DRUG ACTION ON PERIPHERAL VASCULAR SYSTEM[1]

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The present subject is a diverse one and includes a large volume of work published in recent years. Two excellent review articles have provided a broader coverage of the field than that intended by the author. Somlyo & Somlyo (1) have very thoroughly analyzed the effects of pharmacologic agents on isolated vascular smooth muscle, and Mellander & Johansson (2) reviewed in detail control of the resistance and capacitance vessels, including effects of endogenous substances and some drugs. In addition, Henning has amply considered the cardiovascular effects of methyldopa (3). The purpose of this review is to pull together the results of work which have not previously been covered and which I feel are of particular interest to those in the field. Specifically, these subjects comprise recent studies of the peripheral sympathetic actions of angiotensin and possible relationships of the sympathetic nervous system to renal hypertension. A very great effort has been made in the past decade to delineate the sympathetic interactions of angiotensin II, and because of their possible role in renal hypertension this subject will comprise a large part of the review. Recent developments concerning the mechanisms of several antihypertensive agents will also be considered, and the actions of clonidine, several compounds related to clonidine, and the adrenergic neuronal blockers will be discussed. An exhaustive consideration of these selected topics is not the intent of the author and unfortunately all pertinent investigations cannot be included.

PERIPHERAL SYMPATHETIC EFFECTS OF ANGIOTENSIN

It has been known for some time that the cardiovascular response to angiotensin is attributable to direct action on vascular smooth muscle as well as to indirectly mediated peripheral (4–6) and central (7, 8) adrenergic effects. In addition, the octapeptide releases catecholamines from the medulla (9) and aldosterone (10, 11) from the cortex of the adrenal gland. Because these effects of angiotensin could lead to an elevation of blood pressure, and because the usual plasma concentration of angiotensin found in renal hypertension is insufficient to increase blood pressure significantly by a

[1] The author's research cited in the review was supported by U.S.P.H.S. Grant HE 08570.

direct vasoconstrictor effect, these indirect actions of angiotensin have important implications.

In recent years many workers have sought to investigate the interactions of angiotensin with the sympathetic nervous system. A variety of studies have been carried out. On one hand the blood pressure or vascular response to angiotensin itself has been studied before and after sympathetic denervation or drug induced adrenergic blockade. In other instances effects of the polypeptide on responses to nerve stimulation, catecholamine release, and uptake have been explored. In a study of the first type, drug-induced interference with sympathetic function reduced responses to angiotensin II. A large dose of angiotensin, 1 μg/kg, given intravenously caused resistance increases in the vascular beds supplied by the superior mesenteric, subclavian, internal carotid, and renal arteries (12). Administration of the adrenergic blocker phenoxybenzamine reduced greatly the increments in resistance in the superior mesenteric and subclavian vascular beds, but not in the renal or internal carotid beds. These results suggested a sympathetic component of action of angiotensin in only certain vascular regions. Geller & Kendrick (13) found that the degree of reduction in renal blood flow produced by angiotensin injected intraarterially or intravenously in dogs was unaffected by acute sympathetic denervation. Disalvo & Fell (14) confirmed the finding of the lack of any dependency of the renal vasoconstrictor action of angiotensin on the sympathetic innervation. In their study, angiotensin in a range of doses (0.1–2 μg) injected intraarterially produced similar decrements in renal blood flow in the innervated and chronically denervated dog kidney and in the kidney of the reserpine treated animal. Because previous work indicated that the magnitude of the vasoconstrictor response of angiotensin in the dog's hindquarters was proportional to the degree of adrenergic tone in that vascular bed (4), the absence of such an effect in the kidney may be related to the known lack of renal sympathetic tone. Although of pharmacologic interest, the sympathetic influence on responses to intraarterial injections of relatively large doses of angiotensin is of doubtful physiologic importance. Low concentrations of the polypeptide, approaching those achieved physiologically, have been found to augment greatly the vasoconstrictor response to sympathetic nerve stimulation in the renal vascular bed (15). This effect of angiotensin, we believe can play a role in normal renal hemodynamics and in the pathophysiology of the kidney.

