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A STUDY OF THE SYMPATHOMIMETIC ACTION OF GUANETHIDINE ON THE ISOLATED ANOCOCCYGEUS MUSCLE OF THE RAT

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1 Guanethidine, acting on the rat isolated anococcygeus, causes adrenergic neurone blockade (slowly terminated by washing), noradrenaline potentiation and, with higher concentrations, spasm (both rapidly terminated by washing).

2 The spasm is an indirect sympathomimetic action, for it is sensitive to phentolamine and reserpine and shows tachyphylaxis.

3 The concentration of cocaine equieffective with the spasmogenic concentration of guanethidine as an inhibitor of noradrenaline uptake caused much less spasm. Moreover, it did not enhance noradrenaline efflux from anococcygeus loaded with (−)-[^{3}H]-noradrenaline, as guanethidine did.

4 The spasm induced by guanethidine in excess of cocaine is due to guanethidine-evoked noradrenaline release.

Introduction

Guanethidine (3 to 10 × 10^{-7} M) inhibits motor responses of the rat isolated anococcygeus muscle to nerve stimulation, yet potentiates effects of noradrenaline in this tissue. Higher concentrations of guanethidine raise the tone of the anococcygeus muscle, possibly by means of an indirect sympathomimetic action (Gillespie, 1972).

Various workers (Chang, Costa & Brodie, 1965; Obinawu, Stitzel & Lundborg, 1968; Prasad, Shah & Gulati, 1973) have shown that guanethidine is a substrate for the noradrenaline transport mechanism in the cytoplasmic membrane of adrenergic nerve terminals. By competing with noradrenaline for the uptake process, guanethidine might cause an accumulation of spontaneously released noradrenaline in the extracellular space and hence evoke spasm of the anococcygeus muscle. Alternatively, guanethidine might facilitate the release of noradrenaline from the nerve terminals (Maxwell, Plummer, Schneider, Povalski & Daniel, 1960).

The aim of the present study was to determine whether spasmogenic effects of guanethidine in anococcygeus muscle could be attributed to an indirect sympathomimetic action and, if so, whether this resulted simply from inhibition of noradrenaline uptake or also involved facilitation of transmitter release.

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Methods

Tissue bath experiments

Male Sprague–Dawley rats (350–400 g) were stunned and killed by decapitation. The anococcygeus muscles were removed from the animals and suspended in organ baths containing Krebs solution of the following composition (mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 11.1. This solution was gassed with 95% O_2 and 5% CO_2 and maintained at 37°C throughout the experiment.

A pair of ring electrodes (i.d. 0.5 cm and 1.0 cm apart) were positioned around the tissue to permit field stimulation of the intramural nerves. The initial resting tension imposed upon the tissue was 0.5 gram. Responses of the tissue to field stimulation or to drug addition were recorded isometrically by the use of a force/displacement transducer as input to a multichannel potentiometric pen recorder.

In all experiments one annococcygeus muscle was used as a test preparation whilst the contralateral muscle was used as a concurrent control.

Time course of the neurone blocking action of guanethidine

Both the test and control preparations were subjected to field stimulation for 20 s every 3 minutes. Pulses were of just supramaximal voltage and 0.5 ms duration. Frequency-response curves were constructed by increasing pulse frequency in twofold steps from 1 up to 64 hertz. The maximal response to field stimulation was invariably obtained at a pulse frequency of 64 Hz and this response was chosen to assess the time course of the neurone blocking action of guanethidine. The test and control preparations were thus stimulated for 20 s every 3 minutes. When the responses became approximately constant in amplitude, guanethidine (5×10^{-6} M) was added to the test preparation. Stimulation of both preparations was continued over the next 30 minutes. At this time the guanethidine was removed from the test preparation. Stimulation of both preparations was continued for a further 60 minutes.

Time course of potentiation of noradrenaline by guanethidine

Concentration-effect curves to (−)-noradrenaline (2.5×10^{-8} M to 10^{-3} M) were constructed with both test and control tissues. Drug contact time was 30 s and the intervals between successive doses were adjusted so that the preparation had fully relaxed before the next noradrenaline challenge. These experiments showed that the minimally effective concentration of noradrenaline was almost invariably 10^{-7} M. Potentiation of noradrenaline by guanethidine was measured as the percentage increase in the amplitude of the response to this concentration of noradrenaline.

