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Dual actions of some *N*-(1-phenylethyl)guanidines on the nictitating membrane of conscious cats

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After 6 hr, high doses (10 or 20 mg/kg) of some adrenergic neuron blocking drugs derived from *N*-(1-phenylethyl)guanidine produce relaxation of the nictitating membranes of conscious cats which is less than that produced by small doses (2·5 or 5 mg/kg). After 24 hr, there were marked responses to some drugs that had produced little effect after 6 hr. High doses of the drugs contract the nictitating membranes of cats treated with pempidine or (−)-*N*-(1-phenylethyl)guanidine; low doses do not. This contraction opposes the relaxation evoked by the adrenergic neuron blockade and could account for the anomalous dose-response relationships. The contraction may result from localized release of noradrenaline from the nictitating membrane.

ALTHOUGH adrenergic neuron blocking drugs generally relax the nictitating membranes of conscious cats (Exley, 1957), some may, under certain conditions, contract the nictitating membranes (Fielden, Roe & Willey, 1964; Fielden & Green, 1966a). This paper describes the two actions of some *N*-(1-phenylethyl)guanidines on the nictitating membranes of cats.

Experimental

The guanidines have been described earlier (Fielden, Green & Willey, 1965); doses refer to sulphates.

Drugs were given in sterile 0·9% saline by subcutaneous injection into the flank. From photographs of the eyes, taken at intervals, the percentage relaxation of the nictitating membranes was calculated. The eye was never fully covered by the membrane, the maximal relaxation being between 65 and 70%. Cats were used repeatedly, leaving at least a week between experiments.

In some cats the right superior cervical ganglion, together with a length of postganglionic nerve, was removed under pentobarbitone anaesthesia; sometimes the left cervical sympathetic nerve was also cut low in the neck. The animals were used 7 to 21 days after the operation.

Results

The nictitating membranes of conscious cats relaxed after administration of *N*-(1-*o*-tolylethyl)guanidine, *N*-(1-*m*-tolylethyl)guanidine, *N*-(1-*p*-tolylethyl)guanidine or *N*-(1-*p*-chlorophenylethyl)guanidine. Fig. 1 shows the degree of relaxation in cats 6 hr after injecting doses from 1·25 to 20 mg/kg. For every compound there was a dose producing a maximal effect, larger doses causing less effect or sometimes none. Such a phenomenon did not occur with xylocholine bromide at doses from 1 to 40 mg/kg. Responses were sometimes different 24 hr after drug administration. Cats which showed no overt effects 6 hr after doses of 10 or 20 mg/kg of some of the drugs had relaxed nictitating membranes

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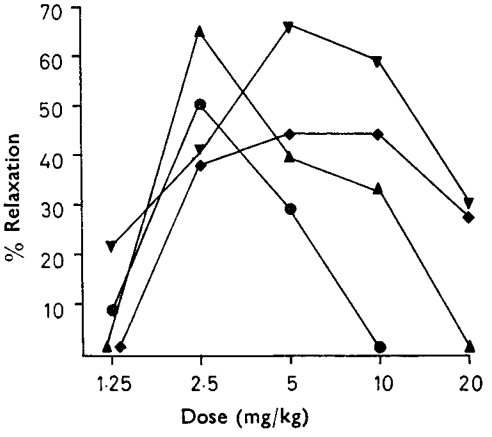

FIG. 1. Dose-6 hr-response curves for some *N*-(1-phenylethyl)guanidines. Response is the relaxation (%) of the nictitating membranes of conscious cats. Drugs were injected subcutaneously. A maximum response with these drugs is 65–70% relaxation. ▲—▲, *N*-(1-*o*-tolylethyl)guanidine sulphate. ●—●, *N*-(1-*m*-tolylethyl)guanidine sulphate. ◆—◆, *N*-(1-*p*-tolylethyl)guanidine sulphate. ▼—▼, *N*-(1-*p*-chlorophenylethyl)guanidine sulphate.

on the following day. This was particularly so after *N*-(1-*o*-tolylethyl)-guanidine. In contrast, 10 or 20 mg/kg of the *m*-tolyl compound did not relax the membranes either 6 or 24 hr after injection. These findings are summarized in Table 1.

TABLE 1. RELAXATION (%) OF THE NICTITATING MEMBRANES OF CATS 6 AND 24 HR AFTER VARIOUS DOSES OF SOME *N*-(1-PHENYLETHYL)GUANIDINES (R–CH(Me)NHC(NH)NH_2). A MAXIMUM EFFECT WITH THESE DRUGS IS 65–70% RELAXATION

R	Dose (mg/kg) (of sulphate)	Nictitating membrane relaxation %	
		6 hr	24 hr
o-Tolyl	2·5	65	25
	10	35	55
	20	0	60
m-Tolyl	2·5	50	5
	10	0	0
	20	0	0
p-Tolyl	2·5	40	15
	10	45	10
	20	30	20
p-Chlorophenyl ..	2·5	40	55
	10	60	65
	20	30	65