Depending on the experimental conditions evidence has been presented for and against a sympathetic component to the overall blood pressure response to angiotensin. In the pithed rat, Finch & Leach (16) demonstrated that the secondary phase of a biphasic blood pressure response elicited by intravenous administration of angiotensin (200–500 mμg) was abolished by alpha adrenergic and ganglionic blocking drugs and bethanidine. The authors concluded that since relatively large doses of angiotensin were necessary to produce this sympathetic effect, it is unlikely to be significant. Schumann & Guther (17) reported that in rats the pressor potency of an-

giotensin relative to norepinephrine, which was normally 10, was decreased to between 3 and 5 by prior treatment with doses of methyldopa and reserpine shown to deplete catecholamines. Reserpine treatment for 7 consecutive days was found to decrease more persistently the blood pressure response of conscious rats to angiotensin than to norepinephrine, which initially suggested a sympathetic effect of angiotensin (18). A subsequent study revealed that other agents that blocked the sympathetic nervous system failed to reduce the pressor effect of angiotensin and led the authors to believe that reserpine was acting to depress the response to angiotensin directly (19). In another study in rats, enhancement of the angiotensin pressor response was produced by norepinephrine and other adrenergic amines (20). The explanation for this potentiated response was that there is an interaction between angiotensin and other amines with the alpha receptor, resulting in an augmented contraction of vascular smooth muscle. A progressive blood pressure rise in dogs (21) and rabbits (22) elicited by subpressor doses of angiotensin has also been reported. This very interesting and possibly important response has been attributed to a peripheral and central sympathetic effect. Thus it can be concluded that sympathetic interactions can contribute to the systemic blood pressure response to angiotensin in the dog, rat, and rabbit, but again, when large doses are necessary to demonstrate such an effect, it is of doubtful significance.

The mechanism of sympathetic action of angiotensin has been examined in a number of investigations on catecholamine release and uptake. Kiran & Khairallah studied the release of labeled norepinephrine from rabbit aortae previously exposed to H^3-NE (23). When superfused with angiotensin II amide (1-asparagine-5 valine angiotensin) in a concentration of 1.8 μg/ml an augmented release was noted. Curiously, twice this concentration of angiotensin II (1-aspartic acid-5 valine angiotensin) was necessary to elicit a comparable effect. In aortae from guinea pigs and rats Schumann & Guther found that angiotensin II amide in a lower concentration, 50 mμg/ml, induced contractile responses partially attributable to an indirect catecholamine releasing action (17). Hughes & Roth found that a large concentration, 0.5–2 μg/ml, was needed to augment basal release of a labeled store of catecholamine (24). The same authors, however, were able to demonstrate a short-lived (1–2 min) release of labeled amine from the coeliac artery with lower concentrations (10–100 mμg/ml) of angiotensin, to the author's knowledge the lowest concentration yet shown to liberate norepinephrine directly. A similar catecholamine releasing action of angiotensin in vivo was reported by Peach & Ford (25). An infusion of 0.1 μg/kg/min of the polypeptide increased the plasma catecholamine level and slightly elevated the myocardial content in dogs. The source of released catecholamine was believed to be the adrenergic nerve endings rather than the adrenal gland. Because in general a much higher concentration of angiotensin is required for eliciting the direct release of norepinephrine than that necessary for augmentation of release during adrenergic nerve discharge (vide infra), the former action appears to be relatively unimportant.

Evidence for facilitation of release of norepinephrine by angiotensin during electrical stimulation of the adrenergic nerve supply was obtained in the dog's renal and cutaneous vascular beds (15, 26). The basal level of catecholamine in the venous effluent of these beds was unchanged during intraarterial infusion of angiotensin II (0.1–1 μg/min), whereas the release during stimulation was increased by approximately 50%. In the case of the kidney the calculated concentration of angiotensin in the blood that could evoke this effect was found to be 1.4 mμg/ml (27). It was concluded from these results that angiotensin acted in some manner to facilitate transmitter release, which brings about potentiation of the neurally elicited vasoconstrictor response. Two other groups of investigators have reported a similar facilitating effect of angiotensin on adrenergic transmitter release in other tissues and have further clarified the mechanism of this action. Facilitation of neurally elicited release of labeled norepinephrine but not its metabolites from the rabbit portal vein was evoked by angiotensin in a concentration range of 10–400 mμg/ml of superfusion medium (24). The percent of increase above basal release was nearly doubled at the lower frequencies of stimulation but not at 5 or 10 Hz. Even in the presence of cocaine, 4 μg/ml, which blocked reuptake, the potentiation of release by angiotensin was of a similar magnitude and indicated that the mechanism of this effect was not interference with uptake of catecholamine. Facilitated release was even more prominent in the coeliac artery, in which nerve-stimulated catecholamine efflux was augmented 30–150% by a concentration range of 0.1–1 mμg/ml of angiotensin. Results basically in agreement with the above were presented by Starke et al (28). Angiotensin in even lower concentrations (0.128–1.28 mμg/ml) augmented adrenergic transmitter release from the perfused rabbit heart during nerve stimulation at 5 Hz. Interestingly, the threshold concentration of angiotensin capable of producing this effect was lower at constant flow than at constant pressure perfusion. Furthermore, the inotropic response to nerve stimulation was increased in presence of angiotensin only when constant flow perfusion was employed. Since they found that blockers of uptake did not interfere with the potentiated transmitter release, these workers also concluded that angiotensin exerts an action on the adrenergic neuron unrelated to an effect on the uptake process.

