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ADRENERGIC NEURONE BLOCKING ACTIVITY OF SOME ARALKYLGUANIDINES

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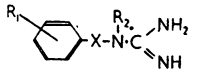
The ability of aralkylguanidines, such as *N*-benzyl-*N'N"*-dimethylguanidine, to cause adrenergic neurone blockade has been previously reported (Boura, Copp, Green, Hodson, Ruffell, Sim, Walton & Grivsky, 1961; Boura & Green, 1963). However, little has been published on aralkylguanidines in which only one of the amino-nitrogen atoms is substituted. We have synthesized and examined a series of compounds of the general structure:



where R_1 is a hydrogen or chlorine atom, or a methyl or methoxyl group; R_2 is a hydrogen atom or a methyl group; and X is a straight- or branched-chain alkyl group. In this series we have been chiefly concerned with the influence of the structure and stereochemistry of the alkyl group X on adrenergic neurone blocking activity; but we have also studied, although to a lesser extent, the effect of substitution in the benzene nucleus. The tests for adrenergic neurone blocking activity were: failure, in anaesthetized cats, of the nictitating membrane to respond to postganglionic stimulation of the cervical sympathetic nerve; relaxation of the nictitating membranes of conscious cats; and suppression of the inhibition, produced by stimulation of the sympathetic nerves, of the pendular movements of isolated preparations of rabbit ileum (Finkleman, 1930). In all three tests there are wide variations in activity with small changes in structure, and some of the compounds are among the most potent adrenergic neurone blocking agents yet described. Of particular interest are the two optical isomers of *N*-(1-phenylethyl)guanidine (general formula, $R_1=R_2=H$, $X=-CH(CH_3)-$). These compounds show a similar order of activity on the Finkleman preparation, but in cats only the (−)-isomer has high activity and the (+)-isomer antagonizes the blocking action of the (−)-isomer. The pharmacology of these two compounds is described in greater detail than that of the other compounds studied.

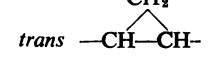
METHODS

Compounds

Structures, empirical formulae, uncorrected melting points (m.p.) and analytical data are listed in Table 1. A few of the compounds have been described previously: *N*-benzylguanidine sulphate by Davis & Elderfield (1932), m.p. 204° C (*cf.* 206° C, Table 1, compound I), *N*-phenethylguanidine sulphate by Braun (1933), m.p. 175–177° C (*cf.* 178–181° C, compound II), *N*-(3-phenylpropyl)guanidine sulphate by

TABLE 1

STRUCTURES, PHYSICAL PROPERTIES AND ANALYTICAL DATA FOR THE ARALKYLGUANIDINE SALTS

	Compound No.	Structural variation in general formula	Empirical formula	Melting point (°C)	Analysis C Calc.	C Found	H Calc.	H Found	N Calc.	N Found
Group I		*Alkyl group* –X–								
N-(*Phenylalkyl*)-*guanidines* (R_1=R_2=H)	I	$-CH_2-$	$C_8H_{11}N_3$, $\frac{1}{2}H_2SO_4$	206						
	II	$-CH_2.CH_2-$	$C_9H_{13}N_3$, $\frac{1}{2}H_2SO_4$	178–181						
	III	$-CH_2.CH_2.CH_2-$	$C_{10}H_{15}N_3$, $\frac{1}{2}H_2SO_4$	176–177						
	IV	$-CH(CH_3)-$	$C_9H_{13}N_3$, HNO_3	124–125	47·8	47·9	6·2	6·6	24·8	24·6
	V	$(+)-CH(CH_3)-$	$C_9H_{13}N_3$, HNO_3	91–92	47·8	48·0	6·2	6·4	24·8	24·6
	VI	$(-)-CH(CH_3)-$	$C_9H_{13}N_3$, HNO_3	91–92	47·8	47·8	6·2	6·2	24·8	24·6
	VII	$-CH(C_2H_5)-$	$C_{10}H_{15}N_3$, HNO_3	93–94	50·0	50·4	6·7	6·7	23·3	23·2
	VIII	$-CH(CH_3).CH_2-$	$C_{10}H_{15}N_3$, $\frac{1}{2}H_2SO_4$, H_2O	217	49·2	49·6	7·4	7·3	17·2	17·3
	IX	*trans* 	$C_{10}H_{13}N_3$, $\frac{1}{2}H_2SO_4$	216–217	53·6	53·6	6·3	6·4	18·7	18·9
Group II		*Ring substituent* R_1								
N-*Benzylguanidines* (R_2=H, X=$-CH_2-$)	X	*p*–CH_3	$C_9H_{13}N_3$, $\frac{1}{2}H_2SO_4$	229–230	50·9	50·8	6·7	6·8	19·8	19·9
	XI	*p*–CH_3	$C_8H_{10}ClN_3$, $\frac{1}{2}H_2SO_4$	225–226	41·3	41·2	4·8	4·9	18·1	18·0
	XII	2,4-$(CH_3)_2$	$C_{10}H_{15}N_3$, $\frac{1}{2}H_2SO_4$, $\frac{1}{4}H_2O$	233–235	52·1	49·7	7·4	7·6	17·5	17·8
Group III	XIII	*p*–CH_3	$C_{10}H_{15}N_3$, $\frac{1}{2}H_2SO_4$	285	53·1	53·1	7·1	7·2	18·6	18·7
N-(1-*Phenylethyl*)-*guanidines*	XIV	$(+)$-*p*–CH_3	$C_{10}H_{15}N_3$, $\frac{1}{2}H_2SO_4$	275–277	53·1	52·9	7·1	7·2	18·6	18·2
	XV	$(-)$-*p*–CH_3	$C_{10}H_{15}N_3$, $\frac{1}{2}H_2SO_4$	269	53·1	53·2	7·1	7·1	18·6	18·4
(R_2=H, X=$-CH(CH_3)-$)	XVI	*p*–Cl	$C_9H_{12}ClN_3$, $\frac{1}{2}H_2SO_4$	279–280	43·8	43·7	5·3	5·3	17·0	16·9
	XVII	*p*–CH_3O	$C_{10}H_{15}N_3O$, $\frac{1}{2}H_2SO_4$	245–246	49·6	49·5	6·7	6·6	17·4	17·3
	XVIII	*o*–CH_3	$C_{10}H_{15}N_3$, $\frac{1}{2}H_2SO_4$	286–287	53·1	52·9	7·1	7·2	18·6	18·7
	XIX	*m*–CH_3	$C_{10}H_{15}N_3$, $\frac{1}{2}H_2SO_4$	265–268	53·1	52·8	7·1	7·1	18·6	18·4
	XX	2,4-$(CH_3)_2$	$C_{11}H_{17}N_3$, $\frac{1}{2}H_2SO_4$	276–277	55·0	55·1	7·6	7·7	17·5	17·3
Group IV		R_1 R_2 X								
Miscellaneous	XXI	*p*–Cl H $-CH(C_2H_5)-$	$C_{19}H_{14}ClN_3$, $\frac{1}{2}H_2SO_4$, $\frac{1}{2}H_2O$	240–243	44·5	44·8	6·0	6·0	15·6	15·5
	XXII	*p*–CH_3O H $-CH_2.CH(CH_3)-$	$C_{11}H_{17}N_3O$, $\frac{1}{2}H_2SO_4$	210	51·5	51·5	7·1	7·2	16·4	16·2
	XXIII	H CH_3 $-CH_2-$	$C_9H_{13}N_3$, $\frac{1}{2}H_2SO_4$	252–253						

