Brit. J. Pharmacol. (1963), **20**, 171–177.

THE ACTION OF GUANETHIDINE WITH PARTICULAR REFERENCE TO THE SYMPATHETIC NERVOUS SYSTEM

BY

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(*Received October* 16, 1962)

It has been suggested that guanethidine can release and then deplete postganglionic sympathetic nerve endings of noradrenaline. However, no release of noradrenaline from postganglionic nerve endings or from the adrenal medulla by guanethidine was found by direct experiment. Although release of noradrenaline from postganglionic sympathetic nerve endings in response to nerve stimulation was rapidly reduced and finally abolished by guanethidine, the drug did not appear to affect the release of catechol amines from the adrenal medulla in response to splanchnic nerve stimulation. The nature of the action of guanethidine is discussed, and it is concluded that it blocks the effect of postganglionic sympathetic nerve stimulation by interfering with the synthesis of transmitter and that it also has a direct sympathomimetic effect.

Intravenous injection of guanethidine has three main effects in the anaesthetized cat. First, there is an initial rise of blood pressure followed by a slow fall; the control value is regained 0.5 to 2 hr later, and the pressure eventually settles somewhat lower. Second, the effect of postganglionic sympathetic nerve stimulation is abolished. Third, the blood pressure responses to intravenous injections of adrenaline and noradrenaline are potentiated.

Although these actions have not been fully explained, it has been suggested that guanethidine releases transmitter from, and then depletes, the postganglionic sympathetic stores. The present experiments were designed to test this hypothesis.

METHODS

Cats were anaesthetized with chloralose (80 mg/kg) after induction with ethyl chloride and ether. Femoral arterial blood pressure was recorded using a mercury manometer.

Collection and assay of vasopressor activity in venous blood

Spleen. The methods for stimulation of the splenic nerves and collection of blood samples were those of Brown & Gillespie (1957). Close-arterial injections into the spleen were made through a cannula in the stump of either the hepatic or the left gastric artery.

Adrenal gland. The intestines and spleen were removed and the central end of the tied left renal vein was cannulated with polyethylene tubing. The left genital vein was tied. Blood from the left adrenolumbar vein was diverted into the cannula by an occlusion ligature proximal to the junction of the renal and adrenolumbar veins.

Assay. All samples were assayed for noradrenaline on the blood pressure of the pithed rat. The method of assay and the precautions taken to prevent the development of vasopressor activity unrelated to the transmitter in the blood were those of Brown & Gillespie (1957). Small amounts of guanethidine in the plasma interfered with the assay. For this reason no assay was carried out if the whole animal had been given guanethidine. The technique was to give a close-arterial injection into the organ under investigation and, after a short period of venous occlusion, then to collect the first sample of venous blood from the organ and discard it. Subsequent samples could be assayed satisfactorily.

Splanchnic nerve stimulation. Whenever these nerves were to be stimulated, they were approached through a midline incision. The major splanchnic nerves were freed and tied, and shielded platinum electrodes placed on the nerves distal to the tie with the cathode placed peripherally.

Drugs. A standard dose of 20 mg of guanethidine hemisulphate (Ciba) was given intravenously; this is equivalent to a dose of 6 to 10 mg/kg for the cats used. In some experiments phenoxybenzamine, *N*-phenoxyisopropyl-*N*-benzyl-β chloroethylamine (Dibenyline, S. K. & F.), in a dose of 10 mg/kg was used as a sympathetic blocking agent.

RESULTS

Effect of guanethidine on sympathetic nerve stimulation and on the release of transmitter. Stimulation of the postganglionic fibres of the superior mesenteric or splenic nerves raised blood pressure, an effect which was abolished by guanethidine. A typical response to splenic nerve stimulation at a frequency of 10 shocks/sec