There are conflicting views about whether or not angiotensin is capable of blocking the active transport of catecholamines into the adrenergic neuron. Several workers have obtained evidence for such an action (29–32) and in some cases propose that this accounts for the sympathetic potentiation produced by the polypeptide. This postulate was based partly on the following findings. Palaic & Khairallah demonstrated that the presence of angiotensin in the medium used to perfuse the rat's brain reduced the tissue uptake of H^3-NE and its subsequent washout (29). Release of H^3-NE from the brain elicited by vagal stimulation was increased by a large dose of angiotensin (5 μg/ml) (30). These authors accounted for the results of both these studies by a blocking effect of angiotensin on uptake; however, the augmented release they observed could as easily be ascribed to facilitation

of the process of release. Other evidence for blockade of uptake by angiotensin consists of the results of experiments carried out by Peach et al employing the perfused rabbit heart (32) and by Panisset & Bourdois utilizing the perfused cat mesenteric bed (31). Binding of H^3-NE by these tissues was diminished in presence of angiotensin in the perfusion medium in the concentration of 0.05–0.2 mμg/ml in the former and 100 mμg/ml in the latter study. A number of reports have indicated, however, that uptake of catecholamines by various tissues is not consistently decreased by angiotensin in a wide range of doses or concentrations. In the report of Hughes & Roth (24) previously alluded to, exposure of the rabbit portal vein, rat heart, and coeliac artery to 50 and 500 mμg/ml of angiotensin resulted in no decreased uptake of H^3-NE. There was, in fact, an increased uptake of amine when the portal vein was exposed to the polypeptide for 15 min before addition of H^3-NE. When both angiotensin and H^3-NE were incubated simultaneously, there was a small decrease (24%) in amine accumulation by the portal vein but this was not found in the other tissues that they used. Employing a preparation similar to that previously discussed (32) Schumann et al (33) found no reduction in the amount of unlabeled norepinephrine bound by the rabbit heart or cleared from the perfusion medium in presence of angiotensin in a range of concentrations from 0.01 mμg/ml to 1.3 μg/ml. Only at the enormous concentration of 13 μg/ml was a significant decrease in uptake demonstrated, and this amounted to only 19.5%. In a later study Starke extended these studies to include the effect of angiotensin on C^{14} labeled norepinephrine (34). With 1 and 100 mμg/ml of angiotensin in the perfusion medium there was no significant change in norepinephrine removed from the medium or bound in the heart, although there was a tendency for increased cardiac accumulation with the higher concentration. The largest dose employed, 10 μg/ml, did reduce uptake by about 29%. In our laboratory we too have failed to show a blockade of catecholamine uptake in isolated cutaneous canine blood vessels by angiotensin in the concentration range of 0.01–1 μg/ml (35). We also found the reverse effect, an increase in uptake of labeled norepinephrine in presence of the agent. I would conclude as have others (24, 33) that the augmented adrenergic transmitter release and potentiated end organ response brought about by angiotensin is not attributable to blockade of the reuptake process, but to some other effect of this agent on the adrenergic neuron. The finding of Starke (34) that tyramine-induced release of norepinephrine from the perfused rabbit heart is not enhanced by angiotensin indicates that the facilitatory effect is exerted only on a specific store of catecholamine in the neuron. Although no direct evidence is available to explain the mechanism of this action of angiotensin, the author has felt, and it has been suggested by others (24, and Palaic & Panisset, personal communication), that perhaps an effect of the polypeptide on cell membrane permeability could be involved in the facilitatory action. There have been reports indicating that angiotensin increases sodium and water transport in several tissues (36, 37), and it is conceivable that such an action may be exerted on the membrane of the adrenergic neu-

ron or the adrenergic vesicle. Well documented autonomic actions of angiotensin on the central nervous system, sympathetic ganglia, and adrenal medulla also exist, but are beyond the scope of the present review.

