ADRENERGIC NEURONE BLOCKING AGENTS[1]

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This review is concerned with substances that depress peripheral adrenergic nerve function by preventing release of adrenergic nerve transmitters. It does not deal with the so-called adrenergic blocking or adrenolytic agents; these are antagonistic towards the adrenergic transmitter substances, epinephrine and norepinephrine, after their release. Only brief reference is made to substances like reserpine which deplete stores of transmitter before affecting adrenergic neurone function [Muscholl & Vogt (1)]. The best known of the adrenergic neurone blocking agents are choline 2,6-xylyl ether (known also as xylocholine and as TM 10), bretylium, and guanethidine; but others, of equal or greater potency and specificity, have been described more recently. We are concerned here only with their pharmacology and especially with that which has an important bearing on their mechanism of action; details of mechanism remain unknown, in keeping with the lack of complete knowledge of the intricacies of the sympathetic postganglionic mechanisms. Earlier reviews of similar type have been published by Aviado, (2) Green, (3) and Maxwell (4), but the material available for consideration has grown rapidly. It has not been our policy to exclude material covered in earlier reviews, though we have attempted, where appropriate, to put most emphasis on the more recent observations.

Reviews dealing with the therapeutic use of adrenergic neurone blocking agents, particularly for the treatment of hypertensive disease, for which purpose these compounds have made an important contribution to medicine, are available elsewhere [Pickering, Cranston & Pears (5); Bock & Cottier (6); Mendlowitz (7); Brest & Moyer (8); Hoobler (9)]. We have confined our consideration of this aspect to a brief description of the pharmacological principles underlying the use of adrenergic neurone blocking agents and to the mention of a few clinical papers pertaining to the application of the newer drugs. Those requiring information on methods for the pharmacological evaluation of adrenergic neurone blocking agents in the laboratory will find that we have endeavoured to cater specifically to them in a recently published laboratory manual [Green & Boura (10)].

The history of adrenergic neurone blocking agents is short. That pertain-

[1] The survey of literature pertaining to this review was concluded in April, 1964.

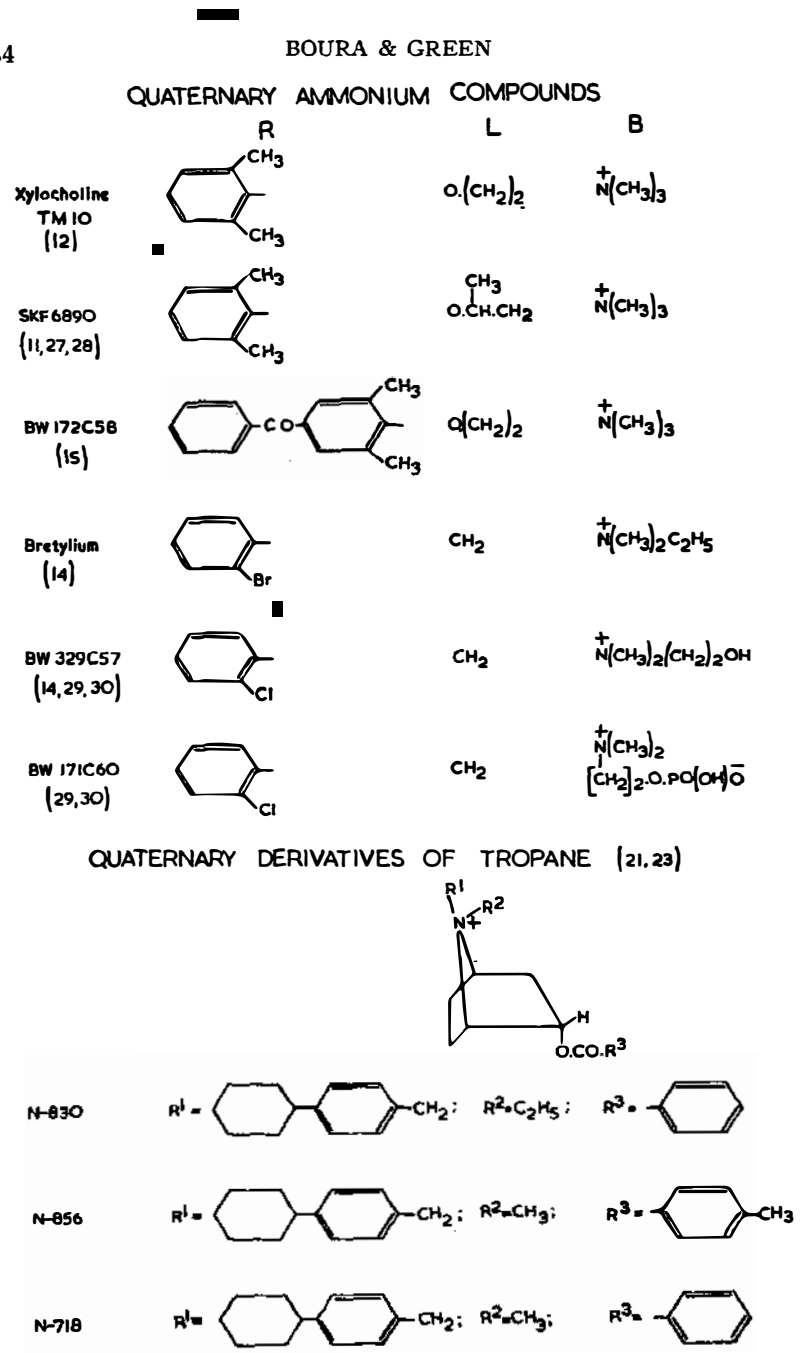

FIG. 1

OTHER BASES

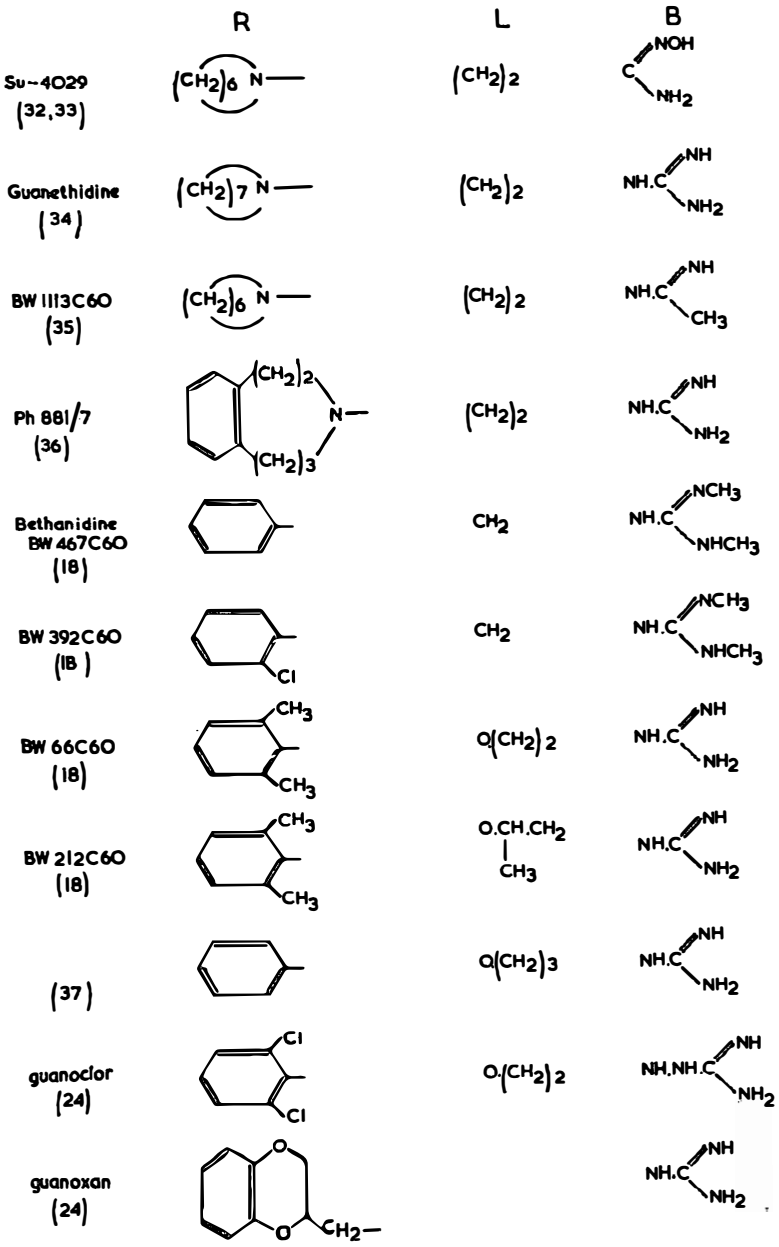

FIG. 1 (*continued*)