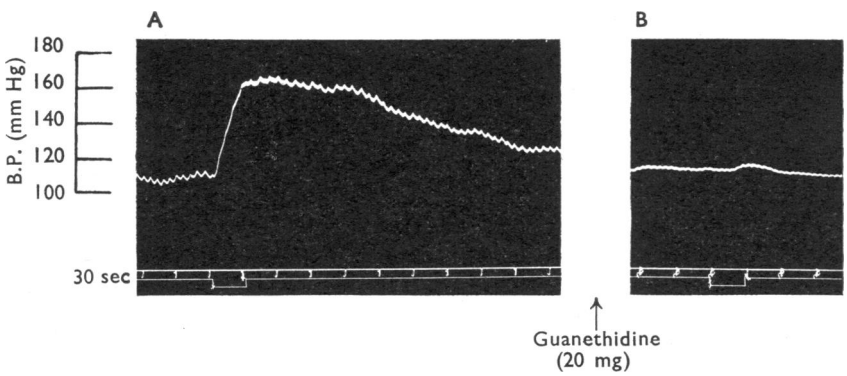

Fig. 1. Cat, male, 3.4 kg. Chloralose anaesthesia. Record of the femoral blood pressure. A and B: the effects of stimulation (at signals) of the splenic nerves with 300 stimuli at 10 shocks/sec. Between A and B, 20 mg of guanethidine was injected intravenously.

for 30 sec, raising the blood pressure by 50 mm Hg, is seen in Fig. 1, A. Guanethidine was then given and, during the next 40 min, stimulation of the splenic nerves was repeated at 10 min intervals. On no occasion was there any rise in blood pressure (Fig. 1, B), nor was there any visual evidence of contraction of the spleen.

In six experiments the amount of noradrenaline appearing in the venous blood from stimulation of the splenic nerves was determined using a standard stimulation of 200 stimuli at 30 shocks/sec. Stimulation was repeated at 10 min intervals and, between the second and third periods of stimulation, guanethidine was given into the spleen by close-arterial injection and held there for 1 min by occluding the venous outflow. A typical result from these experiments is shown in Fig. 2, when the first and second amounts of noradrenaline released were 920 and 925 pg/stimulus respectively.

Then 2 mg of guanethidine was injected intra-arterially into the spleen and, after venous occlusion for 1 min, 5 ml. of blood was collected and discarded so that guanethidine did not enter the general circulation. Release of noradrenaline in response to splenic nerve stimulation 10 min after the injection was reduced to 180 pg/stimulus and, after another 10 min, to 35 pg/stimulus. Later stimulations produced little or no detectable release of noradrenaline (Fig. 2).

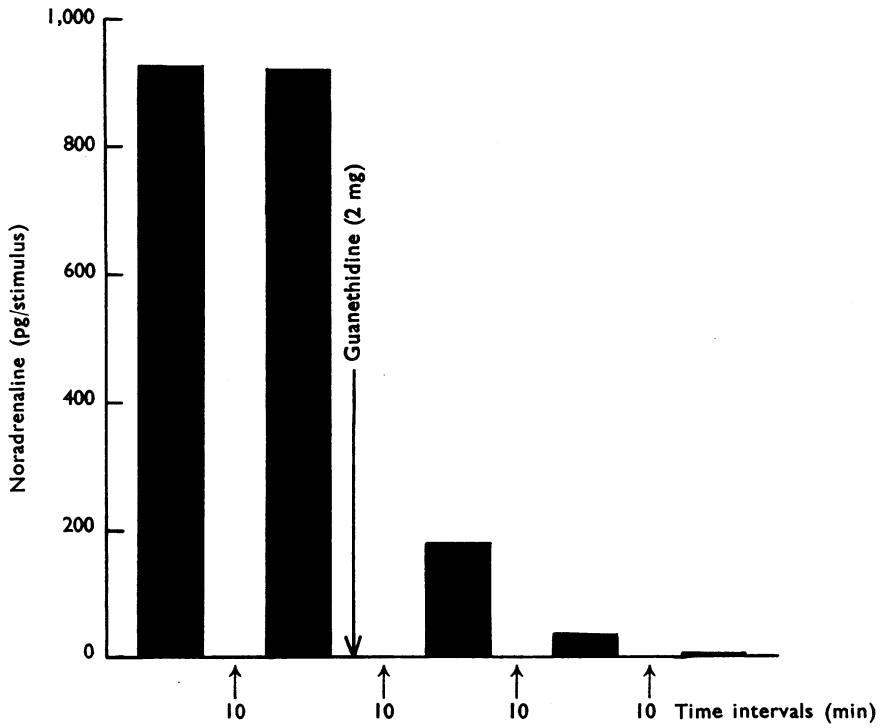

Fig. 2. Outputs of noradrenaline (ordinate, pg/stimulus) from the spleen in response to stimulation of the splenic nerves with trains of 200 stimuli at a frequency of 30 shocks/sec. Between the second and third stimulations, 2 mg of guanethidine was given to the spleen by close-arterial injection.

