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STUDIES ON THE ANTAGONISM BETWEEN THE OPTICAL ISOMERS OF *N*-(1-PHENYLETHYL)GUANIDINE

BY

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Although it is commonplace in pharmacology to find pairs of optical isomers which differ widely in their potency as agonists, or as antagonists to some particular action, it is rare to find one optical isomer of a compound which will both prevent and reverse the effects of the other. One instance of this has recently been found in *N*-(1-phenylethyl)guanidine (Fielden, Green & Willey, 1965). The (−)-isomer of this compound is a potent adrenergic-neurone blocking drug in conscious and anaesthetized cats. The (+)-isomer is inactive in conscious cats and has only weak blocking activity in anaesthetized cats, but it antagonizes the powerful blocking action of the (−)-isomer in both. (+)-*N*-(1-Phenylethyl)guanidine not only overcomes the adrenergic-neurone blockade produced by its (−)-isomer but will also prevent the adrenergic-neurone blocking action of other drugs, such as guanethidine (Fielden & Green, 1965). In this paper we have attempted to characterize this antagonistic action further, and to elucidate its mechanism.

METHODS

Compounds. The *N*-(1-phenylethyl)guanidines were prepared as described by Fielden *et al.* (1965). Guanethidine sulphate and reserpine were kindly provided by Ciba Laboratories (Horsham, Sussex) and pempidine tartrate by May & Baker (Dagenham, Essex). With the exception of reserpine, drug doses are expressed in terms of the appropriate salt.

Experiments in cats. Drugs were given in sterile solution by subcutaneous injection into the flank. The percentage relaxation of the nictitating membranes was calculated from photographs of the eyes taken at suitable intervals after the injections. The maximum response was a relaxation of between 65 and 70%. In some cats a short section of the right cervical sympathetic nerve, or the right superior cervical ganglion, together with a short length of postganglionic nerve, was removed during pentobarbitone anaesthesia 7 to 21 days before injection of the drugs.

Experiments in mice. Drugs were dissolved in 0.9% saline and injected subcutaneously in a volume of 10 ml./kg into male mice (weight range 24 to 30 g). Ptosis was estimated by direct observation on a 0 to 8 scale (Rubin, Malone, Waugh & Burke, 1957). Each eye of every mouse in groups of six was given a score between 0 and 4, depending on whether the eye was fully open, ¼, ½, ¾, or fully closed. The final score was expressed as the average value per mouse. Noradrenaline was extracted with butanol and heptane from the pooled hearts from groups of six mice and assayed fluorimetrically after oxidation with potassium ferricyanide (Fielden & Green, 1965). In each day's experiment, control groups of mice were treated with saline alone. The heart-noradrenaline content of mice treated with drugs was calculated as a percentage of that in the appropriate controls.

RESULTS

Experiments in cats

The adrenergic-neurone blocking action of racemic *N*-(1-phenylethyl)guanidine on conscious cats was uncertain, and there was no consistent relationship between the dose of drug and the degree of relaxation of the nictitating membranes. A small dose (5 or 10 mg/kg) sometimes produced a slight relaxation but this could not be increased by raising the dose, even up to 50 mg/kg. (+)-*N*-(1-Phenylethyl)guanidine caused no relaxation at any dose up to 50 mg/kg, but the nictitating membranes were readily and fully relaxed by the (−)-isomer. A maximal response was regularly evoked by 5 mg/kg, and usually by half this amount. The duration of action depended on the dose, but the relaxation caused by 5 mg/kg lasted over 24 hr.

The experiment in Fig. 1 confirms that previous treatment with (+)-*N*-(1-phenylethyl)-guanidine prevents the adrenergic-neurone blocking action of the (−)-isomer. Two

