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THE ACTION OF GUANETHIDINE ON THE ADRENAL MEDULLA OF THE CAT

BY

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Much information has been accumulated concerning the action of guanethidine at peripheral adrenergic neurones (Boura & Green, 1965). Less is known about the direct effects of guanethidine on release of catecholamines from the adrenal medulla. Philippu & Schümann (1962) have shown that guanethidine can inhibit release of catecholamine from perfused bovine adrenal glands elicited by carbachol, nicotine, and phenylethylamine. Although the injection of high concentrations of guanethidine directly into the gland could augment catecholamine release (Philippu & Schümann, 1962), the inhibitory activity of guanethidine on catecholamine release from isolated dog and bovine adrenal glands was found to be more dominant than was its stimulatory activity (Athos, McHugh, Fineberg & Hilton, 1962 ; Philippu & Schümann, 1962).

In the present study, the action of guanethidine on the isolated perfused cat adrenal gland was investigated in order to gain further understanding of the mechanisms by which this agent exerts its inhibitory actions on catecholamine release. In addition, it was hoped that this study would enable us to gain further understanding of the role of the adrenal medulla in the pharmacological actions of guanethidine observed *in vivo*.

METHODS

The acutely denervated left adrenal gland of the cat was perfused *in situ* according to the method of Douglas & Rubin (1961). Perfusion was carried out at room temperature with normal Locke solution or Locke solution containing 56 mM KCl (10 times normal), with the NaCl reduced by an equivalent amount to maintain isotonicity. $CaCl_2$ was added to give a final concentration of either 0.5, 2.0, 3.0 or 12 mM. All solutions were equilibrated with 95% oxygen and 5% carbon dioxide and had a pH close to 7.0.

The perfusate was acidified and subsequently assayed for catecholamine (adrenaline and noradrenaline) by a modification (Rubin & Jaanus, 1966) of the trihydroxyindole photofluorometric method of Anton & Sayre (1962). Outputs were expressed as total catecholamines (μg adrenaline plus noradrenaline base/min).

Calculation of inhibitory activity

The inhibitory activity of guanethidine on the response to a given secretogogue was calculated on the basis of the extent to which guanethidine depressed the ratio of the second response to that of the first. Thus, for a given secretogogue the second 2 min response as a percentage of the first

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was as follows (mean ± S.E.): acetylcholine (6×10^{-6}M) 71.6±2.1% ($n=8$); phenylethylamine (8×10^{-4}M) 54.7±4.3% ($n=6$); amphetamine (7×10^{-5}M) 65.1±4.1% ($n=4$); and calcium (0.5 mM) 39.7±1.8% ($n=7$). These concentrations of each agent elicited approximately equal rates of secretion when initially introduced (Rubin & Jaanus, 1967). Values were plotted on probit plots to obtain linear-dose response curves, and the ED50s for inhibition by guanethidine of the activity of each secretogogue were calculated according to the method of least squares (Finney, 1964). For further details of this method see Jaanus, Miele & Rubin (1967).

Drugs used

Guanethidine sulphate was generously supplied by Ciba (Summit, New Jersey, U.S.A.). The following drugs were also used: acetylcholine chloride, *d*-amphetamine hydrochloride, hexamethonium chloride, and atropine sulphate (Nutritional Biochemical); phenylethylamine (Eastman Chemical). The concentrations of all agents are given in terms of moles/l.

