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THE EFFECTS OF SOME ARALKYLGUANIDINES IN MICE

BY

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Although transmission at postganglionic adrenergic nerve endings can be blocked by a wide variety of guanidine derivatives (Mull, Egbert & Dapero, 1960; Boura, Copp, Green, Hodson, Ruffell, Sim & Walton, 1961; Barron, Bavin, Durant, Natoff, Spickett & Vallance, 1963; Augstein & Green, 1964), considerable specificity is often found among guanidine derivatives with closely related structures. The adrenergic neurone blocking activity of *N*-aralkylguanidines in cats has been shown (Fielden, Green & Willey, 1965) to depend markedly on the nature of the alkyl side-chain, on substitution in the aromatic nucleus, and on the stereochemistry of individual pairs of optical isomers. In the present paper we show that *N*-aralkylguanidines display a similar pattern of specificity when their adrenergic neurone blocking activity is assessed in mice by the production of ptosis, and we have attempted to relate this specificity to some biochemical action.

METHODS

Drugs. The *N*-aralkylguanidines were prepared as described previously (Fielden *et al.*, 1965). Guanethidine sulphate was kindly supplied by Ciba Laboratories, Horsham. All dosages are given in terms of the weight of sulphate or nitrate salt.

Animal experiments. The drugs were dissolved in 0.9% saline and injected in a volume of 10 ml./kg into male mice (weight range 24 to 30 g). The extent of ptosis was estimated by direct observation. For determination of noradrenaline content, the hearts from groups of six mice were pooled. The animals were killed by cervical dislocation, the hearts were removed, rinsed with water, blotted, placed in small polyethylene bags and frozen in a mixture of acetone and solid carbon dioxide. The tissue was stored in solid carbon dioxide overnight, then converted into a pellet using a die and punch (Callingham & Cass, 1963). The pellet was weighed, then dropped into a mixture of butanol (30 ml., purified as described by Shore & Olin, 1958), sodium chloride (3 to 4 g), ascorbic acid (0.2%, 0.1 ml.) and 0.01 N-hydrochloric acid (2.9 ml. minus the weight in g of the heart pellet). The tissue pellet was ground up and the mixture was shaken for 1 hr. After centrifugation, 20 ml. of the butanol layer was added to purified heptane (35 ml.) and 0.01 N-hydrochloric acid (2.5 ml.). This mixture was shaken for 5 min, then centrifuged. The acid layer was pipetted off and the noradrenaline was assayed fluorimetrically, using 2-mercaptopropionic acid as a stabilizing agent for the fluorescence (Palmer, 1963). To 0.4 ml. of the acid layer was added 0.1 ml. of sodium acetate buffer (2 M, *p*H 6) followed by 0.1 ml. of potassium ferricyanide (0.05%). After 3 min, 0.6 ml. of alkaline ascorbate (1 ml. of 2% ascorbic acid solution, 1 ml. of 2-mercaptopropionic acid and 8 ml. of 5 N-sodium hydroxide solution) was added. The fluorescence intensity (activation wavelength 405 mμ, fluorescence wavelength 540 mμ, uncorrected instrumental wavelengths) was read 10 to 30 min later using an Aminco-Bowman spectrophotofluorimeter. Blanks were obtained by reversing the order of addition of the ferricyanide and alkaline ascorbate. In each day's experiment a control group of untreated mice was run in addition to the groups of treated mice.

Monoamine oxidase inhibition. Monoamine oxidase activity was determined by the dinitrophenylhydrazine method (Green & Haughton, 1961). A suspension of guinea-pig liver mitochondria in sodium phosphate buffer (0.1 M, *p*H 7.4) was used as the enzyme source (Green, 1962). 0.4 ml. of various concentrations of the inhibitor were added to the enzyme (2.8 ml. of an approximately 25-fold dilution of the stock enzyme suspension with buffer solution) 15 min before addition of 0.8 ml. of a neutralized solution of tyramine (5 mM) and semicarbazide (62.5 mM). The mixture was shaken at 25° C for 30 min, and then 1 ml. of acetic acid (0.5 N) was added to terminate the reaction. The remaining steps in the assay were as already described (Green & Haughton, 1961).