The possible release of noradrenaline at sympathetic nerve endings by guanethidine. The possibility that the initial pressor effect of guanethidine when given intravenously is due to the release of noradrenaline at sympathetic nerve endings was tested by direct experiment. After one standard test determination of noradrenaline release by stimulation of the splenic nerve had shown that the preparation was in good condition, 2 mg of guanethidine was given by close-arterial injection into the spleen. The venous outflow was occluded for 1 min and then sampling was started in the absence of nerve stimulation. The first sample of blood was rejected. In some experiments sampling was continuous for about 5 min ; in others, samples were collected at regular intervals during the 10 or 15 min after the injections. Guanethidine never increased the vasopressor activity above the earlier control value in the

absence of stimulation. In each of the experiments it was confirmed that the guanethidine had prevented further release of noradrenaline in response to splenic nerve stimulation.

To exclude the possibility that noradrenaline might be released by the guanethidine and then taken up by receptors, the experiments were repeated with cats treated with phenoxybenzamine, which blocks the uptake of noradrenaline by the receptors (Brown & Gillespie, 1957 ; Brown, Davies & Ferry, 1961) and thus any noradrenaline released by the nerve endings should appear in the venous blood. In none of the three experiments did guanethidine release noradrenaline.

These experiments indicate that guanethidine did not suddenly release noradrenaline from sympathetic nerve endings, but a prolonged release of very small amounts of noradrenaline, which could not be detected by our method of assay, cannot be excluded.

The origin of the vasopressor effect of guanethidine. Since the initial vasopressor effect of guanethidine cannot be attributed to a general release of noradrenaline at postganglionic sympathetic nerve endings, the possibility that it is due to release of catechol amines from the adrenal medulla was investigated. Results of experiments in which guanethidine was given to adrenalectomized animals were variable. In one of five experiments, 20 mg of guanethidine did not raise blood pressure, but about 2 min after the injection a slow fall began. In every other instance guanethi-

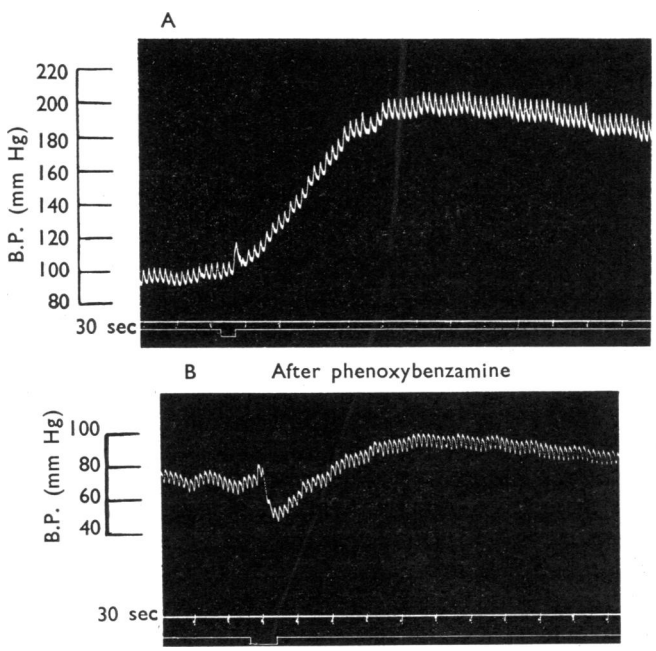

Fig. 3. Records of the femoral blood pressure. The effects of intravenous injections of 20 mg of guanethidine at the signals. A: cat, male, 2.6 kg. Chloralose anaesthesia. B: cat, female, 2.2 kg. Chloralose anaesthesia. Phenoxybenzamine (10 mg/kg) was injected before recording began.

dine greatly raised blood pressure. Although the initial parts of the pressor effects often had different time courses, the peak of the blood pressure rises was always about 3 min after the injections. The vasopressor action of guanethidine is therefore not dependent on an intact adrenal medulla. This rise in pressure after guanethidine could be repeated several times with the same animal.