Braun & Randell (1934), m.p. 173–174° C (*cf.* 176–177° C, compound III), *N*-(1-phenylethyl)guanidine nitrate by Bobeck (1931), m.p. 123–124° C (*cf.* 124–125° C, compound IV), *N*-(*p*-chlorobenzyl)guanidine sulphate by Short, Biermacher, Dunnigan & Leth (1963), m.p. 220–222° C (*cf.* 225–226° C, compound XI) and *N*-benzyl-*N*-methylguanidine sulphate by Buck, Baltzly & Ferry (1942), m.p. 253° C (*cf.* 252–253° C, compound XXIII).

Preparation of the guanidines

The *N*-aralkylguanidine salts listed in Table 1 were prepared from the corresponding amines by heating them in water with a slight molar excess of *S*-methylthiuronium sulphate (Philips & Clarke, 1923). The aralkylguanidine sulphate generally crystallized out on cooling the reaction mixture, but occasionally either this did not happen or it gave a very impure product. In these cases the guanidine was first isolated from the reaction mixture as a bicarbonate salt, which was then reconverted into a sulphate or nitrate. The following preparation of (−)-*N*-(1-phenylethyl)guanidine nitrate (Table 1, compound VI) is typical. (−)-1-Phenylethylamine (15 g), *S*-methylthiuronium sulphate (16.8 g) and water (30 ml.) were heated on a water-bath. Methyl mercaptan, which was absorbed in a cooled trap containing charcoal impregnated with cupric chloride (Hill & Wolfrom, 1947), began to be evolved at about 70° C. The temperature of the water-bath was raised to 100° C during about 1 hr and maintained at that temperature for a further 30 min. When cool, no crystals had appeared in the reaction mixture. It was consequently treated with potassium bicarbonate (12 g) in water (60 ml.). The precipitate was filtered off, sucked partly dry and then added carefully to hot, stirred dilute nitric acid (2 N, 60 ml.). On cooling, (−)-*N*-(1-phenylethyl)guanidine nitrate (10 g, m.p. 90–92° C) crystallized out. Recrystallization from water gave the pure product, m.p. 92–93° C.

Instead of using the free amine it is sometimes preferable to use an amine salt, particularly where the free base is not very stable. In this case, the reaction mixture must be initially basified with concentrated aqueous ammonia solution, and at the end of the reaction neutralized with sulphuric acid. *trans*-*N*-(2-Phenylcyclopropyl)guanidine sulphate was prepared in this way from *trans*-2-phenylcyclopropylamine sulphate.

The amines used for the preparation of compounds I to IV, IX to XII, XXII and XXIII were available commercially. The α-substituted benzylamines required for making compounds VII, XIII and XVI to XXI were prepared by a Leuckart reaction from the appropriate phenyl alkyl ketone (Ingersoll, 1943a). 2-Phenylpropylamine, the amine used for the preparation of compound VIII, was prepared by reduction with sodium amalgam of hydrotropic aldehyde oxime.

