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# A COMPARATIVE STUDY OF THE NORADRENALINE-DEPLETING AND SYMPATHETIC-BLOCKING ACTIONS OF GUANETHIDINE AND (−)-β-HYDROXYPHENETHYLGUANIDINE

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(−)-β-Hydroxyphenethylguanidine is the most active of a series of aralkylguanidines studied for their ability to lower the noradrenaline content of sympathetically-innervated tissues. It is several times more potent in this respect than guanethidine, but, unlike guanethidine, it produces only weak impairment of sympathetic transmission. In this paper we have compared the mechanisms of noradrenaline depletion by guanethidine and (−)-β-hydroxyphenethylguanidine, and studied the relationship between noradrenaline depletion and sympathetic blockade. The nature of the adrenergic neurone blocking action of guanethidine is discussed in the light of these results.

## METHODS

### *Experiments with mice and rats*

The drugs were dissolved in 0.9% saline and injected subcutaneously in a volume of 10 ml./kg into male mice (w. 24 to 30 g) or in a volume of 1 ml./kg into male rats (w. 200 to 250 g). The extent of ptosis was estimated by direct observation on a 0 to 8 scale (Rubin, Malone, Waugh & Burke, 1957; Fielden & Green, 1966). Noradrenaline was assayed by fluorimetry after extraction with butanol from the pooled hearts from groups of six mice or from individual rat hearts (Fielden & Green, 1965a). In each day's experiment, control animals were given saline alone and the heart-noradrenaline content of treated animals was calculated as a percentage of that of the controls. We reported previously (Fielden & Green, 1965a) that a small amount of ascorbic acid was added to the hearts before homogenization in dilute hydrochloric acid. Although in most of our experiments this addition was advantageous, in a few sets of experiments better recoveries of noradrenaline from the tissues were obtained if the ascorbic acid was omitted. The reason for this variability is unknown, and before starting any new series of experiments we have found it advisable to do a few trial runs to ascertain whether higher recoveries were obtained with or without the ascorbic acid.

Doses are expressed in terms of amine or guanidine salt. This was always the sulphate except where otherwise stated.

### *Experiments with anaesthetized cats*

Cats were anaesthetized with ethyl chloride and ether, followed by intravenous chloralose (100 mg/kg). Blood pressure was recorded from the left femoral artery by a Devices Blood Pressure Transducer and Pen Recorder (M8). The pulse rate was determined from high speed records, or

by using the pressure pulse to drive a ratemeter, the output of which was displayed on one channel of the recorder and on a digital electronic counter (Philips PW 4032). The right stellate ganglion was exposed, the preganglionic trunk cut, and electrodes placed round the inferior cardiac nerve. Contractions of the right nictitating membrane were recorded by a frontal writing lever on smoked paper, or on one channel of the Devices Recorder by means of a linear motion transducer. The load was between 3 and 4 g, and the transducer was calibrated in mm with a micrometer. Electrodes were placed on the preganglionic cervical sympathetic nerve. Stimulation of the nerves was by rectangular pulses of 0.5 msec duration, 5 to 10 V amplitude and, unless stated otherwise, at a frequency of 50/sec.

For noradrenaline estimations, the hearts and spleens were removed, washed in distilled water, and dried on filter paper. Portions of 0.8 to 1 g were cut from the left ventricle of the heart or from the whole spleen. Both nictitating membranes were excised and the cartilagenous borders removed. The samples were frozen and the noradrenaline was extracted and assayed as for mouse hearts. Ascorbic acid (2% 0.05 ml.) was added to the hearts before homogenization but not to the spleens or nictitating membranes.

## RESULTS

### *Effects of guanethidine and (−)-β-hydroxyphenethylguanidine in mice*

The noradrenaline content of mouse hearts at various times after administration of guanethidine or (−)-β-hydroxyphenethylguanidine is shown in Fig. 1. Maximal depletion was not achieved until 2 to 4 hr after injection, irrespective of dose, but the larger doses of both drugs ultimately caused more extensive and prolonged loss of noradrenaline. Depletion was slightly faster after (−)-β-hydroxyphenethylguanidine than after a dose of guanethidine sufficient to give the same ultimate degree of depletion. The noradrena-