To test whether guanethidine has some vasopressor effect unrelated to the sympathetic nervous system, its action was investigated in animals treated with phenoxybenzamine. The immediate effect of guanethidine in such animals was hypotension, followed by a small slow rise in pressure. This response was reproducible. Fig. 3 compares the effects of injections of 20 mg of guanethidine into a normal animal (A) and into one treated with phenoxybenzamine (B).

The hypotension in response to guanethidine after phenoxybenzamine is reminiscent of the reversal of the pressor effect of adrenaline by phenoxybenzamine. Bilateral adrenalectomy was carried out in an animal which had received phenoxybenzamine and which responded to guanethidine with a fall in blood pressure ; this fall remained unchanged, showing that it was not due to release of catechol amines by the adrenal medulla.

The differential action of guanethidine on the adrenal medulla and on postganglionic sympathetic nerve endings. Experiments to test directly whether guanethidine releases catechol amines from the adrenal medulla proved unsatisfactory, for the presence of guanethidine in the venous blood, coupled with the small sample volumes, made assay difficult. It was possible, however, to demonstrate large amounts of vasopressor activity in the adrenal venous blood following splanchnic nerve stimulation, and neither close-arterial injection of guanethidine into the adrenal gland nor intravenous administration to the whole animal appeared to affect this release. In the absence of splanchnic nerve stimulation, there was no evidence that guanethidine released vasopressor substances from the adrenal medulla. The assay technique used did not differentiate between adrenaline and noradrenaline.

The effect of guanethidine on the blood pressure response to splanchnic nerve stimulation illustrates the differential action that it has upon the adrenal medulla and upon postganglionic sympathetic nerve endings. Stimulation of the left major splanchnic nerve caused the characteristic rise in blood pressure, consisting of a faster component due to vasoconstriction in the splanchnic bed and a slower component due to the release of vasopressor substances from the adrenal medulla. When 20 mg of guanethidine was given and the stimulation was repeated, the fast component of the response was absent and the slow component was potentiated. In some of these experiments the adrenal glands were later removed and then stimulation of the left splanchnic nerves caused little or no rise in blood pressure.

DISCUSSION

Much work has been done on content of catechol amines in organs after the administration of guanethidine. It is generally agreed that, within 1 to 2 hr of an injection of guanethidine, the amount of noradrenaline is reduced in the spleen, heart and intestines of cats, rabbits and rats (Sheppard & Zimmerman, 1959 ; Cass, Kuntzman & Brodie, 1960 ; Cass & Spriggs, 1961). This depletion is soon apparent,

but the maximum depletion of 80 to 90% is not reached for 4 to 18 hr. However, the effect of sympathetic nerve stimulation is abolished within a few minutes. It seems unlikely, therefore, that the two events are related. The present experiments show that the abolition of the effect of nerve stimulation is due to the failure of the postganglionic sympathetic endings to release noradrenaline and is not related to the receptor mechanism of the effector cell, which gives a potentiated response to injected noradrenaline.

The action of guanethidine on postganglionic sympathetic nerve endings might consist of two phases. First, it rapidly prevents release of transmitter, as the present work has shown, and second, if the organ content of catechol amines is a reflexion of the amount of transmitter in the nerve endings, a relatively slow depletion follows.

It has been postulated that the transient vasopressor effect of guanethidine is due, in part at least, to the liberation of catechol amines from some source, probably in the heart or blood vessels (Gillis & Nash, 1961). The present experiments do not support this view since there was no evidence of the release of any substantial amount of transmitter at the sympathetic nerve endings tested. It is possible that guanethidine caused a prolonged release of small amounts of transmitter which were not detected by the assay technique. This possibility would be compatible with the time course of the depletion of tissue catechol amines, but the quantities involved could play little part in the initial vasopressor effect of guanethidine. In addition, Maxwell, Plummer, Povalski & Schneider (1960) have presented indirect evidence that there is no increase in the blood level of catechol amines after guanethidine.

