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A calcium requirement for release of ^{3}H-guanethidine by sympathetic nerve stimulation

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Cat colons were labelled with ^{3}H-guanethidine by close intra-arterial injection. Two hr later the colons were removed and the vascular bed was perfused with Krebs solution containing 0, 2·5 or 5 mM calcium. The spontaneous efflux of ^{3}H-guanethidine was not changed by alterations in the calcium concentration, but stimulation of the post-ganglionic sympathetic nerves failed to enhance the output of ^{3}H-guanethidine unless calcium was present in the perfusion fluid. It is concluded that extracellular calcium is essential for release of ^{3}H-guanethidine by sympathetic nerve stimulation and that the neural release of the inactive transmitter substitute is related to the pharmacological action of the drug.

RECENTLY, evidence has been accumulating that the antihypertensive drug guanethidine possesses some of the properties associated with the series of β-hydroxylated phenylethylamines believed to act as "false" transmitters, in the sense postulated by Day & Rand (1963). Two of the obvious requirements of a "false" neuro-humoral transmitter are firstly, uptake into adrenergic neurones and secondly, release by sympathetic nerve stimulation. Both requirements have recently been fulfilled for guanethidine (Costa, Chang & Brodie, 1964; Chang, Costa & Brodie, 1965; Boullin, Costa & Brodie, 1966a). Another essential factor might be a dependence upon extracellular calcium.

It is well-known that this cation is required for release of acetylcholine from cholinergic nerves (Harvey & MacIntosh, 1940), and for release of catecholamines from the adrenal medulla (Douglas & Rubin, 1961); it has also been demonstrated recently that calcium is required for the release of noradrenaline from sympathetic nerve endings in the cat colon (Boullin & Brodie, 1965; Boullin, 1966). The data presented in this communication indicate that extra-cellular calcium is required for the release of ^{3}H-guanethidine from sympathetic nerve fibres by electrical stimulation.

Experimental

GENERAL

Experiments were made with a preparation of the isolated cat colon, perfused through the vascular bed from the inferior mesenteric artery to the colic vein, and described in detail elsewhere (Boullin, Costa & Brodie, 1966b). Briefly, the procedure was as follows: cats weighing 2–4 kg were anaesthetised with pentobarbitone and dissected to expose the inferior mesenteric artery to the colon. 0·5 mg (35 μc) ^{3}H-guanethidine sulphate (specific activity 17·4 mc/mM) was injected into the artery so that the drug passed into the vascular bed of the colon. After 2 hr the colon was removed for perfusion.

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PERFUSION OF THE COLON

The vascular bed was perfused at constant flow (1·2–2·4 ml/min). The perfusion medium was Krebs solution (Gillespie & MacKenna, 1961). The calcium concentration was varied from 0 to 2·5 mM or 5 mM and 1 mM disodium edetate was always included in the calcium-free solutions. It was added to the 2·5 or 5 mM calcium solutions as stated in Results.

During perfusion, the venous effluent from the vascular bed of the colon was collected at 2 min intervals before, during and after sympathetic nerve stimulation. The post-ganglionic sympathetic nerve fibres accompanying the inferior mesenteric artery were stimulated supra-maximally for 5 min with trains of 3000 rectangular impulses of 1 msec duration at a frequency

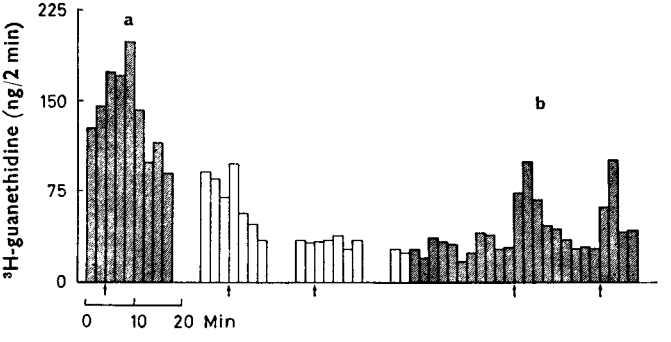

FIG. 1. Effects of changes in calcium concentration on the output of ^{3}H-guanethidine from the vascular bed of the isolated cat colon. Each histogram represents the output of drug in venous effluent from the colon collected over 2 min. Hatched histograms refer to output during perfusion with Krebs solution containing 2·5 mM calcium (without EDTA at a; with 1 mM EDTA at b) and open histograms to output during perfusion with calcium-free solution. At the arrows the nerve fibres were stimulated with 3000 impulses at 10/sec. Experimental data were obtained 40 min after commencement of perfusion.