1-Phenylethylamine was resolved with malic acid and tartaric acid (Ingersoll, 1943b) and 1-*p*-tolylethylamine with camphoric acid (Ingersoll & Burns, 1932). The optically active guanidines, compounds V, VI, XIV and XV, were prepared in the normal manner from the corresponding optically active amines. Optical rotations were determined using sodium light and an ETL-NPL Automatic Polarimeter, Type 143A (Ericsson Telephones). The *N*-(1-phenylethyl)guanidine salts were made up in 0.1 M solution in water and had molecular rotations at 23.5° C of +50° (V) and −49.5° (VI). The *N*(1-*p*-tolylethyl)guanidine salts were made up in water at a concentration of 0.025 M and had molecular rotations at 26.5° C of +56° (XIV) and −54° (XV).

Pharmacological methods

The methods used were similar to those described by Fielden, Roe & Willey (1964). All doses are expressed as weights of salts.

Experiments on anaesthetized cats. Anaesthesia was usually induced with ether and maintained with chloralose (80 to 100 mg/kg, intravenously). Blood pressure was recorded from a femoral artery with a mercury manometer. Drugs were injected into a cannulated femoral vein. Contractions of the nictitating membrane were recorded with an isotonic frontal-writing lever. Heart rate was recorded with a Thorp impulse counter triggered by the R wave of the electrocardiogram, or by a fluid switch operated by a Cushny myocardiograph.

The effect of the optical isomers of *N*-(1-phenylethyl)guanidine on the noradrenaline content of splenic venous blood, collected during stimulation of the splenic nerves, was studied by the method of Brown &

Gillespie (1957). The splenic nerves were stimulated with 50 shocks/sec for 10-sec periods and the blood was collected for 15 sec from the start of the stimulation. The pressor activity of the plasma was assayed on the blood pressure of pithed rats with (−)-noradrenaline bitartrate as the standard.

Blood flow through the femoral artery was recorded with a modified version of the density flowmeter (Dawes, Mott & Vane, 1953). Sympathetic vasoconstrictor responses were produced by stimulating the lumbar sympathetic nerve trunk at a point distal to the 4th lumbar ganglion.

The effect of stimulation of the chorda tympani or cervical sympathetic nerves on salivary secretion was studied by cannulating the right submaxillary duct and allowing the saliva to displace saline from a reservoir through a fine-bore drop tube.

The response of the uterus to hypogastric nerve stimulation was recorded by fixing the cervical end of the right horn *in situ* and attaching the other end to a heavily loaded lever.

Experiments on unanaesthetized cats. Changes in the sympathetic tone were observed at intervals following subcutaneous injection of the aralkylguanidines by measuring on photographic records the fraction of the eye covered by the nictitating membrane.

Experiments on the rabbit isolated ileum. The effect of the aralkylguanidines was studied on the inhibition, produced by mesenteric nerve stimulation, of the pendular movements of the rabbit isolated ileum. The nerve was stimulated with 50 shocks/sec for 30 sec every 10 min.

RESULTS

Structure-activity relationships

Table 2 summarizes the activities of the compounds studied (structures in Table 1) on the three test systems used. In all three tests the adrenergic neurone block, once produced, is long-lasting and in neither the Finkleman nor the anaesthetized cat nictitating membrane preparations does recovery occur within the duration of the experiment. Consequently, only one drug can be tested on any one preparation. As there is some variability in the

TABLE 2

ADRENERGIC NEURONE BLOCKING ACTIVITIES OF SOME *N*-ARALKYLGUANIDINES

The intravenous dose of compound required to abolish the response of the nictitating membrane to postganglionic stimulation within the specified time interval is shown in the first section. The second section shows the subcutaneous dose required to cause 30% relaxation of the nictitating membranes of conscious cats when measured 6 hr after administration of the drug (the compounds marked with an asterisk were given intravenously). The final section shows the concentration required to abolish the response of the rabbit ileum to periarterial nerve stimulation

Nictitating membrane preparation			Conscious cat		Finkleman preparation	
Dose (mg/kg)	Time interval for abolition of response (min)	Compounds	Dose (mg/kg)	Compounds	Concentration (μg/ml.)	Compounds
2	0–30	VI, XV	1·25–2·5	VI, XV, XVI, XVIII, XIX	0·3	XIV, XV, XVIII
5	0–10	XIII, XIV, XVI, XVII, XX	2·5–5	VII, XIII, XIV, XVII	1	IV, V, VI, XVI, XVII
10	0–10	IV, XII, XVIII, XIX	5–10	XII, XX	3	X, XIII, XIX, XX
10	10–120	II, VII, X, XXII	10–25		10	II, III, XI, XII, XXI
25	0–120	I, III, VIII, XI, XXIII	25–50	X	30	I, VII, VIII
			Inactive at 25	I*, II*, III*, IX, XXI, XXII	100	XXII, XXIII
			Inactive at 50	IV, V, VIII, XI, XXIII	Inactive at 100	IX

results obtained on different preparations, small differences in potency cannot be established without using impractically large numbers of animals. Because of this limitation, for each of the three tests the compounds have been arranged into groups of approximately equal potency.

