GUANETHIDINE AFTER TWENTY YEARS: A PHARMACOLOGIST'S PERSPECTIVE

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Introduction

I had the good fortune of having been one of the midwives at the pharmacological birth of guanethidine (Maxwell *et al.*, 1960) and also of having chronicled its life story as both of us arrived at our 'mature' years (Maxwell & Wastila, 1977). As a result I have had the impetus to develop a 'unifying view' of this drug, the pharmacology of which is simultaneously both simple and complex. Before presenting this view, however, I will discuss what seems to me to be the important actions of guanethidine. This drug has proven to be a useful tool in the elucidation of the basic physiology and biochemistry of the adrenergic nerve terminal, while in turn, such basic findings have helped to explain the intricacies of the pharmacology of guanethidine. Practically the complete gamut of the pharmacological activities of guanethidine that are

elicited *in vivo* following acute administration of moderate doses can be understood in terms of its actions at adrenergic nerve terminals. Guanethidine exerts all of the following actions:

1. *Inhibition of the release by action potentials of a fraction of the transmitter stored in the nerve terminals*

The term 'release' means the passage out of the neurone of an amount of physiologically-active transmitter which is sufficient to be measured or to produce a response in effector cells. The quantity of noradrenaline released usually does not diminish the overall magnitude of the store of noradrenaline since it is normally rapidly replaced by synthesis or by

Figure 1 Effect of guanethidine on the release of [³H]-noradrenaline in the spleen. Cats were given 200 μ Ci/kg [³H]-noradrenaline i.v. Splenic nerves were stimulated at a frequency of 30/s for a total of 300 stimuli. Guanethidine (1 mg) was injected into the splenic artery. Each column represents [³H]-noradrenaline (in m μ Ci) released during a 1-min collection period. ▨ tritiated noradrenaline measured before stimulation; ■ 1 min during and after stimulation. [From Hertting, G., Axelrod, J. & Patrick, R.W. (1962). *Br. J. Pharmac.*, 18, 161–166 with permission.]

uptake as described in 3. This inhibition of the release normally evoked by action potentials is the primary useful effect of these drugs and has led to the term 'adrenergic neurone blockade'. As can be seen in Figure 1 upon stimulation of the splenic nerves of cats which had previously received tritiated noradrenaline, large amounts of the tritiated noradrenaline were released into venous blood. Following guanethidine administration subsequent stimulation of the splenic nerve failed to release tritiated noradrenaline (Hertting *et al.*, 1962).

2. Release of transmitter from stores in nerve endings

Release is sufficient following intravenous administration of guanethidine to lead to sympathomimesis in many effector cells. Guanethidine caused a rise in the tritiated noradrenaline in the splenic outflow following its injection into the splenic artery (Figure 1). Release is a result of a direct action of the compound at the nerve endings and is presumably independent of electrical activity in the neurones. This action is short compared to the duration of neurone blockade.

3. Inhibition of the capacity of the nerve terminal to take up and/or to bind transmitter from the extracellular fluid surrounding the nerve terminals and effector cells

Uptake and binding into nerve terminals is a primary mechanism for terminating the physiological actions of noradrenaline in many adrenergic neuroeffector junctions (Iversen, 1967). The uptake mechanism is operative regardless of whether the noradrenaline reaches the region of the nerve endings as a result of local release or as a consequence of exogenous administration.

4. Depletion of noradrenaline stored within effector organs

Guanethidine brings about measurable depletion in the content of stored noradrenaline. This depletion is slow and of long duration relative to the phenomenon of release. Depletion is probably due in part to release as described in (2), inhibition of uptake as described in (3) and in larger part to 'damage' to storage vesicles and the subsequent intraneuronal metabolism of the transmitter.

5. Inhibition of drug-induced release of transmitter from nerve endings

Several phenylethylamines, typified by tyramine, release noradrenaline from adrenergic nerve terminals. This release is blocked by guanethidine. Blockade of tyramine-induced release is a protracted action.