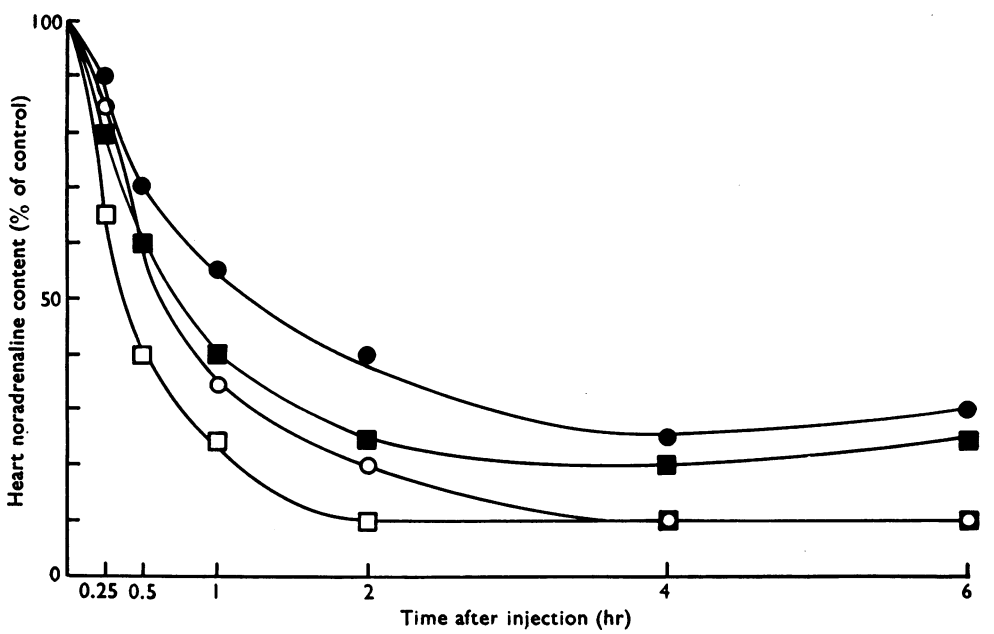


Fig. 1. The noradrenaline content of the hearts from groups of six mice was measured at various times after subcutaneous injection of guanethidine sulphate 4 mg/kg (●) or 20 mg/kg (○), or of (−)-β-hydroxyphenethylguanidine sulphate at 1 mg/kg (■) or 5 mg/kg (□).

line levels 18 hr after injection varied among groups of mice studied on different occasions, but at least 30% recovery in the heart-noradrenaline content had always occurred by this time after both drugs.

Dose-response curves for depletion of mouse-heart noradrenaline by guanethidine and (−)-β-hydroxyphenethylguanidine 4 hr after injection are given in Fig. 2.

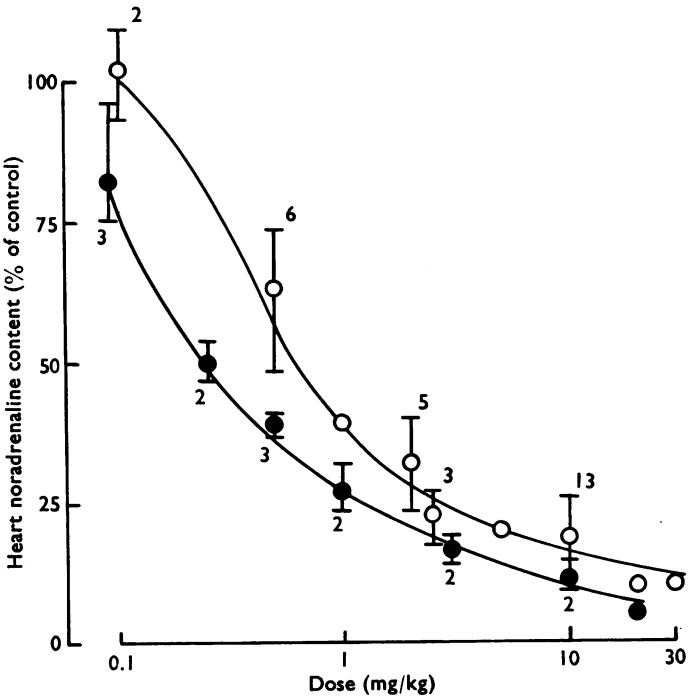


Fig. 2. The noradrenaline content of the hearts from groups of six mice was measured after various doses of guanethidine sulphate (○) or (−)-β-hydroxyphenethylguanidine sulphate (●). The mice were killed 4 hr after subcutaneous injection of the drug. Where more than one group was studied at any dose the vertical bars show the range of results for the number of groups indicated.