ing to xylocholine is reviewed by Bain (11) in whose laboratory the compound was discovered. This compound was shown, by Hey & Willey (12), to suppress certain responses to sympathetic postganglionic nerve stimulation without antagonising responses to epinephrine or norepinephrine; other aspects of its pharmacology and notably the suppression of the release of transmitter were demonstrated by Exley (13). The chemical development of this pharmacological lead, by the exploration of the properties of related substances, was aimed primarily at finding a substance that lacked the acetylcholine-like action of xylocholine which prohibited its application clinically. This target was first reached in bretylium [Boura, Copp & Green (14)] and the 4-benzoyl analogue of xylocholine [Boura et al., (15)] each of which possessed the required specificity of action. Bretylium became the first adrenergic neurone blocking agent to be used commonly in man after its first clinical trial by Rosenheim and his co-workers [Boura et al. (16)]. The research leading to the discovery of guanethidine was quite independent, again arose by accident, and has been described by Maxwell (17). This compound has enjoyed world-wide therapeutic use for several years, its application, unlike that of bretylium, not having been restricted by development of unacceptable tolerance to the drug action. The more important of the newer adrenergic neurone blocking agents include bethanidine, one of a series of benzylguanidines [Boura et al. (18)]. Its clinical importance stems from the findings that, unlike guanethidine, it does not cause diarrhoea in a significant proportion of patients nor does it continue to block sympathetic pathways for long after discontinuing drug administration [Montuschi & Pickens (19); Smirk (20)]. The other new compounds of special interest are 3α-O-benzoyl-N-*p*-cyclohexylbenzyltropinium (Br), whose properties were first described by Dóda, György & Nádor (21), and guanoxan and guanoclor [Lawrie et al. (22); Peart & MacMahon (23)] both synthesised by Augustine & Green (24).

Noteworthy Compounds Affecting Adrenergic Nerve Mechanisms

The large number of adrenergic neurone blocking agents, so far discovered, all possess a chemical structure (Figure 1) which may be considered to comprise three units: (*a*) a highly basic unit B which includes quaternary ammonium cationic heads, amidoximes, guanidines, aminoguanidines, and amidines; (*b*) a connecting chain or link L; and (*c*) a ring R which may or may not contain one or more additional but weaker basic groups.

Structure-activity relationships were reviewed by Schlittler, Druey & Marxer in 1962 (25) and by Copp in 1964 (26). The compounds which have aroused the greatest pharmacological interest are shown in Figure 1. This includes the typical adrenergic neurone blocking agents and also phenoxypropylguanidine and the forerunner of guanethidine (Su-4029) which do not directly affect transmitter release but produced hypotension, probably by depleting catechol amine stores (32, 33, 37).

Inhibition of the Release of Adrenergic Nerve Transmitter

Whereas many adrenergic neurone blocking agents have been shown to suppress the responses of various systems to adrenergic nerve stimulation, while either not affecting or increasing responses to injected epinephrine or norepinephrine, direct evidence indicating that the depression is due to inhibition of the release of the transmitters, epinephrine, or norepinephrine, from adrenergic nerve terminals is relatively scant. Evidence of this nature has, however, been obtained by measuring the release of norepinephrine into the splenic vein during stimulation of the splenic nerve in anesthetised cats. Inhibition of release in this test situation was demonstrated for xylocholine by Exley (13) and confirmed by Nasmyth & Andrews (38). Similar results were obtained by this method in studies of bretylium [Boura & Green (39)], BW 172C58 [Boura & Green (Unpublished results)], guanethidine [Abercrombie & Davies (40)], and bethanidine [Boura & Green (41)]. In these studies, norepinephrine was assayed biologically using pithed rats. The results agreed well with experiments reported by Hertting, Axelrod & Patrick (42) in which isotopically labelled norepinephrine was used. This was first injected into the cats and time then allowed for its uptake by tissues; elevations of the radioactivity of splenic venous blood, caused by stimulation of the splenic nerve, were reduced by bretylium or guanethidine.

Our only reference to a direct demonstration of inhibition of transmitter release, in systems other than the spleen, is to studies in which bretylium suppressed release from the stimulated cardioaccelerans nerve in the dog [Gilmore & Siegel (43)].

Adrenergic Neurone Blockade

The adrenergic neurone blocking agents referred to above suppress adrenergic nerve functions, irrespective of whether the response to nerve stimulation is motor or inhibitory, and the changes produced are, in many ways, comparable to those following postganglionic sympathectomy. Their effects have been demonstrated in studies of a variety of smooth muscle responses in isolated organs and *in situ*. Suppression by BW 392C60 of the mobilisation of glucose and fatty acids in adrenalectomised rats exposed to cold [Gilgen et al. (44)], changes caused by guanethidine in deposition of cholesterol and lipid in vascular tissues [Bein (45); Loustalot, Schuler & Albrecht (46)], and brief hypocholesterolaemia following bretylium [Chruschiel (47)] have also been reported.

Differences in sensitivity to the depressant action of these drugs of different adrenergic nerves in the same species have been small and only occasionally have major differences in sensitivities among species been suspected. For example, cats, dogs, and men show similar sensitivity to the blocking action of bretylium, guanethidine, and bethanidine, but man was much less sensitive than the cat or dog to the blocking action of BW 172C58 [Boura et al. (15)]. It is generally found that the effects of the known adrenergic neurone blocking agents take several minutes to reach their peak even

after intravenous administration or direct application to isolated smooth muscle preparations, and that the blockade shows high persistence; but BW 172C58 is relatively rapid acting and far less persistent than the others [Boura et al. (15)].

Demonstration of blockade in vitro.—Effects on inhibitory responses to sypathetic nerve stimulation have been studied, most commonly using rabbit duodenum which shows a fall in tone and arrest of pendular movements when the periarterial nerves are stimulated. This response is readily inhibited by xylocholine [Bain (11)], bretylium [Boura & Green (39)], guanethidine [Day & Rand (48); Day (49)], bethanidine [Boura & Green (41) and many of their analogues. Adrenergic neurone blockade also occurred in similar preparations from kittens [Bentley (50)] and in guinea pig jejunum when the tone was increased by the presence of histamine [Szerb (51)]. Blockade of excitatory responses of isolated smooth muscle to postganglionic sympathetic stimulation has been demonstrated using contractions of rabbit uterus [Boura & Green (39)] and guinea pig vas deferens (41, 50, 52), vasoconstriction in perfused rabbit ears (39, 41, 53), pilomotor erection in the skin of cats [Hellman (54)], and positive inotropic and chronotropic responses in various species (48, 53, 55). Adrenergic neurone blocking agents also depress motor responses to direct electrical stimulation of guinea pig vas deferens (56, 57) and rabbit aortic strips (58).

Demonstration of blockade in vivo.—The compounds have inhibited all adrenergically mediated responses to peripheral nerve stimulation that have been examined. In the cat or dog, responses such as contractions of the nictitating membrane and spleen, piloerection, tachycardia, and vasoconstriction were blocked after xylocholine (12, 13), bretylium (39, 43, 59, 60, 61), guanethidine (45, 59, 62, 63), bethanidine (41), or the tropinium derivative, N 718 (31). Inhibitory responses, such as relaxation of the cat nongravid uterus, are also blocked (13, 15).

Specificity of action within sympathetic postganglionic nerve trunks.—Sympathetic postganglionic cholinergic mechanisms are not affected by moderate amounts of the best known adrenergic neurone blocking agents. Anticholinergic effects, occurring at several sites, have been described but are relatively brief compared to the long lasting depression of adrenergic nerve function. Indeed, during stimulation of sympathetic postganglionic nerves, the specific depression of their adrenergic component by these drugs causes the appearance in many preparations of cholinergic effects previously masked by the adrenergically mediated response.