Cats in 2 groups of 5 were given 5 mg/kg of (—)-*N*-(1-phenylethyl)-guanidine [a potent adrenergic neuron blocking drug (Fielden & others, 1965)], alone, or together with 20 mg/kg of *N*-(1-*o*-tolylethyl)guanidine or *N*-(1-*m*-tolylethyl)guanidine or (+)- or (—)-*N*-(1-*p*-tolylethyl)guanidine. Whereas the nictitating membranes were 65% relaxed 6 hr after the

(−)-*N*-(1-phenylethyl)guanidine itself, or after the mixture with the (−)-isomer of the *p*-tolyl compound, there was little or no response to the other drug combinations. Twenty-four hr after giving the drugs the membranes of all the cats were about 30% relaxed.

In another experiment, cats in 2 groups of 4 were given 5 mg/kg of (−)-*N*-(1-phenylethyl)guanidine, alone, or with 2·5, 5 or 10 mg/kg of *N*-(1-*o*-tolylethyl)guanidine. The 10 mg/kg dose greatly reduced the response to the first mentioned compound, the 5 mg/kg dose had little effect and the 2·5 mg/kg dose none.

The experiment was repeated using pempidine tartrate (5 mg/kg) instead of (−)-*N*-(1-phenylethyl)guanidine. This time the relaxation of the membranes caused by the pempidine was completely prevented by 10 mg/kg of the *o*-tolyl compound; the other doses had little or no effect.

Eight cats were treated with (−)-*N*-(1-phenylethyl)guanidine (5 mg/kg) and 8 with pempidine tartrate (5 mg/kg). One hr later, when the nictitating membranes had relaxed, 2 cats from each group were given 2·5 or 20 mg/kg of one of the following: *N*-(1-*o*-tolylethyl)guanidine, *N*-(1-*m*-tolylethyl)guanidine or (+)- or (−)-*N*-(1-*p*-tolylethyl)guanidine. Both doses of the (−)-isomer of the latter compound had no effect. The 20 mg/kg dose of the other drugs fully retracted the membranes for at least 5 hr. The lower dose had no such actions.

In 2 cats 7 to 21 days after removal of the right superior cervical ganglion and preganglionic section of the left cervical sympathetic nerve, 20 mg/kg of *N*-(1-*o*-tolylethyl)guanidine completely retracted the left nictitating membrane for at least 5 hr, but had no effect on the right membrane.

Discussion

Except for (−)-*N*-(1-*p*-tolylethyl)guanidine, high doses of the ring-substituted *N*-(1-phenylethyl)guanidines prevent the nictitating membranes relaxing after an adrenergic neuron blocking agent or a ganglion blocking drug. Similarly high, but not low, doses contract the nictitating membranes of cats pretreated with pempidine or (−)-*N*-(1-phenylethyl)-guanidine.

The anomalous dose-response curves in Fig. 1 are therefore the result of the drugs having two opposing actions, but with different "threshold" doses. Since the compounds are potent adrenergic neuron blocking drugs, low doses relax the nictitating membranes; but, as the dose is increased, the membrane contracting action begins to predominate and so the degree of membrane relaxation decreases.

These drugs differ, therefore, from the more specific antagonists of adrenergic neuron blockade such as those described by Fielden & Green (1966b).

The time-response relationships in Table 1 suggest that the membrane contracting action of the *o*-tolyl and *p*-chlorophenyl compounds is shorter-lasting than the adrenergic neuron blocking action, so that 24 hr after a large dose only the blocking action remains. Since after *N*-(1-*m*-tolylethyl)guanidine and *N*-(1-*p*-tolylethyl)guanidine, there was no increase

in response overnight, the duration of the contracting action must be similar to that of the blocking action. With the *p*-tolyl compound, at least, the contracting action is due largely to the dextrorotatory isomer.

Although these ring-substituted *N*-(1-phenylethyl)guanidines cause slight loss of noradrenaline from tissues (Fielden & Green, 1965), the nictitating membrane contraction is unlikely to result from a general release of noradrenaline, since the *o*-tolyl compound, at least, does not retract the nictitating membrane relaxed by removal of the superior cervical ganglion, and thus made extremely sensitive to catecholamines (Lockett, 1950). Such a lack of response also precludes a direct muscle stimulant action. Ganglion stimulation is unlikely, since these drugs act in pempidine-treated cats. Since, however, removal of the superior cervical ganglion results within 48 hr in almost complete loss of noradrenaline from the nictitating membrane, but section of the preganglionic nerve does not (Kirpekar, Cervoni & Furchgott, 1962), the contraction induced by these drugs seems to be dependent on the integrity of the nerve endings and possibly on their stores of noradrenaline. The compounds may, therefore, cause contraction by a localized release of noradrenaline from the nictitating membrane.

The results described in this paper have some relevance when testing drugs for adrenergic neuron blocking activity in cats, when to induce a response it may, paradoxically, be necessary to reduce and not to increase the dose.

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