6. Destruction of adrenergic neurones

Under appropriate dosing regimens and conditions, guanethidine can be made to destroy adrenergic neurones in experimental animals.

Discussion

Interactions of guanethidine with adrenergic neurones

Uptake and accumulation. It was demonstrated that slices of rat heart accumulated guanethidine and that this occurred by two separate processes (Schanker & Morrison, 1965). One required oxygen and was saturable, suggesting a specialized transport process. The second process was nonspecific tissue binding. Additional evidence supporting two types of accumulation has been presented for the rat heart and spleen (Chang *et al.*, 1965) and for the rat heart (Mitchell & Oates, 1970).

Guanethidine was shown to be taken up and retained in sympathetic nerves by a process involving a high affinity for noradrenaline storage sites. Chang *et al.* (1964) and Brodie *et al.* (1965) demonstrated that this specific accumulation in rat heart could be inhibited or reversed by reserpine treatment (which selectively affects the storage sites for noradrenaline in adrenergic nerves), whereas the accumulation in skeletal muscle was not affected.

Mitchell & Oates (1970) confirmed the uptake of guanethidine into adrenergic neurones in rat heart. They also demonstrated that the related adrenergic neurone-blocking agents, bethanidine and debrisoquine, were also accumulated against a concentration gradient by an oxygen-requiring, temperature-dependent, saturable uptake system. In addition, the uptake of tritiated bethanidine into rat heart slices was competitively inhibited by adrenergic amines (noradrenaline, metaraminol, and tyramine); other neuronal blocking agents (guanethidine, bretylium, and debrisoquine); and by the tricyclic antidepressant, desmethylinipramine (Mitchell & Oates, 1970).

Noradrenaline and related catecholamines were also known to be taken up across adrenergic neuronal membranes by a saturable, energy-dependent, active transport system, the so-called 'amine-pump' (Dengler *et al.*, 1961; Whitby *et al.*, 1961; Iversen, 1963; Lindmar & Muscholl, 1964; Hillarp & Malmfors, 1964; Bogdanski & Brodie, 1966; Carlsson, 1966; Giachetti & Shore, 1966; Iversen & Kravitz, 1966; Berti & Shore, 1967). This uptake mechanism of the adrenergic neurone membrane, which is a major mechanism for inactivating noradrenaline released from nerve terminals, was depressed by cold, heat, cardiac glycosides, and low Na^+ levels. Tricyclic antidepressants and adrenergic neurone blockers were, likewise, potent competitive in-

inhibitors of this uptake mechanism for noradrenaline and 1-metaraminol in adrenergic neurone membranes (Hertting *et al.*, 1961; Burgen & Iversen, 1965; Carlsson & Waldeck, 1965a, b; Iversen, 1965; Berti & Shore, 1967; Callingham, 1967; Maxwell *et al.*, 1969).

The competition for the uptake site in adrenergic neurones exhibited by the various adrenergic neurone blockers, the adrenergic amines, cocaine, and the tricyclic antidepressants indicated very clearly that the adrenergic neurone blockers were taken up by the same amine pump in adrenergic neurone membranes that takes up noradrenaline.

The amine pump. As pointed out by several authors the uptake mechanism for noradrenaline in the cell membrane of adrenergic neurones exhibits the properties of an active transport system, but the complexity of the biological systems studied does not allow direct rigorous proof of this (Thoenen *et al.*, 1968; Mitchell & Oates, 1970). It is usually considered to be a carrier-mediated process. The evidence for this concept is based on stereochemical selectivity of the process, dependence on temperature and energy metabolism, uphill transport, saturation kinetics and competitive inhibition kinetics. For purposes of the present discussion we will consider the system as a carrier-mediated process.