Dopamine-β-oxidase inhibition. Dopamine-β-oxidase was extracted from bovine adrenal medullae and assayed by the spectrophotometric method (van der Schoot, Creveling, Nagatsu & Udenfriend, 1963) using the reaction conditions described previously (Green, 1964). Various concentrations of inhibitor were incubated at *p*H 6.4 and 37° C for 10 min with the enzyme and cofactors before the addition of sufficient tyramine to give a final substrate concentration of 1 mM.

RESULTS

Ptosis and heart noradrenaline levels

The effect of the *N*-aralkylguanidines (20 mg/kg, subcutaneously) on mouse heart noradrenaline levels 6 or 24 hr after injection is shown in Table 1. Results for guanethidine are included for comparison. The adrenergic neurone blocking action in the same animals was roughly assessed by noting the extent of ptosis. Ptosis was recorded 2 hr, as well as 6 and 24 hr, after the drug. The noradrenaline contents of the hearts of treated mice were calculated as a percentage of the noradrenaline in the hearts of a control group of untreated mice run at the same time. Untreated mice had a heart noradrenaline content of 0.64 γ/g$\pm$0.06 (mean and standard deviation, fifteen groups). The ptosis readings are only approximate, and little significance can be attached to variations of less than 20% in the heart noradrenaline content determined on a single group of mice. Hence, for both measurements, a simplified scoring system was used for tabulating the results. For the ptosis, scores of +, ++ or +++ signify average eyelid closure approximately 0.25, 0.5 or 0.75, respectively. For the depletion, scores of +, ++ or +++ signify 20 to 40, 40 to 60 or over 60%, respectively, reduction in noradrenaline content.

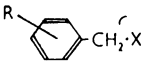
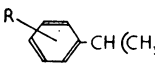
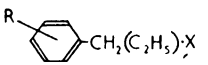
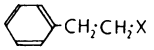
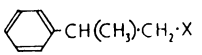
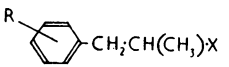
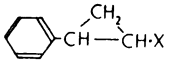
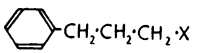
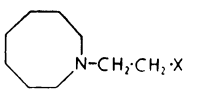
The extent of ptosis often varied widely between individual mice, but a reproducible average value was generally obtained when the same experiment was repeated on different days. Sometimes, however, there were erratic and unexplained variations. For example, 20 mg/kg of guanethidine almost invariably produced marked and prolonged ptosis, but on one occasion several groups of mice treated with this dose of drug showed no more than slight ptosis. Similarly, (+)-*N*-(1-phenylethyl)guanidine normally produced no significant ptosis, yet in a few experiments slight to moderate ptosis appeared. Unexpected changes in the duration of ptosis were also sometimes found. For example, the effect of 20 mg/kg of (−)-*N*-(1-phenylethyl)guanidine or (−)-*N*-(1-*p*-tolylethyl)guanidine usually lasted 6 to 12 hr, but occasionally recovery was much quicker and ptosis virtually vanished in under 6 hr. These erratic variations generally occurred in all the groups of animals injected on a particular day, and anomalous results of this kind could almost always be detected by some inconsistency with previous or subsequent work. They have been ignored in compiling Table 1.

As can be seen from Table 1, the onset of ptosis was always fast. Maximal activity was almost always displayed within 2 hr, and the effect was usually wearing off within 6 hr.

