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ACTIONS OF BRETYLIUM AND GUANETHIDINE ON THE UPTAKE AND RELEASE OF [^{3}H]-NORADRENALINE

BY

G. HERTTING,* J. AXELROD AND R. W. PATRICK

From the Laboratory of Clinical Science, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland

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The effect of bretylium and guanethidine has been studied on the uptake and the spontaneous and reserpine-induced release of [^{3}H]-noradrenaline in the rat heart and in the splenic nerve endings of the cat. Bretylium and guanethidine inhibited the uptake by the heart of circulating [^{3}H]-noradrenaline. Bretylium blocked spontaneous and reserpine-induced release of [^{3}H]-noradrenaline, while guanethidine caused release and partially antagonized the reserpine-induced release. Both compounds produced a transient liberation of [^{3}H]-noradrenaline from splenic nerves, but blocked the release of the [^{3}H]-catechol amine following stimulation of the splenic nerve.

Bretylium and guanethidine can produce hypotension in animals and in man (Boura & Green, 1959; Maxwell, Mull & Plummer, 1959; Maxwell, Plummer, Povalski & Schneider, 1960; Page & Dustan, 1959; Brest, Duarte, Glantz & Moyer, 1960). Both compounds block or abolish the effect of stimulation of pre- or post-ganglionic sympathetic nerves and enhance the action of injected catechol amines (Boura & Green, 1959; Maxwell *et al.*, 1960). Bretylium blocks the release of noradrenaline from the spleen after stimulation of the splenic nerve (Boura & Green, 1959). Guanethidine, on the other hand, depletes endogenous catechol amines from tissue stores (Sheppard & Zimmerman, 1959; Cass, Kuntzman & Brodie, 1960).

It has been demonstrated previously that a wide variety of drugs cause or prevent the release of [^{3}H]-noradrenaline (Axelrod, Whitby & Hertting, 1961; Hertting, Axelrod & Whitby, 1961; Axelrod, Hertting & Patrick, 1962). In this report, the effect of bretylium and guanethidine on the uptake and release by the rat heart of [^{3}H]-noradrenaline and on the release of [^{3}H]-noradrenaline from unstimulated and stimulated cat spleen will be described.

METHODS

Male Sprague-Dawley rats weighing 160 to 180 g were divided into groups of 6 and given 10 μc of 7-[^{3}H]-(±)-noradrenaline (20 mc/mg) for 100 g into the tail vein. Dosage of bretylium tosylate and of guanethidine sulphate and time of administration are shown in Tables 1 and 2. Rats were killed by decapitation and the hearts removed immediately, weighed and homogenized in 10 ml. of ice-cold 0.4 N-perchloric acid. [^{3}H]-Noradrenaline was assayed by the method of Whitby, Axelrod & Weil-Malherbe (1961). Endogenous noradrenaline was

* Present address: Department of Pharmacology, University of Vienna, Vienna, Austria.

determined by the procedure of Crout, Creveling & Udenfriend (1961). Monoamine oxidase was estimated by the method of Lovenberg, Levine & Sjoerdsma (1961).

To examine the action of bretylium and guanethidine on the release of [^{3}H]-noradrenaline from the spleen before and after stimulation of the splenic nerve, a preparation previously described was used (Hertting & Axelrod, 1961). Cats were given 200 μc/kg of [^{3}H]-noradrenaline (20 mc/mg) intravenously and the spleen was isolated 4 to 5 hr later, placed in a saline bath at 37° C and perfused with non-radioactive blood from a donor cat. The venous outflow from the spleen contained a small amount of [^{3}H]-noradrenaline due to spontaneous release. Upon stimulation of the splenic nerve, large amounts of [^{3}H]-noradrenaline were found in the outflow. Blood was collected during 1-min intervals, an aliquot of the plasma was deproteinized with perchloric acid and assayed for [^{3}H]-noradrenaline. A total of 300 stimuli (10 V, 2 msec duration at 30/sec) were applied through bipolar platinum electrodes to the splenic nerve. Four experiments were performed with guanethidine and one with bretylium.

RESULTS

Effect of bretylium and guanethidine on uptake and release of [^{3}H]-noradrenaline by the rat heart

When given to rats before the injection of [^{3}H]-noradrenaline, both bretylium and guanethidine reduced the amount of the radioactive noradrenaline in the heart, guanethidine causing a more pronounced effect. The administration of guanethidine after the [^{3}H]-noradrenaline had become bound to tissues resulted in a reduction of the [^{3}H]-catechol amine. The [^{3}H]-noradrenaline content of the heart after bretylium, on the other hand, was greater than that of the control animals. After 24 hr only a trace of [^{3}H]-noradrenaline was present in the heart after guanethidine treatment, while the values in the bretylium-treated animals were almost twice those of the controls. These results are shown in Table 1.

