Br. J. Pharmac. Chemother. (1968), 32, 350–359.

OPTICAL ISOMERS OF β-HYDROXYPHENETHYLGUANIDINE: EFFECTS ON HEART NORADRENALINE AND ON SYMPATHETIC BLOCKADE

BY

R. FIELDEN AND A. L. GREEN*

From the Research Institute, Smith Kline & French Laboratories Ltd., Welwyn Garden City, Hertfordshire

(*Received October* 11, 1967)

Although (−)-β-hydroxyphenethylguanidine is more potent than guanethidine as a depletor of noradrenaline from peripheral sympathetically innervated tissues, it causes only weak impairment of sympathetic transmission (Fielden & Green, 1967). We have shown previously (Fielden & Green, 1965, 1966a) that phenethylguanidine and some related compounds which have only weak sympathetic blocking activity can prevent and sometimes reverse the effects of more potent adrenergic neurone blocking drugs. We have now studied the interaction with a variety of sympathetic blocking drugs of the two optical isomers of β-hydroxyphenethylguanidine. A comparison is also made in this paper between the noradrenaline-depleting properties of these isomers. The results are considered to provide further evidence of a dissociation between the mechanisms of noradrenaline depletion and adrenergic neurone blockade.

METHODS

Experiments with mice

Drugs were dissolved in 0.9% saline and given subcutaneously in a volume of 0.1 ml./10 g to groups of six or ten male mice (weight 24–30 g). The extent of ptosis was estimated on a 0–8 scale (Rubin, Malone, Waugh & Burke, 1957; Fielden & Green, 1966b). Pupil diameters were measured with a Watson binocular stereoscopic microscope (magnification 6.25) with a graticule in one eye-piece. Noradrenaline was injected into a tail vein through a fine polyethylene catheter, and changes in pupil size were monitored continuously during the injection.

Mouse heart noradrenaline was estimated fluorimetrically after extraction with butanol (Fielden & Green, 1967).

Experiments with cats

Cats were anaesthetized with ethyl chloride and ether, followed by intravenous chloralose (100 mg/kg). Drugs were injected into a cannulated femoral vein. Blood pressure was recorded from the left femoral artery—either on smoked paper using a mercury manometer, in which case contractions of the nictitating membranes were recorded with a frontal writing lever; or on a Devices 8-channel recorder with a Devices blood pressure transducer, when the membrane contractions were recorded with an isotonic linear-motion transducer. The preganglionic cervical

* Present address: Department of Biochemistry, University of Strathclyde, Glasgow, Scotland.

sympathetic nerves were stimulated through shielded platinum electrodes with rectangular pulses of 0.5 msec duration, 5–10 V amplitude, and, unless otherwise stated, at a frequency of 50/sec. In most experiments the nerves were stimulated for periods of 15 sec every 2 min. Preganglionic stimulation was used rather than postganglionic stimulation to give preparations which remained stable for many hours.

In the experiments with unanaesthetized cats (weight 1.5–2.0 kg), the drugs (dissolved in about 2 ml. of sterile 0.9% saline) were injected subcutaneously. The relaxation of the nictitating membranes was determined from photographs taken at intervals after each injection.

Drugs

The preparation of β-hydroxyphenethylguanidine sulphate and its optical isomers (Green, Fielden, Bartlett, Cozens, Eden & Hills, 1967), and of (−)-*N*-(1-phenylethyl)guanidine sulphate, *N*-[1-(2,4-xylyl)ethyl] guanidine sulphate and *N*-benzyl-*N*-methylguanidine sulphate (Fielden, Green & Willey, 1965) have been previously described. Other drugs were obtained commercially. All doses refer to the salts, except for reserpine and noradrenaline. Our reasons for using (−)-*N*-(1-phenylethyl)-guanidine and *N*-[1-(2,4-xylyl)ethyl]guanidine in preference to better known adrenergic neurone blocking drugs in the antagonism experiments have been given elsewhere (Fielden & Green, 1966a).