TABLE 1

PTOSIS AND HEART NORADRENALINE DEPLETION PRODUCED BY GUANIDINE DERIVATIVES IN MICE

The mice were killed 6 or 24 hr after 20 mg/kg of drug (subcutaneously). Ptosis and noradrenaline depletion are scored on the following scales: for ptosis 0=negligible, +=slight, ++=moderate and +++=marked; for noradrenaline content (% of normal) 0>80, +=60–80, ++=40–60 and +++<40

Parent compound	Structure [X=–NH.C(:NH).NH$_2$]	Ring substituent R	Salt	Ptosis after 2 hr	Ptosis after 6 hr	Ptosis after 24 hr	Depletion after 6 hr	Depletion after 24 hr
N-Benzylguanidine		H	Sulphate	0	0	0	0	+
		p-CH_3	Sulphate	+	0	0	0	+
		p-Cl	Sulphate	+	0	0	+	+
		2,4-$(CH_3)_2$	Sulphate	++	+	0	0	0
N-(1-Phenylethyl)guanidine		H	Nitrate	+	+	0	0	+
		H (+)-isomer	Nitrate	0	0	0	+	+
		H (−)-isomer	Nitrate	++	++	0	+	+
		p-CH_3	Sulphate	+++	++	0	0	+
		p-CH_3 (+)-isomer	Sulphate	+++	+++	0	0	+
		p-CH_3 (−)-isomer	Sulphate	+++	++	0	0	0
		o-CH_3	Sulphate	+++	++	0	+	0
		m-CH_3	Sulphate	++	+	0	0	0
		p-Cl	Sulphate	+++	+++	+	++	+++
		p-CH_3O	Sulphate	+++	++	0	0	0
		2,4-$(CH_3)_2$	Sulphate	+++	++	0	0	0
N (1-Phenylpropyl)guanidine		H	Nitrate	+	0	0	+	0
		p-Cl	Sulphate	0	0	0	+	0
N-Phenethylguanidine			Sulphate	0	0	0	+++	++
N-(2-Phenylpropyl)guanidine			Sulphate	0	0	0	++	0
N-(α-Methylphenethyl)guanidine		*p*-CH_3O	Sulphate	0	0	0	0	+
N-(2-Phenylcyclopropyl)guanidine			Sulphate	0	0	0	+	++
N-(3-Phenylpropyl)guanidine			Sulphate	0	0	0	++	+
Guanethidine			Sulphate	+++	+++	+	+++	++

Only two of the compounds, *N*-(1-*p*-chlorophenylethyl)guanidine and guanethidine, produced ptosis lasting 24 hr. The effect of 20 mg/kg of *N*-(1-*p*-chlorophenylethyl)guanidine was in fact detectable up to 48 hr after injection.

The most potent of the aralkylguanidines as a depleting agent was *N*-phenethylguanidine, a compound which produced no ptosis after 2, 6 or 24 hr. In a more detailed study of this compound it was found that even 3 mg/kg would produce over 60% depletion in 4 hr, yet in doses up to 30 mg/kg no more than marginal ptosis could be detected.

The absence of correlation between ptosis and depletion is further illustrated by Fig. 1 in which is shown the dependence of ptosis and heart noradrenaline depletion on the dose of guanethidine or *N*-(1-*p*-chlorophenylethyl)guanidine 4 hr after injection. In the experiments shown in all the Figures, a slightly different method of scoring ptosis was used.

