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A comparison of aralkylamines and aralkylguanidines as antagonists of adrenergic neurone blockade

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Structure-activity relationships have been studied for aralkylamines and aralkylguanidines which restore the responses of the nictitating membranes to nerve stimulation in anaesthetised cats given sympathetic blocking drugs. This reversing action was largely specific for adrenergic neurone blockade; blockade of sympathetic ganglia or of α-adrenergic receptors was unaffected. (+)-Amphetamine was the most active amine and *N*-benzyl-*N*-methylguanidine was the most active guanidine. In mice, ptosis resulting from adrenergic neurone blockade was much more readily prevented or abolished by the aralkylamines and aralkylguanidines than was ptosis caused by other types of sympathetic blocking agent. The most potent antagonist of ptosis was *N*-(2-phenylcyclopropyl)guanidine which was about ten times as active as amphetamine. The relative antagonistic potencies of 2 amines and 8 guanidines were virtually identical for all types of adrenergic neurone blocking drug, regardless of whether or not they cause noradrenaline depletion. The prevention of guanethidine-induced ptosis was always accompanied by some reduction in the extent of heart-noradrenaline depletion, but the minimum dose of antagonist required to prevent ptosis completely was always lower than that required to eliminate depletion.

AMPHETAMINE and some other sympathomimetic amines are known to counteract adrenergic neurone blockade produced by xylocholine, bretylium or guanethidine (Bain & Fielden, 1956; Day, 1962). We have shown (Fielden, Green & Willey, 1965; Fielden & Green, 1965) that adrenergic neurone blockade could also be prevented, and in some circumstances reversed, by certain aralkylguanidines. The mechanism of this reversing action for one particular compound, (+)-*N*-(1-phenylethyl) guanidine, has been discussed in detail elsewhere (Fielden & Green, 1966). In the present paper we have compared a variety of aralkylamines and aralkylguanidines as antagonists to different types of adrenergic neurone blocking agent in cats and mice in an attempt to obtain further insight into the mechanism of adrenergic neurone blockade.

Experimental

METHODS

Cats were anaesthetised with chloralose (100 mg/kg, intravenously). The contractions of both nictitating membranes were recorded on smoked paper by frontal writing levers (magnification 15 times, load 3–4 g). The preganglionic cervical sympathetic nerves were periodically stimulated, through shielded platinum electrodes, with 200 rectangular pulses (0·5 msec duration, 10 to 15 V amplitude) at frequencies of 1, 3, 10 or 30/sec. Drugs were dissolved in 0·9% saline and injected through a cannula into a femoral vein.

Groups of 6 male mice (weight range 20 to 30 g) were injected subcutaneously with the drugs dissolved in sufficient 0·9% saline to give a volume of 10 ml/kg. Ptosis was estimated by direct observation on a 0 to 8 scale (Rubin, Malone, Waugh & Burke, 1957; Fielden & Green,

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1966). The hearts from the 6 mice were pooled and the noradrenaline was extracted with butanol and assayed fluorimetrically (Fielden & Green, 1965). The heart-noradrenaline content of treated mice was calculated as a percentage of that in control groups treated with saline alone.

The aralkylguanidines were prepared as described by Fielden & others (1965). Doses are expressed in terms of free amine or guanidine base except where the salt is specifically mentioned.

Results

STRUCTURE-ACTIVITY RELATIONSHIPS FOR ANTAGONISM OF ADRENERGIC NEURONE BLOCKADE IN CATS

Antagonistic potency was assessed in anaesthetised cats from reversal of the blocking action of (−)-*N*-(1-phenylethyl)guanidine sulphate (2 mg/kg) on the responses of the nictitating membranes to sympathetic nerve stimulation. This drug is a powerful adrenergic neurone blocking agent in anaesthetised cats, but, unlike many related drugs, it has little contracting action of its own on the nictitating membranes (Fielden & others, 1965), hence it is well suited to quantitative reversal studies. A typical experiment is shown in Fig. 1. The response of the nictitating membranes at all rates of stimulation up to 30 pulses/sec was greatly reduced 1 hr after the (−)-*N*-(1-phenylethyl)guanidine. The antagonist was then injected intravenously and the responses of the membranes were re-examined 30 min later. If recovery was incomplete, additional doses of antagonist were given. The percentage recovery was generally much the same at all rates of stimulation, but with large doses of the most potent amines, the responses to low rates of nerve stimulation after recovery from block were sometimes greater than those observed before injection of the adrenergic neurone blocking drug. These amines did not potentiate responses to nerve stimulation when given alone. The relative potency of each antagonist was estimated from the total dose required to cause 50% recovery in the responses to stimulation at 10 pulses/sec. Each drug was tested on the contractions of both membranes in 2 or 3 cats. The doses listed in Table 1 are only approximate, since some variations occurred with different cats. *N*-Phenethylguanidine (IIIb) and