On the anaesthetized cat nictitating membrane preparation, activity is assessed in terms of the intravenous dose of drug required to abolish, in the specified time, the response to postganglionic stimulation of the cervical sympathetic nerve. Activity in conscious cats is assessed in terms of the subcutaneous dose of drug required to cause 30% relaxation of the nictitating membranes in 6 hr; that is, approximately half maximal relaxation. The activity on the isolated rabbit ileum is assessed by the minimum concentration of the drug which abolishes the response to periarterial nerve stimulation.

Although all results are expressed in terms of weight per ml. or per kg, the molecular weight range of the compounds studied is relatively small and no significant change in relative activities would be found if these were expressed on a molar basis.

In the column of results for anaesthetized cats, compounds V, IX and XXI are omitted. Compound V does have some blocking action at a low dose (approximately 50% at 2 mg/kg), but increasing the dose does not appear to increase the extent of the block. Furthermore, because of the strong membrane contraction produced by the drug itself, it is impossible to say whether complete block is ever produced. Compound IX is lethal at 10 to 25 mg/kg but at these doses gives no indication of block. Compound XXI causes approximately 50% reduction of the response in 30 min at 5 mg/kg, but this dose eventually causes death. With the exception of the unsubstituted straight-chain *N*-(ω-phenylalkyl)guanidines (that is, compounds I, II and III), all compounds which inhibit the response of the nictitating membrane to nerve stimulation either do not affect the responses to adrenaline or, more generally, increase such responses. Compounds I, II and III antagonize the response of the nictitating membranes to adrenaline.

Of the *N*-benzylguanidines, only *N*-(2,4-dimethylbenzyl)guanidine (XII) shows significant activity in conscious cats, although *N*-(*p*-methylbenzyl)guanidine (X) is fairly active in anaesthetized cats.

In contrast to the *N*-benzylguanidines, substituted *N*-(1-phenylethyl)guanidines are potent adrenergic neurone blocking drugs. However, the racemic unsubstituted compound, *N*-(1-phenylethyl)guanidine (IV), is almost inactive in conscious cats, as is the (+)-isomer (V), but the (−)-isomer (VI) is very active both in conscious cats and on anaesthetized cat preparations. On the other hand, both isomers of *N*-(1-*p*-tolylethyl)guanidine (XIV and XV) are active drugs, as is the racemic mixture (XIII).

In experiments on conscious cats, the extent of membrane relaxation is normally assessed after 6 hr. At this time, some of the ring-substituted analogues of racemic *N*-(1-phenylethyl)guanidine (XIII, XVI–XIX) show anomalous dose/response lines. Although marked relaxation is apparent with small doses of the drug, the extent of membrane relaxation is much less with larger doses. However, with intermediate doses, relaxation is more marked 24 to 48 hr after the drug, at a time when the effect of smaller doses has virtually disappeared. This effect is very marked with compound XIX; 2.5 mg/kg causes a maximal relaxation after 6 hr which then persists for about 24 hr, but 6 hr after 5 mg/kg there is only slight membrane relaxation, and after 10 mg/kg there is none. Maximal

relaxation does, however, appear 24 to 48 hr after 5 or 10 mg/kg. With compounds XVI or XIII this delayed onset of relaxation is only apparent when doses of 20 mg/kg or more are injected.

Compound XIII has been resolved and the two optical isomers studied. The (−)-isomer (XV) shows a normal dose/response relationship, although at 20 mg/kg there is a slight decrease in the response compared with that produced by 10 mg/kg. In contrast, however, the (+)-isomer (XIV) causes no effect 6 hr after doses above 5 mg/kg, although this dose and 10 mg/kg cause marked effects at 24 hr; 20 mg/kg is without apparent effect at either time.

Pharmacology of the optical isomers of N-(1-*phenylethyl*)*guanidine*

Effects on the nictitating membrane. Intravenous injection of 2 mg/kg of (−)-*N*-(1-phenylethyl)guanidine (VI) abolishes the contraction of the nictitating membrane to stimulation of the postganglionic cervical sympathetic nerve, without affecting the tone of the unstimulated membrane. These effects differ markedly from those produced by intra-

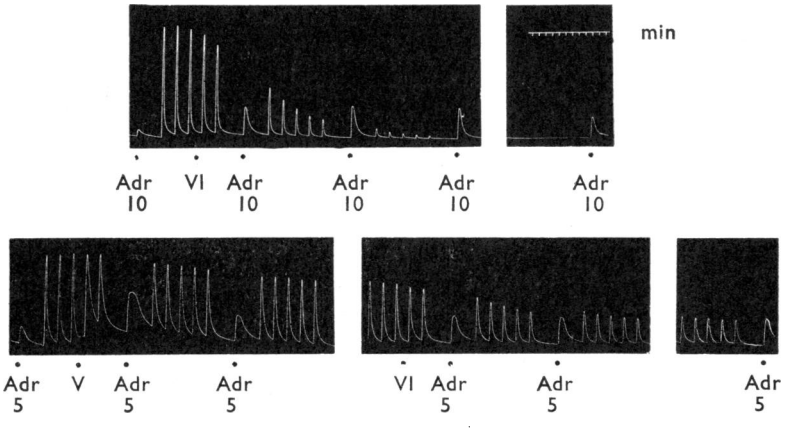


Fig. 1. Records of the contractions of the nictitating membranes of two cats treated with atropine and anaesthetized with chloralose. Adrenaline was injected intravenously (5 or 10 μg) at Adr. The postganglionic cervical sympathetic nerves were stimulated for 10 sec every 2 min except when adrenaline was injected. The upper records show the abolition of the responses to electrical stimulation by 2 mg/kg of compound VI with an increase in the response to adrenaline. Interval between the two upper traces, 45 min. The lower records show the slight diminution of the response to electrical stimulation by 2 mg/kg of compound V together with a direct stimulant action on the nictitating membrane, and also demonstrates the weaker blocking action of 2 mg/kg of the (−)-isomer (VI) when preceded by the (+)-isomer (V). Intervals between the lower traces, 20 and 65 min respectively.