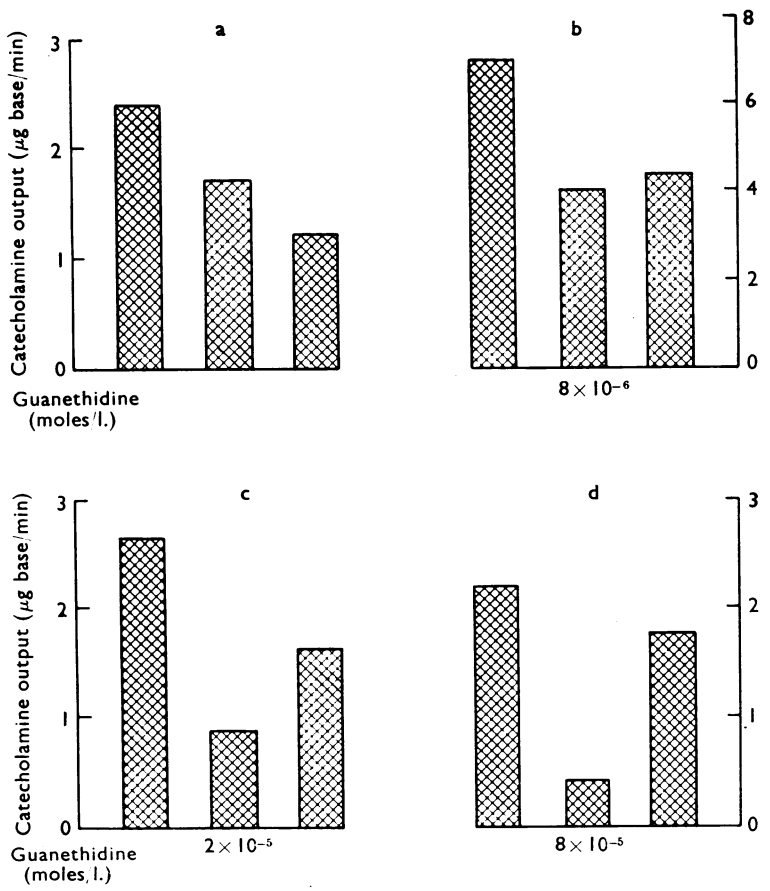

Fig. 1. Inhibition of acetylcholine-evoked secretion by guanethidine. Adrenal glands were perfused with Locke solution with or without varying concentrations of guanethidine. Every 7 min, acetylcholine (6×10^{-6}M) was added to the perfusion fluid for 2 min. The vertical bars represent the catecholamine outputs obtained during the 2 min period when acetylcholine was present. Each second response was obtained in the presence of guanethidine, except for Fig. 1a which represents the control outputs in the complete absence of guanethidine. Each set of three responses was obtained from a different preparation. The control rates of secretion just before the addition of acetylcholine are not shown but were usually <0.2 μg/min.

RESULTS

Inhibitory activity of guanethidine

The addition of increasing concentrations of guanethidine to Locke solution caused a graded inhibition of catecholamine release evoked by acetylcholine (ACh) (Fig. 1). The inhibitory action of guanethidine on the response to ACh (6×10^{-6}M) was observed in the concentration range of 4×10^{-6}M to 8×10^{-5}M, and an ED50 of 2.4×10^{-5}M was calculated (Fig. 2). Over this range of concentrations, guanethidine inhibited the secretion of adrenaline and noradrenaline to approximately the same extent.

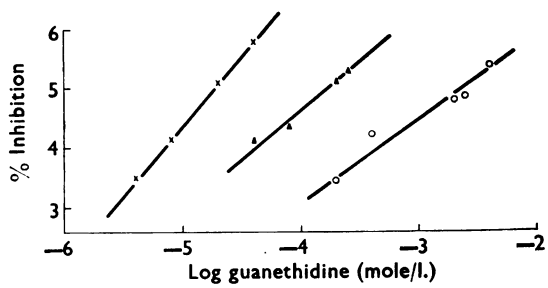

Fig. 2. Guanethidine inhibition of catecholamine secretion evoked by 6×10^{-6}M acetylcholine (×), 8×10^{-4}M phenylethylamine (▲) and 0.5 mM calcium (○). In experiments where acetylcholine or phenylethylamine was used to elicit secretion, the procedure was the same as described in Fig. 1. In experiments which employed calcium as the secretogogue, adrenal glands were perfused with calcium-free Locke solution containing 56 mM KCl for 5 min and then calcium (0.5 mM) was added for 2 min; perfusion was then switched to calcium-free high K^+ Locke solution plus guanethidine for 5 min and the 2 min stimulation with calcium (0.5 mM) repeated. Each point was obtained from a different preparation. See METHODS for means of determining per cent inhibition, which is plotted in probit units.