As phenoxybenzamine converted the pressor effect of guanethidine into a depressor response, adrenaline may be involved, possibly as a result of activity of the adrenal medulla. Experiments to test this point directly, although not completely satisfactory, gave no indication that this was so. In addition the depressor effect of guanethidine in an animal treated with phenoxybenzamine persisted after adrenalectomy.

The initial vasopressor effect of guanethidine, therefore, seems unlikely to be due to the release of adrenaline or noradrenaline from sympathetic nerve endings or the adrenal medulla. Guanethidine probably has some direct sympathomimetic action, as has been suggested by Abboud & Eckstein (1961).

Cass *et al.* (1960) found that, whereas guanethidine reduced the noradrenaline content of the spleen and intestines in cat and rabbit, it had no effect on the noradrenaline content of the adrenal medulla. Hertting, Axelrod & Whitby (1961) observed that guanethidine reduced the uptake of [H^3]-noradrenaline by the heart and spleen of the cat, but the uptake by the adrenal medulla was unaltered. Thus guanethidine affects the metabolism of noradrenaline at sympathetic nerve endings but not in the adrenal medulla. The present experiments in which the splanchnic nerves were stimulated support this differential action, as the hypertensive response attributed to vasoconstriction was abolished, whereas that attributed to the release of catechol amines from the adrenal medulla was unaltered. This could not be due to block of ganglionic transmission, because Maxwell, Plummer, Schneider, Povalski & Daniel (1960) showed that, although some depression of

ganglionic transmission occurred after guanethidine, recovery was complete within 30 min.

It was concluded that guanethidine appeared to have two distinct actions. First, it prevented the synthesis of transmitter, and this resulted eventually in depletion, possibly by the slow release of any preformed transmitter. Second, the drug had a direct sympathomimetic effect.

We thank Mr R. A. Dwyer for technical assistance, Ciba Laboratories for supplying guanethidine, and the Royal Society for a grant defraying part of the expenses of this work.

REFERENCES

ABBOUD, F. M. & ECKSTEIN, J. W. (1961). Observations on the mechanisms of the local vasodilator effect of guanethidine seen in man and in dog. *Circulation*, **24**, 873.

BROWN, G. L., DAVIES, B. N. & FERRY, C. B. (1961). The effect of neuronal rest on the output of sympathetic transmitter from the spleen. *J. Physiol. (Lond.)*, **159**, 365–380.

BROWN, G. L. & GILLESPIE, J. S. (1957). The output of sympathetic transmitter from the spleen of the cat. *J. Physiol. (Lond.)*, **138**, 81–102.

CASS, R., KUNTZMAN, R. & BRODIE, B. B. (1960). Norepinephrine depletion as a possible mechanism of action of guanethidine (SU 5864), a new hypotensive agent. *Proc. Soc. exp. Biol. (N.Y.)*, **103**, 871–872.

CASS, R. & SPRIGGS, T. L. B. (1961). Tissue amine levels and sympathetic blockade after guanethidine and bretylium. *Brit. J. Pharmacol.*, **17**, 442–450.

GILLIS, C. N. & NASH, C. W. (1961). The initial pressor actions of bretylium tosylate and guanethidine sulphate and their relation to release of catecholamines. *J. Pharmacol. exp. Ther.*, **134**, 1–7.

HERTTING, G., AXELROD, J. & WHITBY, L. G. (1961). Effect of drugs on the uptake and metabolism of H^3-norepinephrine. *J. Pharmacol. exp. Ther.*, **134**, 146–152.

MAXWELL, R. A., PLUMMER, A. J., POVALSKI, H. & SCHNEIDER, F. (1960). Concerning a possible action of guanethidine (SU-5864) in smooth muscle. *J. Pharmacol. exp. Ther.*, **129**, 24–30.

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

SHEPPARD, H. & ZIMMERMAN, J. (1959). Effect of guanethidine (SU-5864) on tissue catecholamines. *Pharmacologist*, **1**, 69.