venous injection of 2 mg/kg of the (+)-isomer (V), which does not abolish the response to stimulation of the postganglionic cervical sympathetic nerve and which usually causes a well-sustained contraction of the nictitating membrane (see Fig. 1). The response to intravenous injection of adrenaline is increased by both compounds.

Similar results are obtained when the relaxation of the nictitating membrane in conscious cats is used as an indicator of adrenergic neurone blocking activity. Maximal relaxation of the nictitating membrane follows subcutaneous injection of 2.5 mg/kg of compound VI,

whereas injection of up to 50 mg/kg of compound V causes no relaxation. Moreover, it has been demonstrated, in conscious and in anaesthetized cats, that administration of the (+)-isomer antagonizes the adrenergic neurone blocking activity of the (−)-isomer. In one experiment, 5 mg/kg of compound VI, which is capable of causing relaxation of the nictitating membrane for over 24 hr, were administered subcutaneously to four conscious cats. Three of the cats were immediately injected with compound V (1.0, 2.5 or 5 mg/kg), the remaining cat serving as a control. The nictitating membranes of the cat receiving only the (−)-isomer rapidly relaxed, but there was no apparent effect on the cat receiving equal parts of the two isomers and the membranes of the other two cats relaxed only slightly. In other experiments, when the (+)-isomer (5 mg/kg) was given to cats previously treated with 5 mg/kg of the (−)-isomer, the relaxed membranes quickly retracted. Similar effects on the anaesthetized cat are illustrated in Fig. 1. The upper trace shows the adrenergic neurone blocking action of 2 mg/kg of the (−)-isomer; and the lower trace shows that this dose causes much less effect when preceded by 2 mg/kg of the (+)-isomer.

Cardiovascular effects. Intravenous injection of compound V (2.5 mg/kg) causes a prolonged rise in the arterial blood pressure and an increase in the heart rate of supine anaesthetized cats. This is in contradistinction to compound VI (2 mg/kg), which usually causes a prolonged fall in the blood pressure and only a transient increase in the heart rate. The blood pressure responses to intravenous injections of adrenaline, noradrenaline or dimethylphenylpiperazinium are increased by both isomers, whereas the response to tyramine is reduced.

Injection of compound VI (1 mg/kg) causes a transient reduction in the blood flow in the femoral artery of anaesthetized cats, probably as a result of the lowered blood pressure.

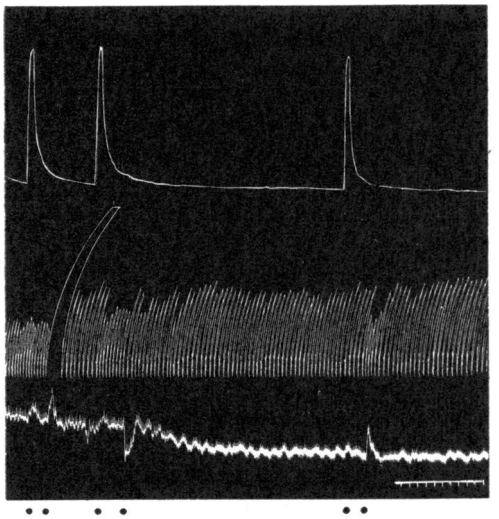


Fig. 2. Records of the blood pressure, blood flow in the left femoral artery, and the tone of the right nictitating membrane of a cat anaesthetized with chloralose. At CS, stimulation of the preganglionic cervical sympathetic nerve at 50 shocks/sec for 15 sec; at LS, stimulation of the left lumbar sympathetic nerve. Compound VI (1 mg/kg) abolished the decreased blood flow resulting from nerve stimulation but had little effect on the contractions of the nictitating membrane.

Reduction of the blood flow in the femoral artery resulting from stimulation of the lumbar sympathetic chain is blocked by this dose. This is illustrated in Fig. 2, which also shows that the response of the nictitating membrane to preganglionic stimulation is only slightly affected although the vascular response to stimulation of the sympathetic chain is completely abolished. However, 1 mg/kg of compound V causes only a slight and transient block of the decreased blood flow on sympathetic stimulation, 5 mg/kg being required to cause complete block.

The injection of compound VI (2 mg/kg) abolishes the hypertension caused by stimulation of the periarterial mesenteric nerves without affecting the pressor response to stimulation of the left splanchnic nerves. On the other hand, the (+)-isomer is without effect at this dose.

Compound VI, but not compound V, reduces the pressor response to carotid occlusion and the tachycardia which follows stimulation of the nervi accelerantes. Fig. 3 illustrates the effect of 0.1 to 0.5 mg/kg of compound VI on the cardiac response to stimulation of the nervi accelerantes, and to intravenous injection of adrenaline.