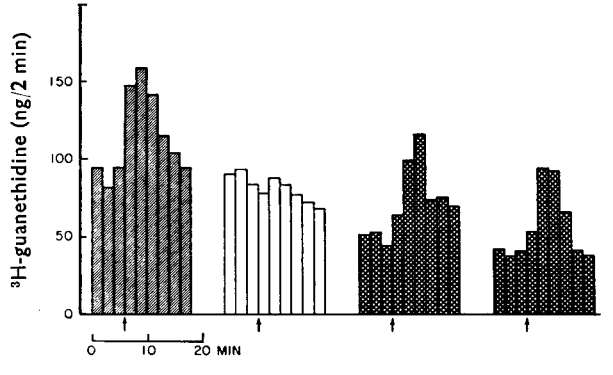

FIG. 2. Experimental details as Fig. 1 except that the cross-hatched histograms represent the output of guanethidine during perfusion with Krebs solution containing 5 mM calcium and 1mM EDTA Hatched histograms refer to 2·5 mM calcium only, while open histograms refer to calcium-free 1mM EDTA.

of 10/sec. 1 ml of each sample of the venous effluent was assayed for total radioactivity by liquid scintillation spectrometry.

The data presented are based on experiments with 6 cats.

Results

Boullin & others (1966a) have described the pattern of efflux of ^{3}H-guanethidine from the vascular bed of the isolated cat colon during perfusion with Krebs solution containing 2·5 mM calcium. Sympathetic nerve stimulation at 10 impulses/sec caused an increase in output of radioactive guanethidine that was independent of the spontaneous efflux of drug that occurred in the absence of nerve stimulation; successive periods of nerve stimulation released similar amounts of guanethidine irrespective of whether the spontaneous output was high or low.

In the present experiments it was found that sympathetic nerve stimulation only increased the efflux of ^{3}H-guanethidine when calcium was present in the perfusion medium. During perfusion with calcium-free Krebs solution containing 1 mM disodium edetate, the spontaneous output of radioactivity was unchanged, but the response to nerve stimulation was abolished. When the cation was replaced, in the presence of disodium edetate, the response to nerve stimulation was restored. In addition there was also a small increase in the spontaneous output occurring shortly after the replacement of calcium, and lasting for several minutes (Fig. 1). Similar results were obtained when double the normal concentration of calcium was added to the perfusion fluid after perfusion with calcium-free solution (Fig. 2).

Discussion

The data indicate that extracellular calcium is required for the release of tritiated guanethidine in the same way as it is required for the release of tritiated noradrenaline from the isolated cat colon in response to sympathetic nerve stimulation (Boullin & Brodie, 1965; Boullin, 1966). In both instances replacement of the calcium ion evokes a small increase in the spontaneous release of labelled compound. Calcium also appears to exert a direct releasing action on ^{14}C-guanethidine bound in tissue slices (Boullin, unpublished). However, provided the cation is present, increase in the calcium ion concentration does not enhance the efflux of guanethidine or noradrenaline (Boullin, 1966) released in response to sympathetic nerve stimulation. Apart from these and other similarities (Boullin & others, 1966a), the essential difference between guanethidine and noradrenaline, the authentic transmitter, is that guanethidine does not possess affinity for adrenergic receptors, since noradrenaline is still effective when adrenergic blockade is maximal (Boura & Green, 1962) and phenoxybenzamine does not augment the output of radioactive guanethidine after sympathetic nerve stimulation (Boullin, unpublished). Therefore guanethidine may be termed an inactive transmitter substitute.

It is well-known, particularly from the work of Cass & Spriggs (1961), that there is no close correlation between the onset of adrenergic blockade and depletion of catecholamines from tissues. In experiments with the cat spleen, Hertting, Axelrod & Patrick (1962) showed that there was a transient release of ^{3}H-noradrenaline immediately after guanethidine was administered. At this time the output of noradrenaline to sympathetic nerve stimulation was blocked (Hertting & others, 1962; Abercrombie & Davies, 1963). Thus it appears that guanethidine is bound and produces adrenergic blockade without any substantial noradrenaline loss. In the light of these observations and the present data it seems that guanethidine has two actions: it prevents release of the authentic transmitter noradrenaline and also behaves as an inactive substitute. These actions may or may not be related.

Guanethidine differs from the false transmitters such as α-methylnoradrenaline, metaraminol and octopamine in that it does not possess affinity for postsynaptic receptors. After neural release, therefore, guanethidine is either lost into the general circulation, or recaptured into the binding sites.

The duration of the pharmacological action of guanethidine may depend upon the time taken for depletion of the intra-cellular stores of the drug following release by nerve impulses and loss of the transmitter substitute into the general circulation. If this is so, then for a given dose of guanethidine, the adrenergic blockade may be related to the level of sympathetic nervous activity.

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