TABLE 1. REVERSAL BY ARALKYLAMINES AND ARALKYLGUANIDINES OF ADRENERGIC NEURONE BLOCKADE PRODUCED BY (−)-*N*-(1-PHENYLETHYL)GUANIDINE

X and R in Ph·X·NRZ		Dose (mg/kg) causing 50% reversal of block			
X	R	Amine (Z = H)	Cpd No.	Guanidine (Z = $C\langle^{NH}_{NH_2}$)	Cpd No.
$-CH_2-$	H	No effect at 6	Ia	3	Ib
$-CH_2-$	Me	30% recovery at 10	IIa	0·2–0·4	IIb
$-(CH_2)_2-$	H	No effect at 6	IIIa	Blocks (see text)	IIIb
$-(CH_2)_3-$	H	No effect at 13	IVa	No effect at 6	IVb
(+)-CH(Me)–	H	4	Va	Blocks (see text)	Vb
$-CH(Me)CH_2-$	H	0·4	VIa	1·5–3	VIb
(+)-CH_2CH(Me)–	H	0·01–0·02	VIIa	10% recovery at 2	VIIb
CH_2 / –CH——CH–	H	0·05–0·1	VIIIa	0·5–1	VIIIb

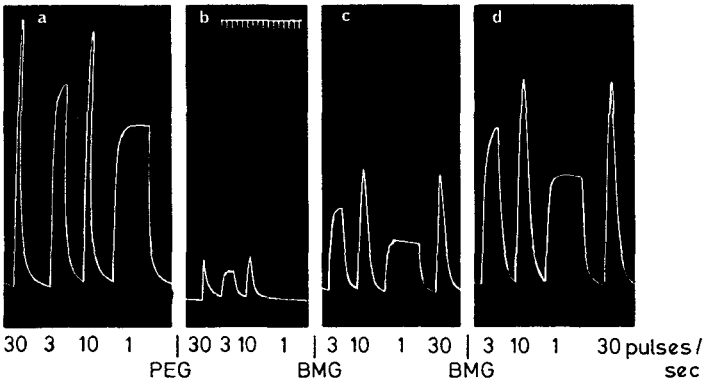
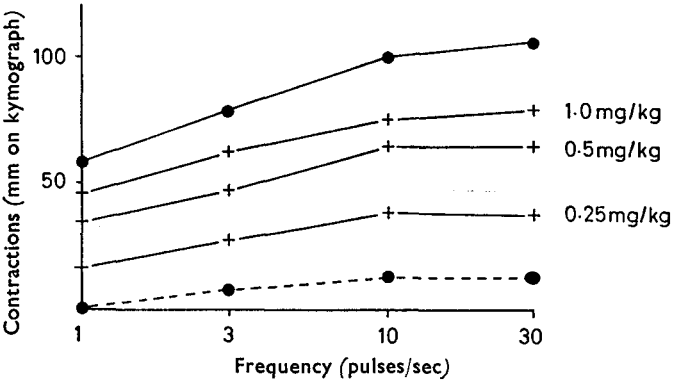


FIG. 1. A. Effect of drugs on contractions of the nictitating membranes produced by stimulation of the cervical sympathetic nerve in cats anaesthetised with chloralose. 200 Rectangular pulses (15V, 0·5 msec) were applied at frequencies of 1, 3, 10 or 30/sec. (a) Contractions of the right nictitating membrane before drugs. (b) 1 hr after 2 mg/kg of (−)-*N*-(1-phenylethyl)guanidine sulphate (PEG). Panels (c) and (d) show the responses 30 min after two consecutive doses of 0·25 mg/kg of *N*-benzyl-*N*-methylguanidine sulphate (BMG). (Time: 30 sec).



B. Mean contractions (mm on kymograph) of both membranes:— ●——● responses before drugs, ●- - -● responses 1 hr after PEG, +——+ responses 30 min after subsequent consecutive injections of 0·25, 0·25 and 0·5 mg/kg of BMG, giving the total doses specified on the graphs.

(+)-*N*-(1-phenylethyl)guanidine (Vb) exert some blocking action of their own in anaesthetised cats, hence reversal could not be conclusively demonstrated. However, we have shown previously that (+)-*N*-(1-phenylethyl)guanidine will prevent the blocking action of the (−)-isomer in anaesthetised cats if given either beforehand or simultaneously with it (Fielden & others, 1965). The parent amine of (−)-*N*-(1-phenylethyl)-guanidine, namely (−)-1-phenylethylamine, produced 50% reversal at about 1 mg/kg.