Exley (13) stimulated the peripheral end of the lumbar sympathetic trunk of the anesthetized cat and found that although xylocholine blocked piloerection caused by stimulation of the adrenergic fibres, it had no effect on sweat secretion caused by stimulation of the cholinergic fibres. Similar findings have been reported for bretylium (39), bethanidine (41), and the parabenzoyl analogue of xylocholine (15). Boura & Green (39, 41) addi

tionally noticed that, following administration of either bretylium or bethanidine to cats, the tachycardia caused by stimulation of the cardioaccelerans changed to bradycardia which in turn was blocked by atropine. Similar findings using guanethidine on isolated atria of cats were reported by Day & Rand (48). Likewise, in vascular beds, vasodilation often replaces the vasoconstrictor response to sympathetic nerve activation after adrenergic neurone blocking agents are given. Such was the case after injecting bretylium intra-arterially in the human forearm [Blair et al. (64)], and after injecting guanethidine into the hind limb of the dog [McCubbin, Kaneko & Page (65); Bogaert, DE Schaepdryver & Vleeschhower (66)]; analogous observations were made in rabbit ears after giving guanethidine [Holton & Rand (67)] and in the paw of the cat after bethanidine (41).

Day & Rand (48) found that guanethidine abolished inhibitory responses of the rabbit isolated intestine to stimulation of the periarterial mesenteric nerve to reveal atropine-sensitive motor responses. Although they did not find a similar reversal after bretylium or xylocholine, Bentley (50) reported that bretylium caused the appearance of a contractile response to mesenteric nerve stimulation if the animals had been pretreated with amine-depleting doses of reserpine. He suggested that the reversal of effect after either bretylium or guanethidine was attributable to the concomitant stimulation of parasympathetic fibres, and further, that reserpine augmented transmission in the peripheral vagal ganglia. The absence of reversal of the response of isolated rabbit ileum to periarterial nerve stimulation in the presence of bretylium is, however, partly explained by its anticholinergic action. Reversal has been found occasionally when the preparation was washed after bretylium treatment [R. D. Robson (unpublished experiments)] presumably because the anticholinergic action, in contrast to the adrenergic neurone blockade, lacks persistence.

Just as some sympathetic postganglionic nerve trunks contain cholinergic fibres, so also does the vagus supplying the guinea pig stomach carry adrenergic fibres whose presence is revealed by administering atropine and which are blocked by bretylium [Greeff, Kasperat & Osswald (68)].

General pattern of cardiovascular changes.—Immediately following administration of adrenergic neurone blocking agents, particularly when using the intravenous route, adrenergic depression may be masked by sympathomimetic effects; only when the latter have disappeared may the results of adrenergic neurone blockade be manifest.

In cats and dogs, bretylium lowers the arterial blood pressure and causes marked orthostatic hypotension, while depressing various cardiovascular reflexes (39, 61, 69). Pressor responses caused by chemoreceptor excitation, following anoxia or injection of sodium cyanide, are reduced, as also are those caused by medullary stimulation following cerebral ischemia or carotid artery occlusion. Guanethidine lowers arterial blood pressure and reduces the cardiac index and pulse pressure in dogs [Maxwell et al. (62)]; reflex

vasopressor reactions, such as those elicited either from the carotid sinus or by noxious stimuli, are inhibited (62). Similar observations have been made in cats, dogs, and monkeys given bethanidine and BW 392C60 (41) and in cats and dogs given other agents [Dóda et al. (31)]. In the rat, bretylium, guanethidine, and xylocholine block the hypertensive response to physostigmine (70, 71, 72); this response is said to be due to central activation of adrenergically mediated peripheral pathways.

Depression of cardiovascular reflexes occurs with doses similar to those that suppress responses to peripheral adrenergic nerve stimulation, whichever adrenergic neurone blocking agent is used, and hence probably is due to blockade of peripheral pathways. Supporting this conclusion is the finding that the increased rate of traffic in the splanchnic nerves of cats or dogs when central vasomotor centres were stimulated was not diminished by guanethidine [Maxwell, Schneider & Plummer (73); Kaneko, McCubbin & Page (74)]; also, the electrical activity in the carotid sinus nerves of cats was not diminished (73).

Because adrenal medullary function is not depressed, moderate doses of adrenergic neurone blocking agents do not completely abolish those centrally mediated cardiovascular pressor reflexes which are in part due to release of catechol amines from the adrenal medulla. Thus, a combination of adrenergic neurone blockade and adrenalectomy was required to abolish, completely, rises in blood pressure caused by a number of procedures which activated centrally mediated cardiovascular reflexes (39, 41, 69, 75). Intravenous injection of the ganglion stimulant dimethylphenylpiperazinium (DMPP) or peripheral stimulation of the splanchnic nerve in anesthetized cats or dogs causes a biphasic pressor response. The initial rapid component attributable to stimulation of postganglionic vasomotor fibres is suppressed by adrenergic neurone blockade, whereas the second delayed component, resulting from activation of the adrenal medulla, is not reduced but often is increased (39, 40, 41, 69, 75). When the adrenals are removed, responses to splanchnic nerve stimulation or intravenous DMPP are abolished (39, 41, 69). Pressor responses to 4-(*m*-chlorophenylcarbamoyloxy)-butynyl-trimethylammonium (McNeil A-343) are apparently mainly attributable to activation of atropine-sensitive receptors in sympathetic ganglia [Roszowski (76)] and are reduced by adrenergic neurone blockade to a greater extent than are those caused by DMPP. Residual effects mediated via the adrenal medulla persist (10, 76, 77, 78). Pressor responses to other ganglion stimulants, including acetylcholine, nicotine, and trimethylammonium, are modified in analogous ways (31, 39, 75).

Unleashing of parasympathetic influences.—Drugs that remove the attenuating influence of adrenergic innervation may be expected to unleash cholinergic influences on various systems. This probably explains the increased bowel activity commonly found in animals given adrenergic neurone blocking agents. Guanethidine, however, has a relatively greater cathartic action than bretylium in rats (71) and more than bethanidine in guinea pigs

(41). This may be explained by the more complete suppression of responses to low rates of nerve stimulation (page 191) or the release of endogenous 5-hydroxytryptamine by guanethidine (71). Diarrhoea occurs soon after xylocholine administration and may be referred largely to the parasympathomimetic action(12, 79).

Vernikos-Danellis & Zaimis (80) found that bradycardia, caused by peripheral vagal stimulation in cats, was increased after bretylium or guanethidine. This may be attributed to blockade of the cardiac adrenergic innervation. Campos & Friedman (81) made a similar observation using xylocholine and drew attention to earlier findings that indicated the important contribution made by accelerator fibres in the phenomenon of vagal escape. Augmentation of vagal bradycardia after adrenergic neurone blockade might also be partly the result of facilitation of transmission in the vagal ganglia; facilitation has been observed in other ganglia (page 194).

Acid secretion in cats' stomachs is not increased during daily administration of guanethidine, suggesting that adrenergic nerves do not attenuate vagal-induced acid secretion [Emås (82)].

Adrenergic neurone blockade at various frequencies of nerve stimulation.—Bretylium depresses the slope of the regression line which relates the log frequency of stimulation applied to the cervical sympathetic nerve (pre- or post-ganglionic) and the magnitude of the isotonic contraction of the nictitating membranes in cats (39). In contrast, guanethidine and reserpine preferentially abolish responses to low rates of nerve stimulation and cause a roughly parallel shift of the regression curve to the right (59, 83). Similar differences between bretylium and guanethidine have been found in studies of contraction of the spleen in cats and vasoconstriction in the femoral vascular bed in cats and perfused rabbit ears, although no difference was detected in studies of the inhibitory response of isolated rabbit ileum to periarterial nerve stimulation [Green & Robson 84)].

The effect of bethanidine on the response of the nictitating membrane to sympathetic nerve stimulation was intermediate between those of guanethidine and bretylium; the slope of the regression line was depressed, but the effects of low rates of stimuli were preferentially inhibited (41). Analogous observations were made in studies of the responses of some other smooth muscles, but differences between the effects of bethanidine and guanethidine on vasoconstrictor responses of the femoral vascular bed to different rates of lumbar sympathetic stimulation were trivial (84).

Modification of adrenergic neurone blockade by other drugs.—A number of compounds antagonise the depressant actions of adrenergic neurone blocking agents. Norepinephrine reduces their effect but is not strictly an antagonist; it does not antagonise their depressant action on responses to adrenergic nerve stimulation (49, 65). In contrast, dopamine, *in vitro*, prevented or enhanced recovery from the blocking action of xylocholine (11, 49), bretylium (3, 49), and guanethidine (3, 49), whereas other catechol amines or precursors of norepinephrine were ineffective (11, 49). An antagonistic action

of dopamine was not found in the intact animal (49).