Both test and control tissues were challenged with noradrenaline 10^{-7} M at 6 min intervals until responses were approximately constant. The test preparation was then exposed to guanethidine (5×10^{-6} M) and the noradrenaline standards were applied to both preparations every 6 min for 30 minutes.

The guanethidine was then removed from the test preparation and both tissues were subjected to the noradrenaline challenge at 6 min intervals for the next 36 minutes.

The sympathomimetic effects of guanethidine

Guanethidine (2.5×10^{-6} M to 8×10^{-5} M) often caused spasm of the isolated anococcygeus muscle. Sometimes this contraction did not reach a maximum until 15 min had elapsed. Individual responses tended to wax and wane (see Figure 2), tachyphylaxis was very marked (particularly at the lower end of the concentration range) and it proved very difficult to demonstrate whether the contraction induced by guanethidine was concentration-dependent. Attempts to construct concentration-effect curves for guanethidine were abandoned. Instead a standardized exposure to guanethidine 5×10^{-5} M for 15 min every 30 min was adopted. The responses obtained were used as an index of guanethidine's spasmogenic activity.

Concentration-effect curves for noradrenaline (10^{-7} to 10^{-3} M) were constructed with both test and control tissues. Guanethidine 5×10^{-5} M was then applied to each tissue for 15 minutes. The contraction elicited by guanethidine was expressed as a percentage of the maximal response to noradrenaline for that tissue. The test preparation was incubated with phentolamine (10^{-7} M) for 30 min and then again challenged with noradrenaline and guanethidine in the presence of the phentolamine. At the same time the control preparation was rechallenged with noradrenaline and guanethidine in the absence of antagonist.

Experiments with tissue from reserpine-treated rats

Rats were injected with reserpine (5 mg/kg i.p.) 18 h before they were killed. Control rats received injections of 0.9% w/v NaCl solution (saline). Tissues from both test and control animals were subjected to field stimulation (1 to 64 Hz), the construction of log concentration-effect curves for tyramine (2×10^{-6} M to 1.2×10^{-4} M) and noradrenaline (10^{-7} to 10^{-3} M) and to a guanethidine challenge (5×10^{-5} M for 15 minutes). Responses to all agencies were expressed as a percentage of the maximal response to noradrenaline for that tissue.

Comparison of the spasmogenic action of guanethidine and cocaine

The 16 anococcygeus muscles from 8 rats were assigned to one of 4 treatment blocks, balanced with respect to side of origin and order of dissection. On each muscle a noradrenaline log concentration-effect curve was first obtained and at the end of the experiment the noradrenaline maximum response was repeated. Between these events were interposed four 15 min treatments with guanethidine (G, 5×10^{-5} M) or cocaine (C, 2.64×10^{-5} M) separated by 15 min of washing. The 4 blocks were G G G G, G C G C, C G C G and C C C C.

Radioisotope studies

Each anococcygeus muscle, as it was dissected free from the animal, was suspended in 20 ml Krebs solution, bubbled with 95% O_2 and 5% CO_2 and maintained at 37°C, until all dissection was complete (30–90 minutes). Tissues were then assigned to treatments according to a block design, balanced with re-