Although all the potent amines in Table 1 are primary amines, reversal of block also occurred readily with some secondary and tertiary amines;

for example, *N*-methylamphetamine produced 50% reversal at about 0·05 mg/kg, and *NN*-dimethylamphetamine at about 0·15 mg/kg.

Reversal experiments with guanethidine as the blocking drug gave essentially similar results, although additional effects sometimes arose which rendered interpretation more difficult. Blockade by guanethidine sulphate (4 mg/kg) was half-reversed by 0·05 to 0·1 mg/kg of (+)-amphetamine (VIIa) or by 0·5 mg/kg of *N*-benzyl-*N*-methylguanidine (IIb). However, although *N*-(2-phenylpropyl)guanidine (VIb) (0·5–2 mg/kg) or *N*-(2-phenylcyclopropyl)guanidine (VIIIb) (0·5–2mg/kg) caused some restoration of responses after guanethidine, there was, at the same time, a pronounced and sustained contraction of the unstimulated nictitating membranes which made it impossible to assess quantititatively the response to nerve stimulation. These sustained contractions did not occur when either of these two drugs was injected into cats which had not had guanethidine.

(+)-Amphetamine and *N*-benzyl-*N*-methylguanidine failed to restore contractions of the nictitating membranes blocked by pempidine (5 mg/kg) or by phenoxybenzamine (2·5 mg/kg).

STRUCTURE-ACTIVITY RELATIONSHIPS FOR ANTAGONISM OF PTOSIS IN MICE

Antagonism experiments in mice were done to compare the potency of aralkylguanidines as antagonists of those adrenergic neurone blocking agents which also deplete the peripheral tissues of noradrenaline, such as guanethidine, with their potency against blocking agents which do not markedly lower noradrenaline levels, such as (−)-*N*-(1-phenylethyl)-guanidine. As the latter has only a short-lasting action in mice, the related, more persistent drug, *N*-[1-(2,4-xylyl)ethyl]guanidine (Fielden & Green, 1965), was used instead. Table 2 shows the relative potencies of eight aralkylguanidines and two aralkylamines in preventing ptosis when given subcutaneously to mice together with 10 mg/kg of guanethidine sulphate or *N*-[1-(2,4-xylyl)ethyl]guanidine sulphate. We have shown previously (Fielden & Green, 1965) that adrenergic neurone blockade in mice can be assessed from the extent of ptosis. None of the aralkylguanidines used as antagonists caused significant ptosis in mice when

TABLE 2. PREVENTION BY ARALKYLGUANIDINES AND ARALKYLAMINES OF PTOSIS PRODUCED BY ADRENERGIC NEURONE BLOCKADE IN MICE

Antagonist $Ph{\cdot}X{\cdot}N(R){\cdot}C(:NH)NH_2$			Approximate dose of drug causing 50% reduction in ptosis score when injected together with 10 mg/kg of	
X	R	Cpd No.	Guanethidine sulphate	*N*-[1-(2,4-Xylyl)ethyl]-guanidine sulphate
$-CH_2-$	H	Ib	3	2
$-CH_2-$	Me	IIb	0·4	0·2
$-(CH_2)_3-$	H	IIIb	2	0·6
$-(CH_2)_3-$	H	IVb	>20	20
(+)-$-CH(Me)-$	H	Vb	2·5	1
$-CH(Me)CH_2-$	H	VIb	0·3	0·1
(+)-$-CH_2CH(Me)-$	H	VIIb	15	not tested
	H	VIIIb	0·12	0·08
(+)-Amphetamine		VIIa	1·0	0·7
2-Phenylcyclopropylamine		VIIIa	3·0	4·0

given alone at doses up to 20 mg/kg, although some of them produced slight ptosis at doses higher than this.

Similar results to those in Table 2 were obtained when the antagonists were given after the blocking drug, as in the experiments with cats. *N*-(2-Phenylcyclopropyl)guanidine sulphate (VIIIb) (0·5 mg/kg) or *N*-benzyl-*N*-methylguanidine sulphate (IIb) (1·5 mg/kg) injected 1 hr after 10 mg/kg of guanethidine sulphate or *N*-[1-(2,4-xylyl)ethyl]guanidine sulphate reduced the ptosis score within a further 2 hr from between 5 and 6 to less than 2.