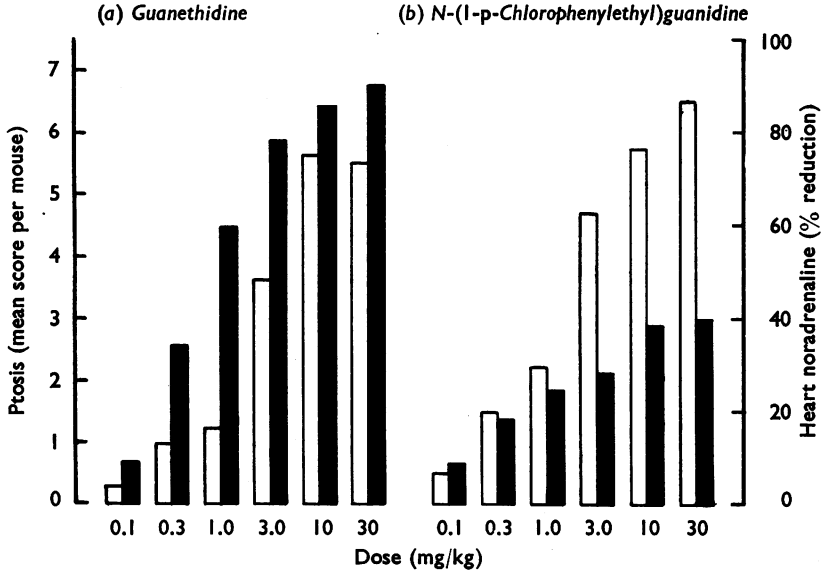


Fig. 1. Effect of dose of guanethidine (*a*) or *N*-(1-*p*-chlorophenylethyl)guanidine (*b*) on the extent of ptosis (open columns) and heart noradrenaline (closed columns) 4 hr after subcutaneous injection. Each observation was on a group of six mice.

Each eye of each mouse was assessed separately on a scale of 0 to 4 and the final result is given as an average value per mouse. This procedure is essentially that of Rubin, Malone, Waugh & Burke (1957). Although a maximum score of 8 is theoretically possible, complete eyelid closure of all the mice in a group was never approached with any dose of any of the drugs used.

With the two pairs of optical isomers, *N*-(1-phenylethyl)guanidine and *N*-(1-*p*-tolylethyl)guanidine, the depletion was in all cases small, but the extent of ptosis varied with the isomer and the time after administration. This is illustrated in Fig. 2 which shows the extent of ptosis at various times after 5 mg/kg of (+)-, (−)- and (±)-*N*-(1-*p*-tolylethyl)-guanidine. The (−)-isomer was much the shortest acting, and the racemic mixture produced an effect approximately equivalent to the mean of that produced by the two isomers. In another experiment in which 20 mg/kg of the (−)- or (+)-isomer were injected, both

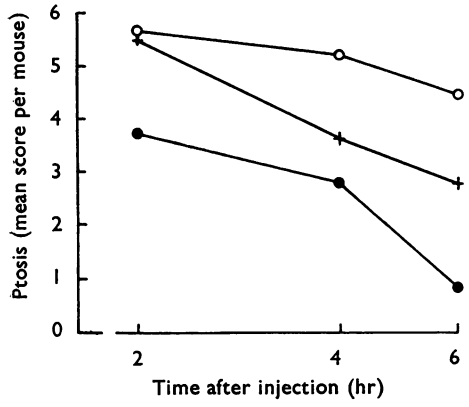


Fig. 2. Effect of time after subcutaneous injection on the extent of ptosis produced by (−)-*N*-(1-*p*-tolylethyl)guanidine (●), (+)-*N*-(1-*p*-tolylethyl)guanidine (○), or the racemic mixture (+). All three drugs given at 5 mg/kg to groups of ten mice.

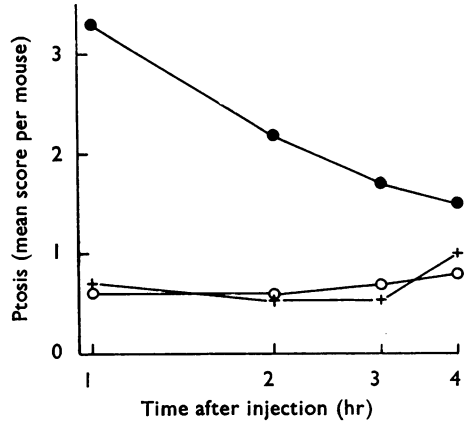


Fig. 3. Effect of time after subcutaneous injection on the extent of ptosis produced by (−)-*N*-(1-phenylethyl)guanidine (10 mg/kg) (●), (+)-*N*-(1-phenylethyl)guanidine (10 mg/kg) (○), or the racemic mixture (20 mg/kg) (+). Each observation was on a group of ten mice.