A given concentration of guanethidine produced less inhibition in the presence of a higher concentration of ACh (Fig. 3). For example, against 3×10^{-5}M ACh, 2×10^{-5} and 4×10^{-5}M guanethidine produced only a 16% and 48% inhibition—compared with a 54% and 78% inhibition by the same concentration of guanethidine against the response to ACh 6×10^{-6}M. The inhibition of ACh-evoked secretion by guanethidine could be antagonized by excess calcium (Fig. 3). Thus the addition of 12 mM calcium to the perfusion medium in the continued presence of a concentration of guanethidine which depressed the ACh-response by 50%, restored the catecholamine output to a value greater than the output on initial stimulation (Fig. 3b). At higher concentrations of guanethidine, excess calcium also produced some antagonism of the inhibition (Fig. 3c) but to a lesser degree than it did at the lower concentrations of inhibitor. Like the guanethidine inhibition, the depression of the ACh-response by atropine plus hexamethonium was also partially antagonized by excess calcium (Fig. 3d).

Guanethidine in concentrations which produced a greater than 50% inhibition of the response to ACh (6×10^{-6}M) produced an almost identical inhibition of catecholamine secretion elicited by amphetamine (7×10^{-5}M) (Fig. 4c, d). At lower concentrations of guanethidine, however, amphetamine-evoked secretion was not depressed but, in fact, was potentiated (Fig. 4b). In three experiments the mean value of the second stimulation by

amphetamine, in the presence of 8×10^{-6}M guanethidine, was $93.4 \pm 10.2\%$ of the first. In the absence of guanethidine, the second response to amphetamine was 65.1% of the first. This represented a 43% potentiation of the response to amphetamine, compared with a 19.7% inhibition of the response to ACh (6×10^{-6}M) by the same concentration of guanethidine.