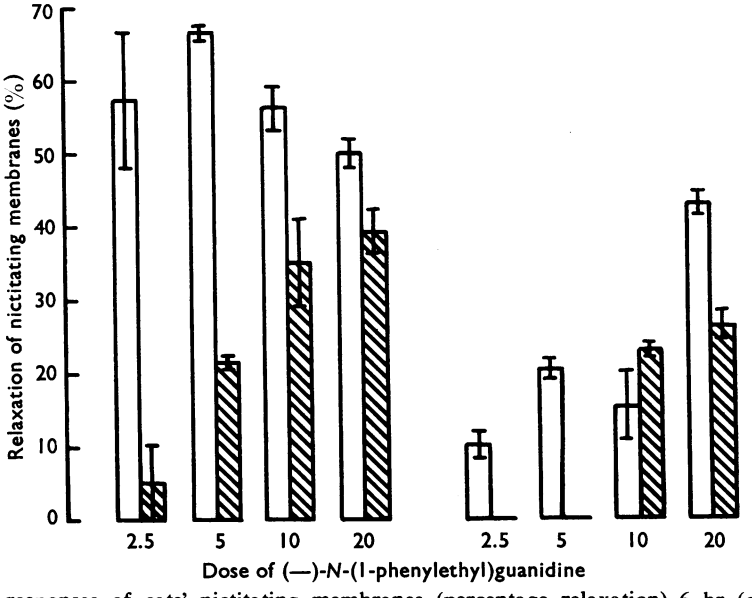

Fig. 1 Mean responses of cats' nictitating membranes (percentage relaxation) 6 hr (open columns) and 24 hr (shaded columns) after subcutaneous injection of 2.5, 5, 10 or 20 mg/kg of (−)-*N*-(1-phenylethyl)guanidine nitrate. The right-hand side of the figure shows results from cats previously treated with 5 mg/kg (subcutaneously) of (+)-*N*-(1-phenylethyl)guanidine nitrate, and the left-hand side the results from animals given only the (−)-isomer. The ranges are shown by the vertical bars.

groups of four cats were used. The four cats in one group were each given 5 mg/kg of (+)-*N*-(1-phenylethyl)guanidine, and both groups were then given (−)-*N*-(1-phenyl-ethyl)guanidine (2.5, 5, 10 or 20 mg/kg). A week later the experiment was repeated with the groups reversed. The results show that (+)-*N*-(1-phenylethyl)guanidine markedly reduced the adrenergic-neurone blocking action of an equal dose of the (−)-isomer, but that this antagonism could be partly overcome by raising the dose. A similar, but less pronounced, antagonism occurred when the cats had been first treated with 2.5 mg/kg of (+)-*N*-(1-phenylethyl)guanidine.

Cats treated with 5 mg/kg of (+)-*N*-(1-phenylethyl)guanidine also failed to respond to xylocholine (5 mg/kg) or guanethidine (5 mg/kg).

In contrast to the above results with adrenergic-neurone blocking agents, treatment of cats with (+)-*N*-(1-phenylethyl)guanidine did not prevent the relaxation of the nictitating membranes produced by pempidine (5 mg/kg), but the development of the full response to pempidine was delayed for 2 to 4 hr.

(+)-*N*-(1-Phenylethyl)guanidine will not only prevent the relaxation of the nictitating membranes produced by the (−)-isomer, but will also reverse it (Fielden *et al.*, 1965). Fig. 2 shows that (+)-*N*-(1-phenylethyl)guanidine will also cause partial reversal of pempidine-induced relaxation, but in contrast to what is found with relaxation caused

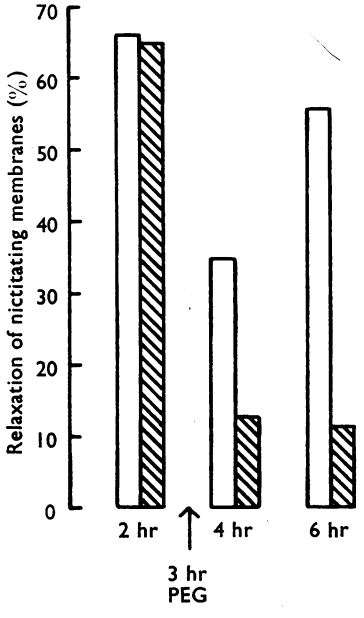

Fig. 2 Responses (percentage relaxation) of cats' nictitating membranes at intervals following the subcutaneous injection of 5 mg/kg of pempidine tartrate (open columns) or of 5 mg/kg (−)-*N*-(1-phenylethyl)guanidine nitrate (shaded columns), and, 3 hr later, of (+)-*N*-(1-phenylethyl)-guanidine nitrate (PEG, 10 mg/kg, at arrow).