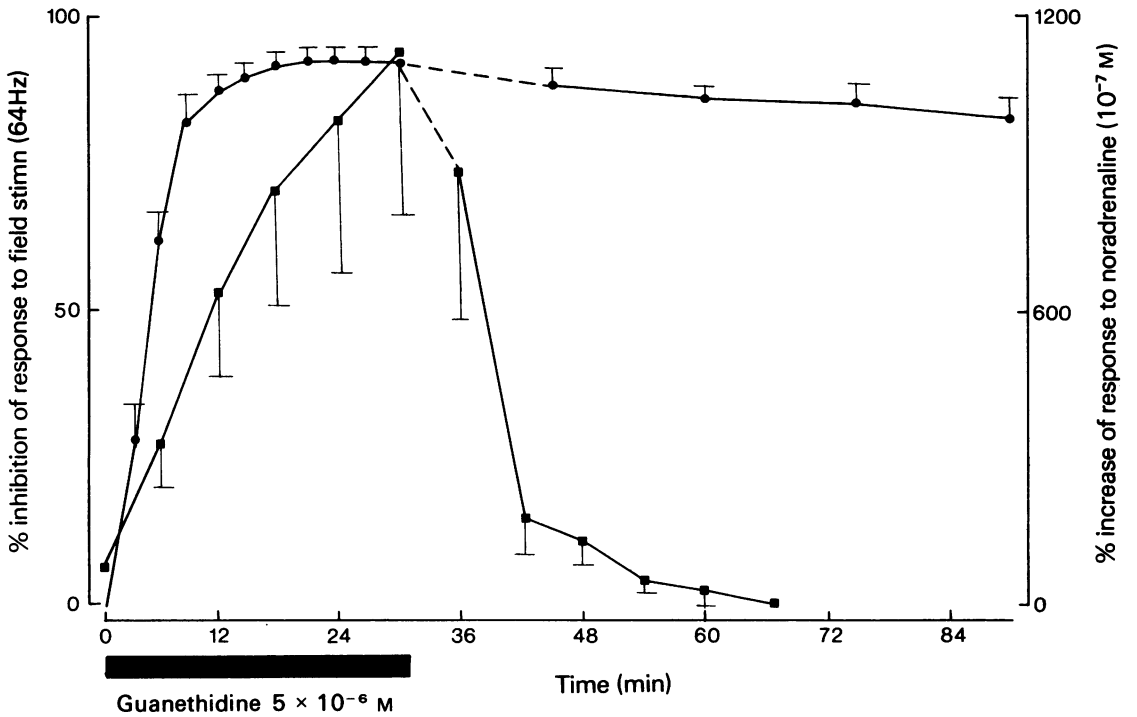

Figure 1 Time courses of the onset and decline of the effects of guanethidine, 5 × 10^{-6} M, on the responses of the rat anococcygeus to field stimulation at 64 Hz and 0.5 ms (left vertical axis) and (−)-noradrenaline, 10^{-7} M (right vertical axis). (●): Field stimulation, n = 8; (■): noradrenaline, n = 6. Vertical bars represent s.e. mean.

spect to side of origin and order of dissection. Each treatment included exposure of the tissue to (−)-[^{3}H]-noradrenaline and ascorbic acid (5.7 × 10^{-4} M), and washing for varying periods. Each tissue was then blotted, weighed and incubated (65°C) with 0.3 ml Soluene 350 (Packard) for 90 min or until completely digested. Sufficient 0.5 N HCl to neutralize the alkalinity of the Soluene and 10 ml phosphor (27.5 g PPO, 0.5 g dimethyl POPOP, 3333 ml toluene, 1667 ml Triton-X-100) were added and the radiotracer content assayed by liquid scintillation spectrometry.

Time course of noradrenaline accumulation

Eight tissues each were exposed for 2, 4, 8, 16 or 32 min to labelled noradrenaline 5 × 10^{-8} M and then washed for 2 minutes.

Concentration-dependence of noradrenaline accumulation

Eight tissues each were exposed for 4 min to 0.5, 1.25, 2.5, 3.75 or 5 × 10^{-7} M labelled noradrenaline and then washed for 2 minutes.

Inhibition of noradrenaline uptake with retention by guanethidine and cocaine

Six tissues each were exposed for 30 min to guanethidine (5 × 10^{-5} M) or cocaine (1.06 or 5.28 × 10^{-5} M, concentrations chosen from the result of a preliminary assay) while the 18 contralateral muscles functioned as controls. After the preincubation all were exposed to labelled noradrenaline 10^{-7} M for 8 min and then washed for 17 minutes.

Effect of guanethidine and cocaine on noradrenaline efflux

Eight tissues each were exposed for 32 min to labelled noradrenaline 10^{-7} M and then washed (5 ml) at 2, 5, or 10 min intervals for a total of 77 minutes. Washing was performed with guanethidine (5 × 10^{-5} M) or cocaine (2.64 × 10^{-5} M). Aliquots of all washings were collected for radioassay. The 16 contralateral tissues functioned as controls, being washed with Krebs solution.

Tissue content/time curves were resolved into two exponential components by the curve peeling method (Riggs, 1963).