RESULTS

Effect of β-hydroxyphenethylguanidine on mouse heart noradrenaline

Figure 1 shows the noradrenaline content of mouse hearts determined 4 hr after varying doses of the two optical isomers of β-hydroxyphenethylguanidine and of the

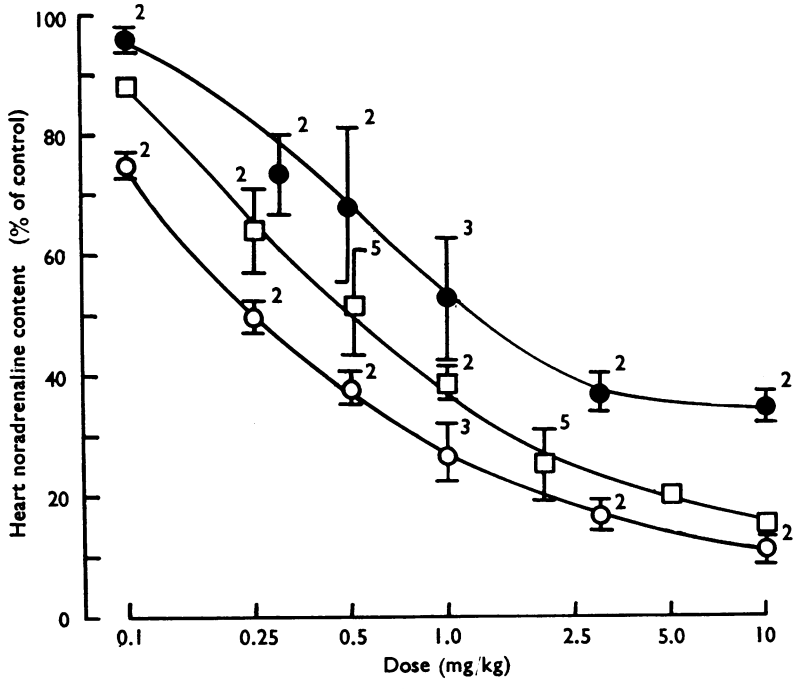

Fig. 1. Various doses of (+)-, (−)-, or (±)-β-hydroxyphenethylguanidine sulphate were given subcutaneously to groups of six mice. The mice were killed 4 hr later for assay of their heart noradrenaline. When more than one group was studied at any dose the vertical bars show the range of results for the number of groups indicated. -□-, Racemic mixture; -●-, (+)-isomer; -○-, (−)-isomer.

racemic mixture. Depletion by the (−)-isomer has been shown to be maximal at this time (Fielden & Green, 1967). Approximate doses causing 50% depletion were 0.25 mg/kg for (−)-β-hydroxyphenethylguanidine, 1.0 mg/kg for the (+)-isomer and 0.5 mg/kg for the racemic mixture. Guanethidine under these conditions causes 50% loss of heart noradrenaline at about 0.7 mg/kg.

Effect of β-hydroxyphenethylguanidine on the ptosis caused by sympathetic blocking drugs in mice

(+)- or (−)-β-Hydroxyphenethylguanidine up to 10 mg/kg does not cause significant ptosis, although slight closure of the eyelids may occur after higher doses (Green *et al.*, 1967).

The effect of simultaneous administration of each of the optical isomers of β-hydroxyphenethylguanidine (10 mg/kg) on the ptosis induced by various types of sympathetic blocking drug is shown in Table 1. When given alone, all four blocking drugs caused

TABLE 1

EFFECT OF β-HYDROXYPHENETHYLGUANIDINE (HPG) ON PTOSIS PRODUCED BY SYMPATHETIC BLOCKING DRUGS IN MICE

Groups of six mice were injected subcutaneously with the sympathetic blocking drug together with (+)- or (−)-HPG sulphate 10 mg/kg. At the time shown, sympathetic blockade was assessed from the mean ptosis scores for each group. At these times the sympathetic blocking drugs by themselves gave ptosis scores between 5 and 7.

Sympathetic blocking drug	Dose (mg/kg)	Mean ptosis score after sympathetic blocking drug: Plus (+)-HPG, Time after injection (hr) 1	Plus (+)-HPG, Time after injection (hr) 4	Plus (−)-HPG, Time after injection (hr) 1	Plus (−)-HPG, Time after injection (hr) 4
N-[1-(2,4-xylyl)ethyl]-guanidine sulphate	10	0	0·2	2·2	4·0
	20	0	0·9	5·0	6·0
Guanethidine sulphate	10	0·3	0·5	5·1	5·2
	20	0·2	1·2	5·4	5·8
Phenoxybenzamine hydrochloride	2	5·2	5·3	6·2	6·2
	10	6·3	6·5	6·1	6·9
Reserpine	0·1	—	0·8	—	2·8
	0·3	—	5·0	—	4·8

marked and prolonged ptosis. (−)-β-Hydroxyphenethylguanidine had little effect on the response to guanethidine or to the higher dose of *N*-[1-(2,4-xylyl)ethyl]guanidine, a potent adrenergic neurone blocking agent which does not cause depletion of tissue noradrenaline. In contrast, (+)-β-hydroxyphenethylguanidine greatly reduced or abolished the ptosis caused by both adrenergic neurone blocking drugs. Both optical isomers weakly antagonized ptosis induced by reserpine, but neither affected the ptosis caused by phenoxybenzamine.