Although noradrenaline disappeared from the tissues relatively slowly after injection of guanethidine into mice, adrenergic neurone blockade, as assessed from the extent of ptosis, developed rapidly. Ptosis was appreciable within 15 min of the injection of 4 mg/kg guanethidine, was at its maximum level within 1 hr, and had diminished considerably within 6 hr. In contrast, ptosis was not detectable after less than about 30 mg/kg (−)-β-hydroxyphenethylguanidine and 100 mg/kg were required to produce ptosis comparable in intensity to that caused by 2.5 to 5 mg/kg guanethidine. Furthermore, the onset of ptosis was much slower after (−)-β-hydroxyphenethylguanidine than after guanethidine, not reaching its maximum until 4 to 6 hr after injection of the drug.

Numerous compounds are known to reduce the depleting action of guanethidine or reserpine on heart noradrenaline. Table 1 compares the protective action of a variety of such drugs against the depletion of mouse-heart noradrenaline by (−)-β-hydroxyphenethylguanidine, guanethidine and reserpine.

TABLE 1

EFFECT OF DRUGS ON THE DEPLETION OF MOUSE-HEART NORADRENALINE BY (−)-β-HYDROXYPHENETHYLGUANIDINE, GUANETHIDINE AND RESERPINE

The antagonist (20 mg/kg, except for the amphetamines which were given at 5 mg/kg) was injected subcutaneously into groups of six mice, either alone, or together with (−)-β-hydroxyphenethylguanidine sulphate (2·5 mg/kg), guanethidine sulphate (10 mg/kg) or reserpine (0·3 mg/kg). The mice were killed 4 hr later and their hearts were removed and pooled for noradrenaline assay. Each result is the mean for the number of groups shown in square brackets. The range is given in parentheses. Pempidine was used as the tartrate, the other antagonists as sulphates

| | Heart noradrenaline content (% of control) | | | |
|---|---|---|---|---|
| Antagonist | Antagonist alone | Antagonist + β-hydroxyphenethylguanidine | Antagonist + guanethidine | Antagonist + reserpine |
| None | 100 | 11 (7–15) [3] | 20 (16–26) [4] | 4 (0– 7) [6] |
| (+)-Amphetamine | 76 (68– 79) [5] | 43 (40–45) [2] | 60 (51–70) [2] | 5 (4– 6) [3] |
| (−)-Amphetamine | 93 (89–102) [4] | 42 (40–43) [2] | 48 (47–48) [2] | not tested |
| *N*-Benzyl-*N*-methylguanidine | 88 (75– 98) [6] | 64 (62–65) [2] | 75 (72–78) [3] | 18 (16– 19) [2] |
| *N*-[1-(2,4-Xylyl)ethyl]guanidine | 94 (72–117) [6] | 56 (54–57) [2] | 66 (62–71) [2] | 99 (90–110) [3] |
| *N*-(*p*-Methoxy-α-methylphenethyl)guanidine | 87 (77– 95) [6] | 36 (29–43) [2] | 64 (55–76) [5] | 45 (16– 60) [4] |
| Pempidine | 91 (76–112) [4] | 12 (12–12) [2] | 18 (16–19) [2] | 7 (4– 12) [3] |

*Effect of monoamine oxidase inhibition on depletion of rat-heart noradrenaline by (−)-β-hydroxyphenethylguanidine and guanethidine*

The ability of monoamine oxidase inhibitors to diminish guanethidine-induced depletion of rat-heart noradrenaline is well established. We have recently shown (Fielden & Green, 1965b) that, when iproniazid is used as the inhibitor, there is a fair correlation between the protection afforded against depletion and the extent to which the heart monoamine oxidase is inhibited. Table 2 shows that similar protection is afforded against (−)-β-hydroxyphenethylguanidine-induced depletion of rat-heart noradrenaline when iproniazid is injected 20 hr beforehand. The previously published results obtained with guanethidine are included for comparison. Iproniazid alone under these conditions had a negligible effect on rat-heart noradrenaline.

TABLE 2

EFFECT OF IPRONIAZID ON THE NORADRENALINE-DEPLETING ACTIONS OF (−)-β-HYDROXYPHENETHYLGUANIDINE AND GUANETHIDINE

Various doses of iproniazid phosphate were injected subcutaneously into rats 20 hr before the subcutaneous injection of (−)-β-hydroxyphenethylguanidine sulphate (2·5 mg/kg) or guanethidine sulphate (10 mg/kg). The rats were killed after a further 4 hr and their hearts were removed for noradrenaline assay. The number of rats is shown in square brackets and the range in parentheses

| Dose of iproniazid phosphate (mg/kg) | Monoamine oxidase inhibition (%) | Heart noradrenaline content (% of control) | |
|---|---|---|---|
| | | (−)-β-Hydroxyphenethylguanidine | Guanethidine |
| 0 | 0 | 10 (6–14) [3] | 21 (14– 32) [8] |
| 20 | 55 | 25 (23–27) [2] | 46 (35– 62) [4] |
| 40 | 75 | 57 (50–66) [3] | 71 (62– 88) [5] |
| 80 | 90 | 76 (64–89) [3] | 91 (77–105) [4] |