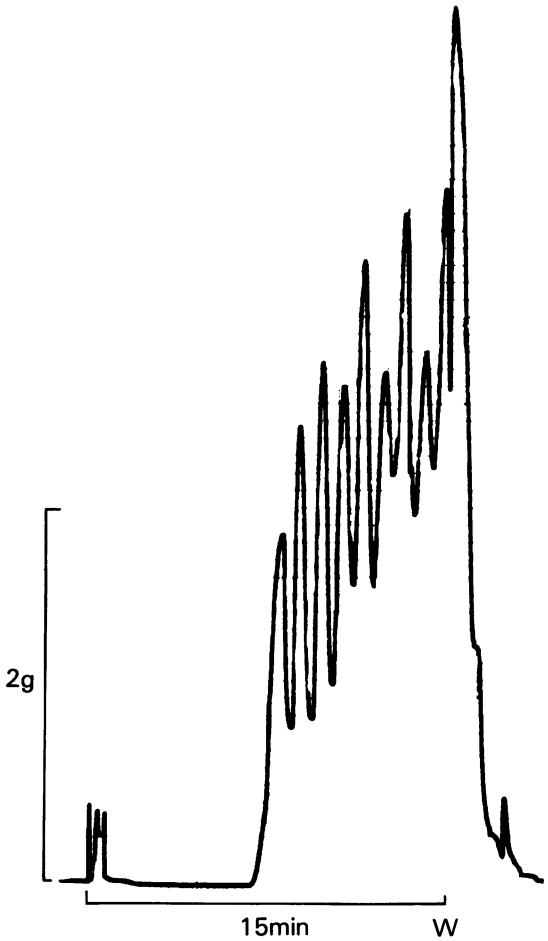

Figure 2 Representative spasmogenic response of rat isolated anococcygeus muscle to guanethidine 5×10^{-5} M. The tissue was exposed to the drug for 15 min as indicated by the horizontal bar. The drug was then washed (W) from the tissue.

All drugs used were obtained from commercial sources and included: (+)-ascorbic acid, cocaine hydrochloride, guanethidine sulphate, (−)-[^{3}H]-noradrenaline (Amersham, radiochemical purity >97%), (−)-noradrenaline bitartrate monohydrate, reserpine and tyramine hydrochloride. Concentrations are expressed in terms of the active ionic species.

Results

Time course of the neurone blocking action of guanethidine

The neurone blocking action of guanethidine was evident 3 min after its addition to the bathing medium (Figure 1). The peak effect (a reduction in the maximal response to field stimulation of 92%) was attained at 18 min and was maintained until the drug was removed from the bath at 30 minutes.

Recovery from the neurone blocking action of guanethidine was slow. The maximal response to field stimulation was still reduced by 82% 1 h after the removal of the drug from the bath.

The maximal responses of control tissues to field stimulation did not differ significantly ($P > 0.1$) from the initial response at any time during the experiment.

Time course of potentiation of noradrenaline by guanethidine

There was an increase in the response of the tissue to the noradrenaline standard (10^{-7} M) immediately guanethidine (5×10^{-6} M) was added to the bath (Figure 1). The enhancement of the response to the noradrenaline standard was still increasing after 30 min exposure to the guanethidine but was rapidly reversible after removal of the guanethidine.

In control preparations no significant changes in response to the noradrenaline standard were observed over 90 minutes.

The sympathomimetic effects of guanethidine

Figure 2 shows a typical spasmogenic response to guanethidine (5×10^{-7} M). Phentolamine (10^{-7} M) was a very effective antagonist ($P < 0.0002$, $n = 10$), reducing the spasm from 68 ± 10% to 9 ± 7% (mean ± s.e.) of the previous noradrenaline maximum response while the control response was unaltered ($P \simeq 0.3$). Phentolamine (10^{-7} M) also almost completely inhibited the response to noradrenaline (10^{-3} M).

Tissues taken from animals that had received reserpine exhibited much smaller responses to field stimulation and tyramine than those taken from animals that had received saline injections (see Figure 3). Treatment of animals with reserpine abolished the response of the anococcygeus muscle to guanethidine (5×10^{-5} M). The sensitivity to noradrenaline of tissues from reserpine-treated animals did not differ from that of tissues from saline pretreated animals ($P > 0.1$).