The amine pump has been referred to as a 'promiscuous pump' in that it will not only process noradrenaline but will concentrate adrenergic neurone blocking agents as well as a variety of other ring-substituted bases and is blocked by tricyclic antidepressants and cocaine (Oates *et al.*, 1971). However, this notion of promiscuity may represent oversimplification in thinking about interactions between small organic molecules and biological macromolecules. It is probably more to the point that the known substrates for the pump can all be described as having a moderately large hydrophobic group connected through a one- or two-carbon chain to a positively charged basic group. The best substrates are phenylethylamine derivatives such as noradrenaline. Compounds having larger rings, shorter or longer chains or quaternary or guanidinium cationic heads rather than protonated primary or secondary amines are poorer substrates. Thus, in the rabbit aortic strip the approximate relative affinities for the pump as judged by ID_{50} values were noradrenaline = 25, amphetamine = 10, guanethidine = 6, and bretylium = 1 (Maxwell, unpublished observations). All of these substrates bind to the pump receptor, but their affinities differ greatly. It is unlikely that any receptor system accepts only one specific substrate or agonist. Certainly, the α -adrenergic receptor accepts agonists as widely divergent as noradrenaline, naphazoline, and tuaminoheptane. Likewise, the cholinergic, muscarinic receptor receives agonists as widely divergent as acetylcholine, muscarine, and pilo-

carpine. If one considers inhibitors of the uptake mechanism one finds that the best inhibitors are generally bulkier molecules than are the substrates. Desmethylinipramine is a very potent inhibitor of the noradrenaline uptake mechanism with an affinity of approximately 1500 relative to 1 for bretylium. Cocaine is also a fair inhibitor of uptake (potency relative to bretylium = 40). Both of these substances are multi-ringed compounds. Likewise, the α -adrenergic receptor blocking agents phentolamine, piroxan and azapetine are multi-ringed compounds as are the anticholinergics atropine, procyclidine and a long list of anticholinergics (see *e.g.* Cutting, 1969). It seems likely that these receptors have ancillary binding sites that are used by inhibitors but are not used by the substrates. For further discussion see Maxwell & Wastila (1977) and Maxwell & White (1978).

The competition between tricyclic antidepressant drugs and guanethidine (or related neurone-blocking agents) for the amine pump produces one of the more dramatic drug interactions in man, where it is observed that the tricyclic antidepressants can drastically negate the hypotensive effects of guanethidine. Desmethylinipramine was first reported to prevent the neuronal blockade due to guanethidine *in vivo* by Stone *et al.* (1964) and Kaumann *et al.* (1965). It has also been reported to antagonize the antihypertensive action of guanethidine in man by Leishman *et al.* (1963) and Mitchell *et al.* (1967). Skinner *et al.* (1969), Meyer *et al.* (1970), and Fann *et al.* (1971a, b) have noted similar troublesome interactions when tricyclic compounds were administered to patients receiving bethanidine and debri-soquine. Carlsson & Waldeck (1965b) and Mitchell *et al.* (1967), in an attempt to explain the reversal of neurone blockade with antidepressant drugs, were the first to postulate that desmethylinipramine acted by blocking the uptake of guanethidine into adrenergic neurones.

Pressor amines, such as amphetamine or ephedrine, since they can compete with guanethidine for entry into neurones via the amine pump and once inside act to release noradrenaline, also antagonize its hypotensive actions.

Action on storage granules. Obianwu *et al.* (1968) found that tritiated guanethidine injected intravenously into mice was preferentially retained in a high-speed particulate fraction (100,000 g) of the heart known to be high in noradrenaline content. One hour after administration, the ratio *guanethidine in particulate/guanethidine in particulate + supernatant* was 0.56, which indicated a high affinity for the same particulate fraction that stored noradrenaline.

Maitre & Staehelin (1971) compared the subcellular distribution of tritiated guanethidine with that of tritiated noradrenaline following intravenous

injections of these agents into the rat. The microsomal pellet (105,000 g) obtained from a heart homogenate was subjected to density gradient centrifugation. Tritiated guanethidine was present in the same narrow band of particles that contained the tritiated noradrenaline, thus demonstrating directly that guanethidine was taken up into the same granules that stored noradrenaline. In reserpine-pretreated rats the uptake of tritiated guanethidine and noradrenaline was greatly reduced as compared with untreated controls.