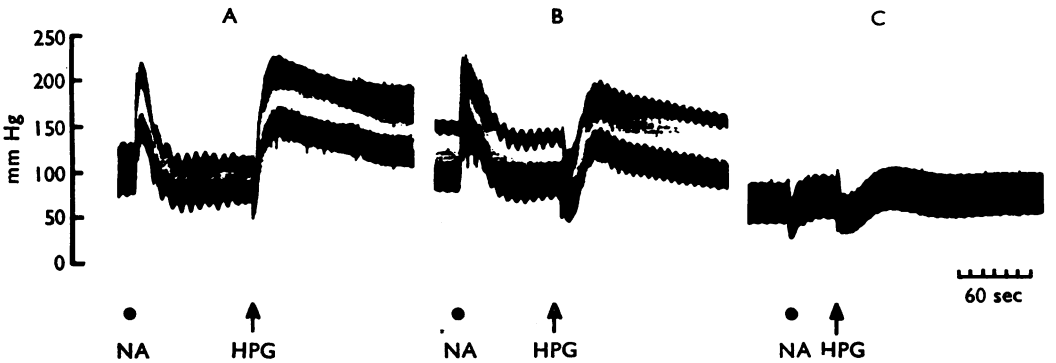


Fig. 3. Record of the blood pressure of a cat (male, 2.3 kg) anaesthetized with chloralose. Noradrenaline (5 $\mu$g) or (−)-$\beta$-hydroxyphenethylguanidine sulphate (2.5 mg/kg) were injected at NA or HPG. Panel B shows the result 1 hr after A. Phenoxybenzamine hydrochloride (5 mg/kg) was injected 30 min after B. Panel C shows the result 30 min later.

*Effects of (−)-β-hydroxyphenethylguanidine and guanethidine in cats*

Intravenous injection of 2.5 mg/kg (−)-$\beta$-hydroxyphenethylguanidine into anaesthetized cats produced a marked pressor response and increase in pulse pressure. These effects were prevented by phenoxybenzamine hydrochloride (5 mg/kg, intravenously) (Fig. 3) or by giving reserpine (2 mg/kg, intraperitoneally) 24 hr beforehand. In some cats the intensity of this pressor effect slowly declined on repeated injection of the drug. However, in one cat, the rise in blood pressure was still maintained after five injections at hourly intervals.

This dose (2.5 mg/kg) of (−)-$\beta$-hydroxyphenethylguanidine had no effect on the responses of the nictitating membrane to periodic stimulation of the cervical sympathetic nerve, or on the response of the heart rate to stimulation of the inferior cardiac nerve.

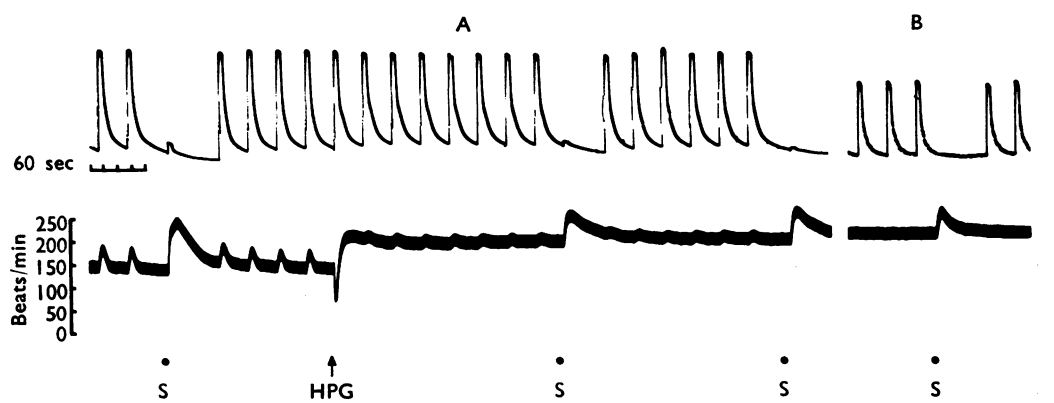


Fig. 4. Record of the contractions of the right nictitating membrane (upper trace) and the heart rate of a cat (male, 4.1 kg) anaesthetized with chloralose. The cervical sympathetic nerve was stimulated for 15 sec every 2 min except when the inferior cardiac nerve was stimulated for 15 sec (at S). The stimulation frequency was 50 pulses/sec. Panel A shows the effect of (−)-$\beta$-hydroxyphenethylguanidine sulphate (2.5 mg/kg) injected at HPG. Further doses of this drug (2.5 and 5 mg/kg) were injected 30 min and 75 min after the initial dose. Panel B shows the effect 2 hr after the third dose.