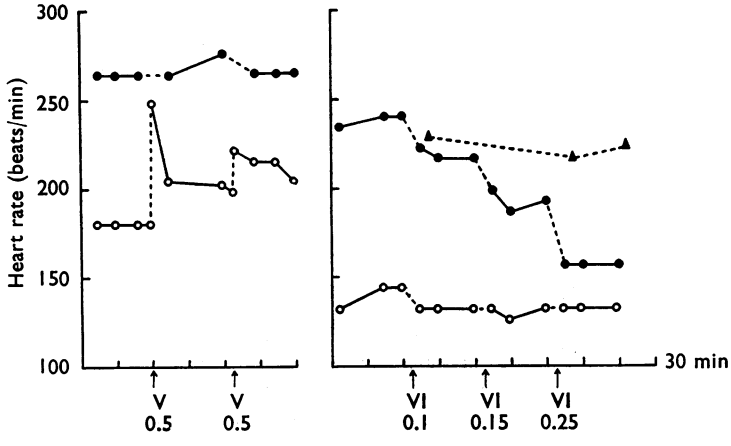


Fig. 3. Heart rate in anaesthetized cats. ○——○ Resting heart rate; ●——● effect of stimulation of the nervi accelerantes; ▲——▲ effect of adrenaline (10 μg). Compound V (0.5 mg/kg, intravenously) followed by a further dose (0.5 mg/kg) had no effect on the response to stimulation of the nervi accelerantes, although each dose caused cardiac acceleration. Compound VI (total dose, 0.5 mg/kg) virtually abolished the response to sympathetic stimulation, without affecting the response to adrenaline. Abscissa, 30-min intervals; doses in mg/kg.

Effect on sympathetic inhibitory responses. Relaxation of the nonpregnant cat uterus on stimulation of the hypogastric nerve is partially blocked by 1 mg/kg, and almost abolished by 2.5 mg/kg of compound VI (Fig. 4), whereas the inhibition caused by adrenaline is not affected. Compound V is also active but less so than compound VI; 2 mg/kg causes some reduction of the response to nerve stimulation, and 10 mg/kg almost abolishes the response. Differences are also apparent between the (+)-isomer and the (−)-isomer in the response of the uterus to the drug itself; compound VI relaxes the uterus, whereas compound V causes a contraction.

The inhibition of the pendular movements of the rabbit isolated ileum by stimulation of the mesenteric nerves is abolished by both these compounds, at concentrations of 1 to

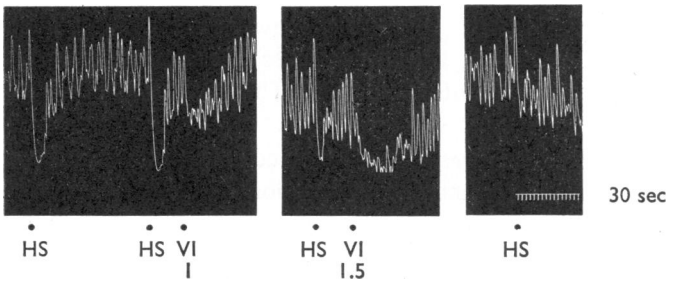


Fig. 4. Record of the uterine tone of a nonpregnant cat anaesthetized with chloralose. At HS, stimulation of the hypogastric nerves at 50 shocks/sec for 15 sec. The first record shows the effect of 1 mg/kg of compound VI; the second record shows the effect of a further 1.5 mg/kg injected 1 hr later; and the final record the effect of hypogastric nerve stimulation 30 min after the second dose. The response to sympathetic nerve stimulation was inhibited by compound VI, which itself relaxed the uterus.

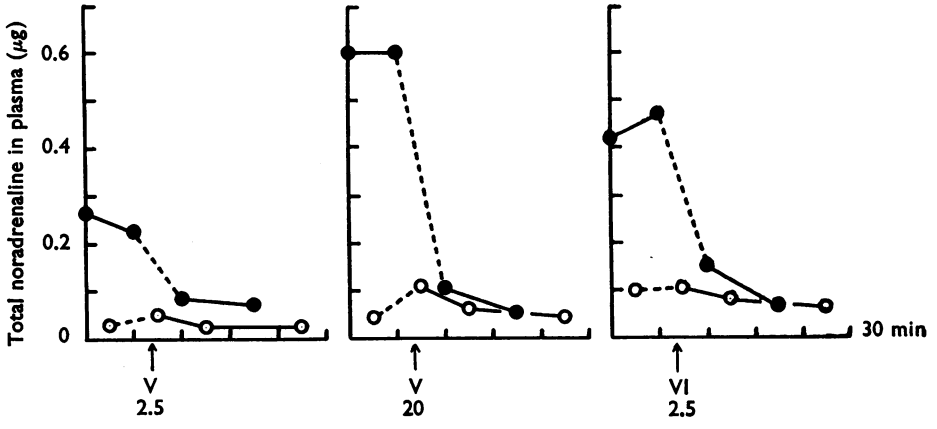


Fig. 5. The noradrenaline contents of blood samples collected from the splenic veins of cats anaesthetized with chloralose. O——O Represents resting samples collected for periods of 15 sec; ●——● represents samples collected during stimulation (50 shocks/sec) of the splenic nerves for 10 sec and for the following 5 sec. These graphs show that 2.5 mg/kg of compound VI completely inhibits the release of noradrenaline, whereas 2.5 mg/kg of compound V only partially inhibits the release. Total inhibition is achieved by 20 mg/kg of compound V. Abscissa, 30-min intervals; doses in mg/kg.