In summary, available evidence demonstrates that guanethidine is taken up by and concentrated in adrenergic neurones because it is a substrate for the amine pump in adrenergic neurones. The amine pump has the normal physiological function of recapturing noradrenaline secreted by adrenergic nerve terminals. Once inside the cell guanethidine is sequestered in the same granules that store noradrenaline and can be released by stimulation of adrenergic nerves. Substances that inhibit the amine pump (e.g., tricyclic antidepressants, neuroleptics, and cocaine) or that are competitive substrates (e.g., tyramine, ephedrine) inhibit the entry of guanethidine into the neurones and thereby act to negate its neurone-blocking action.

Structural and ultrastructural changes in neurones. In animals, moderately high doses of guanethidine given parenterally for a prolonged period or high doses given for a shorter period have destructive effects on the sympathetic nervous system.

Intraperitoneal administration of guanethidine in doses up to 5 mg/kg for 18 weeks did not affect the ultrastructure of the rat sympathetic ganglion cells (Burnstock *et al.*, 1971; Jensen-Holm & Juul, 1971). These doses were effective in producing sympatholytic effects. However, administration of 5 mg/kg guanethidine i.p. for 18 weeks to rats has been reported to lead to long-lasting damage to adrenergic nerves in the male reproductive tract, although adrenergic nerves in other parts of the body were not affected (Gannon *et al.*, 1971). Gerkens (1974a) noted that treatment of rats for 19 weeks with 5 mg kg⁻¹ day⁻¹ guanethidine i.p. reduced the contractile responses of the vas deferens to electrical stimulation to 25% of control, potentiated the response to noradrenaline, and depleted endogenous noradrenaline. Within 1 week after discontinuation of guanethidine treatment, the responses to electrical stimulation increased to 60% of control but showed no further increase. Potentiation of the response to noradrenaline persisted for 14 weeks and endogenous levels of noradrenaline were still greatly reduced after 7 weeks. Gerkens (1974b) also reported that after 4 weeks treatment with 5 mg kg⁻¹ day⁻¹ guanethidine i.p. the pressor response evoked by stimulation of the spinal sympathetic outflow were reduced by 55% and

after 19 weeks treatment they were reduced by 69%. However, after cessation of treatment the responses increased to near normal within 3 weeks.

Jensen-Holm & Juul (1968, 1970a) described the occurrence of slowly reversible or partly reversible structural changes in sympathetic ganglion cells of the rat as a result of treatment for 5 to 14 days with high doses (20 mg/kg) of guanethidine. Chromatolysis of ganglion cells was observed accompanied by infiltration of a large number of small lymphocyte-like cells and macrophages. A pronounced loss of specific and nonspecific cholinesterase activity and catecholamines was also seen. The dry weight and protein content of the sympathetic ganglia were increased substantially (Jensen-Holm & Juul, 1970b). This dosage regimen also elicited pronounced swelling of the mitochondria of the superior cervical ganglion cells. The mitochondria also assumed round or irregular shapes (Jensen-Holm & Juul, 1971). The membranes were intact but an unfolding of the crests was seen. Irregularization of the endoplasmic reticulum occurred and an increased number of dense bodies was observed. A large increase in lymphocytes was evident. The changes were reversible, with partial recovery occurring by 22 and 56 days following the cessation of treatment. The pre- and post-ganglionic nerve fibres, the synaptic vesicles, and the synapses were unaffected.

Intraperitoneal administration of 25 or 30 mg kg⁻¹ day⁻¹ of guanethidine to rats for 6 weeks resulted in widespread sympathectomy, which persisted over long periods (Burnstock *et al.*, 1971). Most of the cell bodies in the superior cervical ganglia were completely degenerated. The few remaining were severely damaged. The mitochondria were the main site of neurone damage. They were swollen, and the cristae were lost or severely damaged and of irregular outline.