Further injections of 2.5 and 5 mg/kg of the drug reduced these responses but did not abolish them (Fig. 4). In another experiment, five injections of 2 mg/kg at hourly intervals suppressed the nictitating membrane responses by only 25%.

A single injection of 10 mg/kg (−)-β-hydroxyphenethylguanidine produced variable results. In some cats there was a marked contracture of the nictitating membranes immediately after injection, which took up to 2 hr to subside; in others this contracture was less noticeable. Over a total of 4 to 6 hr there was usually a gradual reduction in the responses to periodic stimulation of the cervical sympathetic nerve, but this reduction rarely exceeded 30%. In one cat, about 75% blockade was reached after 1 hr. This block was slowly abolished by (+)-amphetamine (0.5 mg/kg given in three divided doses).

Intravenous injection of 2.5 mg/kg guanethidine also produced a pressor response, but this was accompanied by a substantial decrease in the contraction of the nictitating membrane to stimulation of the cervical sympathetic nerve and in the tachycardia evoked by stimulation of the inferior cardiac nerve. An additional dose of 2.5 mg/kg guanethidine, or higher single doses (5 mg/kg or above), completely blocked both these responses to sympathetic nerve stimulation. Figure 5 shows that the suppression of sympathetic transmission induced by 2.5 mg/kg guanethidine was rapidly abolished by 0.125 mg/kg (+)-amphetamine.

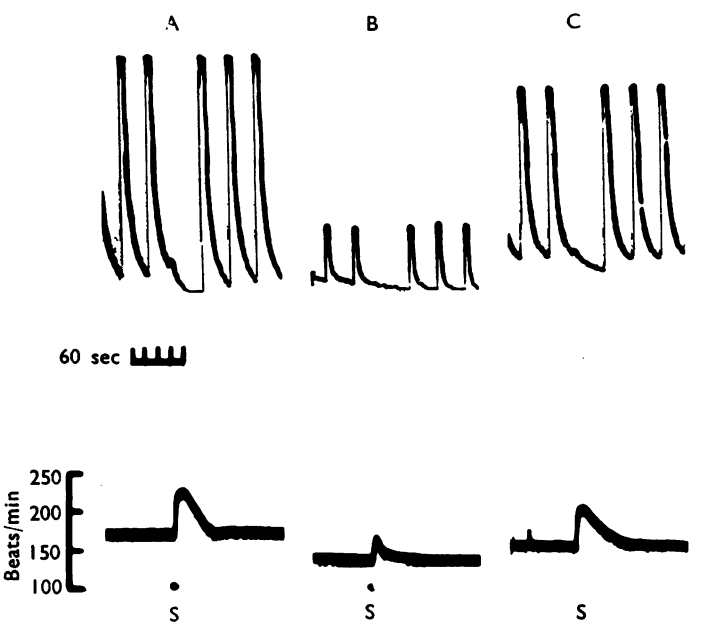


Fig. 5. Record of the contractions of the nictitating membrane (upper trace) and the heart rate of a cat (female, 2.1 kg) anaesthetized with chloralose and treated with atropine sulphate (0.5 mg/kg). The cervical sympathetic nerve was stimulated for 15 sec every 2 min except when the inferior cardiac nerve was stimulated for 15 sec (at S). The stimulation frequency was 50 pulses/sec. Panel A shows responses before drug injection. Panel B shows responses 30 min after intravenous guanethidine sulphate (2.5 mg/kg). (+)-Amphetamine sulphate (0.125 mg/kg) was then injected. Panel C shows the responses after a further 30 min.