Nasmyth & Andrews (38) found that cocaine reduced the depressant action of xylocholine on the response of the spleen and nictitating membranes of cats to sympathetic nerve stimulation, the action being greater when the cocaine was given first. The antagonism was associated with partial restoration of transmitter release. These studies have been extended to show that cocaine antagonises blockade in isolated rabbit ileum and cat nictitating membranes produced by xylocholine (49), bretylium (39, 49), guanethidine (49), and bethanidine (41).

Day (49) found that several indirectly-acting sympathomimetic amines antagonised the blocking action of xylocholine, bretylium, and guanethidine on the responses of cat nictitating membrane and isolated rabbit intestine to sympathetic nerve stimulation; dexamphetamine, mephenteramine, hydroxyamphetamine, ephedrine, and phenethylamine were active on both preparations, but tyramine was active only *in vitro*. Bethanidine blockade is also antagonised by amphetamine (41).

Antagonism of guanethidine by dexamphetamine was shown also in studies of the pressor responses to carotid occlusion or central vagal stimulation in anaesthetized dogs, and of the effects of daily administration of guanethidine on the responses of the nictitating membranes in cats [Day & Rand (85, 86)], and in studies using the vas deferens of the guinea pig (83). Further, the parallel shift to the right of the curve relating the response of the cat nictitating membrane to the frequency of nerve stimulation (page 191) was reversed by amphetamine. In isolated rabbit ileum, stimulated through its adrenergic nerves, amphetamine again behaved as a competitive antagonist of guanethidine (83).

Inhibition of monoamine oxidase does not antagonise adrenergic neurone blockade; using the cat nictitating membrane and isolated rabbit ileum, antagonism was produced only by inhibitors that have sympathomimetic properties (phenelzine, pheniprazine, tranylcypromine) but not by others (iproniazid, nialamide) [Day (49)]. Blockade by guanethidine or bretylium of the isolated hypogastric nerve-vas deferens (guinea pig preparation) was reversed by norepinephrine, histamine, or 5-hydroxytryptamine [Bentley (50)]. Matsumoto & Horita (87) likewise found that many sympathomimetic amines antagonised depression by bretylium of contractions of the nictitating membrane; imipramine had a similar effect. They postulated that this may be attributable to the property common to these compounds of potentiating catechol amines by inhibition of their general uptake by tissues. This property would allow subliminal concentrations of transmitter released during nerve stimulation to reach threshold levels despite bretylium administration.

Adrenergic neurone blockade in man.—In man, adrenergic neurone blocking agents cause hypotension and block many adrenergically mediated responses. Some differences in the magnitude of the various effects produced by the different agents have been found. Bretylium lowers the arterial blood pressure in man and causes exertional and orthostatic hypotension (16,

88, 89), has a variable effect on cardiac output (88, 89, 90), and blocks reflex vasoconstriction mediated by adrenergic nerves (90–93), but has little effect on reflex vasodilation believed to be associated with sympathetic postganglionic cholinergic mechanisms [Blair et al. (64)].

Guanethidine causes hypotension together with bradycardia; the latter is abolished by atropine [Laurence & Rosenheim (91); Page & Dustan (94)] and is more pronounced after guanethidine than after bretylium. Cardiac output falls or is little altered [Taylor & Donald (88); Richardson & Wyso (95); Kirkendall & Wilson (96)]. Vasomotor reflexes were reduced after administering guanethidine [Richardson & Wyso (95); Kirkendall et al. (97)]. The blood pressure overshoot following tilting of the patient from the supine to the vertical position was reduced, but a later post-tilt overshoot persisted; the latter may have been referrable to amines released from the adrenal medulla [Richardson & Wyso (95)].

Many unwanted effects of the drugs, such as orthostatic and exertional hypotension, syncope, miosis, nausea, loss of sphincter control, and failure of ejaculation, can be ascribed to the adrenergic depression, although differences between the drugs in the extent of these various effects have been reported.

Clinical reports suggest that bretylium may cause rather greater orthostatic and exertional hypotension than guanethidine but less bradycardia (88, 91, 98, 99). We have postulated [Boura & Green (59)] that such differences could result from bretylium having a relatively greater depressant action on high rates of nerve stimulation and guanethidine a more depressant action on low rates (page 191). Sympathetic traffic would be minimal in the supine position and greatest during exercise.

The increased bowel activity found after bretylium [Laurence & Rosenheim (91); Turner (100)], guanethidine [Kirkendall & Wilson (96)], guanoxan [Peart & MacMahon (23)], and guanoclor [Lawrie et al. (22)], like the similar observation in animals, may be explained by adrenergic neurone blockade releasing parasympathetic influences over the alimentary tract. Diarrhoea occurs more frequently with guanethidine than with bretylium (96) and is remarkably rare with bethanidine in man (19, 20, 101, 102). These differences parallel those in experimental animals and may be explained similarly (page 190).

That amphetamine and similar agents are antagonistic to adrenergic neurone blocking agents in man, as in experimental animals, is suggested by the observations that patients were unresponsive to the hypotensive action of bretylium while taking amphetamine [Wilson & Long (103)], and that orthostatic hypotension caused by guanethidine was abolished by methylamphetamine [Laurence & Rosenheim (91)].

Action at Cholinergic Sites

All adrenergic neurone blocking agents examined in detail have shown a variety of actions at cholinergic sites. Generally, these are seen only with application to isolated organs or, briefly, immediately following large intra-

venous doses when their prominence is probably related to high extracellular drug concentration and not to cumulation in nerve tissue, as with adrenergic neurone blockade (page 203). Xylocholine shows muscarinic, nicotinic, ganglion blocking, and neuromuscular paralysing actions at doses near to those that affect adrenergic transmission [Bain (11); Exley (13)], but these effects are much less marked with many other adrenergic neurone blocking agents.

Transitory ganglion blockade follows intravenous administration of bretylium [Boura & Green (39); Gertner & Romano (104)], guanethidine [Bein (45); Maxwell et al. (62); Gertner & Romano (104)], and bethanidine [Boura & Green (41)]. A very weak nicotine-like effect also follows the close arterial injection of large doses of bretylium in the superior cervical ganglion (39). In isolated smooth muscle preparations, high concentrations of bretylium cause muscarinic effects [Boura & Green (39); Gokhale et al. (105)], but anti-muscarinic actions have also been described (105, 106, 107). Antagonism by guanethidine of acetylcholine-induced contractions of rabbit ileum and guinea pig tracheal chains was thought, on the other hand, to be due to a ganglionic effect (105), although antagonism of acetylcholine on guinea pig rectum and toad bladder was apparently exerted on the muscle [Boyd et al. (107)]. Large intravenous doses of bretylium cause brief atropine-sensitive depressor effects [Yelnosky & Mortimer (108)].

Transmission in sympathetic ganglia may be facilitated following the brief ganglionic blockade. We first suspected this during the examination of a large number of adrenergic neurone blocking agents in anaesthetized cats. Responses of the nictitating membranes to preganglionic nerve stimulation were often reduced slightly less than those responses to contralateral post-ganglionic stimulation. Enhancement of ganglionic transmission has since been found to follow administration of reserpine, bretylium, and antagonists of epinephrine at α-receptors [Costa et al. (109)]. These actions may be due to depletion, immobilisation, or antagonism of norepinephrine which might attenuate transmission in autonomic ganglia.