Juul & Sand (1973) reported that desmethylinipramine largely prevented the accumulation of guanethidine in the superior cervical ganglia and almost completely prevented the morphological effects of guanethidine in the ganglia. Reserpine only lowered the content of guanethidine in the ganglia to a small degree and likewise only slightly protected the ganglia from morphological changes. These authors concluded that the cytotoxic effects of guanethidine were due to its accumulation in ganglion nerve cells outside the noradrenaline storage granules, as opposed to a mainly intragranular localization in peripheral sympathetic terminals.

Malmquist & Oates (1968) demonstrated that guanethidine at 10⁻³ M and higher, as well as other adrenergic neurone-blocking agents in high concentrations, inhibited oxidative phosphorylation in rat isolated liver mitochondria *in vitro*. Since the destructive effect of guanethidine on adrenergic neurones is first associated with prominent changes in mito-

chondria, it seems highly likely that such destruction is secondary to inhibition of intraneuronal, oxidative phosphorylation by guanethidine when it is concentrated in adrenergic neurons. It is not known if neuronal destruction occurs in man. However, guanethidine has been in wide clinical use for over 20 years without reports of its producing irreversible hypotension or other effects attributed to irreversible sympathetic inhibition. Jandhyala *et al.* (1975) treated dogs with $2.5 \text{ mg kg}^{-1} \text{ day}^{-1}$ p.o. for 7 to 8 months. This dose approximated the upper limit of the human therapeutic dose range. Ventricular function curves obtained in treated and placebo groups were virtually identical. Chronic guanethidine administration did not produce adverse effects on myocardial function.

Hypotheses regarding the mechanism of neurone blockade

Subcellular pool depletion hypothesis. The finding that, following the administration of guanethidine, adrenergic neurone blockade preceded depletion of the noradrenaline content suggested that neuronal blockade was not a direct result of the general depletion of amines (Cass & Spriggs, 1961; Gaffney *et al.*, 1962; Sanan & Vogt, 1962; Gaffney *et al.*, 1963; Spriggs, 1966). According to Chang *et al.* (1967a) and Abbs & Robertson (1970) this did not necessarily mean that the neurone-blocking effect of guanethidine was unrelated to the storage mechanism of the transmitter amine. They felt that some small, but necessary, compartment might be depleted. Chang *et al.* (1967a) pointed out that guanethidine caused a significant decrease in the tritiated noradrenaline of the particulate subcellular fraction of rat heart and vas deferens within 30 min following its administration. At this time, depletion of total noradrenaline had not yet reached significance. Bhagat (1964), on the other hand, reported rapid depletion (within 45 min) of only the supernatant noradrenaline in rat heart following guanethidine. Approximately three times as long a time was required for depletion of particulate fractions.

Abbs & Dodd (1974) found that 5 mg/kg i.v. guanethidine decreased the noradrenaline content of the high-speed particulate (100,000 g) and supernatant fractions of splenic homogenates. These changes were temporally correlated with neurone blockade. Dexamphetamine prevented and antagonized neurone blockade produced by guanethidine. The restoration of nerve function was temporally correlated with a selective repletion of the noradrenaline content of the supernatant fraction. High doses of guanethidine (15 mg/kg i.v.) selectively depleted noradrenaline in only the supernatant fraction of the spleen. The depletion was temporally correlated with neurone blockade.

Since these several workers had differing results with the same and with different tissues, (a) there may be technical difficulties to overcome or (b) it appears that these early subcellular effects produced by guanethidine may be unrelated to neurone blockade. The possibility that a small but select pool of noradrenaline within the neurone-terminal is involved in the mode of action of guanethidine is still a viable idea, but one for which no consensus of evidence exists.