Relationship of Sympathetic Nervous System to Renal Hypertension

There have been a number of investigations performed recently that have dealt with alterations in sympathetic and vascular responsiveness in renal and other types of experimental hypertension. Discussion will be mainly limited to those studies involving renal hypertension. The methods employed to produce renal hypertension differ among these investigations and it should be kept in mind that although a procedure that results in high blood pressure was carried out to compromise the kidney, we may be dealing with hypertensive conditions with different characteristics. For example, there is an important distinction between whether the contralateral kidney is left intact or removed in hypertensive animals with renal artery occlusion, since the ischemic kidney in the former case has the capacity to release more renin than in the latter (38). Addition of a high salt diet or saline injections to animals used in these experiments also decreases the ability of the kidney to release renin. The possibility exists, too, that hypertension due to compression of the kidney with cellophane or by other means may not be identical to that produced by occlusion of the renal artery.

Baum & Shropshire (39) compared vasoconstrictor responses elicited by sympathetic nerve stimulation and exogenously administered norepinephrine in the perfused hindquarters of normal rats and animals made hypertensive by figure-of-eight renal ligation and contralateral nephrectomy. The responses to nerve stimulation were significantly greater in the hypertensive group only at the two week interval postoperatively (acute phase of hypertension) and were not different during later intervals of 6 and 12 weeks. The responses to norepinephrine on the other hand were significantly greater in the hypertensives only at the 6 and 12 week intervals, which probably represent intervals during the chronic phase of the hypertension. Finch (40) examined blood pressure responses to pithed normotensive and hypertensive rats and vasoconstrictor responses of mesenteric vessels taken from these animals, at a single interval postoperatively (at 8 weeks or later). Hypertension was induced in one group of animals by renal artery occlusion accompanied by unilateral nephrectomy, and in a second group by DOCA and salt administration. The systemic pressor responses to norepinephrine, tyramine, and DMPP were greater than normal in both groups of hypertensive rats whereas the pressor response to stimulation of the total sympathetic outflow was larger only in the DOCA hypertensive rats. Increased reactivity of the mesenteric vessels to norepinephrine and sympathetic nerve stimulation, however, was obtained in both groups of hypertensives. In the renal hypertensives, responses to sympathetic stimulation at frequencies greater than 12 Hz were potentiated, whereas responses to stimulation at 6 Hz were not. Haeusler & Haefely (41) found greater responsiveness to mesenteric vessels of renal hypertensive rats to norepinephrine

but not to nerve stimulation (25 Hz) at 6–8 weeks postoperatively. Their rats were made hypertensive by renal compression and salt administration. In contrast, they found that in genetic hypertensive rats responses of these vessels to norepinephrine and nerve stimulation, as well as to KCl, were all greater than in normotensives. It would appear from these studies that the mesenteric and hindquarter vessels of renal hypertensive rats become hyperresponsive to norepinephrine and other vasoactive substances, especially during the chronic phase of the process, and that this may occur in other types of experimental hypertension as well. Whether this is attributable solely to hypertrophy of the medial layer of the resistance vessels which is believed to occur in spontaneously hypertensive rats (42) is not yet known. No clear-cut evidence in rats of increased responsiveness of the sympathetic nervous system, i.e. either increased transmitter release or higher discharge rate of sympathetic fibers, which could be attributable to an effect of angiotensin, has been presented. Henning, however, interpreted his results of a lower than normal norepinephrine content in the heart and femoral muscle of renal hypertensives treated with alpha-methyl-paratyrosine, a blocker of norepinephrine synthesis, as being indicative of greater impulse traffic in the sympathetic nerves of hypertensive animals (43).