The antagonism displayed by these compounds is generally selective for ptosis resulting from adrenergic neurone blockade. This is illustrated for *N*-benzyl-*N*-methylguanidine in Table 3. However, ptosis

TABLE 3. PREVENTION BY *N*-BENZYL-*N*-METHYLGUANIDINE OF PTOSIS PRODUCED BY VARIOUS SYMPATHETIC BLOCKING DRUGS

Drug	Dose (mg/kg)	Time after injection (hr)	Ptosis score	
			Drug alone	Drug + *N*-benzyl-*N*-methylguanidine sulphate (5 mg/kg)
Xylocholine bromide	50	1	5·8	0
Guanethidine sulphate	20	2	6·2	1·0
Pempidine tartrate	20	1	4·0	3·3
Pentolinium tartrate	10	1	4·8	3·5
Phentolamine methanesulphonate	10	1	4·7	4·8
Phenoxybenzamine hydrochloride	10	1	5·3	5·2
Reserpine	0·25	4	5·7	3·0
Chlorpromazine hydrochloride	2·5	1	6·3	5·7

caused by other types of drug may be affected to some extent. As shown in Table 3, *N*-benzyl-*N*-methylguanidine caused some reduction in the ptosis produced by reserpine or by ganglion blocking agents. A rather more marked effect on ganglion blockade was found with (+)-amphetamine or *N*-(2-phenylpropyl)guanidine (VIb), both of which produce a distinct exophthalmos in mice. However, doses of these drugs sufficient to reduce the extent of ptosis do not significantly decrease the mydriasis caused by ganglion blocking agents.

COMPARISON OF EFFECTS ON GUANETHIDINE-INDUCED PTOSIS AND HEART-NORADRENALINE DEPLETION

As shown in Table 4, the reduction in the extent of ptosis, which occurs 4 hr after the subcutaneous injection of some aralkylguanidines into mice together with guanethidine sulphate (10 mg/kg), was accompanied by a decrease in the extent of heart-noradrenaline depletion. Larger doses (5–20 mg/kg) of *N*-(2-phenylcyclopropyl)guanidine (VIIIb) or *N*-benzyl-*N*-methylguanidine (IIb) almost completely eliminated the noradrenaline depletion as well as preventing ptosis.

When given after guanethidine, both (+)-amphetamine and *N*-benzyl-*N*-methylguanidine abolished ptosis without any dramatic recovery occurring in the heart-noradrenaline levels. Thus 6 hr after 10 mg/kg of guanethidine sulphate, mice had a ptosis score of 4–5 and noradrenaline levels about 20% of normal. In mice given (+)-amphetamine sulphate

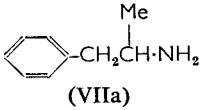
TABLE 4. EFFECT OF ARALKYLGUANIDINES ON GUANETHIDINE-INDUCED PTOSIS AND NORADRENALINE-DEPLETION IN MICE
Where more than one group of mice was used, the range of results is given in parentheses

Drug	Cpd No.	Dose (mg/kg)	Heart-noradrenaline content (% of control)		Ptosis score
			Drug alone	Drug + guanethidine	Drug + guanethidine
None		—	100(82–112)	20(18–21)	5·3(4·5–6·3)
N-(2-Phenylcyclopropyl)guanidine sulphate	VIIIb	0·1	77	35(34–36)	3·1(3·0–3·2)
		0·5	70	59(53–64)	0·2(0·0–0·3)
N-(2-Phenylpropyl)guanidine sulphate	VIb	0·1	98	25(25–25)	3·9(3·5–4·3)
		0·5	86	37(35–39)	0·8(0·5–1·0)
N-Benzyl-*N*-methylguanidine sulphate	IIb	0·3	75	30(29–30)	3·9(3·8–4·0)
		1·5	75	43(43–43)	0·4(0·3–0·5)
N-Phenethylguanidine sulphate	IIIb	2·5	49	32(32–32)	2·5(2·5–2·5)
		20	38	34(32–36)	0·5(0·3–0·7)

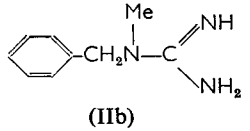
(5 mg/kg) or *N*-benzyl-*N*-methylguanidine sulphate (5 or 20 mg/kg) 4 hr after the guanethidine, ptosis had disappeared completely within a further 2 hr, but the noradrenaline level was still less than 30% of normal.