Figure 2 shows that when given to mice 1 hr after *N*-[1-(2,4-xylyl)ethyl]guanidine (20 mg/kg), (+)-β-hydroxyphenethylguanidine (10 mg/kg) permanently abolished the ptosis, whereas the (−)-isomer caused only a temporary reduction in the response. Similar results were obtained when guanethidine (20 mg/kg) was used instead of *N*-[1-(2,4-xylyl)ethyl]guanidine.

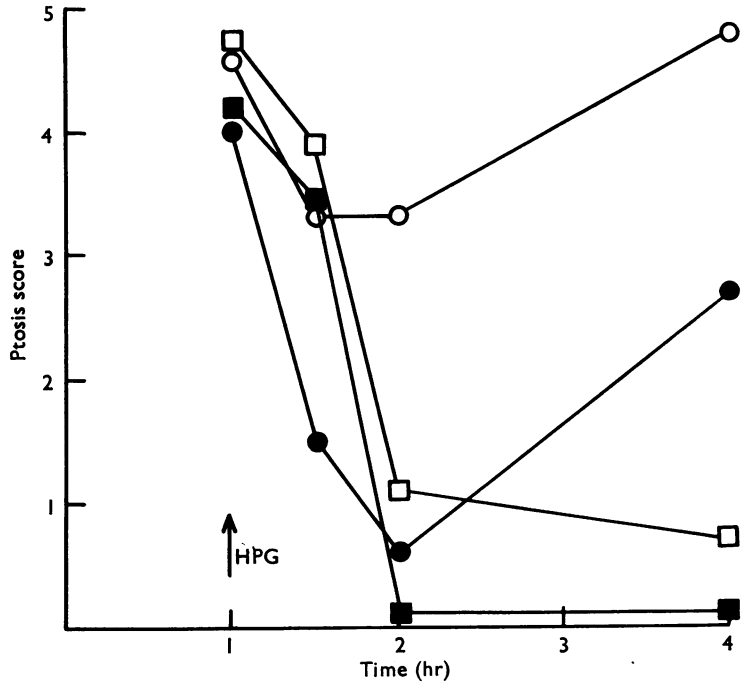

Fig. 2. Four groups of ten mice were given *N*-[1-(2,4-xylyl)ethyl]guanidine sulphate 10 mg/kg subcutaneously. The ptosis score was recorded 1 hr later, and the mice were then given (at arrow) (−)-β-hydroxyphenethylguanidine sulphate 10 mg/kg (-○-) or 20 mg/kg (-●-) or (+)-β-hydroxyphenethylguanidine sulphate (HPG) 5 mg/kg (-□-) or 10 mg/kg (-■-). Ptosis was measured after a further 0.5, 1 or 3 hr. Mice given *N*-[1-(2,4-xylyl)ethyl]guanidine sulphate 10 mg/kg alone had a ptosis score of about 5, lasting for more than 4 hr.

Pempidine (20 mg/kg) and phentolamine (20 mg/kg) caused marked ptosis in mice, but it was shorter lasting than that produced by the two adrenergic neurone blocking drugs. (+)-β-Hydroxyphenethylguanidine (10 mg/kg) had no effect on ptosis caused by phentolamine and exerted only weak antagonism to pempidine. When (−)-β-hydroxyphenethylguanidine (10 mg/kg) was given together with pempidine or phentolamine, there was no reduction in the intensity of ptosis, but instead marked ptosis persisted for much longer than when either pempidine or phentolamine was given alone.

Interaction of β-hydroxyphenethylguanidine with sympathetic blocking drugs in cats

Effects in conscious cats. The subcutaneous injection of (+)-β-hydroxyphenethylguanidine (20 mg/kg) did not relax the nictitating membranes. The (−)-isomer (5–25 mg/kg) caused erratic responses, and sometimes partial relaxation occurred (Fielden & Green, 1967).