TABLE 1

EFFECT OF BRETYLIUM AND GUANETHIDINE ON UPTAKE AND RELEASE OF [^{3}H]-NORADRENALINE BY THE RAT HEART

Groups of 6 rats were given 10 μc [^{3}H]-noradrenaline/100 g intravenously before or after the drugs. Bretylium and guanethidine were given intramuscularly

Drug	Dose mg/kg	Time drug given before or after [^{3}H]-noradrenaline (min)	Time rats killed after [^{3}H]-noradrenaline (hr)	[^{3}H]-Noradrenaline in heart (mμc/g±SEM)
None	—	—	2	193±12
Bretylium	20	30 before	2	151±5 ($P<0.05$)
Bretylium	20	30 after	2	268±12 ($P<0.01$)
Guanethidine	20	30 before	2	28±2·2 ($P<0.001$)
Guanethidine	20	30 after	2	146±12 ($P<0.05$)
None	—	—	24	38±3
Bretylium	20	30 after	24	71±6 ($P<0.001$)
Guanethidine	20	30 after	24	1·9±0·2 ($P<0.001$)

Twelve rats were given 20 mg/kg of bretylium intramuscularly, twice at 24 hr intervals; they were killed 48 hr after the initial injection and the hearts examined for endogenous catechol amine content. The concentration of endogenous noradrenaline and the monoamine oxidase activity in the bretylium-treated rats were the same as in untreated controls.

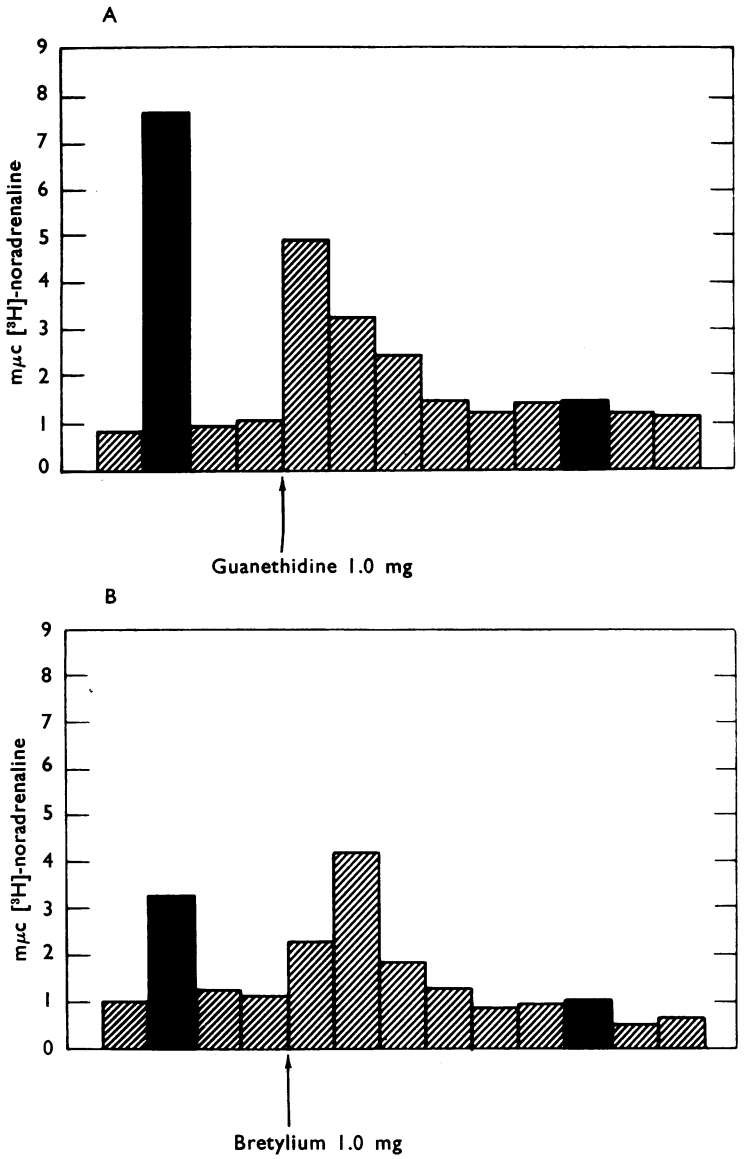

Fig. 1. Effect of guanethidine (A) and bretylium (B) on the release of [³H]-noradrenaline in the spleen. Cats were given 200 μc/kg [³H]-noradrenaline intravenously and the spleen was prepared as described in the text. Splenic nerves were stimulated at a frequency of 30/sec for a total of 300 stimuli. Guanethidine (1 mg) or bretylium (1 mg) was injected into the splenic artery. Each column represents [³H]-noradrenaline (in mμc) released during a 1-min collection period. Hatched columns represent [³H]-noradrenaline measured before stimulation and solid columns for 1 min, during and after stimulation.