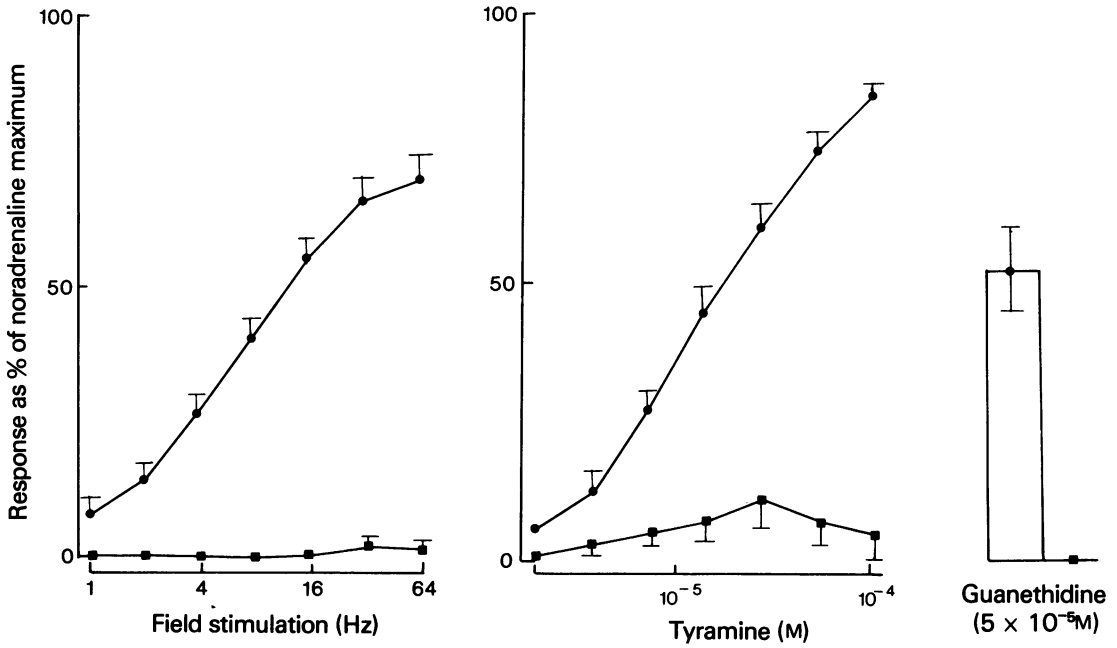

Figure 3 Effect of reserpine pretreatment on the responses (expressed as % of noradrenaline maximum) of rat anococcygeus muscle to (a) field stimulation, (b) tyramine, and (c) guanethidine (5 × 10^{-5} M for 15 minutes). (●): Saline pretreated controls, n = 19; (■): reserpine pretreated (5 mg/kg, 18 h before rats were killed), n = 13. Vertical bars represent s.e.

Comparison of the spasmogenic effects of guanethidine and cocaine

Analysis of variance revealed that the blocks of tissues did not differ in their responses to noradrenaline. Cocaine (2.64 × 10^{-5} M) was a much less active spasmogen (6 ± 5% of the noradrenaline maximum) than was guanethidine (5 × 10^{-5} M, 50 ± 11%) on first exposure. With four exposures tachyphylaxis to guanethidine was very evident, though the second response was not significantly smaller than the first. Cocaine did not cause cross tachyphylaxis with guanethidine.

Radioisotope studies

The tissue to medium ratio (T/M) of radioactivity increased with time of incubation with (−)-[^{3}H]-noradrenaline, rapidly at first and then more slowly, to reach 3.56 ± 0.75 ml/gram.

Noradrenaline accumulation showed a typical saturable dependence on (−)-noradrenaline concentration. The best fitting rectangular hyperbola had a mean Michaelis constant of 2.7 × 10^{-7} M and a mean maximum initial velocity of uptake of 99 pmol g^{-1} min^{-1}.

The uptake-with-retention of labelled noradrenaline was inhibited 79.3 ± 2.7% by guanethidine (5 × 10^{-5} M), 72.6 ± 8.1% by cocaine (1.06 × 10^{-5} M) and 84.7 ± 2.2% by cocaine (5.28 × 10^{-5} M). Interpolation suggests that cocaine 2.64 × 10^{-5} M is equipotent in this respect with guanethidine 5 × 10^{-5} M.

The two control tissue content/time curves did not differ, nor did the curve obtained during washout with cocaine (2.64 × 10^{-5} M) differ significantly from them. The curve obtained during washout with guanethidine (5 × 10^{-5} M) shows a significant and selective difference (Table 1), the slope of the slow phase of efflux was increased ($P < 0.001$).

Discussion

Guanethidine evokes a slowly increasing and phasic spasm of the isolated anococcygeus muscle of the rat which is rapidly reversed by washing the tissue. In this latter respect the spasmogenic action bears no resemblance to the adrenergic neurone blocking action but does resemble the noradrenaline potentiating action.