As shown in Table 2 both isomers reduced the relaxation caused by the potent adrenergic neurone blocking drug (−)-*N*-(1-phenylethyl)guanidine (Fielden, Green & Willey, 1965). The (+)-isomer was the more active.

TABLE 2

EFFECT OF β-HYDROXYPHENETHYLGUANIDINE (HPG) ON THE ADRENERGIC NEURONE BLOCKING ACTION OF (−)-*N*-(1-PHENYLETHYL)GUANIDINE (PEG) IN CONSCIOUS CATS

(+)- or (−)-HPG sulphate (10 mg/kg) was given subcutaneously to cats at various times before or after subcutaneous injection of PEG sulphate (5 mg/kg). The percentage relaxation of the nictitating membranes was noted 4–6 hr after injection of the PEG (PEG alone causes 60–70% relaxation 4–6 hr after injection).

Time interval between injections of HPG and PEG	% relaxation produced by PEG	
	Plus (+)-HPG	Plus (−)-HPG
HPG given together with PEG	0	20
HPG 24 hr before PEG	0	25
HPG 72 hr before PEG	30	70
HPG 2 hr after PEG	0	30

Effects in anaesthetized cats. (+)-β-Hydroxyphenethylguanidine (up to 10 mg/kg) did not reduce the contractions of the nictitating membranes to sympathetic nerve stimulation, but, as shown in Fig. 3, specifically prevented the blockade of responses produced by adrenergic neurone blocking drugs. The upper record in Fig. 3 shows the extent of

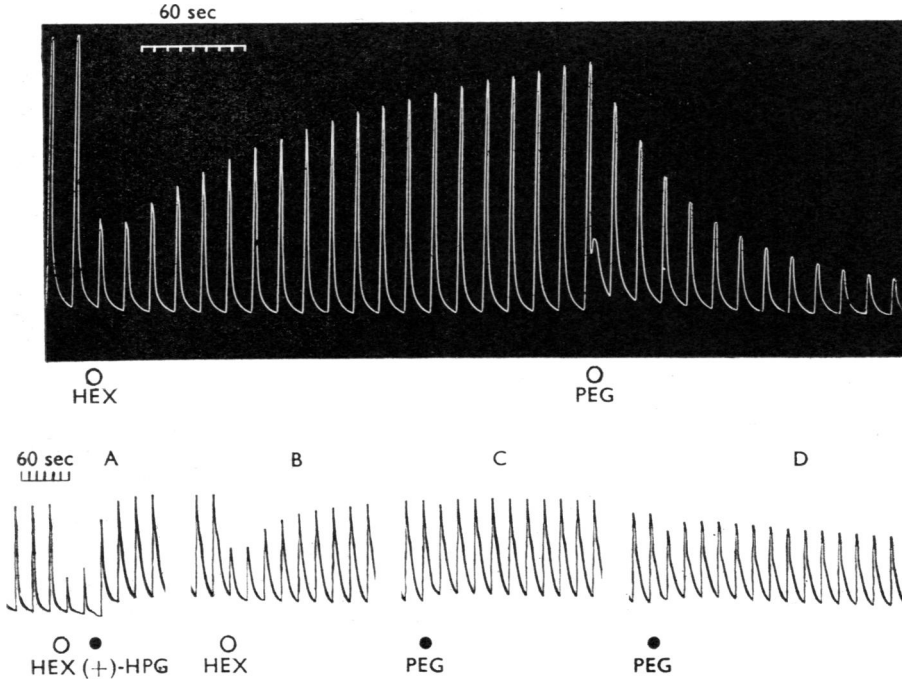

Fig. 3. Records of the contractions of the right nictitating membranes of two cats anaesthetized with chloralose. The right cervical sympathetic nerves were stimulated for 15 sec every 2 min with rectangular pulses at a frequency of 50/sec. The upper trace shows the effects in one cat of hexamethonium bromide (HEX) 2 mg/kg and of (−)-*N*-(1-phenylethyl)guanidine sulphate (PEG) 2 mg/kg. The lower trace shows, in a second cat, the effect of (+)-β-hydroxyphenethylguanidine sulphate [(+)-HPG] 5 mg/kg 5 min after hexamethonium bromide 2 mg/kg (panel A), and the response to a second dose of hexamethonium bromide (2 mg/kg) 20 min after the first (panel B). Panel C shows the effect of (−)-*N*-(1-phenylethyl)guanidine sulphate 2 mg/kg 60 min after the (+)-β-hydroxyphenethylguanidine, and panel D of 4 mg/kg 3 hr after the antagonist. Between panels C and D a second dose of (−)-*N*-(1-phenylethyl)guanidine sulphate was given 1 hr after the first dose.