Neuromuscular blockade is caused by high doses of bretylium [Boura & Green (39); Dixit, Gulati & Gokhale (110); Green & Hughes (111)], guanethidine (110, 111, 112), and bethanidine [Boura & Green (41)]. Boura & Green (39) examined only the early effect of bretylium, finding that the paralysis in chicks was curare-like, but that neuromuscular paralysis in mammalian preparations was not antagonised by an anticholinesterase. Dixit, Gulati & Gokhale (110) revealed a second delayed depressive action of greater persistence with both bretylium and guanethidine. They likened the neuromuscular paralysis to presynaptic blockade by procaine. Responses of the frog rectus to acetylcholine were potentiated by bretylium and low concentrations of guanethidine; but higher concentrations of guanethidine reduced the responses [Gokhale et al. (105)], perhaps by a direct action on smooth muscle (see below). In the same preparation, bretylium antagonised the depressant action of *d*-tubocurarine (105), but in rat diaphragms,

d-tubocurarine and decamethonium both acted additively with bretylium or guanethidine [Dixit, Gulati & Gokhale (110)]. Green & Hughes (111) found that the blockade of the muscle response to indirect stimulation caused by bretylium had much in common with that following hemicholinium or triethylcholine. The degree of blockade increased sharply with the frequency of nerve stimulation and choline was antagonistic *in vitro*. In contrast, the frequency dependence of the blocking action of guanethidine resembled that with *d*-tubocurarine. The differences in frequency dependence may be likened to those at adrenergic sites (page 191). Vernikos-Danellis & Zaimis (80) observed that doses of bretylium or guanethidine below those causing neuromuscular blockade gradually diminished the maximum twitch tension of the cat tibialis anterior muscle. Electromyograms were abnormal in these experiments and likewise in some men given bretylium [Evanson & Sears (99); Campbell & Montuschi (113)]; it has been suggested that adrenergic neurone blockade depresses recovery of exercised striated muscle [Zaimis (114)]. This may be related to depression of mobilisation of free fatty acids and glucose (page 187). In curarised preparations, responses to direct muscle stimulation were inhibited weakly by bretylium and powerfully by guanethidine [Green & Hughes (111); Kroneberg & Stoepel (112)].

Sympathomimesis

Sympathomimetic effects commonly occur following administration of adrenergic neurone blocking agents and become prominent with high dose levels. Although this action is shown by all known adrenergic neurone blocking agents, sympathomimetic potency is unrelated to adrenergic blocking activity. Whereas xylocholine causes early sympathomimetic effects in cats [Hey & Willey (12); Exley (13)], together with increased plasma catechol amine levels [Willey (116)], its para benzoyl analogue (BW 172C58), although approximately ten times as potent as an adrenergic neurone blocking agent, has only weak sympathomimetic properties [Boura et al. (15)]. Other examples of dissociation of the two properties have been described (31, 36, 115).

The sympathomimetic effect of bretylium in the anaesthetized cat is manifested by hypertension, tachycardia, contractions of the nictitating membrane and spleen, increased peripheral vascular resistance, mydriasis, and piloerection [Boura & Green (39)]. The prominence of the pressor action varies greatly in different animals, depending probably on the level of sympathetic tone. A large rise in blood pressure occurs rapidly after intravenous injection of bretylium in some dogs but not in others [Boura & Green (39); Aviado & Dill (69)]. Other manifestations of sympathomimesis include tachycardia, increased coronary flow, positive inotropism [Aviado & Dill (69); Gilmore & Siegel (43)], contraction of the nictitating membrane, and vasoconstriction in the femoral vascular bed, and nasal mucosa [De Beer & Wnuck (personal communication)]. Rats respond initially to intravenous bretylium by a rise in blood pressure [Gillis (117); Gillis & Nash

(118)], and subcutaneous administration produces a powerful diuretic response similar to that of epinephrine and norepinephrine but differing, in that excretion of potassium is greater [Green & Sim (119)]. Sympathomimetic effects also occur in man following bretylium (64, 88, 91), the most prominent manifestation being vasoconstriction with intra-arterial injection [Blair et al. (64)]. The pressor action is accentuated in some patients with phaeochromacytoma but not in others [Laurence & Rosenheim (91)].

Bretylium causes vasoconstriction in the perfused rabbit ear [Boura & Green (39)], contracts rabbit aortic strips [Kirpekar & Furchgott (55)]; and exerts positive inotropic effects in perfused hearts [Davey & Farmer (120)], in isolated electrically-driven atria of the guinea pig (55), and in spontaneously beating atria of the rat [Bhagat & Shideman (121)] and guinea pig [Boyd, Chang & Rand (52)].

That the sympathomimetic effects of bretylium are attributable mainly to peripheral release of catechol amines from adrenergic tissues was first suggested by Boura & Green (39) after finding that such effects were prominent in spinal cats, persisted after adrenalectomy or ganglionic blockade, and occurred in isolated smooth muscle preparations. This conclusion is supported by the literature already reviewed and by the finding that appropriate blocking agents of α- and β-receptors suppress the various manifestations of sympathomimesis (3, 108, 117, 118, 122, 123). In good agreement are experiments showing that prior administration of depleting doses of reserpine markedly reduced the pressor response to bretylium in rats [Gillis & Nash (118)] and also reduced the sympathomimetic responses of rabbit aortic strips and guinea pig atria [Kirkpekar & Furchgott (55)], the responses being partially restored by norepinephrine. Findings in dogs [Yelnovsky & Mortimer (108)] and spinal cats [Gokhale, Gulati & Kelkar (123)] were similar; the additional observation that the sympathomimesis was blocked by cocaine (123) again pointed to release of norepinephrine from adrenergic nerve terminals. Direct evidence of release of tritium-labelled norepinephrine, from spleen is reported by Hertting, Axelrod & Patrick (42). Similarly, Gilmore & Siegel (43) found that bretylium released catechol amines into the coronary venous blood of dogs.

The sympathomimetic effects following intravenous injection of guanethidine into animals include pressor responses, contractions of the nictitating membranes, and positive inotropic and chronotropic actions (62, 65, 94, 118, 121, 124, 125, 126). Guanethidine hypertension in anesthetized dogs is due mainly to peripheral vasoconstriction and partly to increased cardiac output [Bogaert, De Schaepdryver & de Vleeschhouwer (66)]. Pressor effects can occur with intravenous doses in man (88, 127), and vasoconstriction follows intra-arterial injection (128). Pressor effects can be prominent in patients with phaeochromocytomas [Genest (129)].

That the sympathomimesis is due to release of catechol amines from the adrenergic nerve endings or adjacent chromaffin tissue is well supported. The pressor action of guanethidine persists after ganglion blockade [Maxwell

et al. (62)] or adrenalectomy [Kroneberg & Schümann (126)] but not after an antagonist of epinephrine at α-receptors (Maxwell (17)]. Maxwell et al. (130) found that the sympathomimetic action of guanethidine on the nictitating membranes of cats was at least as great after acute denervation as after chronic denervation, despite the enormously greater sensitivity to norepinephrine after chronic denervation. This suggests that the sympathomimesis is due to local release of catechol amines at adrenergic nerve endings, as tissue stores of amines become depleted after denervation. Furthermore, after depleting tissue catechol amine with reserpine, guanethidine showed less sympathomimetic effect on the rabbit non-gravid uterus [Bein (45)], the dog heart lung preparation [Gaffney (125); Krayer, Alper & Paasonen (131)], and the blood pressure of the rat [Gillis & Nash (118)] and dog [McCubbin, Kaneko & Page (65); Butterfield & Richardson (124)]. Moreover, in isolated guinea pig hearts, tachyphylaxis to the positive inotropic action of guanethidine occurred at a time when the tissue had lost about half its norepinephrine content [Davey & Farmer (120)], and a similar effect may account for tachyphylaxis to the pressor response in cats [Bartlet (132)]. Furthermore, guanethidine produces effects similar to those of the indirectly acting amines in the isolated guinea pig atrium-effects which likewise are abolished by cocaine [Kroneberg & Schümann (126)]. Guanethidine caused weak contractions of some strips of rabbit aorta but like cocaine, produced strong, sustained contractions in the presence of norepinephrine [Maxwell et al. (133)]; cocaine, guanethidine, and methylphenidate inhibited the capacity of one another to contract strips exposed to norepinephrine (133). The hypertensive effect was more prominent after giving inhibitors of monoamine oxidase [Kadzielawa (134)] or O-methyltransferase [Wylie (135)]. More direct evidence for the release of norepinephrine into the circulation of cats and dogs by guanethidine has been reported (42, 123, 136).

Although it has been reported that either splenectomy or adrenalectomy accelerates tachyphylaxis to the pressor response to guanethidine in anesthetized cats [Kadzielawa (134)], other investigators found no major change in the pressor response after adrenalectomy in cats, rats, or dogs (75, 126, 137). In dogs, no release of catechol amines from the adrenal medulla was found [Athos et al. (138)], although guanethidine's capacity to release catechol amines from the amine-containing granules of bovine adrenal medulla has been demonstrated [Phillippu & Schümann (139)]. However, failure to detect a rise in plasma amine levels is not proof of lack of amine release, since the tests may be too insensitive and, moreover, a rise will occur only when the rate of release exceeds the rate of metabolic destruction. It has been suggested that guanethidine also possesses direct stimulating properties on α and β receptors (40, 128, 130, 140), but some of the observations on which this conclusion is based could be explained by the persistence of the catechol amine-releasing action of guanethidine despite reserpine administration (140) or release but nondetection of norepinephrine (40).