Effect of bretylium and guanethidine on release of [^{3}H]-noradrenaline by reserpine by the rat heart

Reserpine caused a marked reduction of [^{3}H]-noradrenaline in the rat heart after 4 hr. Guanethidine also lowered [^{3}H]-noradrenaline, while bretylium caused an elevation (Table 2). When bretylium was given before reserpine, the releasing action of reserpine was diminished considerably. Although guanethidine itself released [^{3}H]-noradrenaline, it significantly reduced the releasing action of reserpine (see Table 2).

TABLE 2

EFFECT OF BRETYLIUM AND GUANETHIDINE ON THE [^{3}H]-NORADRENALINE-RELEASING ACTION OF RESERPINE IN THE RAT HEART

The animals were killed 4 hr after the administration of [^{3}H]-noradrenaline; 20 mg/kg of guanethidine or of bretylium were given intramuscularly 30 min after and 1 mg/kg reserpine 2 hr after the [^{3}H]-catechol amine administration. Six animals were used for each group. The following means were found to be significantly different: guanethidine, bretylium and reserpine versus controls ($P<0{\cdot}001$); reserpine versus combined bretylium and reserpine ($P<0{\cdot}001$); reserpine versus combined guanethidine and reserpine ($P<0{\cdot}01$)

Drug	[^{3}H]-Noradrenaline mμc/g heart±SEM
Control	202±9
Guanethidine	70±8·5
Bretylium	262±7·8
Reserpine	21±5
Guanethidine and reserpine	50±4·5
Bretylium and reserpine	125±7·5

Effect of guanethidine and bretylium on release of [^{3}H]-noradrenaline from unstimulated and stimulated spleen

The venous outflow from spleens of cats which had received [^{3}H]-noradrenaline was assayed for [^{3}H]-catechol amine content. Upon stimulation of the splenic nerve, large amounts of [^{3}H]-noradrenaline were released into the venous blood (Fig. 1). After the injection of guanethidine or of bretylium into the splenic artery, a rise in [^{3}H]-noradrenaline was observed in the splenic outflow for 3 to 5 min in the absence of stimulation. However, after the administration of either drug, stimulation of the splenic nerve failed to release [^{3}H]-noradrenaline. At the end of each experiment, the spleen was examined for [^{3}H]-noradrenaline. About 500 mμc of [^{3}H]-noradrenaline was found in the spleen and less than 60 mμc had been released during the entire course of the experiment.

DISCUSSION

It has been shown that circulating noradrenaline is taken up, bound and retained at or near the sympathetic nerve endings (Axelrod, Weil-Malherbe & Tomchick, 1959; Whitby *et al.*, 1961; Hertting, Axelrod, Kopin & Whitby, 1961). Upon stimulation of the sympathetic nerves, this catechol amine is released (Hertting & Axelrod, 1961). Our results here show that guanethidine and bretylium had actions in connexion with uptake and release of [^{3}H]-noradrenaline, some of which were similar and others of which were different. Both compounds appeared to prevent the uptake of the [^{3}H]-catechol amine by the rat heart, the effect of guanethidine being quantitatively greater than that of bretylium. However, once the [^{3}H]-noradren-

aline had been bound in the heart tissue, guanethidine released [^{3}H]-noradrenaline while bretylium inhibited the spontaneous release of the hormone. Guanethidine caused a slow but continuous release of [^{3}H]-noradrenaline for long periods of time, so that, after 24 hr, negligible amounts of [^{3}H]-noradrenaline are present in the heart. Both drugs, when injected into the splenic artery, produced a transient rise in blood pressure (Boura & Green, 1959 ; Maxwell *et al.*, 1960) as well as an immediate but brief release of [^{3}H]-noradrenaline.

Bretylium inhibited markedly [^{3}H]-noradrenaline release by reserpine. This effect was similar to that produced by mono-amine oxidase inhibitors (Spector, Kuntzman, Shore & Brodie, 1960 ; Axelrod *et al.*, 1962), yet bretylium did not inhibit mono-amine oxidase in the doses used here. Mono-amine oxidase inhibitors have been shown to elevate the endogenous adrenaline concentration in the heart (Pletscher, 1958), but bretylium did not. These differences could be explained by the fact that mono-amine oxidase inhibitors impair the release of noradrenaline but not its uptake, while bretylium blocked both the release and the uptake. Consequently with the former drug there is an increase in catechol amine while with the latter there was no net change.