sympathetic blockade produced by hexamethonium (2 mg/kg) and, when the hexamethonium blockade had disappeared, by (−)-*N*-(1-phenylethyl)guanidine (2 mg/kg). The lower record shows that although (+)-β-hydroxyphenethylguanidine (5 mg/kg) hastened the recovery of the responses of the nictitating membrane after hexamethonium, it did not prevent the blocking action of a second dose of hexamethonium. In contrast, the subsequent injection of (−)-*N*-(1-phenylethyl)guanidine (2 mg/kg) now had no effect on the contractions of the nictitating membrane to sympathetic nerve stimulation. Even after a total of 8 mg/kg of (−)-*N*-(1-phenylethyl)guanidine the responses to stimulation were only reduced by 30%.

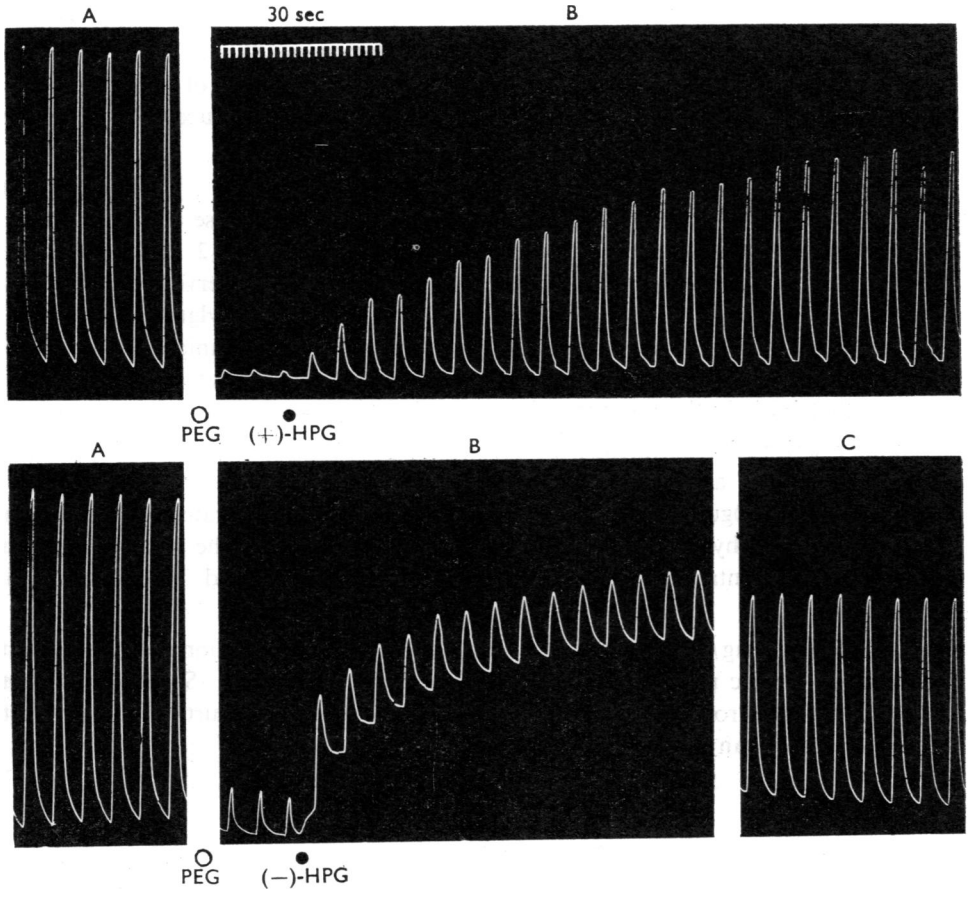

Fig. 4. Records of the contractions of the right nictitating membranes of two cats anaesthetized with chloralose. The cervical sympathetic nerves were stimulated for 15 sec every 2 min with rectangular pulses at a frequency of 50/sec. Contractions before drugs are shown in panels A, and the initial contractions in panels B were those 30 min after (−)-*N*-(1-phenylethyl)guanidine sulphate (PEG) 2 mg/kg, β-hydroxyphenethylguanidine sulphate (HPG) 5 mg/kg were then given. The upper trace shows the recovery of contractions after the (+)-isomer and the lower trace recovery after the (−)-isomer. Panel C (lower trace) shows the contractions 90 min after the end of panel B.