by (−)-*N*-(1-phenylethyl)guanidine, this reversal is only short-lived. It is of interest that racemic and (−)-*N*-(1-phenylethyl)guanidine have comparable potency to the (+)-isomer in causing this brief partial contraction of the pempidine-relaxed nictitating membrane. Furthermore, when 5 or 10 mg/kg of (−)-*N*-(1-phenylethyl)guanidine was given to cats 2 hr after a previous dose of 5 mg/kg there was also some contraction of the relaxed nictitating membrane, but this was only slight and transient.

Some additional experiments were done with cats whose nictitating membranes were relaxed by cutting one cervical sympathetic nerve, or by removing one superior cervical ganglion.

(+)-*N*-(1-Phenylethyl)guanidine (2.5 or 5 mg/kg) was injected into a cat whose right nictitating membrane had been relaxed by section of the preganglionic cervical sympathetic nerve. At 1 hr later the right nictitating membrane had completely retracted, and remained so for at least 6 hr, but by the following day it had relaxed again. The racemic mixture behaved similarly. (−)-*N*-(1-Phenylethyl)guanidine also retracted the right nictitating membrane, but relaxed the left one. Other cats, whose right cervical sympathetic nerves had been cut, were given pempidine (5 mg/kg) and then, 1 hr later, (+)- or (−)-*N*-(1-phenylethyl)guanidine (5 mg/kg). The nictitating membranes on the right retracted for over 6 hr, but those on the left gave only small, short-lived contractions. (+)-*N*-(1-Phenylethyl)guanidine (5 or 10 mg/kg) had little effect, however, on the right nictitating membrane of a cat whose right superior cervical ganglion had been removed 14 days earlier.

Thus (+)-*N*-(1-phenylethyl)guanidine has a weak and transient contracting action on innervated nictitating membranes, a powerful action after preganglionic sympathetic nerve section, and little effect after postganglionic section.

Experiments in mice

We have shown (Fielden & Green, 1965) that adrenergic-neurone blockade can be assessed in mice from the extent of ptosis, and that ptosis caused by (−)-*N*-(1-phenyl-

TABLE 1

EFFECT OF (+)-*N*-(1-PHENYLETHYL)GUANIDINE ON PTOSIS CAUSED BY SYMPATHETIC BLOCKADE

(+)-*N*-(1-Phenylethyl)guanidine nitrate (20 mg/kg) was injected subcutaneously into groups of six mice together with 10 mg/kg of (−)-*N*-(1-phenylethyl)guanidine nitrate, (±)-*N*-[1-(2,4-xylyl)ethyl]guanidine sulphate or guanethidine sulphate, or with pempidine tartrate (20 mg/kg) or reserpine (0·3 mg/kg). Ptosis was recorded on a 0 to 8 scale 1 hr after (−)-*N*-(1-phenylethyl)guanidine or pempidine, or 4 hr after the other three drugs. Each result is the mean for the number of groups shown in square brackets. Where appropriate the range is given in parentheses

Drug	Extent of ptosis produced by: Drug alone	Drug + (+)-*N*-(1-phenylethyl)-guanidine
(−)-*N*-(1-Phenylethyl)guanidine	3·2 [1]	0·5 [1]
(±)-*N*-[1-(2,4-Xylyl)ethyl]guanidine	4·5 (4·2–4·8) [2]	0·8 (0·6–1·0) [3]
Guanethidine	5·0 (4·3–5·5) [6]	1·5 (1·2–1·8) [2]
Pempidine	4·2 (3·7–4·7) [4]	3·9 (3·7–4·0) [2]
Reserpine	5·1 (4·8–5·5) [4]	3·2 (2·8–3·5) [4]

TABLE 2

PREVENTION OF HEART-NORADRENALINE DEPLETION INDUCED BY GUANETHIDINE OR RESERPINE

(+)-*N*-(1-Phenylethyl)guanidine nitrate (20 mg/kg) was injected subcutaneously into groups of six mice, either alone or together with guanethidine sulphate (10 mg/kg) or reserpine (0·3 mg/kg). The mice were killed 4 hr later for noradrenaline assay. Each result is the mean for the number of groups shown in square brackets. The range is given in parentheses