Bretylium, guanethidine and mono-amine oxidase inhibitors have been shown to prevent the effects of sympathetic nerve stimulation. Mono-amine oxidase inhibitors block ganglionic transmission (Gertner, 1961 ; DaCosta & Goldberg, 1961), while bretylium and guanethidine prevent the effects of postganglionc stimulation (Boura & Green, 1959 ; Maxwell *et al.*, 1960). Bretylium and guanethidine also blocked the release of [^{3}H]-noradrenaline when sympathetic nerves were stimulated. Drugs which block the passage of sympathetic nerve impulses appear to interfere with the releasing action of reserpine. These observations suggest that the release of noradrenaline by reserpine depends to some degree on sympathetic nerve impulses.

REFERENCES

AXELROD, J., HERTTING, G. & PATRICK, R. W. (1962). Inhibition of H^3-norepinephrine release by monoamine oxidase inhibitors. *J. Pharmacol. exp. Ther.* In the press.

AXELROD, J., WEIL-MALHERBE, H. & TOMCHICK, R. (1959). The physiological disposition of H^3-epinephrine and its metabolite metanephrine. *J. Pharmacol. exp. Ther.*, **127**, 251–256.

AXELROD, J., WHITBY, L. G. & HERTTING, G. (1961). Effect of psychotropic drugs on the uptake of H^3-norepinephrine by tissues. *Science*, **133**, 383–384.

BOURA, A. L. A. & GREEN, A. F. (1959). The actions of bretylium: adrenergic neurone blocking and other effects. *Brit. J. Pharmacol.*, **14**, 536–548.

BREST, A. N., DUARTE, C., GLANTZ, G. & MOYER, J. H. (1960). Preliminary observations on the antihypertensive properties of guanethidine. *Current Therapeutic Research*, **2**, 17–21.

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.

CROUT, J. R., CREVELING, C. R. & UDENFRIEND, S. (1961). Norepinephrine metabolism in rat brain and heart. *J. Pharmacol. exp. Ther.*, **132**, 269–277.

DACOSTA, F. M. & GOLDBERG, L. I. (1961). Depression of ganglionic transmission by MO-911, (N-methyl-N-benzyl-2-propynylamine hydrochloride) a non-hydrazine monoamine oxidase inhibitor. *Fed. Proc.*, **20**, 318.

GERTNER, S. B. (1961). The effects of monoamine oxidase inhibitors on ganglionic transmission. *J. Pharmacol. exp. Ther.*, **131**, 223–230.

HERTTING, G. & AXELROD, J. (1961). The fate of H^3-noradrenaline at the sympathetic nerve endings. *Nature (Lond.)*, **192**, 172–173.

HERTTING, G., AXELROD, J., KOPIN, I. J. & WHITBY, L. G. (1961). Lack of uptake of catecholamines after chronic denervation of sympathetic nerves. *Nature (Lond.)*, **189**, 66.

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**, 146–153.

LOVENBERG, W., LEVINE, R. & SJOERDSMA, A. (1961). Sensitive assay of monoamine oxidase activity: application to sympathetic ganglia and heart. *Fed. Proc.*, **20**, 318.

MAXWELL, R. A., MULL, R. P. & PLUMMER, A. J. (1959). [2-Octa-hydro-1-azocinyl)-ethyl]-guanidine sulfate (Ciba 5864-SU), a new synthetic antihypertensive agent. *Experientia*, **15**, 267.

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.

PAGE, I. H. & DUSTAN, H. P. (1959). A new, potent antihypertensive drug. Preliminary study of [2-(octahydro-1-azocinyl)-ethyl]-guanidine sulfate (guanethidine). *J. Amer. med. Ass.*, **170**, 1265–1271.

PLETSCHER, A. (1958). Einfluss von isopropyl-isonikotinsäurehydrazid auf den Katecholamingehalt des Myocards. *Experientia*, **14**, 73–74.

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

SPECTOR, S., KUNTZMAN, R., SHORE, P. A. & BRODIE, B. B. (1960). Evidence for release of brain amines by reserpine in presence of monoamine oxidase inhibitors: implication of monoamine oxidase in norepinephrine metabolism in brain. *J. Pharmacol. exp. Ther.*, **130**, 256–261.

WHITBY, L. G., AXELROD, J. & WEIL-MALHERBE, H. (1961). The fate of H^3-norepinephrine in animals. *J. Pharmacol. exp. Ther.*, **132**, 193–201.