(−)-β-Hydroxyphenethylguanidine had a similar, but weaker antagonistic action, and (−)-*N*-(1-phenylethyl)guanidine (2 mg/kg) caused only a 30% reduction of the contractions of the nictitating membrane when given after (−)-β-hydroxyphenethylguanidine (5 mg/kg); however, after a second dose of 2 mg/kg the responses to nerve stimulation were almost abolished. Unlike the (+)-isomer, (−)-β-hydroxyphenethylguanidine did not speed up the recovery from a hexamethonium blockade.

The adrenergic neurone blocking action of guanethidine was also antagonized by β-hydroxyphenethylguanidine. In untreated, anaesthetized cats the contractions of the nictitating membrane to nerve stimulation were completely abolished by guanethidine (2.5–5.0 mg/kg). But, in a cat given five doses of (+)-β-hydroxyphenethylguanidine (2 mg/kg) at hourly intervals, although the subsequent injection of guanethidine (10 mg/kg) caused an immediate abolition of the nictitating membrane responses to nerve stimulation, within 5–10 min these had recovered to about 65% of normal. In a cat similarly treated with the (−)-isomer, guanethidine 10 mg/kg reduced the responses of the nictitating membrane to 30% of normal.

Besides preventing the adrenergic neurone blocking action of (−)-*N*-(1-phenylethyl)-guanidine, both isomers of β-hydroxyphenethylguanidine also reverse it. Figure 4 (upper trace) shows that in a cat given (−)-*N*-(1-phenylethyl)guanidine (2 mg/kg) the greatly reduced contractions of the nictitating membrane to periodic nerve stimulation were rapidly restored towards the normal level by (+)-β-hydroxyphenethylguanidine (5 mg/kg). The lower trace in Fig. 4 shows that (−)-β-hydroxyphenethylguanidine seemed to act similarly, but a dose of 5 mg/kg also caused such a marked contracture of the nictitating membrane that initially the responses to nerve stimulation were masked.

In another series of experiments, the cervical sympathetic nerves were stimulated with bursts of 200 pulses at frequencies of 1, 3, 10 or 30/sec. The blockade caused by (−)-*N*-(1-phenylethyl)guanidine 2 mg/kg was half reversed at all rates of stimulation by (+)-β-hydroxyphenethylguanidine (about 4 mg/kg). Because of the membrane contracture, the intrinsic antagonistic activity of the (−)-isomer could not be determined quantitatively.

Phentolamine (3 mg/kg) reduced the amplitude of the contractions of the nictitating membrane to periodic nerve stimulation to about 40% of normal. Subsequent injection of (−)- or (+)-β-hydroxyphenethylguanidine (5 mg/kg) caused a further slight reduction in response rather than an increase.

DISCUSSION

Both the (+)- and (−)- isomers of β-hydroxyphenethylguanidine deplete the noradrenaline from mouse hearts. When assessed from the doses necessary to cause 50% depletion 4 hr after injection, the (−)-isomer is about four times as potent as the (+)-isomer. At this time, however, even 30 mg/kg of the (+)-isomer failed to induce more than 70% depletion, whereas 30 mg/kg of either the (−)-isomer or the racemic mixture produced a loss of more than 90% of the heart noradrenaline.

The action of sympathetic blocking drugs can be antagonized by both isomers of β-hydroxyphenethylguanidine in mice and in cats, but the precise nature of the antagonistic effect is species dependent, and also varies with the character of the blocking drug.