Sympathomimetic effects occur also with bethanidine [Boura & Green

(41)], BW 392C60 (41), the N-*p*-cyclohexylbenzyltropinium derivatives [Doda et al. (31)], and other adrenergic neurone blocking agents (15, 18, 35, 141).

Effects on Tissue Amine Stores

It is with respect to changes in tissue catechol amine stores that the greatest differences between adrenergic neurone blocking agents are thought to exist. In many short-term experiments, bretylium has not affected or has elevated the quantity of norepinephrine found in the heart, spleen, and intestine of rats, guinea pigs, or rabbits (71, 120, 142, 143), although a small but highly significant reduction was found in the submaxillary glands of rats between 8 and 24 hr, but not at 4 hr, after administering large doses of bretylium [Benmiloud & von Euler (144)]. Very little is known, however, about the degree of adrenergic neurone blockade produced in the above species under the conditions used. In cats, daily subcutaneous administration for 14 days of 30 mg per kg caused a marked depletion of the norepinephrine content of the heart, spleen, and superior cervical and stellate ganglia; the daily amount given was greater than that used in other species by many investigators, but is less than that required to maintain continuous full suppression of adrenergic neurone function [Boura & Green (59); Green (145)]. With 10 mg per kg daily, there was a tendency towards elevation of catechol amine levels in sympathetic ganglia after 3 days of drug administration [McCoubrey (146)]. It, therefore, remains possible that full adrenergic neurone blockade by bretylium in cats and other species results in depletion; this may follow a similar time course to that after sympathectomy [Kirkpekar, Cervoni & Furchgott (147)]. In rats, such reduction of catechol amines as was observed developed slowly, as after sympathectomy [Benmiloud & von Euler (144)].

Guanethidine depleted catechol amines from artery walls, heart, spleen, or intestine in rats, rabbits, guinea pigs, cats, and dogs (66, 71, 124, 131, 148–153). Lowered norepinephrine levels were found after guanethidine in the sympathetic ganglia of cats and rabbits [Sanon & Vogt (153)]. Depletion of transmitter is not the cause of adrenergic neurone blockade but follows later (65, 71, 154).

The catechol amine content of the adrenal medulla is not depleted by doses of bretylium that lower the norepinephrine content of adrenergic nerve tissue [McCoubrey (146)], this being in keeping with differences in selective accumulation of the drug (page 204) and with the relatively smaller effect on adrenal function. Similarly, to lower the level by 50 per cent in the adrenal medulla of rats with guanethidine required about 80 times the dose necessary for depletion of the spleen [Kuntzman et al. (155)].

Reports concerning the effects of guanethidine on amine levels in the brain allow little generalisation. Whereas no change was found in the norepinephrine or 5-hydroxytryptamine contents of rabbit brain (149, 155),

a temporary decrease of norepinephrine may have occurred in rat brain [Kroneberg & Schümann (126)]. Furthermore, in cats and rabbits, hypothalamic norepinephrine was decreased in some but not all experiments [Sanon & Vogt (153)], and when guanethidine was given daily for a week hypothalamic norepinephrine was lowered in cats but not in rats [Dagirmanjian (156)]. Effects on brain 5-hydroxytryptamine are also variable, the levels being lowered by guanethidine in large (greater than 200 g) but not in small animals (140 to 160 g) [Ferrini & Glasser (157)]. No single explanation of these various differences is satisfactory, but as guanethidine is almost completely ionised at physiological pH it is not expected to pass readily into brain tissue.

The relative effects of other adrenergic neurone blocking agents on tissue catechol amines vary considerably. Twenty-four hours after single adrenergic neurone blocking doses of bethanidine or BW 392C60, the norepinephrine found in the iris of cats was elevated (41), whereas the amidine (BW 1113C60), like guanethidine, caused a marked depletion (35). On the other hand, multiple large daily doses of bethanidine but not of BW 392C60 lowered the norepinephrine (or pressor amine) content of the heart or spleen of cats [Boura et al. (18)] and rats [Costa et al. (158)]. Of further interest is the finding that guanoxan and guanoclor deplete norepinephrine not only from heart and spleen but also from adrenal glands and hypothalamus [Lawrie et al. (22); Peart & MacMahon (23); Davey & Reinert (159)]. Xylocholine also depleted the pressor amine content of rat adrenals [Coupland & Exley (160); Coupland (161)].

In a variety of test situations in various species, bretylium greatly reduced the depletion of norepinephrine from heart, spleen, liver, salivary gland, and sympathetic ganglia produced by reserpine (78, 144, 146, 150, 162–164) or guanethidine (144, 150, 155, 163–165). Further, the rate of loss of catechol amines after reserpine was less in animals given guanethidine [Benmiloud (151)].

Reserpine-induced release of [^{3}H]-norepinephrine was blocked by bretylium and partly antagonised by guanethidine [Hertting, Axelrod & Patrick, (42)]. Reduction of the norepinephrine-depleting action of reserpine occurred also after bethanidine, of which the ortho chloro derivative, BW 392C60, is particularly powerful in this respect [Kuntzman et al. (155); Costa et al. (158)]. Failure of bretylium to prevent depletion of brain norepinephrine [Ryd (162)] may be explained by lack of penetration into the central nervous system [Boura et al. (166)]. Antagonism of reserpine-induced blephorospasm followed intraventricular injection of bretylium [Norton & Colville (167)].

These observations show that adrenergic neurone blocking agents have both stabilising and depleting effects on norepinephrine stores, and that the relative prominence of each effect varies in different compounds. Elevations of tissue norepinephrine may, in part, be due to a conservation of transmitter analogous to that during neuronal rest following preganglionic nerve

section or administration of ganglion blocking agents [Brown, Davies & Ferry (168)]. Inhibition of spontaneous release of [^{3}H]-norepinephrine has been observed after bretylium [Hertting, Axelrod & Patrick (42)]. Enzymes concerned with the biogenesis of norepinephrine are not inhibited by adrenergic neurone blocking agents (page 204) and depletion of norepinephrine is not due solely to losses during sympathomimesis. Indeed, when isolated perfused guinea pig hearts were repeatedly exposed to guanethidine or bretylium, the positive inotropic responses produced gradully declined; but, whereas there was some loss of norepinephrine with guanethidine, there was elevation with bretylium [Davey & Farmer (120)]. Much of the depletion *in vivo* caused by guanethidine or bretylium may be attributed to inhibition of norepinephrine uptake (42, 121, 169) which, together with a continuous leakage of norepinephrine, would result in a mounting deficit Such may also be the major cause of reserpine depletion [von Euler & Lishajko (170)]. Hertting, Axelrod & Patrick (42) also found that bretylium inhibited norepinephrine uptake, but the negative findings of Bhagat & Shideman (121) suggest that its effect is weaker than that of guanethidine. The loss of norepinephrine from rat submaxillary glands following denervation was reduced by bretylium but not by guanethidine [Benmiloud & von Euler (144)].

Alternatively, differences in the depleting action of adrenergic neurone blocking agents can be related to their different inhibitory effects on monoamine oxidase. It was known, as early as 1956, that xylocholine and several analogues inhibited this enzyme [Brown & Hey (171)] and bretylium has a similar effect [McCoubrey (146) Dvornik et al. (172)]. Bethanidine has the same order of activity as bretylium, and BW 392C60 is a much more active, competitive, and reversible inhibitor [Kuntzman & Jacobsen (173)], whereas guanethidine is relatively ineffective (172, 173). Hence, the possibility exists that the adrenergic neurone blocking agents that cause conservation of norepinephrine are those that inhibit monoamine oxidase within adrenergic nerves. It is known that bretylium and bethanidine reach high concentrations in adrenergic nerves (page 204), and it is accepted that the monoamine oxidase of the nerves may not behave identically with respect to inhibitors as the enzyme on which the blocking agents have been tested. Many monoamine oxidase inhibitors are known to elevate tissue norepinephrine and inhibit depletion by reserpine [Green (3)], and several reduce guanethidine-induced depletion of norepinephrine (121, 164, 165) and modify sympathomimetic responses to adrenergic neurone blocking agents.[2] An alternative explanation of the relative stabilising and depleting actions of the different compounds is mentioned on page 206. We have related depression of tissue

[2] It is of interest in this connection that a number of monamine oxidase inhibitors show weak depressant effects on adrenergic nerve function [Gessa, Cuenca & Costa (165); Gessa (174)].

norepinephrine to effects on the nerve-frequency sympathetic-response curves (page 191) in earlier publications (41, 59, 84).