A concentration of phentolamine demonstrably effective against both exogenous noradrenaline and

Table 1 The coefficients of the double exponential decline in tissue content of radioactivity (tissue content$_t$ = $Ae^{-k_1 t} + Be^{-k_2 t}$) after incubation of the anococcygeus in (−)-[^{3}H]-noradrenaline for 32 min in Krebs solution

	A		k_1		B		k_2	
	mean (ml/g)	*s.e.*	*mean (min^{-1})*	*s.e.*	*mean (ml/g)*	*s.e.*	*mean (min^{-1})*	*s.e.*
Cocaine control	1.60	0.24	0.134	0.009	9.58	0.90	0.00260	0.00021
Cocaine 2.64×10^{-5} M	2.07	0.30	0.130	0.009	8.83	0.99	0.00237	0.00019
Guanethidine Control	1.47	0.19	0.110	0.012	9.78	1.96	0.00283	0.00034
Guanethidine 5×10^{-5} M	1.17	0.13	0.144	0.023	10.56	1.78	0.00633*	0.00024

Control tissues were washed with Krebs' solution while test tissues were washed with drug solution.
* $P < 0.001$ compared with control.

intramural nerve stimulation also greatly reduced guanethidine's ability to evoke spasm thus identifying this spasm as a sympathomimetic effect.

Gillespie (1972) suggested that the sympathomimetic action of guanethidine was indirect, being mediated by endogenous noradrenaline, rather than direct, involving an agonist action of guanethidine at the α-adrenoceptors. We have two pieces of evidence which support this suggestion. A reserpine pretreatment demonstrably effective against both tyramine and intramural nerve stimulation and selective in that noradrenaline responses are not inhibited, also abolishes the spasm evoked by guanethidine. The anococcygeus muscle shows marked tachyphylaxis to the spasmogenic action of guanethidine.

Further experiments were designed to distinguish between two indirect sympathomimetic mechanisms for the spasmogenic action 1) a cocaine-like potentiation of noradrenaline spontaneously released from the intramural nerves into the extracellular fluid and 2) an induced release of stored noradrenaline with or without its subsequent potentiation. The method we employed to distinguish between these mechanisms rests upon the comparison of the properties of cocaine and guanethidine, tested in concentrations equieffective as inhibitors of the uptake of noradrenaline into adrenergic nerves.

The tissue's content of radioactive material after incubation with low concentrations of (−)-[^{3}H]-noradrenaline was first shown to have some of the properties of uptake into adrenergic nerves (typical time course, substrate concentration-dependence and susceptibility to inhibition by cocaine). Our standard spasmogenic concentration of guanethidine inhibited the uptake-with-retention of radioactive material by about 80% and a bracketing assay was used to identify cocaine 2.64×10^{-5} M as equiactive in this respect. This concentration of cocaine caused much less spasm of the anococcygeus than did guanethidine, did not show cross tachyphylaxis with guanethidine in causing spasm and did not increase the rate of efflux of radioactive material from anococcygeus muscles previously incubated in (−)-[^{3}H]-noradrenaline. Guanethidine, on the other hand, did cause a selective increase in the rate of the slow phase of such efflux which we interpret as a release of noradrenaline from adrenergic nerves.

We conclude that the spasm of rat anococcygeus muscle which guanethidine evokes in excess of cocaine is due to the release of neuronal noradrenaline and that the weaker spasmogenic action of cocaine has its origin purely in the potentiation of spontaneously released noradrenaline.

Note that significantly higher concentrations of guanethidine are required for its spasmogenic action than are needed for adrenergic neurone blockade or noradrenaline potentiation. The ready termination by washing of two of these actions sets them apart from the adrenergic neurone blockade which therefore occurs at sites exchanging less readily with the extracellular fluid.

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References

CHANG, C.C., COSTA, E. & BRODIE, B.B. (1965). Interaction of guanethidine with adrenergic neurons. *J. Pharmac. exp. Ther.*, **147**, 303–312.

GILLESPIE, J. S. (1972). The rat anococcygeus muscle and its response to nerve stimulation and to some drugs. *Br. J. Pharmac.*, **45**, 404–416.

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

OBIANWU, H.O., STITZEL, R. & LUNDBORG, P. (1968). Subcellular distribution of ^{3}H amphetamine and ^{3}H guanethidine and their interaction with adrenergic neurons. *J. Pharm. Pharmac.*, **20,** 585–594.

PRASAD, C.M., SHAH, D.S. & GULATI, O.D. (1973). Some factors affecting the neuron blocking action of guanethidine, xylocholine, bretylium and debrisoquin, *Jap. J. Pharmac.*, **23,** 805–811.

RIGGS, D.S. (1963). *The Mathematical Approach to Physiological Problems.* Baltimore: Williams and Wilkins.

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