Amphetamine receptor site hypothesis. It was reported by Wilson & Long (1960) that the hypotensive action of guanethidine in man was reduced by simultaneous treatment with dexamphetamine. Day & Rand (1962) demonstrated that dexamphetamine reversed the adrenergic neurone-blocking action of guanethidine in anaesthetized cats and dogs. It was suggested by Day & Rand (1963) that the antagonism between guanethidine and dexamphetamine was competitive and that both drugs interacted with the receptor concerned with the blocking action of guanethidine in sympathetic neurone terminals. Chang *et al.* (1965) presented evidence that they interpreted as showing that amphetamine selectively displaced guanethidine from its specific binding sites in adrenergic neurones and it thereby reversed neuronal blockade. Methamphetamine is also known to prevent accumulation of guanethidine in rabbit hearts (Matsumoto & Horita, 1963).

Gerken *et al.* (1969) demonstrated that cocaine was also effective in reversing the adrenergic neurone blockade in rabbit isolated ileum, ear artery, and spleen. Cocaine was effective in a concentration that reduced noradrenaline uptake to the same extent as a concentration of dexamphetamine which also reversed adrenergic neurone blockade in these tissues. The principle interaction of cocaine with adrenergic neurones is to block the uptake mechanism in the terminals. This result suggested strongly that amphetamine, like cocaine, was antagonizing the effects of guanethidine by inhibiting the uptake of guanethidine. The more rapid recovery from neuronal blockade after amphetamine as compared with recovery after cocaine suggests that release of guanethidine by amphetamine may also play a part. Kirpekar *et al.* (1969) demonstrated that cocaine antagonized the guanethidine-induced blockade of the response of the cat nictitating membrane to stimulation of the cervical sympathetic nerve. Gerken *et al.* (1969) suggested that the rapid reversal of guanethidine-induced neuronal blockade by compounds that prevented its uptake indicated that the 'pool' of guanethidine involved in neuronal block was in facile steady state with the guanethidine outside the neurone. Alternatively, guanethidine may readily leak out of neurones, only to be taken up again by the neuronal pump in a rapid recycling process. In either case, blockade of

the uptake of guanethidine would be expected to result in a net movement of guanethidine away from its site of action and out of the nerve.

Recent work (Giachetti & Hollenbeck, 1976) suggests that because dexamphetamine accelerated the efflux of both radiolabeled guanethidine and noradrenaline from extravesicular (reserpine-resistant) binding sites, the sites may be identical and important to the production of neurone blockade.

The bulk of the evidence, presented above, suggests that, although the 'amphetamine receptor site hypothesis' is an interesting possibility, additional work is required to establish it clearly as a definitive mechanism.

Membrane depolarizing and stabilizing hypotheses and local anaesthetic action. Chang *et al.* (1965) suggested that there was a link between the degree of neurone blockade as measured by appearance of ptosis in the rat, the amount of guanethidine in sympathetic neurones of the rat heart, and the noradrenaline depleted from the rat heart. These authors considered that their evidence indicated that the blockade of adrenergic neurones and the loss of noradrenaline from nerve endings was a consequence of the same primary action of guanethidine and speculated that this action might be a persistent depolarizing of adrenergic terminals.

Evidence that guanethidine did not depolarize sympathetic terminals was presented by Cabrera *et al.* (1966). These authors used the 'collision technique' in cats. With this technique the antidromic impulses that were induced in adrenergic nerve terminals in the heart by injection of drugs into the left atrium were assessed by their capacity to reduce the amplitude of orthodromic impulses arriving in postganglionic fibres (inferior cardiac nerve). The orthodromic impulses were initiated by electrical stimulation of preganglionic fibres. The presence of antidromic impulses indicated that the postganglionic nerve endings had been depolarized. Acetylcholine readily initiated antidromic impulses, but bretylium and guanethidine did not. The failure of these two agents to do so indicated that depolarization of the nerve membrane was not essential for their neurone block. On the contrary, guanethidine and bretylium, like local anaesthetics, were strong antagonists of acetylcholine depolarization, suggesting that guanethidine and bretylium blocked the sympathetic terminals by stabilizing their membranes. Similar findings were presented by Häusler *et al.* (1968), who used a cat Langendorff preparation with its innervation intact. Guanethidine (2×10^{-7} M) completely blocked the antidromal discharges produced by acetylcholine (0.05 mM) in the postganglionic sympathetic innervation of this preparation.