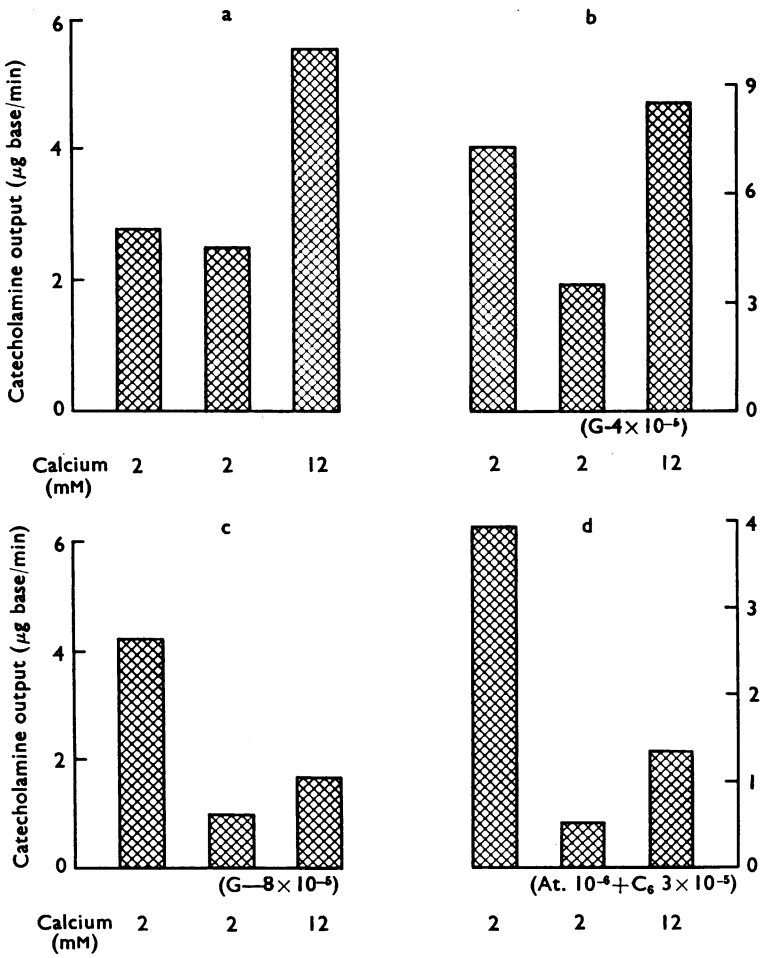

Fig. 3. Antagonism by calcium of guanethidine-inhibited acetylcholine-evoked secretion. Glands were perfused sequentially for 7 min periods with Locke solution, Locke solution plus inhibitor, and then Locke solution plus inhibitor and excess calcium (12 mM). During the last 2 min of each perfusion period acetylcholine (3×10^{-5}M) was added. The vertical columns represent the outputs elicited by acetylcholine. a, Control experiment in the absence of any inhibitor. The inhibitor in b and c was guanethidine and in d atropine plus hexamethonium.

Calcium is a potent medullary secretogogue when added to the perfusion medium in the presence of high K^+ (Douglas & Rubin, 1961; Rubin, Feinstein, Jaanus & Paimre, 1967). Guanethidine was able to depress the secretory response to calcium (0.5 mM) in high K^+ (Fig. 5), and this inhibition could be antagonized by excess calcium (3.0 mM)

(Fig. 5c, d). The inhibition by guanethidine of calcium-evoked catecholamine release was observed only in concentrations 100 times higher than those required to inhibit the secretory response to ACh (Fig. 2), and for guanethidine against calcium an ED50 of 2.4×10^{-3}M was calculated.

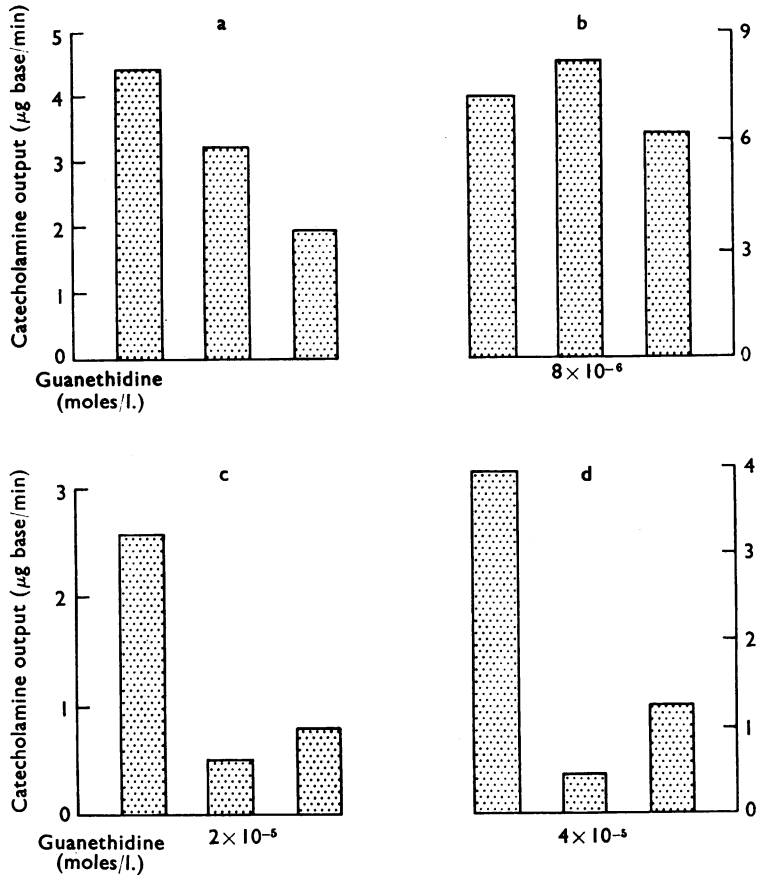

Fig. 4. Inhibition and potentiation of amphetamine-evoked secretion by guanethidine. Glands were perfused alternately with normal Locke solution or Locke solution plus guanethidine for 7 min. During the last 2 min of each perfusion period, amphetamine (7×10^{-5}M) was added. The vertical bars represent outputs obtained during the period when amphetamine was present. a, Control outputs in the absence of guanethidine.