2 μg/ml., without reducing the inhibitory response to adrenaline. In contrast to the antagonistic effects shown by the two isomers on anaesthetized and on conscious cats, no antagonism can be demonstrated on this preparation.

Effect on the noradrenaline output from the spleen. Stimulation of the splenic nerves increases the noradrenaline content of the venous effluent from the spleen. Intravenous injection of compound VI (2.5 mg/kg) or compound V (20 mg/kg) reduces the amount of noradrenaline released so that plasma samples collected 65 min after either drug contain approximately the same amount of noradrenaline as do those collected in the absence of sitmulation (Fig. 5).

Fig. 5 shows that, while both drugs are effective in abolishing the stimulated release of noradrenaline, injection of the (+)-isomer increases the noradrenaline content of the venous effluent collected in the absence of stimulation. This effect is not shown following injection of 2.5 mg/kg of the (−)-isomer.

Effect on parasympathetic nerves. The increased salivary flow in response to stimulation of the chorda tympani nerve, and the contraction of the stomach on stimulation of the ventral oesophageal branch of the vagus nerve, are unaffected by either compound V or compound VI (2 mg/kg). The effects of sympathetic nerve stimulation on the salivary glands and stomach are, however, abolished by these doses. For example, 0.5 to 1 mg/kg of the (−)-isomer or 1 to 2 mg/kg of the (+)-isomer abolish the effects of sympathetic nerve stimulation on salivary flow.

Effects on tissue noradrenaline. At 6 hr after a single subcutaneous injection of compound V or compound VI (20 mg/kg), the noradrenaline content of the hearts of male rats or mice is somewhat lowered, but the reduction (about 20%) is only slight compared with that (about 90%) produced by a similar dose of guanethidine. The noradrenaline was extracted from the tissue essentially by the method of Shore & Olin (1958) and estimated spectrophotofluorimetrically after oxidation with potassium ferricyanide (Euler & Lishajko, 1961).

Other actions. Oral administration of compound VI (200 mg/kg) to conscious male mice causes mydriasis and a reduction in locomotor activity. Ptosis is apparent in both rats and mice after subcutaneous doses of 20 mg/kg of compound VI, but not after 20 mg/kg of the (+)-isomer (V).

Both isomers reduce the response to indirect stimulation of the gastrocnemius muscle of cats anaesthetized with chloralose. The (−)-isomer has about one-tenth, and the (+)-isomer about one-twentieth, of the activity of (+)-tubocurarine. This effect of both isomers is much shorter in duration than that of (+)-tubocurarine. Contractions of the rat isolated diaphragm to supramaximal stimulation of the phrenic nerve are abolished by 0.4 mg/ml. of compound VI, but contractions to direct stimulation of the muscle are unaffected.

Oral administration of compound VI (100 mg/kg) to unanaesthetized dogs causes respiratory distress and muscle inco-ordination; 300 mg/kg is lethal. In male mice, the LD50 of compound V is 17 mg/kg intravenously, and that of compound VI is 30 mg/kg.

Neither compound in concentrations up to 100 μg/ml. shows a spasmogenic effect on the guinea-pig isolated ileum. Compound V does not antagonize the spasmogenic actions of histamine, acetylcholine, dimethylphenylpiperazinium or barium, but 100 μg/ml. of compound VI depress the spasmogenic effects of histamine and dimethylphenylpiperazinium.

DISCUSSION

In general the relative activities (given in Table 2) of the *N*-aralkylguanidines fall into much the same order on the three test systems used—anaesthetized-cat nictitating membranes, conscious-cat nictitating membranes, and the Finkleman preparation. The only striking exception is (+)-*N*(1-phenylethyl)guanidine (V), which is as active as the (−)-isomer (VI) on the Finkleman preparation, but is inactive in conscious cats. Some of the compounds show weak activity in anaesthetized cats, but are inactive in conscious cats at the highest dose tested. This may be because of higher sympathetic tone in conscious animals, or because the weak adrenergic neurone blockade is masked by the intrinsic membrane contracting effect of the compounds.

In the unsubstituted *N*-phenylalkylguanidines pronounced adrenergic neurone blocking activity appears only in the α-substituted *N*-benzylguanidines—notably in (−)-*N*-(1-phenylethyl)guanidine (VI). (±)-*N*-(1-Phenylpropyl)guanidine (VII) is less active than the racemic 1-phenylethyl compound (IV) on anaesthetized cats and on Finkleman preparations, but is more active in conscious cats. There is some indication that in the straight-chain series (I to III) optimal activity is reached at *N*-phenethylguanidine (II). *N*-(2-Phenylpropyl)guanidine (VIII) is virtually inactive and *N*-(2-phenylcyclopropyl)guanidine (IX) is completely inactive. The unsubstituted α-methylphenethyl compound was not prepared, but its *p*-methoxy derivative (XXII) has only weak activity, whereas *N*-(1-*p*-methoxyphenylethyl)guanidine (XVII) is highly active.