Some support for the depolarization mechanism favoured by Chang *et al.* (1965) and Brodie *et al.*

(1965) comes from Misu and his coworkers. Kubo & Misu (1974) demonstrated that in the presence of guanethidine-induced adrenergic neurone blockade in innervated Langendorff preparations, high Na^+ (286 mM) increased while low Na^+ (86 mM) decreased the level of blockade as compared to the blockade seen in normal Na^+ (143 mM). In addition, guanethidine was shown by Hosotani & Misu (1978) to inhibit the increased efflux of noradrenaline out of rabbit ventricular slices caused by low Na^+ (18 mM). These authors interpreted their data to mean that guanethidine increased the permeability of adrenergic nerve endings to Na^+ ions and that its neurone blocking action was secondary to the consequent sustained depolarization of these endings. The significance of these findings in explaining neurone blockade is diminished because the concentrations of guanethidine employed in elucidating these several effects ranged from 4×10^{-6} M to 8×10^{-5} M whereas in the work of Häusler and his associates (1968) stabilizing effects and neurone blockade were reported to occur at 2×10^{-7} M to 10^{-6} M. It is likely that the 'depolarizing' effects postulated by Misu and coworkers are consequent on the higher concentrations which they used.

The idea that guanethidine might exert a depressant action on the adrenergic terminals, which was analogous to the impairment of conduction in a nerve trunk caused by local anaesthetics, was first presented by Boura *et al.* (1960) and Boura & Green (1965). Guanethidine is a relatively weak local anaesthetic agent (Bein, 1960), but is taken up and highly concentrated within the adrenergic nerve terminals because it is a substrate for the amine pump in the terminal neuronal membrane. Further, Häusler (1971) estimated that guanethidine was accumulated within the adrenergic nerve endings as compared with its concentration in the extracellular space by a ratio of 1,000 to 3,000:1.

Mitchell & Oates (1970) and Oates *et al.* (1971) reemphasized that guanethidine and related adrenergic neurone blocking drugs in high concentrations are general inhibitors of nerve and muscle function. Perhaps this is related to their weak local anaesthetic-like actions. Guanethidine administered intravenously to dogs and cats briefly inhibited cholinergic junctions in the sympathetic, parasympathetic, and somatic motor outflow. Transient ganglionic blockade was observed as well as transient blockade of the cardiac response to peripheral vagal stimulation (Boura & Green, 1959). Transient paralysis of neuromuscular transmission has been described in several preparations both *in vivo* and *in vitro* (Boura & Green, 1959; Dixit *et al.*, 1961; Chang *et al.*, 1967b). These blocking actions required high concentrations of the agents *in vitro* and relatively large intravenous doses *in vivo*. *In vivo* these responses were very transient relative to the adrenergic

neurone blockade. The brief duration of these effects was undoubtedly due to the rapidly falling plasma levels of the compounds that occurs after their intravenous administration and the consequent reduction in concentrations at the various synapses and junctions. On the other hand, adrenergic neurone blockade persists because the blocking agents are actively taken up by the amine pump in adrenergic nerves and thereby held in sufficiently high concentrations within the neurone to elicit an effect. Thus, as suggested by earlier work (Boura *et al.*, 1960; Boura & Green, 1963; Schanker & Morrison, 1965), it can be argued that the selective inhibition of the adrenergic neurone, *in vivo*, results solely from the fact that these agents are substrates for the amine pump in adrenergic neurones and not because they interrupt a function specific to adrenergic nerve terminals.

At the present time the 'stabilizing effect' of high concentration of intraneuronal guanethidine seems the best explanation of neurone blockade.