Stimulant activity of guanethidine

Guanethidine, in the range of concentrations from 2×10^{-6} to 4×10^{-4}M, was unable to elicit a consistent or significant increase in the rate of catecholamine secretion. The increase in output, when observed, was seen during either the first 2 min or the second to fourth minute after the addition of guanethidine to the perfusion medium. Only with concentrations of 4×10^{-6}–8×10^{-5}M was any augmentation of output observed, and even in this concentration range the enhancement of catecholamine release was manifest in

only five of ten experiments. In these five experiments, the mean spontaneous output for the 2 min before the introduction of guanethidine was 0.170 ± 0.04 μg/min. During the first 2 min after guanethidine was added the mean catecholamine output rose to 0.376 ± 0.03 μg/min, and then decreased to 0.370 ± 0.07 μg/min during the second to fourth minute of exposure to guanethidine. The maximum stimulation by guanethidine in any of the experiments was observed at a concentration of 4×10^{-6}M, when the spontaneous rate of secretion rose from 0.24 to 0.45 μg/min during the first 2 min after the addition of guanethidine and then to 0.65 μg/min during the second to fourth minute.

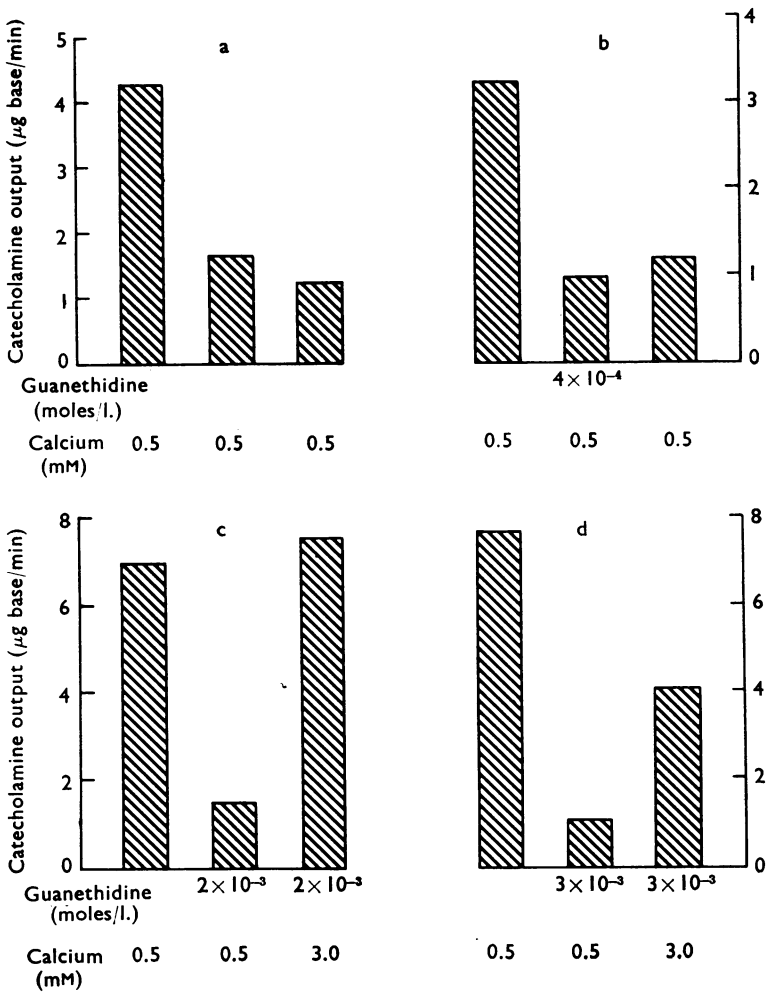

Fig. 5. Inhibition of calcium-evoked secretion by guanethidine. Glands were perfused with calcium-free Locke solution containing 56 mM KCl with or without varying concentrations of guanethidine. At 7 min intervals, calcium (0.5 mM) was added to the perfusion fluid for 2 min. The vertical bars in a depict control outputs in response to calcium in the absence of guanethidine. Guanethidine was present during the 7–14th min of perfusion in b, and during the 7–21st min in c and d. The third responses in c and d were obtained with 3 mM calcium in the continued presence of guanethidine.