Discussion

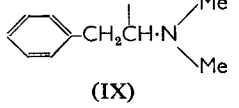
It has been shown that numerous aralkylamines with sympathomimetic properties can reverse adrenergic neurone blockade, but that the restoration of responses to nerve stimulation is not directly related to the sympathomimetic action (Day, 1962). Table 1 shows the effect of variation in the structure of the aralkyl group on the abolition by aralkylamines and aralkylguanidines of adrenergic neurone blockade in anaesthetised cats. Although there is no direct correlation between the aralkyl groups required for optimal activity in the two series—the guanidine derived from the most active amine, (+)-amphetamine (VIIa), had only marginal antagonistic activity, whereas the most potent guanidine, *N*-benzyl-*N*-methylguanidine (IIb), is derived from an almost inactive amine—there is some stereochemical similarity between the most active compounds. This stereochemical similarity is more striking between *N*-benzyl-*N*-methylguanidine (IIb) and *NN*-dimethylamphetamine (IX), which is less active than amphetamine and only slightly more active than *N*-benzyl-*N*-methylguanidine. These results are consistent with both types of compound acting at the same sites on the nerve endings.



(VIIa)



(IIb)



(IX)

The structural requirements for optimal antagonistic activity differ somewhat in mice, but even so, high activity is retained by both classes of

compound. However, (+)-amphetamine is no longer more potent than any of the guanidines, while in the guanidine series itself, the size of the aralkyl group required for maximum activity is increased. *N*-(2-Phenylcyclopropyl)guanidine (VIIIb) and *N*-(2-phenylpropyl)guanidine (VIb) are particularly potent in mice.

The selectivity displayed by these compounds in both cats and mice for counteracting adrenergic neurone blockade in preference to sympathetic blockade produced by other types of drug, suggests that reversal of adrenergic neurone blockade most likely results from displacement of the blocking drug from its binding sites at the nerve endings. Pre-treatment of rats with amphetamine has been shown to prevent the specific uptake of guanethidine into sympathetically-innervated tissues, and, when given subsequent to guanethidine, to displace the latter from its binding sites in these tissues (Chang, Costa & Brodie, 1965). Amphetamine also blocks the specific uptake of bretylium (Brodie, Chang & Costa, 1965).

However, additional actions must also be present. Besides having a weak anti-reserpine activity, which we have attributed elsewhere to monoamine oxidase inhibition (Fielden & Green, 1966), some of the guanidines can also partly relieve the ptosis caused by ganglion blocking agents. (+)-*N*-(1-Phenylethyl)guanidine (Vb) has been shown to produce a short-lasting antagonism of the relaxation of the nictitating membranes produced by pempidine in conscious cats. In contrast, the mydriasis caused by ganglion blockade is not significantly decreased by low doses of these drugs, hence the alleviation of ptosis probably results from an action peripheral to the ganglia. This action may be the local release of noradrenaline at the nerve endings, particularly since ptosis produced by α-receptor blocking drugs is resistant to either amphetamine or the guanidines.

It has been disputed whether guanethidine, which causes a marked loss of noradrenaline from sympathetically-innervated tissues, blocks sympathetic transmission by the same mechanism as drugs, such as xylocholine and bretylium, which do not cause noradrenaline depletion. Brodie and his co-workers (Brodie & others, 1965; Kuntzman, Costa, Gessa & Brodie, 1962) argued that it does not, and that whereas bretylium-like drugs simply block the outflow of noradrenaline from the nerve endings, guanethidine causes adrenergic neurone blockade by persistent activation of the normal noradrenaline-releasing mechanism at the nerve endings, so that no further response can occur on nerve stimulation. The parallelism found between the relative potencies of the amine and aralkylguanidine antagonists in preventing adrenergic nerve block by guanethidine and by *N*-[1-(2,4-xylyl)ethyl]guanidine, which does not cause noradrenaline depletion, suggests that these two types of drug block sympathetic transmission by an action at a common site.

Amphetamine not only prevents the adrenergic neurone blocking action of guanethidine but also reduces the noradrenaline depletion produced by this drug (Chang & others, 1965). Doses of the aralkylguanidines sufficient to decrease the extent of guanethidine-induced ptosis also always lessen the extent of noradrenaline depletion. However, the minimum dose

required to prevent ptosis completely is always lower than that required to eliminate depletion. It has been demonstrated that more than one uptake mechanism for drugs is present on nerve cell membranes (Iversen, 1965), consequently, guanethidine may reach the noradrenaline storage vesicles, from which it ultimately displaces the noradrenaline, by more than one route. One of these may be transference from the binding sites on the nerve cell membrane at which adrenergic neurone blockade is produced, and another an uptake mechanism not directly associated with liberation of the transmitter. If amphetamine and the aralkylguanidines can block the former process without themselves blocking nerve transmission, then the adrenergic neurone blocking action of guanethidine may be completely prevented with only partial loss of the noradrenaline-depleting action.

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