Cocaine and some of the sympathomimetic amines that interact with adrenergic neurone blocking agents in other test situations (pages 191 and 196) reduce the norepinephrine-depleting action of guanethidine, but it should be appreciated that changes in tissue levels are less sensitive indices of release of norepinephrine than are physiological responses. Cocaine blocks guanethidine-induced depletion of heart norepinephrine under some conditions [Callingham & Cass (150)]. Whereas dexamphetamine did not affect the depleting effect of guanethidine on norepinephrine stores in the heart or spleen of rabbits [Day & Rand (83)], methylamphetamine at higher doses did [Matsumoto & Horita (175)]. A tendency for depletion of [^{3}H]-norepinephrine by amphetamine in rat hearts to be less in bretylium-treated animals was not statistically significant [Potter & Axelrod (164)].

Effects on Responses to Sympathomimetic Amines

Whereas brief inhibition of responses to epinephrine or norepinephrine occurs after large intravenous doses of xylocholine (12, 13) or guanoxan [Boura & Green (Unpublished 1960), and (23, 159)] later responses to the catechol amines are increased, as with other adrenergic neurone blocking agents.

In animals of several species including men [Laurence & Nagle (176); Cooper et al. (177)] and in isolated organ preparations, responses to epinephrine and norepinephrine increase rapidly in the presence of xylocholine [Exley (13); Ross et al. (178)], bretylium (39, 55, 70, 80, 179, 180), guanethidine (62, 65, 80, 130, 131, 134, 180–184), and bethanidine [Boura & Green (41)]. These increases occur more rapidly than those after nerve section. They may be attributable to a direct sensitisation of smooth muscle [Vernikos-Danellis & Zaimis (80); Abboud, Eckstein & Wendling (181)], perhaps by lowering of catechol amine background of effector cells or by blockade of access of catechol amines to non-specific take-up receptors. Mechanisms of catechol amine hypersensitisation were reviewed recently [Vane (185); Trendelenberg (186)].

Early increases of sensitivity are small compared with those after daily administration of bretylium or guanethidine. Responsiveness of the nictitating membranes of cats rises to reach a maximum after about 7 days when norepinephrine sensitivity is increased about 100-fold and epinephrine sensitivity is increased about 30-fold [Boura & Green (59) Green (145)]. The time course varies with dosage and the maximal changes produced are similar to those occurring at the same times after sympathectomy. Large increases in sensitivity, again resembling those after postganglionic nerve section, occur after prolonged adrenergic neurone blockade in the spleen of cats [Green & Robson (187)] and the submaxillary glands of rats [Emmelin & Engström (188)]. Increases of pressor (59, 65, 145) or vasoconstrictor ac-

tions of epinephrine and norepinephrine [Green & Robson (187) and Zimmerman & Harris (189)] are, in general, smaller and vary with the test situation. This may be because an increase occurs in β receptor as well as α-receptor responses, as is known to occur in other test situations [Kirkpekar & Furchgott (55); Gokhale & Gulati (179)]; potentiation of a β-vasodilator response would offset the effects of potentiation of an α-receptor vasoconstrictor response. This is relevant to the observation that single doses of bretylium or guanethidine enhance the pressor response to intravenous norepinephrine in man but cause little change in the response of forearm or hand blood flow to intra-arterial norepinephrine (64, 176, 177, 190). Of further interest is that whereas norepinephrine sensitivity is increased to a greater extent than epinephrine sensitivity on the nictitating membrane of cats (which are normally more sensitive to epinephrine,) no important distinction was found in studies of responses that are provoked as readily by either amine [Green & Robson (187)]. Guanethidine potentiated the positive inotropic and chronotropic effects of norepinephrine more than those of epinephrine and had no effect on responses to isoprenaline [Stafford (184)].

Depending on the test situation, bretylium may increase or decrease the responses to sympathomimetic amines that act by releasing endogenous catechol amines (53, 59, 70, 80, 114, 145, 191, 192), as also does guanethidine, though the latter's depressant action is more prominent (59, 62, 80, 121, 130, 134, 182, 183, 191, 192). Bretylium or guanethidine, given daily to cats for various times, depressed the slope of the log dose response curves for the pressor and nictitating membrane contracting effects of intravenous tyramine; sensitivity was apparently related both to norepinephrine sensitivity and the amount released [Boura & Green (59)]. Catechol amine release by tyramine was apparently suppressed to a greater extent by guanethidine than by bretylium. However, on the assumption that sensitivities to the catechol amines released by tyramine were increased as much as those to injected catechol amines, even with bretylium at doses below those required fully to suppress adrenergic nerve function, inhibition of amine release was often substantial. Changes in responses to amphetamine and ephedrine were studied in less detail, but some of the effects observed after bretylium or guanethidine paralleled changes in the tyramine responses (59). In acute experiments in rats, moderate doses of bretylium prolonged, but large doses markedly diminished, responses to tyramine [Léšic & Varagić (70)]. Pressor responses to phenylethylamine or amphetamine were not potentiated, and when catechol amines had been depleted with reserpine, bretylium no longer potentiated the pressor action of tyramine [Schmitt, Schmitt & Depoilly (193)].

Responses to tyramine and amphetamine were inhibited by guanethidine at a time when there was no significant reduction in tissue catechol amine levels (121, 126, 194). This suggests that a critical "pool" of norepinephrine is either lost or stabilised under the influence of guanethidine.

Xylocholine caused an early potentiation of pressor responses to phenylethylamine and tyramine in dogs, exceeding that with bretylium or guanethidine [Ross et al. (178)].

Tolerance

Tolerance to depression of adrenergic function develops during daily administration of bretylium, guanethidine, and bethanidine. This is indicated by a gradual reduction in the degree of nictitating membrane relaxation in cats (16, 59, 145), and of the hypotensive effect in man (19, 96, 101, 195, 196). Studies of nictitating membrane responses suggest that the probable explanation, discussed in detail elsewhere [Boura & Green (59)], lies in the development by smooth muscle of hypersensitivity to catechol amines. In the first instance, this results in hypersensitivity to the released adrenergic transmitter, offsetting the reduction in its release caused by adrenergic neurone blockade. Furthermore, even during full adrenergic neurone blockade, lowered sympathetic tone may not be maintained in consequence of the increased sensitivity to catechol amines from the adrenal medulla. Lastly, the hypersensitivity can, for a time, also result in enhanced sympathomimetic responses to the blocking agents, although these later decline. Tolerance is less dramatic in systems, vascular beds in particular, that acquire lesser hypersensitivity to catechol amines [Green & Robson (187)].

That tolerance is more precipitous with bretylium than with guanethidine may be due to their differing effects on the relation between frequency of nerve stimulation and end organ response (page 191). We have shown that the main factor responsible for the more rapid tolerance of the nictitating membrane to bretylium is probably the relatively small inhibitory action of this drug on transmitter release at low rates of nerve stimulation (59). When hypersensitivity to catechol amines has developed, this allows exaggeration of responses to low rates of nerve stimulation. This finding has a general application although the difference between bretylium and guanethidine found on nictating membranes is greater than in some other adrenergic systems [Green & Robson (84, 187)]. The lesser prominence of tolerance to guanethidine may also be a result of its cumulative action when given daily (59). That effects of the drugs on different rates of nerve stimulation influence the rate of tolerance development is supported further by the results for bethanidine. Its effects on nerve frequency-response curves in cats are intermediate between those of bretylium and guanethidine (41, 84); in man, there has been a more frequent need to increase dosage (to a maximum of twice that used early in treatment) than with guanethidine, but tolerance is not a problem as with bretylium (19, 20).

Selective Distribution

The highly selective distribution of isotopically labelled bretylium, following subcutaneous administration in cats, suggested that this was related

to the specificity of its adrenergic neurone blocking action [Green (145); Boura et al. (166)]. The concentrations accumulated in sympathetic ganglia and their efferent trunks reached some 20 times those in parasympathetic ganglia and cholinergic nerve trunks and were temporally related to adrenergic neurone depression. Persistently high concentrations were found also in tissues known to have a rich adrenergic nerve supply such as spleen and heart, but other tissues, including the adrenal glands, contained lower concentrations of drug and for a relatively shorter time [Boura et al. (166)]. The high concentrations found (as much as 250 μg per g after a 10 mg per kg dose) were sufficient to block conduction in some, but not all, nerve trunks when applied topically, but the greater sensitivity of the nerve endings together with the expectation of easier access of drug to nerve terminals suggests that this is the principal region of adrenergic neurone blockade.