In mice, ptosis produced by adrenergic neurone blocking drugs is prevented and abolished by (+)-β-hydroxyphenethylguanidine, whereas ptosis caused by pempidine is only slightly reduced, and that caused by phentolamine or phenoxybenzamine is unaffected. (−)-β-Hydroxyphenethylguanidine is much less effective than the (+)-isomer in preventing ptosis evoked by adrenergic neurone blocking drugs and causes only a temporary diminution in the intensity of ptosis when given 1 hr after drugs of this kind. Ptosis caused by phenoxybenzamine is unaffected by (−)-β-hydroxyphenethylguanidine, and ptosis caused by the shorter-lasting drugs phentolamine and pempidine is potentiated rather than diminished. Both isomers have only a weak action against ptosis induced by reserpine. The abolition by (+)-β-hydroxyphenethylguanidine of ptosis resulting from adrenergic neurone blocking drugs seems most plausibly attributed to displacement of the blocking drug from its binding sites at the nerve endings (Fielden & Green, 1966a). The temporary reduction in ptosis which follows the injection of the (+)-isomer into mice treated with pempidine, or of the (−)-isomer into mice given adrenergc neurone blocking drugs, needs some other explanation. This could be a facilitation of noradrenaline release, or a blockade of noradrenaline re-uptake, either of which would serve to increase the local concentration of noradrenaline at the effector organs; or there could be an action whereby the effector organs are rendered more sensitive to noradrenaline (Fielden & Green, 1967).

Essentially similar results were obtained for the antagonism of sympathetic blockade in cats. Both (+)- and (−)-β-hydroxyphenethylguanidine reduced the effect of adrenergic neurone blocking drugs on the nictitating membranes, the (+)-isomer being the more potent. Neither isomer affected the sympathetic blockade produced by phentolamine, nor did they prevent hexamethonium from blocking the nictitating membrane responses evoked by preganglionic nerve stimulation. The (+)-isomer, however, when given 5 min after the hexamethonium, accelerated recovery. The mechanism of this action is not known. It is of interest that when given after either isomer of β-hydroxyphenethylguanidine, large doses of guanethidine suppressed the responses of the nictitating membranes to preganglionic nerve stimulation, but only briefly. This is presumably a result of the short-lasting ganglion blocking action of guanethidine (Maxwell, Plummer, Schneider, Povalski & Daniel, 1960), and not of its adrenergic neurone blocking action.

When (−)-β-hydroxyphenethylguanidine was given to anaesthetized cats treated with an adrenergic neurone blocking drug, the reversal of adrenergic neurone blockade, as assessed from the recovery of the nictitating membrane responses to nerve stimulation, persisted, as it did after the (+)-isomer; and for the remainder of the experiment (at least 2 hr) there was no re-establishment of the block. Thus, in cats, the (−)-isomer may share the ability of the (+)-isomer to displace the blocking drug from its binding sites at the nerve endings.

Apart from this exception, the failure to demonstrate marked sympathetic blockade by (−)-β-hydroxyphenethylguanidine in mice or cats (Fielden & Green, 1967) is unlikely to be attributable to some antagonistic property which is also inherent in the structure of the drug. Because (−)-β-hydroxyphenethylguanidine is a potent depletor of noradrenaline, it must be able to reach the noradrenaline storage vesicles by a route which by-passes the cellular sites whose occupation is involved in the production of adrenergic neurone blockade. It is of interest that tyramine, which likewise is capable of producing almost

complete depletion of noradrenaline (Neff, Tozer, Hammer & Brodie, 1965), does not cause adrenergic neurone blockade, nor does it prevent the adrenergic neurone blocking action of guanethidine, bretylium or xylocholine on the cat nictitating membrane (Day, 1962), or prevent ptosis induced by guanethidine in mice (Fielden & Green, unpublished observations). As shown in animals treated with reserpine (Anden & Henning, 1966), nerve transmission can be adequately maintained by an extremely small part of the normal noradrenaline store.

The (+)-isomer differs from the (−)-isomer in being less active as a noradrenaline depletor, but in having a stronger affinity for those sites at the nerve endings concerned with adrenergic neurone blockade, although, like *N*-benzyl-*N*-methylguanidine (Fielden & Green, 1966a), it has almost exclusively antagonistic rather than agonistic actions at these sites. Because high doses of the racemic mixture are capable of depleting noradrenaline as readily as the (−)-isomer, the (+)-isomer can have no marked inhibitory action on the transport mechanism by which the (−)-β-hydroxyphenethylguanidine reaches the noradrenaline storage vesicles, and so, in this respect, it differs from *N*-benzyl-*N*-methylguanidine (Fielden & Green, 1967a).