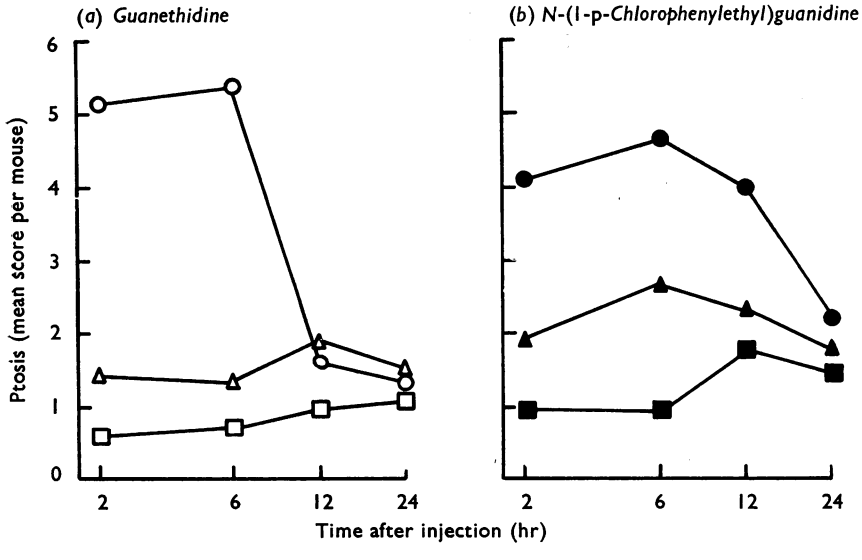


Fig. 4. Effect of giving (+)-*N*-(1-phenylethyl)guanidine or *N*-(2-phenylcyclopropyl)guanidine together with guanethidine (*a*) or *N*-(1-*p*-chlorophenylethyl)guanidine (*b*) on the extent of ptosis at various times after subcutaneous injection. Each observation was on a group of ten mice. ○, Guanethidine (10 mg/kg) alone; △, guanethidine (10 mg/kg) plus (+)-*N*-(1-phenylethyl)guanidine (20 mg/kg); □, guanethidine (10 mg/kg) plus *N*-(2-phenylcyclopropyl)guanidine (20 mg/kg); ●, *N*-(1-*p*-chlorophenylethyl)guanidine (10 mg/kg) alone; ▲, *N*-(1-*p*-chlorophenylethyl)guanidine (10 mg/kg) plus (+)-*N*-(1-phenylethyl)guanidine (20 mg/kg); and ■, *N*-(1-*p*-chlorophenylethyl)guanidine (10 mg/kg) plus *N*-(2-phenylcyclopropyl)guanidine (20 mg/kg).

isomers produced about the same extent of ptosis 1 hr after injection, but the effect of the (−)-isomer was again less persistent. A different result was obtained with the unsubstituted *N*-(1-phenylethyl)guanidine, where both the (+)-isomer and the racemic mixture generally produced negligible ptosis, as illustrated by the experiment in Fig. 3. These results suggested that, as happens in cats (Fielden *et al.*, 1965), the (+)-isomer was antagonizing the adrenergic neurone blockade due to the (−)-isomer. This was confirmed directly by giving the (+)-isomer (20 mg/kg) before a normally highly active dose (20 mg/kg) of the (−)-isomer, when virtually no ptosis was produced. Fig. 4 shows that (+)-*N*-(1-phenylethyl)guanidine will also effectively prevent ptosis due to guanethidine or *N*-(1-*p*-chlorophenylethyl)guanidine. *trans*-*N*-(2-Phenylcyclopropyl)guanidine, which has no adrenergic neurone blocking action of its own, is even more effective as an antagonist of the block due to other compounds. Fig. 4 also illustrates the longer duration of action of *N*-(1-*p*-chlorophenylethyl)guanidine, compared with that of guanethidine.