Drug	Heart noradrenaline content (% of control): Drug alone	Drug + (+)-*N*-(1-Phenylethyl)guanidine
None	100	77 (72–84) [6]
Guanethidine	20 (16–26) [4]	62 (57–67) [2]
Reserpine	4 (0– 7) [6]	13 (8–16) [4]

ethyl)guanidine, ring-substituted *N*-(1-phenylethyl)guanidines or guanethidine can be diminished if (+)-*N*-(1-phenylethyl)guanidine is given at the same time; but, in contrast, (+)-*N*-(1-phenylethyl)guanidine has little effect against ptosis caused by reserpine or pempidine (Table 1).

Both guanethidine and reserpine cause ptosis accompanied by extensive loss of noradrenaline from the tissues. Table 2 shows that (+)-*N*-(1-phenylethyl)guanidine greatly decreases the depletion of mouse-heart noradrenaline produced by guanethidine without having much effect on that caused by reserpine.

DISCUSSION

In conscious cats (+)-*N*-(1-phenylethyl)guanidine antagonizes the adrenergic-neurone blocking action of (−)-*N*-(1-phenylethyl)guanidine. Previous treatment of cats with the (+)-isomer prevents relaxation of the nictitating membranes to subsequent doses of the (−)-isomer, and an established response to the (−)-isomer is rapidly and permanently abolished by the (+)-isomer. It is this antagonism that accounts for the unexpected inactivity of the racemic compound. However, in contrast, the long-lasting relaxation of the nictitating membranes evoked by the ganglion-blocking drug pempidine is largely unaffected by (+)-*N*-(1-phenylethyl)guanidine. Although the latter compound slightly contracts the nictitating membranes of pempidine-treated cats, this action is small and short-lived compared with the complete and permanent abolition of the response to adrenergic-neurone blockade. The antagonism is not confined to an interaction between optical isomers, since (+)-*N*-(1-phenylethyl)guanidine will also prevent relaxation of the nictitating membranes by other adrenergic-neurone blocking drugs, such as xylocholine or guanethidine. Likewise, in mice, the adrenergic-neurone blocking action of a variety of drugs, as assessed from the extent of ptosis, is greatly diminished by simultaneous administration of (+)-*N*-(1-phenylethyl)guanidine, whereas ptosis produced by reserpine or pempidine is only slightly reduced. The marked dependence of the antagonistic action of (+)-*N*-(1-phenylethyl)guanidine on the mechanism by which sympathetic blockade is produced suggests that, although a direct contracting action on the smooth muscle of the nictitating membrane or eyelid may contribute to the antagonism of adrenergic-neurone blockade, it is not the predominant factor.

The precise mode of action of adrenergic-neurone blocking drugs is not yet fully known, but in some way, or ways, they inhibit the release of noradrenaline from the nerve endings during nerve stimulation by an action at, or close to, the nerve terminal (Exley, 1957; Boura & Green, 1959; Hertting, Axelrod & Patrick, 1962; Fielden *et al.*, 1965). The ability of (+)-*N*-(1-phenylethyl)guanidine to reverse a well-established adrenergic-nerve block suggests that it cannot be acting by stopping the uptake of the blocking drug, but probably displaces it from the site at the nerve terminal at which it acts. It is possible to visualize the adrenergic-neurone blocking agent combining with some specific areas on the nerve cell membrane resulting in an electrical or structural change affecting pore size or polarization. Drugs which combine with these same areas without inducing structural or electrical changes, or which produce them to a lesser extent, would then competitively antagonize the adrenergic-neurone blockade. If, in addition, the nerve cell terminal membranes vary somewhat from tissue to tissue, this

could account for the curious fact that on some test systems (+)-*N*-(1-phenylethyl)-guanidine is not an antagonist, but is itself a potent blocking drug (Fielden *et al.*, 1965). A similar explanation was offered by Day (1962) to account for the antagonism of adrenergic-neurone blockade by amphetamine.