Other adrenergic neurone blocking agents investigated [bethanidine (197), BW 172C58, and *o*-bromobenzyltrimethylammonium iodide (198)], showed a selective distribution similar to that of bretylium; and in the case of BW 172C58, a temporal relationship was again found between the concentration in adrenergic nerves and the blockade. The properties of guanethidine suggest that it too may selectively accumulate in tissues with high catechol amine levels; following intravenous injection in rats, guanethidine is rapidly taken up by the heart and bound for 14 hr or more [Bisson & Muscholl (152)], and the rate of decline of tissue concentrations is low [Kuntzman et al. (155)]. The uptake of guanethidine by rabbit hearts was reduced by methylamphetamine [Matsumoto & Horita (175)].

Hypotheses Concerning the Intimate Mechanism of Adrenergic Neurone Blockade

The suggestion that xylocholine blocks adrenergic nerves by inhibiting the biosynthesis of a critical pool of norepinephrine available for immediate release by a nerve impulse (11, 160, 199) has received no support in recent studies. Dopamine β-oxidase from several sources was not inhibited by xylocholine or its β-methyl derivative except when their concentrations were approximately 2×10^{-1} M [Hagen & Zebrowski (200))]. Nor in other studies. DOPAmine β-oxidase from several sources was not inhibited by guanethidine [Creveling et al. (201)]. DOPA decarboxylase is also resistant to bretylium and guanethidine [McCoubrey (146); Chrusciel (202)]. Furthermore, blockade of adrenergic neurones precedes measurable diminution of norepinephrine and may be accompanied by increased norepinephrine levels (pages 198).

Although the suggestion that xylocholine might depress conduction in adrenergic fibres [Hey & Willey (12)] requires some qualification in view of the demonstration that adrenergic neurone blocking doses do not suppress action potentials in postganglionic sympathetic nerve trunks [Exley (13,

203)], the possibility remains that the depressant action on the adrenergic fibre terminals is analogous to the impairment of conduction in a nerve trunk caused by local anaesthetics. This possibility exists also for bretylium and guanethidine, neither of which, at moderate dosage, suppressed postganglionic nerve potentials [Boura (145) and (45, 62, 203, 204)]. The high concentrations of bretylium, bethanidine, and BW 172C60 that accumulate in adrenergic nerves (page 204) are sufficient, when applied topically, to impair conduction in some, but not all, nerves but are fully adequate *in vitro* to suppress a variety of end-organ responses to both adrenergic and cholinergic nerve stimulation (15, 39, 41, 52, 166 and page 193). Nerve terminals are expected both to be more sensitive than the nerve trunks and to accumulate drug more readily. When, in cats, the intravenous dose of BW 172C58, a highly specific and rapidly acting compound, was pushed well beyond amounts needed to abolish end-organ responses to sympathetic nerve stimulation, postganglionic nerve potentials were suppressed [Boura (145)]. Powerful and unusually persistent local anaesthetic actions are shown by xylocholine (12), bretylium (39), BW 172C58 (15), bethanidine (41), and BW 392C60 (unpublished experiments), and even though the assessment of the intradermal effect of guanethidine is complicated by its local irritant action (45), it too appears to have powerful local anaesthetic effects (45, 145). There are other analogies: bretylium, xylocholine, and local anaesthetics have similar effects on the sensitivity of the uterus to stimulants and to calcium in particular [Clegg (205)]; procaine and xylocaine injected intra-arterially affect the responses of the nictitating membranes of cats to sympathetic nerve stimulation in like manner to bretylium [Boura (unpublished observations)]; moreover, sympathetic vasoconstrictor fibres are more sensitive in general to local anaesthetic block than are other fibres [Gaddum (206)].

Burn & Rand (207, 208) suggested that the release of actylcholine at adrenergic nerve terminals is an essential step for the release of norepinephrine and that adrenergic neurone blocking agents act by antagonising this acetylcholine. Findings related to this hypothesis were reviewed by Koelle in 1962 (209) and by Burn in 1963 (210). However, there are many results with adrenergic neurone blocking agents that, in our opinion, seem to deny the suggestion that adrenergic neurone blocking agents act by interfering with an acetylcholine link in adrenergic transmitter release. For example, whereas xylocholine and bretylium depress the "sympathomimetic" action of acetylcholine in isolated heart, this depression contrasts with adrenergic neurone blockade in its lack of persistence [Hukovic (53)]. Similarly, inhibition by bretylium of the nicotinic action of DMPP in the heart of *Tiliqua rugosa* (sleepy lizard) lacks the persistence of adrenergic neurone blockade (de la Lande, Tyler & Pridmore (211)]. One of the main arguments used to support the concept that acetylcholine is involved in adrenergic neurone transmission is that hemicholinium, which is known to

depress both acetylcholine synthesis *in vitro* and cholinergic nerve function in many preparations, also inhibits the smooth muscle responses to stimulation of each of several sympathetic "postganglionic" nerves (209, 210, 212). However, whereas the effects of hemicholinium and adrenergic neurone blocking agents on the response of guinea pig vas deferens to hypogastric nerve stimulation are similar [Bentley (50); Chang & Rand (212)], studies of responses to transmural stimulation of the vas deferens led to the conclusion that the inhibitory action of hemicholinium on the responses to hypogastric nerve stimulation, in contrast to that of adrenergic neurone blockade, was attributable to depression of ganglia within the hypogastric nerve and to depression of smooth muscle contractility [Bentley & Sabine (57)]. All adrenergic neurone blocking agents fully suppress contractions of cat nictitating membranes caused by sympathetic nerve stimulation, whereas hemicholinium does not, either in the isolated preparation [Gardiner & Thompson (213)] or *in situ* [Wilson & Long (214)]. A similar contrast was found in studies of the inhibitory response of rabbit ileum to sympathetic nerve stimulation (50). Other arguments have been advanced against the involvement of acetylcholine in adrenergic nerve transmission (50, 215, 216).

Studies of the storage and release of norepinephrine have made much headway in recent years, and the availability of reserpine and adrenergic neurone blocking agents has added impetus to this research. One of the more convincing discussions of present knowledge is that of Potter & Axelrod (164). Available evidence indicates that the norepinephrine stored in adrenergic nerves is confined to granules of one type. Tyramine and some other indirect acting amines release norepinephrine and this is supposed to come from granules at the perimeter of the fibre terminals. In contrast, amines released by reserpine are found to be mainly deaminated, thus suggesting that they are liberated mainly from granules deep within the fibre, this allowing their metabolism by intracellular monoamine oxidase. All granules seem to release norepinephrine during nerve stimulation (164), and guanethidine also releases a mixture of norepinephrine and products of deamination [Kopin & Gordon (217)]. Many of the findings summarised in this review suggest that bretylium, guanethidine, and other agents first stabilise the granules, perhaps by an action somewhat analogous to their depression of nerve conduction, and at this stage produce adrenergic neurone blockade and counteract the releasing action of reserpine. As the concentration increases, bretylium may cause some displacement of norepinephrine to be manifested as sympathomimesis, but its highly ionised cationic characteristics may limit its penetration of granule membranes, by comparison with guanethidine which causes a marked loss of amines. It is suggested that sympathomimetic effects with different compounds vary with respect to adrenergic neurone blocking potency because of these variations in penetration of granules. Interaction with tyramine and other indirect-acting amines are necessarily complicated because they, like ad-

renergic neurone blocking agents, have the capacity to release norepinephrine, and yet the two pharmacological classes have mutually antagonistic actions. The results summarised on pages 192, 201, and 202 suggest that at low concentration adrenergic neurone blocking agents may synergise with the indirect acting sympathomimetic amines in causing release of norepinephrine but that antagonism is dominant at high concentrations. The situation is complicated further by depletion of tissue catechol amine stores and sensitisation of smooth muscle to released norepinephrine (page 201). Depletion of catechol amine stores cannot be explained entirely by release during the sympathomimesis caused by these drugs but the inhibition of norepinephrine uptake which is a major contributory factor (page 200) may again be due to disturbances of the granule membrane.

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