Enzyme experiments

The potency of the *N*-aralkylguanidines as monoamine oxidase inhibitors is summarized in Table 2, in which is given the extent of inhibition at various concentrations, and, by interpolation, the concentration required to give 50% inhibition. In these experiments the inhibitor was incubated with the enzyme for 15 min before addition of the substrate (1 mM-tyramine) but this incubation is not necessary for inhibition to occur. The effect of

TABLE 2

INHIBITION OF MONOAMINE OXIDASE BY *N*-ARALKYLGUANIDINES

Various concentrations of the *N*-aralkylguanidines (0·4 ml.) were incubated at 25° C and *p*H 7·4 with an approximately 25-fold dilution of the mitochondrial enzyme preparation in phosphate buffer (2·8 ml.) for 15 min before addition of 10 mM-tyramine (0·4 ml.) and 125 mM-semicarbazide (0·4 ml.). The mixture was then shaken in air for 30 min. The approximate concentration of guanidine derivative giving 50% inhibition was obtained by interpolation. Complete structures of all the compounds are given in Table 1

Parent compound	Ring substituent	Inhibition (%) at concentration (μM) 1,000	200	100	40	10	Concentration giving 50% inhibition (μM)
N-Benzylguanidine	H	66	28		12		500
	p-CH_3	93	72		22		100
	p-Cl	85	65		23		100
	2,4-$(CH_3)_2$	94	82		55		30
N-(1-Phenylethyl)guanidine	H	63		35		0	400
	H (+)-isomer	60		31		0	400
	H (−)-isomer	42		14		0	1,000
	p-CH_3	80		43		7	250
	p-CH_3 (+)-isomer	81		37		0	250
	p-CH_3 (−)-isomer	80		32		0	250
	o-CH_3	70	56	47	43	23	100
	m-CH_3	99	57		24		150
	p-Cl	81	62		30		100
	p-CH_3O	85	71		29		100
	2,4-$(CH_3)_2$	89		59		24	60
N-(1-Phenylpropyl)guanidine	H	50		16		2	1,000
	p-Cl	73		31		7	250
N-Phenethylguanidine	H	74		45		15	150
N-(2-Phenylpropyl)guanidine	H	73		32		2	300
N-(α-Methylphenethyl)guanidine	*p*-CH_3O	97		73		29	30
N-(2-Phenylcyclopropyl)guanidine	H	79		29		4	250
N-(3-Phenylpropyl)guanidine	H	79		45		11	150

varying the substrate concentration on the extent of inhibition was examined with only two of the compounds, *N*-phenethylguanidine and (+)-*N*-(1-phenylethyl)guanidine. In both cases inhibition was inversely related to the substrate concentration. For example, *N*-phenethylguanidine (100 μM) gave 30% inhibition in the presence of 50 mM-tyramine and 60% with 5 mM-tyramine. However, this dependence on substrate concentration was not quantitatively consistent with purely competitive inhibition. The variation in the extent of inhibition with inhibitor concentration, as given in Table 2, also suggests some noncompetitive component in the inhibitory action, particularly with compounds such as *N*-(1-*o*-tolylethyl)guanidine which show an unusually shallow concentration/response curve.

A number of compounds, including all the depleting agents, were tested as inhibitors of dopamine-β-oxidase. However, in the presence of 1 mM-tyramine as substrate, none of the following, in concentrations up to 1 mM, gave more than 20% inhibition: guanethidine, (+)- or (−)-*N*-(1-phenylethyl)guanidine, *N*-(1-*p*-methoxy- or -*p*-chloro-phenyl)ethylguanidine, *N*-(1-*p*-tolylethyl)guanidine, *N*-[1-(2,4-xylyl)ethyl]guanidine, *N*-*p*-methylbenzylguanidine, *N*-(*p*-methoxy-α-methylphenethyl)guanidine, *N*-phenethylguanidine or *N*-(2-phenylcyclopropyl)guanidine.