TABLE 3

SYMPATHETIC-BLOCKING AND NORADRENALINE-DEPLETING ACTIONS OF GUANETHIDINE AND (−)-β-HYDROXYPHENETHYLGUANIDINE

Cats were injected subcutaneously with guanethidine sulphate or (−)-β-hydroxyphenethylguanidine sulphate. One cat was given three doses of the latter at 24-hr intervals. Eighteen hr later, any relaxation of the nictitating membranes was noted. The cats were then anaesthetized with chloralose and given atropine sulphate (0·5 mg/kg). Responses were recorded of the right nictitating membrane to stimulation of the cervical sympathetic nerve for 15 sec (50 pulses/sec) and of the heart rate to stimulation of the right inferior cardiac nerve for 15 sec (50 pulses/sec). Both nictitating membranes, part of the spleen and part of the left ventricle of the heart were removed for noradrenaline assay. Five untreated cats were included as controls.

| | | Heart | | | | Nictitating membrane | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Dose (mg/kg) | Resting rate (beats/min) | Stimulated rate (beats/min) | Increase in rate (%) | Noradrenaline content (μg/g) | Relaxation (% of maximal) | Contraction on stimulation (mm) | Noradrenaline content (μg/g) | Spleen noradrenaline content (μg/g) |
| No drug | — | 216 | 276 | 28 | 1·60 | 0 | 6·5 | 1·63 | — |
| | — | 196 | 280 | 43 | 1·31 | 0 | 7·0 | 1·22 | 1·64 |
| | — | 250 | 290 | 16 | 1·39 | 0 | 7·5 | 0·79 | 1·59 |
| | — | 198 | 265 | 34 | 1·20 | 0 | 9·5 | 0·47 | 0·93 |
| | — | 225 | 240 | 7 | 2·25 | 0 | 7·5 | 0·76 | 2·75 |
| | *Mean* | **217** | **270** | **25** | **1.55** | **0** | **7.6** | **0.97** | **1.73** |
| Guanethidine | 10 | 196 | 223 | 14 | 0·40 | 40 | 0 | 0·11 | — |
| | 10 | 190 | 200 | 5 | 0·54 | 0 | 8·0 | 0·25 | 0·46 |
| | 10 | 174 | 184 | 6 | 0·56 | 35 | 2·5 | 0·12 | — |
| | 10 | 166 | 187 | 11 | 0·75 | 45 | 3·0 | 0·07 | — |
| | 15 | 182 | 188 | 3 | 0·26 | 80 | 0 | <0·05 | 0·10 |
| (−)-β-Hydroxyphenethylguanidine | 5 | 172 | 244 | 42 | 0·22 | 0 | 7·5 | <0·05 | 0·21 |
| | 10 | 190 | 270 | 42 | 0·20 | 0 | 7·5 | <0·05 | 0·20 |
| | 20 | 190 | 224 | 18 | 0·05 | 0 | 4 | <0·05 | 0·05 |
| | 5 (3 doses) | 144 | 230 | 60 | 0·08 | 20 | 6·5 | <0·05 | 0·08 |

In conscious cats the subcutaneous injection of 5 mg/kg guanethidine regularly evoked a near-maximal relaxation of the nictitating membranes. Much more erratic responses occurred after 5 to 25 mg/kg (−)-β-hydroxyphenethylguanidine, and there was no clear dependence of the response on the dose of drug. In some cats there was little or no response, whereas in others the membranes partially relaxed after 3 to 6 hr. This relaxation sometimes lasted 24 hr or more, but in only one cat (after 10 mg/kg) did it become more than about half-maximal. In this cat, the relaxation of the membranes was abolished by (+)-amphetamine (1 mg/kg, subcutaneously).

In order to study the relationship between sympathetic blockade and noradrenaline depletion, cats were given guanethidine or (−)-β-hydroxyphenethylguanidine subcutaneously. After 18 hr they were anaesthetized and examined for responses of the heart rate to stimulation of the inferior cardiac nerve and of the nictitating membranes to stimulation of the cervical sympathetic nerve. The hearts, spleens and nictitating membranes were then excised and assayed for noradrenaline. The results are summarized in Table 3. On all three tissues (−)-β-hydroxyphenethylguanidine caused more depletion than did guanethidine. In three out of four cats, 10 mg/kg guanethidine relaxed the nictitating membranes and markedly lowered the responses of these membranes to stimulation. In all four cats the responses of the heart rate to stimulation were reduced. A higher dose (15 mg/kg) caused near-maximal relaxation of the membranes and abolished the responses to sympathetic nerve stimulation. In contrast, after (−)-β-hydroxyphenethylguanidine there was significant relaxation of the membranes in one cat only, and the responses to nerve stimulation were not significantly reduced except after the highest dose (20 mg/kg).