Thus, since with this pair of optical isomers it is possible on the one hand to cause noradrenaline depletion without producing adrenergic neurone blockade, and on the other hand to antagonize adrenergic neurone blockade under conditions in which noradrenaline depletion still occurs, it seems probable that those receptor areas at the nerve endings involved in adrenergic neurone blockade are distinct from those involved in noradrenaline depletion. The specificity requirements for these two areas are presumably similar in many respects, however, so that many drugs may combine with both areas to produce noradrenaline depletion and adrenergic neurone blockade, or to block these two effects, or to produce one and block the other.

SUMMARY

1. (+)-β-Hydroxyphenethylguanidine depletes mouse heart noradrenaline. It has about one quarter of the activity of the (−)-isomer and one half that of the racemic mixture.

2. In mice, (+)-β-hydroxyphenethylguanidine readily prevents and abolishes ptosis caused by adrenergic neurone blocking drugs, but the (−)-isomer causes only slight and short-lived antagonism. (+)-β-Hydroxyphenethylguanidine only weakly antagonizes ptosis caused by pempidine, phenoxybenzamine or reserpine. Ptosis caused by pempidine or phentolamine is prolonged by (−)-β-hydroxyphenethylguanidine.

3. Although both isomers of β-hydroxyphenethylguanidine antagonize the action of adrenergic neurone blocking drugs on the nictitating membranes of cats, the (+)-isomer is more active and its effect lasts longer. Sympathetic blockade by phentolamine or hexamethonium is not antagonized.

4. The results are discussed, and it is concluded that there are two distinct receptor areas at the sympathetic nerve endings, one of which is associated with adrenergic neurone blockade, and the other with noradrenaline depletion.

We wish to thank Mary J. Cozens, P. Cresswell and R. J. Eden for their help with this work.

REFERENCES

ANDEN, N. E. & HENNING, M. (1966). Adrenergic nerve function, noradrenaline level and noradrenaline uptake in cat nictitating membrane after reserpine treatment. *Acta physiol. scand.*, **67**, 498–504.

DAY, M. D. (1962). Effect of sympathomimetic amines on the blocking action of guanethidine, bretylium and xylocholine. *Br. J. Pharmac. Chemother.*, **18**, 421–439.

FIELDEN, R. & GREEN, A. L. (1965). The effects of some aralkylguanidines in mice. *Br. J. Pharmac. Chemother.*, **24**, 408–417.

FIELDEN, R. & GREEN, A. L. (1966a). A comparison of aralkylamines and aralkylguanidines as antagonists of adrenergic neurone blockade. *J. Pharm. Pharmac.*, **18**, 721–728.

FIELDEN, R. & GREEN, A. L. (1966b). The mouse as an experimental animal for the study of drugs producing sympathetic blockade. In *Methods in Drug Evaluation*, ed. Mantegazza, P. & Piccinini, F., pp. 149–157. Amsterdam: North-Holland Publishing Company.

FIELDEN, R. & GREEN, A. L. (1967). A comparative study of the noradrenaline-depleting and sympathetic-blocking actions of guanethidine and (−)-β-hydroxyphenethylguanidine. *Br. J. Pharmac. Chemother.*, **30**, 155–165.

FIELDEN, R., GREEN, A. L. & WILLEY, G. L. (1965). Adrenergic neurone blocking activity of some aralkylguanidines. *Br. J. Pharmac. Chemother.*, **24**, 395–407.

GREEN, A. L., FIELDEN, R., BARTLETT, D. C., COZENS, MARY J., EDEN, R. J. & HILLS, D. W. (1967). New norepinephrine-depleting agents. β-Hydroxyphenethylguanidine and related compounds. *J. med. Chem.*, **10**, 1006–1008.

MAXWELL, R. A., PLUMMER, A. J., SCHNEIDER, F., POVALSKI, H. & DANIEL, A. I. (1960). Pharmacology of [2-(octahydro-1-azocinyl)-ethyl]guanidine sulphate (SU-5864). *J. Pharmac. exp. Ther.*, **128**, 22–29.

NEFF, N. H., TOZER, T. N., HAMMER, W. & BRODIE, B. B. (1965). Kinetics of release of norepinephrine by tyramine. *Life Sci., Oxford*, **4**, 1869–1875.

RUBIN, B., MALONE, M. M., WAUGH, M. H. & BURKE, J. C. (1957). Bioassay of rauwolfia roots and alkaloids. *J. Pharmac. exp. Ther.*, **120**, 125–136.