DISCUSSION

Although ptosis is an indication of sympatholytic action it may result from a variety of causes other than adrenergic neurone blockade. However, in the present series of compounds there is a good correlation between relative activity in mice, as measured by the appearance of ptosis, and the adrenergic neurone blocking action in conscious cats, as measured by relaxation of the nictitating membranes (Fielden *et al.*, 1965). The action in mice is, however, generally much briefer. The most active compounds in both species are the ring-substituted *N*-(1-phenylethyl)guanidines, and the only unsubstituted phenylalkylguanidine to show appreciable activity is (−)-*N*-(1-phenylethyl)guanidine. In the benzylguanidine series, ring substitution shows comparable effects in the two species, activity being negligible in the unsubstituted compound, slight in the monosubstituted compounds and moderate in the 2,4-dimethyl-derivative. A further similarity in the two species is the antagonistic effect of (+)-*N*-(1-phenylethyl)guanidine towards the action of (−)-*N*-(1-phenylethyl)guanidine, which latter produces no effect in animals previously treated with the (+)-isomer. From this discussion it would seem justified to regard ptosis as a reasonable measure of adrenergic neurone blocking activity in the present series of compounds.

The absence of any general correlation between heart noradrenaline depletion and adrenergic neurone blockade is shown by the results in Table 1. Many of the *N*-(1-phenylethyl)guanidines produced marked ptosis unaccompanied by depletion, whereas *N*-phenethylguanidine, which has a depleting action approaching that of guanethidine, produced negligible ptosis even at 30 mg/kg. That *N*-phenethylguanidine is a much more active depleting agent than benzylguanidine or *N*-(3-phenylpropyl)guanidine is consistent with the results of Costa, Kuntzman, Gessa & Brodie (1962) in rats. But these workers also reported that in rats *N*-benzylguanidine and *N*-phenethylguanidine produced marked ptosis. This observation is in contrast to our results in mice or in cats. Guanethidine and *N*-(1-*p*-chlorophenylethyl)guanidine produce both ptosis and depletion; but, as shown

in Fig. 1, guanethidine produces appreciable depletion at doses below those producing significant ptosis, whereas the reverse is true for *N*-(1-*p*-chlorophenylethyl)guanidine. Zaimis (1964) has justly criticized much of the published work which purports to show that noradrenaline depletion underlies the adrenergic neurone blocking action and antihypertensive action of guanethidine, in that the doses of guanethidine used to produce depletion have generally been far higher than those needed to elicit pharmacological effects. However, as is shown in Fig. 1, 50% depletion of mouse heart noradrenaline can be produced by as little as 1 mg/kg of guanethidine. This dose is below that producing significant ptosis and is of the same order as the therapeutic dose for chronic use of guanethidine in man (Julian, 1961).

The antagonism by (+)-*N*-(1-phenylethyl)guanidine of ptosis due to the (−)-isomer does not appear to be directly associated with an effect on noradrenaline levels since (+)-, (−)- and (±)-*N*-(1-phenylethyl)guanidine all produce about the same small degree of depletion (about 20%, 6 hr after 20 mg/kg). This antagonistic action is not confined to optical isomers, since treatment with (+)-*N*-(1-phenylethyl)guanidine also prevents ptosis due to *N*-(1-*p*-chlorophenylethyl)guanidine or guanethidine (Fig. 4). Both optical isomers of *N*-(1-*p*-tolylethyl)guanidine are active adrenergic neurone blocking drugs and differ mainly in having different durations of action (Fig. 2).

Antagonism of ptosis due to (−)-*N*-(1-phenylethyl)guanidine, *N*-(1-*p*-chlorophenylethyl)-guanidine or guanethidine is also found with *N*-(2-phenylcyclopropyl)guanidine, which is a moderate depleting agent (although depleting only slowly) but one which does not itself produce ptosis. As shown in Fig. 4, this drug is in fact rather more potent than is (+)-*N*-(1-phenylethyl)guanidine as an antagonist.

A wide range of adrenergic neurone blocking agents has already been shown to inhibit monoamine oxidase (Dvornik, Kraml, Dubuc, Tom & Zsoter, 1962; Kuntzman & Jacobson, 1963). All the *N*-aralkylguanidines in the present series also have this action (Table 2), but there is little correlation between monoamine oxidase inhibition and adrenergic neurone blockade or heart noradrenaline depletion. In particular, the monoamine oxidase inhibitory action of substituted *N*-(1-phenylethyl)guanidines is generally slightly weaker than that of corresponding *N*-benzylguanidines, whereas the former are much the more active as adrenergic neurone blocking agents. *N*-(*p*-Methoxy-α-methylphenethyl)guanidine, related to amphetamine, is about the most potent of our compounds as a monoamine oxidase inhibitor, yet has only a weak noradrenaline depleting action and no adrenergic neurone blocking action.