DISCUSSION

The most striking effect of guanethidine on the isolated cat adrenal gland is its anti-cholinergic action. The inhibitory action of guanethidine on ACh-evoked catecholamine release was observed in concentrations of the same order as hexamethonium and atropine, which are the classical inhibitors at cholinergic synapses. Guanethidine also produces an initial inhibitory action on medullary catecholamine secretion elicited by nicotinic agents and excess K^+ in the intact cat and dog (Miele, 1966; Hazard *et al.*, 1964). On the other hand, close intra-arterial injection of guanethidine into the cat adrenal gland does not appear to depress secretion evoked by splanchnic nerve stimulation (Abercrombie & Davies, 1963).

Gokhale, Gulati & Kelkar (1963) have shown that the initial pressor response of the cat to guanethidine is unaltered by adrenalectomy and have concluded that the adrenal medulla is not a major site of catecholamine released by guanethidine. The present studies also show that guanethidine possesses very limited ability as a medullary secretogogue, and thus it appears that the adrenal medulla does not contribute significantly to the quantity of catecholamine released by guanethidine. It may be noted, however, that it the intact preparation, medullary secretion may be augmented as a consequence of the peripheral adrenergic blocking actions of guanethidine (Abercrombie & Davies, 1963).

An analysis of the inhibitory action of guanethidine on ACh-evoked catecholamine release showed that the block could be antagonized by a higher concentration of ACh, as well as by excess calcium. The stimulatory action of ACh on medullary chromaffin cells is presumed to involve an increase in membrane permeability which allows extracellular calcium to enter the interior of the chromaffin cell to initiate secretion (Douglas & Rubin, 1961, 1963; Douglas & Poisner, 1962). The inhibition by guanethidine might therefore represent an effect either on the interaction of ACh with receptor sites on the cell membrane, or on the movement of calcium into the cell. The present results suggest that the action of guanethidine on the chromaffin cell is to interfere with the action of ACh. The principal piece of evidence which supports this hypothesis is that guanethidine inhibits calcium-evoked catecholamine secretion only in concentrations some 100 times higher than those required to depress the stimulant activity of ACh.

In previous work it has been shown that specific stimulation of muscarinic receptors in the adrenal medulla of the cat leads to a predominance of adrenaline secretion (Douglas & Poisner, 1965). Furthermore, hexamethonium and certain local anaesthetics primarily depress the noradrenaline secretion elicited by ACh (Jaanus *et al.*, 1967). This latter finding was interpreted to mean that these inhibitory agents act mainly on nicotinic sites of the medullary chromaffin cells. The fact that guanethidine depressed adrenaline and noradrenaline secretion to the same extent suggests that guanethidine can block at both nicotinic and muscarinic sites on the chromaffin cell membrane.

Previous studies have shown that local anaesthetics block calcium-evoked catecholamine release and radio-calcium exchangeability in the adrenal gland (Rubin *et al.*, 1967; Jaanus *et al.*, 1967; Rubin & Miele, 1968). This ability of local anaesthetics to interfere with the secretory activity of calcium can be correlated with local anaesthetic activity as measured in other test systems (see Jaanus *et al.*, 1967; Rubin & Miele, 1968). Guanethidine was a weaker inhibitor of calcium-evoked secretion (ED50 2.4×10^{-3}M) than the weakest of the local anaesthetic agents, which was procaine (ED50 1.5×10^{-3}M).

The great disparity between the ability of guanethidine to depress secretion elicited by ACh and by calcium shows that the primary action of guanethidine on the adrenal medulla differs from that of the local anaesthetics. The dissociation of the primary action of guanethidine from its local anaesthetic effects has recently been reported in sympathetically innervated organs and in C fibres (Rand & Wilson, 1967 ; Watson, 1967).

