for norepinephrine concentrations (table 7). It appears that a hypotensive p.o. dose of 20 mg/kg/day for 10 days did not cause catecholamine depletion, whereas 80 mg/kg/day over a similar period did. A reduction in catecholamines was also

[4] Information contributed by Mr. H. Eisner, Dr. L. Ellenbogen, Mrs. E. Markley and Mrs. M. Wishnick.

TABLE 7

Effect of administering guancydine p.o. daily for 10 days on norepinephrine concentrations in hearts of metacorticoid hypertensive rats[a]

Guancydine	Norepinephrine	
	Concentration	Depletion
mg/kg/day	*μg/g*	%
Vehicle	0.86 ± 0.15	0
20	0.79 ± 0.23	8 ± 26
80	0.44 ± 0.02[b]	49 ± 2

[a] Groups of five rats received either starch or the low or high dose of guancydine. Data are means ± S.D.

[b] Significant change from control at the .05 probability level.

noted in the hearts of normotensive rats given repeated daily p.o. doses of guancydine. Four animals received starch and four received 100 mg/kg of drug for 3 days. The average decrease from control in norepinephrine concentration was 45% ($P < .05$).

TOXICITY.[5] The LD50's and 95% confidence limits in mice 18 hr after p.o. and i.p. administrations of guancydine were 1400 mg/kg (1050–1865) and 322 mg/kg (298–348), respectively. Toxic signs included ataxia and sedation. Prodding the animals caused arousal. Neither ptosis nor the loss of the pinna or corneal reflexes was recorded. Mortality data were also recorded in a group of mice over a 7-day period. Under these conditions, the LD50's and 95% confidence limits after p.o. and i.p. doses were 266 (242–295) and 229 (201–243), respectively. Bradycardia and hypothermia were noted. The LD50's and 95% confidence limits in rats throughout 7 days were 300 mg/kg (266–334) after p.o. dosing and 313 (295–338) after i.p. dosing. The toxic signs were similar to those observed in mice.

Daily doses of 100 mg/kg of guancydine were administered p.o. to three male and three female dogs for 3 months. All of the animals survived and were in good physical condition. No definitive toxic actions were encountered.

DISCUSSION. In the consideration of guancydine's effect in lowering blood pressure, certain modes of action seem relatively insignificant. Since under the described experimental conditions guancydine did not increase blood flow through the lungs or hindlimbs, the drug does not appear to be a nonspecific vasodilator. Likewise, guancydine does not apparently act by ganglioplegia, *beta* or *alpha* adrenergic blockade, cardiac depression or histamine release. Presumptive evidence for the latter is that guancydine produces hypotension in rats whereas histamine liberators do not (Paton, 1957).

Under acute conditions, guancydine does not seem to cause the release of endogenous stores of catecholamines. Repeated administrations of relatively high does, however, reduced norepinephrine concentrations in the hearts of rats. Thus, under certain chronic situations, blockade of adrenergic neurones regulating vascular tone may be involved in the drug's antihypertensive action.

It seems reasonable to assume that a causal relationship exists between the guancydine-induced vasodilation of abdominal blood vessels and the fall in systemic blood pressure which was recorded in anesthetized and conscious animals. In this respect, guancydine resembles hydralazine (Freis *et al.*, 1953) and the vasodilator 31531-Ba (Granata *et al.*, 1967) and differs from guanethidine (Cohn *et al.*, 1963) and hexamethonium (Aviado, 1960), two hypotensive drugs which constrict renal and splanchnic vessels. On the basis of limited regional hemodynamic studies, the administration of guancydine appears to cause a redistribution of blood flow which is dependent on the extent of resistance changes occurring in each vascular bed.

Some of the acute cardiovascular effects of guancydine were partially blocked by the *beta* adrenergic blocking agent, propranolol. Similarily, Brunner *et al.* (1965a, b) noted that pronethalol reduced the hypotensive actions of hydralazine and guanethidine. Although these findings suggest that these three antihypertensive agents possess *beta* adrenergic properties, other interpretations are tenable, *e.g.*, that the propranolol and pronethalol inhibitory effect was nonspecific and unrelated to *beta* adrenergic blockade.

The observation that guancydine inhibits the vasopressor responses to angiotensin and renin without appreciably reducing the vasopressor response to epinephrine was extended by

[5] The majority of these studies were performed by Dr. T. Balazs and associates.

Welter and Grace (1967). In this preliminary study, guancydine lowered the elevated blood pressure of rats receiving infusions of angiotensin over a 30- to 120-min period and converted angiotensin-induced vasoconstriction of the renal bed of anesthetized dogs into a vasodilator response. This latter finding seems particularly noteworthy since the drug did not alter epinephrine-induced renal vasoconstrictor or angiotensin-induced vasopressor responses in dogs. The interrelationship between these findings and guancydine's mode of antihypertensive activity, however, remains obscure. Moreover, elucidation of this problem is complicated by the fact that much remains unknown as to the role of the angiotensin–renin system in the etiology of hypertension (Laragh, 1967).

Guancydine's oral hypotensive actions in hypertensive rats and dogs, apparent lack of tolerance, semiselective blockade of angiotensin, renin and phenethylamine responses and relatively low toxicity suggest a therapeutic use in the management of hypertension. This possibility is being assessed.

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