It is generally accepted that sympathetic blockade by reserpine results from loss of the neurotransmitter, noradrenaline, from sympathetic nerve endings (Carlsson, Rosengren, Bertler & Nilsson, 1957 ; Muscholl & Vogt, 1958). This is not so for guanethidine, which produces sympathetic blockade before extensive depletion of tissue noradrenaline has occurred (Cass & Spriggs, 1961 ; Gaffney, Chidsey & Braunwald, 1963). Guanethidine acts like bretylium in preventing the release of noradrenaline from the cat spleen into the circulation when the splenic nerves are stimulated (Hertting *et al.*, 1962). If the depleting action of guanethidine is secondary to a bretylium-like action at the nerve cell membrane, compounds such as (+)-*N*-(1-phenylethyl)guanidine, which prevent the latter effect by competing with guanethidine for its initial binding sites on the membrane, should also diminish guanethidine-induced noradrenaline release. As shown in Table 2, this is so. Reserpine differs from guanethidine in being weakly basic and very lipid soluble. It would be expected to diffuse freely into the nerve cell, where it causes depletion by a specific action on the noradrenaline storage vesicles (Hillarp & Malmfors, 1964). Consequently, hindrance to passage of reserpine into the nerve endings is unlikely to be an important factor in limiting reserpine-induced ptosis or noradrenaline depletion, and neither of these effects is reduced to any great extent by (+)-*N*-(1-phenylethyl)guanidine. There does, however, appear to be at least some degree of protection. This may possibly result from the weak monoamine oxidase inhibitory activity of (+)-*N*-(1-phenylethyl)-guanidine (Fielden & Green, 1965). It is well known (Carlsson *et al.*, 1957) that established monamine oxidase inhibitors prevent both the sympathetic blockade and the noradrenaline depletion caused by reserpine.

Besides the specific antagonism to adrenergic-neurone blockade discussed above, (+)-*N*-(1-phenylethyl)guanidine also has a weak contracting action on the relaxed nictitating membranes of pempidine-treated cats, a marked contracting action in cats in which the preganglionic sympathetic nerve is cut, but very little contracting action in cats from which the superior cervical ganglion had been removed. These actions could possibly result from local release of noradrenaline from the nictitating membrane. (+)-*N*-(1-Phenylethyl)guanidine does cause some loss of noradrenaline from the tissues (Table 2) and it is known that section of the cervical sympathetic nerve increases the sensitivity of the nictitating membrane to catechol amines (Lockett, 1950). This would explain the greater effect of (+)-*N*-(1-phenylethyl)guanidine on the membranes after preganglionic sympathetic nerve section compared with cats treated with pempidine. The failure of (+)-*N*-(1-phenylethyl)guanidine to contract the membranes after removal of the superior cervical ganglion can also be understood on this basis, since Kirpekar, Cervoni & Furchgott (1962) have shown that whereas section of the preganglionic cervical sympathetic nerve does not change the noradrenaline content of cat nictitating membranes, 1 to 2 days after removal of the superior cervical ganglion the noradrenaline content falls to a low value and does not recover. The brief membrane-contracting action of (−)-*N*-(1-phenylethyl)guanidine in cats treated with pempidine can likewise be accounted for in this way.

SUMMARY

1. In conscious cats (+)-*N*-(1-phenylethyl)guanidine antagonizes the adrenergic-neurone blocking action of (—)-*N*-(1-phenylethyl)guanidine, xylocholine and guanethidine, but not the ganglion-blocking action of pempidine. Likewise, in mice, it markedly decreases ptosis produced by (−)-*N*-(1-phenylethyl)guanidine, or guanethidine, but has little effect on ptosis caused by pempidine or reserpine.

2. (+)-*N*-(1-Phenylethyl)guanidine has a weak depleting action on mouse-heart noradrenaline, but nevertheless greatly diminishes depletion caused by guanethidine. Much less protection is afforded against reserpine-induced noradrenaline depletion.

3. Both (+)- and (−)-*N*-(1-phenylethyl)guanidine cause small, brief contractions of cat nictitating membranes relaxed by pempidine, and have a more marked contracting action when the preganglionic sympathetic nerve is cut. However, they do not contract the membranes relaxed after removal of the superior cervical ganglion. These effects may result from local release of noradrenaline from the nerve endings in the nictitating membranes.

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