Although both ACh and phenylethylamine require the presence of calcium for their activity as medullary secretogogues (Douglas & Rubin, 1961 ; Rubin & Jaanus, 1966), certain more subtle differences in their stimulant actions, as for example, the effects of excess calcium on their secretory activity, suggested that these two agents might release catecholamines from the adrenal medulla by somewhat different mechanisms (Rubin & Jaanus, 1966, 1967). This idea is further supported by the disparity in the ability of guanethidine to depress the secretory response to equipotent concentrations of these two secretogogues. A comparison of the ED50s showed that guanethidine was almost 10 times as active against ACh, as it was against phenylethylamine. On the other hand, the block by guanethidine of the amphetamine response was of the same order as the block of the ACh response, which supports previous data indicating that amphetamine and ACh might bring about medullary catecholamine release by similar mechanisms (Rubin & Jaanus, 1966, 1967). At lower concentrations, however, guanethidine produced an enhancement of amphetamine-induced secretion, which was not seen with ACh. The potentiation of the action of amphetamine as a secretogogue was observed at concentrations of guanethidine which had a small and variable stimulating action. The underlying mechanism of this potentiation is difficult to ascertain because of the ephemeral nature of the secretory activity of guanethidine. Both amphetamine and guanethidine are, however, able to release catecholamines from isolated chromaffin granules (Philippu & Schümann, 1962 ; Schümann & Philippu, 1962), so that catecholamine-containing granules may be the site of the interaction between amphetamine and guanethidine. This idea is supported by evidence that amphetamine and guanethidine can displace one another from intracellular binding sites in peripheral adrenergic effector organs (Chang, Costa & Brodie, 1965 ; Costa, 1966).

It has been suggested that the sequence of events leading to the release of catecholamines from sympathetic post-ganglionic fibres closely parallels the events leading to the release of catecholamines from the chromaffin cell of the adrenal medulla (Burn & Gibbons, 1965 ; Burn & Welsh, 1967). Thus these authors propose that the nerve impulse on reaching the nerve terminal causes the release of ACh, which then acts on the nerve cell membrane to permit the entry of calcium ion which in turn causes the release of noradrenaline. Guanethidine and other neuronal blocking agents are thought to prevent ACh from making the fibre permeable to calcium (Burn & Welsh, 1967). The present findings do indeed demonstrate the potent anticholinergic action of guanethidine on medullary chromaffin cells. These findings might, by inference, be used to support the idea of an anticholinergic mechanism to explain the blocking action of guanethidine on adrenergic nerves, and thus support the cholinergic-link hypothesis. They cannot, however, be said to represent direct evidence in support of the hypothesis.

SUMMARY

1. The effect of guanethidine on catecholamine release from the isolated perfused cat adrenal gland was studied.

2. The addition of guanethidine to Locke solution produced a dose-dependent inhibition of the secretory response to acetylcholine. This inhibition could be antagonized by increasing the concentration of either acetylcholine or calcium.

3. Guanethidine also inhibited the secretory response to concentrations of phenylethylamine and amphetamine which were equipotent to acetylcholine. Guanethidine was one-tenth as effective in depressing the response to phenylethylamine as it was against acetylcholine. With concentrations of guanethidine which produced a greater than 50% depression of amphetamine-evoked secretion, the degree of inhibition closely resembled that obtained with acetylcholine as the secretogogue. At a lower guanethidine concentration, the amphetamine response was potentiated.

4. Guanethidine also produced a dose-dependent inhibition of secretion elicited by calcium in the presence of excess K^+. Concentrations 100-fold higher were, however, required to depress calcium-evoked secretion than those required to depress acetylcholine-evoked secretion.

5. The addition of a wide range of concentrations of guanethidine to Locke solution caused only a variable and extremely small augmentation of catecholamine secretion.

6. The potent anticholinergic action of guanethidine and its extremely weak activity as a stimulant of medullary catecholamine release are discussed in relation to the pharmacological actions of guanethidine on the adrenal medulla *in vivo*.

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