In another cat given 20 mg/kg and anaesthetized 18 hr later, stimulation of the cervical sympathetic nerve with 200 pulses, at rates of 1, 3, 10 or 30/sec, produced contractions of the nictitating membrane which were all within the normal range. In this cat the responses of the nictitating membranes to adrenaline and noradrenaline were enhanced, but the pressor effect of these drugs was normal. The pressor response to tyramine was, however, markedly reduced. In the treated cat 0.5 mg/kg tyramine hydrochloride raised the blood pressure by only 20 mm Hg, whereas in an untreated cat 0.25 mg/kg caused a rise of 130 mm Hg.

## DISCUSSION

(−)-β-Hydroxyphenethylguanidine is several times more active than guanethidine in depleting peripheral sympathetically-innervated tissues of their noradrenaline, but the process of depletion by these two drugs shows many similarities. The noradrenaline depletion produced by them can be diminished by a variety of other compounds having effects at adrenergic nerve endings: (+)- and (−)-amphetamine; *N*-[1-(2,4-xylyl)-ethyl]guanidine, an adrenergic neurone blocking drug (Fielden, Green & Willey, 1965); *N*-benzyl-*N*-methylguanidine, an antagonist to adrenergic neurone blockade (Fielden & Green, 1966); and *N*-(*p*-methoxy-α-methylphenethyl)guanidine, which has little effect on adrenergic transmission but which is a moderately active monoamine-oxidase inhibitor (Fielden & Green, 1965a). Depletion by reserpine is, in contrast, little affected by amphetamine or by *N*-benzyl-*N*-methylguanidine. Pempidine did not reduce depletion by either guanidine but had a marginal protective effect against depletion by reserpine.

Ganglion-blocking agents have been reported to retard reserpine-induced depletion of labelled noradrenaline from rat hearts (Hertting, Potter & Axelrod, 1962). Depletion of rat-heart noradrenaline by both guanidines is partly blocked by pretreatment with iproniazid. The degree of protection is well correlated with the extent of monoamine oxidase inhibition. Although this protection implies that much of the noradrenaline released by these drugs from its storage sites is normally destroyed by deamination, an appreciable amount is probably also liberated as free noradrenaline, since intravenous injection of both drugs produces a marked pressor response which may be prevented by prior administration of an $\alpha$-receptor blocker, phenoxybenzamine, or of reserpine. This has been previously shown for guanethidine by Kadzielawa (1962) and Abercrombie & Davies (1963). Guanethidine-induced release of labelled noradrenaline from perfused rat hearts has been demonstrated directly by Nash, Costa & Brodie (1964).

Despite the similarity between the noradrenaline-depleting actions of guanethidine and (−)-$\beta$-hydroxyphenethylguanidine, these two drugs have markedly different effects on sympathetic transmission. In mice, less than 5 mg/kg guanethidine rapidly produces ptosis, the intensity of which is unrelated to the extent of noradrenaline depletion. Approximately 100 mg/kg (−)-$\beta$-hydroxyphenethylguanidine is required to produce comparable ptosis. Ptosis after (−)-$\beta$-hydroxyphenethylguanidine develops only slowly, not reaching its greatest extent until about 6 hr after injection of the drug. The period of most intense ptosis coincides roughly with that of greatest noradrenaline depletion.

In conscious cats, subcutaneous injection of 5 mg/kg guanethidine regularly evokes a near-maximal relaxation of the nictitating membranes, but (−)-$\beta$-hydroxyphenethylguanidine, at 5 to 25 mg/kg, is erratic in its effect, occasionally producing slight relaxation of the membranes, which may then last a day or more, but often causing no relaxation. In only one cat, given 10 mg/kg, did the extent of relaxation exceed half-maximal.

In anaesthetized cats, intravenous guanethidine at 5 mg/kg or more, given as a single dose or in divided doses, rapidly and completely blocks the contractions of the nictitating membranes produced by stimulation of the cervical sympathetic nerves and the tachycardia produced by stimulation of the inferior cardiac nerve. In contrast, (−)-$\beta$-hydroxyphenethylguanidine (10 mg/kg or more, given as a single dose, or in successive doses of 2 or 2.5 mg/kg at hourly intervals) failed to cause more than partial block of these responses to sympathetic nerve stimulation and often had no effect on them at all.