A 'unifying view'

The proclivity of guanethidine for adrenergic neurones is due to the fact that structurally it fulfills at least the minimal requirements to fit the 'amine pump' in adrenergic neurone membranes. It is, therefore, pumped into the adrenergic neurone against a concentration gradient in a manner similar to the natural substrate for the pump, noradrenaline. As a consequence of this, guanethidine achieves a high concentration in the adrenergic nerve terminals and inhibits the release of noradrenaline which normally occurs when action potentials come down the sympathetic neurones. There are several hypotheses as to the function which guanethidine alters within the nerve terminal. The author believes that the presently-available evidence favours the view that because of its weak local anaesthetic-like action and its being concentrated inside the neurone, guanethidine stabilises the electrical properties of the neuronal membrane from 'inside' and that this is responsible for adrenergic neurone blockade.

That guanethidine is a practical therapeutic agent rather than just a laboratory curiosity is due to its affinity for the amine pump. Although this affinity is relatively low, it is sufficient to permit doses to be administered which give rise to plasma levels that are adequate to ensure uptake and concentration in adrenergic nerve endings but that are too low to produce general depression of excitable membranes. Large doses of guanethidine given by any route, or moderate doses given intravenously, can produce neuromuscular blockade, ganglionic blockade and conduction blockade. These undesirable secondary effects are relatively prolonged when the doses are

given by any route except the intravenous route since a prolonged absorption is thereby involved. They are relatively fleeting when given intravenously due to the rapid rise and subsequent rapid fall in plasma concentration as a result of the distribution of the drugs out of the plasma into tissues. Since the local anaesthetic-like action of guanethidine is readily reversible, the undesirable effects secondary to this action disappear as the plasma levels subside.

The relatively transient, sympathomimetic effect of guanethidine is due to a transient release of noradrenaline from adrenergic nerve endings. Presumably the release is from cytoplasmic sites within the neurone rather than from the storage granules since all adrenergic neurone-blocking agents elicit sympathomimesis but all do not interact with the storage granules.

The capacity of the guanethidine to deplete intraneuronal noradrenaline is a result of its disruptive effect on noradrenaline storage granules and its poor capacity to block intraneuronal monoamine oxidase.

There is considerable evidence that subchronic, parenteral administration of guanethidine into animals, at doses greater than those needed to produce neurone blockade but not great enough to produce serious secondary effects (*e.g.* neuromuscular blockade), can lead to damage or destruction of adrenergic neurones. The evidence at hand suggests quite clearly that this is due to the excessive accumulation of the guanethidine within the neurone with a consequent disruption of mitochondrial function.

Thus both the useful and non-useful pharmacology of guanethidine centres on the fact that in high concentrations it disrupts the functioning of excitable membranes. However high concentrations (*i.e.*, 10^{-4} to 10^{-3} M) are only sustained within adrenergic neurones. Guanethidine apparently alters membrane functioning by exerting a 'local anaesthetic-like' effect. It appears that at somewhat higher concentrations guanethidine may destabilise excitable membranes by rendering them porous to Na^+ and thereby lead to sustained depolarisation. Furthermore, it appears that concentrations of $> 10^{-4}$ M (as can be sustained in adrenergic neurones) may disrupt certain non-excitable membranes, such as those of the noradrenaline storage granules. Lastly in concentrations of 10^{-3} M or greater, coupled with a prolonged exposure period, mitochondrial membranes may be destroyed. These latter two effects which presumably involve non-excitable membranes appear to be less readily reversible than the actions on excitable membranes. One can speculate that with guanethidine we are dealing with a concentration-dependent continuum of inhibition of membrane function, involving high to very high concentrations and increasing time of exposure:

stabilization → depolarization → disruption → destruction
(neurone blockade) (neurone disintegration)

This progression of inhibitory effects on membrane function first becomes noticeable in excitable membranes but eventually becomes apparent in other membranes as well:

The bulk of the experimental data regarding the

interaction of guanethidine with other drugs, *e.g.*, catecholamines, cocaine, amphetamine and tricyclic antidepressants, can be understood on the basis of competition of the various agents with the guanethidine for access to the adrenergic neurone.

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