Introduction of either a chloro-atom or methyl groups into the benzene ring of benzylguanidine causes some, although only slight, increase in activity. Introduction of a chloro-atom or methyl or methoxyl groups into (±)-*N*-(1-phenylethyl)guanidine considerably increases the activity, although in no instance is activity raised significantly above that of (−)-*N*-(1-phenylethyl)guanidine. The most likely reason for this enhancement is illustrated by the activities of the two optical isomers of *N*-(1-*p*-tolylethyl)guanidine. Whereas the two isomers of the unsubstituted compound are markedly different and in fact antagonize one another, the two isomers of the *p*-methyl compound are roughly equiactive and are not antagonistic. Thus the marked rise in activity of the racemic compounds on ring substitution is probably due to a sharp fall in the antagonistic action of the (+)-isomer rather than to an increase in the inherent blocking activity.

Of the compounds tested, the optical isomers of unsubstituted *N*-(1-phenylethyl)-guanidine appear the most interesting and these have been studied in greater detail. The antagonism, which was originally deduced from the low activity of the racemic mixture compared with that of the (−)-isomer, can be shown directly by giving a dose of the (+)-isomer followed by the (−)-isomer. The (+)-isomer, which itself causes little or no blockade of nervous function, will prevent the expected action of an ordinarily active dose of the (−)-isomer.

The potent adrenergic neurone blocking action of the (−)-isomer is apparent on all the sympathetically innervated systems that have been tested. The effects of parasympathetic nerve stimulation are unimpaired by doses which cause adrenergic neurone blockade. Direct evidence that (−)-*N*-(1-phenylethyl)guanidine blocks the release of noradrenaline from sympathetic nerve endings was obtained by measuring the increase in noradrenaline content of splenic venous blood after nerve stimulation. Complete abolition of this increased noradrenaline content is observed following injection of 2.5 mg/kg of the (−)-isomer.

The adrenergic neurone blocking properties of (+)-*N*-(1-phenylethyl)guanidine form a much less consistent picture. This compound has only a relatively weak blocking action on the nictitating membrane of anaesthetized cats. On the other hand, it does produce adrenergic neurone blockade in many other systems although it is usually less active than the (−)-isomer. Thus, 5 mg/kg blocks the vasoconstrictor response to stimulation of the lumbar sympathetic chain, compared with 1 mg/kg of the (−)-isomer; 10 mg/kg abolishes the relaxation of the nonpregnant cat uterus due to stimulation of the hypogastric nerve, compared with 2.5 mg/kg of the (−)-isomer; and 20 mg/kg is required to abolish the

increased output of noradrenaline from the spleen on sympathetic nerve stimulation—that is, approximately ten-times the effective dose of the (−)-isomer. However, both isomers are equiactive on the Finkleman preparation. Like the (−)-isomer, the (+)isomer potentiates the pressor effects of adrenaline, noradrenaline and dimethylphenylpiperazinium and reduces that due to tyramine. Also, like the (−)-isomer, adrenergic neurone blocking doses of the (+)-isomer have no effect on parasympathetic nerves.

(+)-*N*-(1-Phenylethyl)guanidine produces marked sympathomimetic effects which include a rise of blood pressure, tachycardia and contraction of the nictitating membranes, whereas the (−)-isomer causes a fall in the blood pressure, very transient tachycardia and has no effect on the tone of the nictitating membranes. In contrast to the (−)-isomer, the (+)-isomer causes a contraction and not a relaxation of the cat uterus.

On systems, such as the blood pressure, where the two isomers have opposite effects, the racemic mixture generally behaves in a manner intermediate between the two. This may, or may not, indicate antagonism at a single site. However, on the nictitating membrane preparation, the ability of the (+)-isomer to prevent the adrenergic neurone blocking action of the (−)-isomer does suggest that the two drugs may be acting in different ways at a single site.

In a preliminary attempt to see whether these effects are due to differences in action of the two isomers on noradrenaline levels in sympathetically innervated tissues, rats were injected subcutaneously with 20 mg/kg of either compound, killed 6 hr later and the noradrenaline content of the hearts was estimated. Both isomers produced a slight (20%) reduction in the noradrenaline content but this may not be significant. The (−)-isomer caused appreciable ptosis whereas the (+)-isomer did not, showing that in rats, too, the two isomers behave differently. Further work is in progress to try to elucidate the biochemical basis of the differences between these two compounds.

SUMMARY

1. Twenty-three *N*-aralkylguanidines have been synthesized and tested for adrenergic neurone blocking activity on nictitating membranes of conscious or anaesthetized cats, and on Finkleman preparations.

2. Comparable blocking activities were generally shown on all three test systems, the most active compounds being ring-substituted *N*-(1-phenylethyl)guanidines.

3. The optical isomers of *N*-(1-phenylethyl)guanidine also blocked other excitatory and inhibitory responses to sympathetic nerve stimulation and inhibited noradrenaline release on stimulation of cat splenic nerves. (+)-*N*-(1-Phenylethyl)guanidine generally had much weaker blocking activity and could antagonize the potent blocking action of (−)-*N*-(1-phenylethyl)guanidine on nictitating membranes.

4. Neither isomer of *N*-(1-phenylethyl)guanidine significantly reduced rat or mouse heart noradrenaline contents.

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