Cass & Spriggs (1961) suggested that, although the initial adrenergic neurone blocking action of guanethidine is demonstrably not the result of there being inadequate supplies of neurotransmitter in the tissues to maintain function, the depletion of noradrenaline might be responsible for the failure of sympathetic transmission at a later stage in the action of guanethidine. The results in Table 3 show that this is unlikely to be true. Although the impairment of sympathetic transmission in cats studied 18 hr after subcutaneous injection of 10 mg/kg guanethidine was associated with a marked lowering in tissue noradrenaline levels—about 70% in the hearts and over 80% in the nictitating membranes—an even greater lowering in noradrenaline content after treatment with (−)-$\beta$-hydroxyphenethylguanidine (5 or 10 mg/kg) was unaccompanied by significant block of sympathetic transmission. Indeed, the responses of the nictitating membranes

to stimulation were normal despite a loss of over 90% of the noradrenaline. A similar absence of sympathetic blockade, despite depletion of over 95% of the tissue noradrenaline has been noted with (+)-adrenaline and metaraminol (Andén & Magnusson, 1963; Andén, 1964). A higher dose of (−)-β-hydroxyphenethylguanidine (20 mg/kg), which caused more than 95% depletion of noradrenaline from the heart, spleen and nictitating membranes, did cause some reduction in the responses to sympathetic stimulation, but block was nowhere near as intense as that produced by 15 mg/kg guanethidine, which caused rather less depletion of the neurotransmitter. In another cat given 20 mg/kg of (−)-β-hydroxyphenethylguanidine, the responses of the nictitating membranes 18 hr later, at rates of stimulation of 1 to 30 pulses/sec, were not significantly below normal. In this cat the contractions of the nictitating membranes to adrenaline and noradrenaline were enhanced although the pressor effects of these drugs were normal. The pressor response to tyramine was however, markedly lowered. It is noteworthy that in nearly all the cats treated with either guanethidine or (−)-β-hydroxyphenethylguanidine, the resting heart rate was below that of the controls. This may be a consequence of noradrenaline depletion but some other depressant action on the myocardium cannot be excluded.

There is considerable evidence (Chang, Costa & Brodie, 1965) that depletion of noradrenaline by guanethidine is associated with replacement of the noradrenaline by guanethidine in the neurotransmitter storage vesicles. It seems likely from the close similarity between the depleting actions of guanethidine and (−)-β-hydroxyphenethylguanidine that depletion by the latter also results from replacement by the drug of the stored noradrenaline. The weak impairment of sympathetic transmission produced by (−)-β-hydroxyphenethylguanidine may thus conceivably be attributed to this replacement and to consequent inadequate release of noradrenaline, or release of the drug itself as a virtually inactive "false transmitter," on nerve stimulation. However, a bretylium-like adrenergic neurone blocking action cannot be ruled out, especially since amphetamine, a potent antagonist of bretylium (Day, 1962), also abolishes the partial sympathetic blockade caused by (−)-β-hydroxyphenethylguanidine.

Brodie and his colleagues (Kuntzman, Costa, Gessa & Brodie, 1962; Chang *et al.*, 1965) have suggested that the initial sympathetic blocking action of guanethidine results from the sustained stimulation of the normal noradrenaline-releasing mechanism at the nerve-endings, so that no further response could be expected on nerve stimulation. Our results make such an interpretation of the action of guanethidine improbable. Thus, although (−)-β-hydroxyphenethylguanidine appears to deplete noradrenaline in the same way as does guanethidine, it has no immediate blocking action resembling that of guanethidine on sympathetic transmission. It seems more likely, as proposed by Cass & Spriggs (1961), that guanethidine possesses a strong bretylium-like adrenergic neurone blocking action distinct from its depleting action.

## SUMMARY

1. (−)-β-Hydroxyphenethylguanidine is several times more potent than guanethidine in depleting peripheral sympathetically-innervated tissues of their noradrenaline. It also acts rather faster. In mice, 50% depletion of heart noradrenaline is produced in 4 hr by about 0.25 mg/kg of the drug.

2. It is much less active than guanethidine in producing ptosis in mice.

3. Drugs which antagonize the noradrenaline-depleting action of guanethidine also antagonize that of (−)-β-hydroxyphenethylguanidine, but not always that of reserpine.

4. In rats, iproniazid blocks the depleting action of both guanethidine and (−)-β-hydroxyphenethylguanidine to an extent related to the inhibition of heart monoamine oxidase.

5. Unlike guanethidine, (−)-β-hydroxyphenethylguanidine has weak and very erratic sympathetic blocking actions in conscious or anaesthetized cats.

6. In cats (−)-β-hydroxyphenethylguanidine lowers the noradrenaline in the hearts, spleens or nictitating membranes 18 hr after injection more than does an equal dose of guanethidine. In contrast to guanethidine, it has little effect on sympathetic transmission despite the loss of over 90% of the tissue noradrenaline.

7. It is concluded that the sympathetic blocking action of guanethidine is distinct from its noradrenaline depleting action.

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