The general pattern of inhibitory action agrees quite well with that obtained by Kuntzman & Jacobson (1963), who used mouse heart mitochondria instead of guinea-pig liver mitochondria as a source of enzyme and 5-hydroxytryptamine instead of tyramine as substrate, and that found by Dvornik *et al.* (1962) who used rat, cat or guinea-pig liver mitochondria with kynuramine as substrate. In the compounds without a ring substituent, the presence of a two-carbon chain between the phenyl- and guanidino-groups bestows greater activity than is found in compounds with only one carbon atom separating these two groups. In both the *N*-benzylguanidine and *N*-(1-phenylethyl)guanidine series ring substitution enhances activity, the most active compounds being in each case the 2,4-dimethyl derivative. *N*-(2-Phenylcyclopropyl)guanidine is a relatively weak inhibitor and differs markedly in this respect from its parent amine, 2-phenylcyclopropylamine, which is one of the most

potent monoamine oxidase inhibitors known (Sarkar, Banerjee, Ise & Zeller, 1960). Kuntzman & Jacobson (1963) reported that inhibition of monoamine oxidase in mouse heart mitochondria by *N*-*o*-chlorobenzyl-*N'N"*-dimethylguanidine was reversible, competitive with 5-hydroxytryptamine and did not require previous incubation with the enzyme to get maximal inhibition. Our results with *N*-phenethylguanidine and (+)-*N*-(1-phenylethyl)guanidine agree with these findings, although we did not find inhibition of tyramine oxidation to be quantitatively consistent with fully competitive inhibition.

Some preliminary work on the mechanism of action of the adrenergic neurone blocking agent, choline 2,6-xylyl ether (xylocholine), suggested that this drug might act by inhibiting the hydroxylation of dopamine to noradrenaline (Bain & Fielden, 1957). It has since been shown that xylocholine does not significantly inhibit dopamine-β-oxidase in chromaffin granules from ox adrenal glands or in human phaeochromocytoma tissue (Hagen & Zebrowski, 1962). Eleven of the present series of compounds, including compounds which did or did not deplete heart noradrenaline, or which did or did not produce adrenergic neurone blockade, have been tested as inhibitors of partly purified dopamine-β-oxidase from ox adrenal glands, but no significant inhibition was produced by any of them.

Our investigation has thus shown that the appearance of ptosis in mice provides a simple and useful guide to the adrenergic neurone blocking action of compounds of the guanidine type, but we have been unable to relate this action to tissue noradrenaline depletion, nor to inhibition of monoamine oxidase or dopamine-β-oxidase.

SUMMARY

1. The effect of twenty-two *N*-aralkylguanidines on the noradrenaline content of mouse hearts has been compared with their ability to produce ptosis.

2. The extent of ptosis correlated well with relaxation by the same compounds of cat nictitating membranes, but not with heart noradrenaline depletion. The most potent depleting agent, *N*-phenethylguanidine, did not cause ptosis, whereas the most effective ptosis-producing compounds, ring-substituted *N*-(1-phenylethyl)guanidines, generally did not deplete.

3. Ptosis due to (−)-*N*-(1-phenylethyl)guanidine, *N*-(1-*p*-chlorophenylethyl)guanidine or guanethidine was markedly diminished in mice previously treated with (+)-*N*-(1-phenylethyl)guanidine or *N*-(2-phenylcyclopropyl)guanidine.

4. All the *N*-aralkylguanidines inhibited monoamine oxidase, the most potent being *N*-(2,4-dimethylbenzyl)guanidine and *N*-(*p*-methoxy-α-methylphenethyl)guanidine. No correlation was found between monoamine oxidase inhibition and ptosis or heart noradrenaline depletion.

5. Dopamine-β-oxidase was not inhibited by these compounds.

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