Results of several studies in dogs are also of interest in regard to sympathetic effects in renal hypertension. Zimmerman et al found that in dogs made hypertensive by unilateral or bilateral renal artery occlusion the vasoconstrictor response to high frequency (20 Hz) sympathetic nerve stimulation and to norepinephrine were greater than in normotensive animals in the cutaneous but not muscle vascular bed (44). The pattern of transmitter release in the cutaneous bed as reflected by norpinephrine liberated into the venous effluent during nerve stimulation was also altered in the hypertensive animals. Peak release during sympathetic stimulation was greater and occurred sooner in the hypertensive than in the normotensive animal. These results indicate hyperreactivity of the blood vessels and suggest increased responsiveness of the adrenergic innervation in selected vascular regions in renal hypertensive animals. It is conceivable that angiotensin may have triggered these peripheral effects. Brody et al analyzed the difference between reflex vasoconstrictor and vasodilator responses as well as the vasoconstrictor responses to nerve stimulation in the hind limb of normotensive and hypertensive dogs (45). They found that the responses to sympathetic nerve stimulation at 5, 10, and 20 Hz but not to norepinephrine were greater in the hypertensive than in the normotensive animal. The difference between the responses in these two groups was even larger than that obtained in the above study. It is interesting that the pattern of potentiation of responses to nerve stimulation and not to norepinephrine more typifies that found during angiotensin administration (6, 15, 26), yet in this study the hypertension produced by renal artery occlusion and contralateral nephrectomy probably results in lower renin levels than renal artery occlusion alone. They also found no difference between the two groups of animals in the sustained fall in hind limb vascular resistance following acute sympathetic denervation,

which indicated no greater resting adrenergic tone in the hypertensives. The ability of the hypertensive animal to undergo reflex vasodilatation of the hind limb was impaired; a finding which has also been made in the rat (46). Moerman et al also found greater vasoconstrictor responses to nerve stimulation at 6 Hz in the perfused spleen of hypertensive dogs than those obtained in the normotensive's spleen (47). Their results indicated, however, that greater vessel reactivity rather than augmented transmitter release was the factor (48) responsible for this difference. The results of these studies in the canine species suggest that stimuli that would evoke an intense sympathetic discharge (5 Hz or greater) could produce in the renal hypertensive animal a greater than normal increase in blood pressure and one that would be less counteracted by baroreceptor reflexes than in normals.

The influence of the sympathetic nervous system on the development of renal hypertension in rats has also been examined. Rats that had been immunosympathectomized (49–51), or sympathectomized with 6-OH dopamine (50), did not differ from normals in becoming hypertensive following renal artery occlusion (with or without contralateral nephrectomy). The chronic phase of the hypertension of the immunosympathectomized rats, however, differed from that of the intact animals. In the studies of Dorr & Brody (49) and Ayitey-Smith & Varma (51) the blood pressure of the sympathectomized but not the innervated rats had returned to a normotensive level after 28 and 50 days, respectively, following the operative procedure. Presence of the sympathetic innervation, therefore, appears necessary for the maintenance of renal hypertension in rats.

Immunization Against Angiotensin II

The possible participation of the renin-angiotensin system in the production of renal hypertension has had a long and stormy history and will not be considered in this review. Page & McCubbin have amply covered this and other important aspects of renal hypertension in their book on the subject (52). With procedures now available for immunizing animals (either actively or passively) against angiotensin II, a means of antagonizing vascular effects of the polypeptide exists and a study of the role of angiotensin in renal hypertension can now be more readily accomplished. This technique was made use of by a number of investigators. Aars & Eide (53), Johnston et al (54), and Macdonald et al (55) all found that rabbits with effective levels of angiotensin II antibodies developed renal hypertension at the same rate and to the same degree as untreated rabbits. These animals did not exhibit significant pressor responses to injected renin and angiotensin in normally effective doses. In the latter two studies (54, 55) it was also demonstrated that active immunization of rabbits with established hypertension did not lower their blood pressure. Johnston et al (54) also compared the renin levels in immunized and nonimmunized hypertensive rabbits to determine if there was hyperproduction of renin in the immunized animals as compensation to the blockade of the effects of angiotensin. They found no significant difference. Hedwall (56) injected anti-angiotensin plasma (that

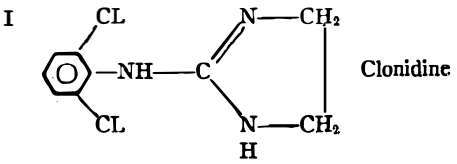
taken from angiotensin II immunized rabbits), which had an inhibitory effect on responses to angiotensin, into renal hypertensive rats and obtained no greater depressor effect than that observed with normal plasma. The results of these studies suggest that the renin-angiotensin system may not play a direct role in the etiology of renal hypertension. Further work by Aars, Eide & Akre revealed that in rabbits immunized against angiotensin II no increase in renal sympathetic nerve activity was elicited by angiotensin as had normally been found in untreated rabbits (57). This renal sympathetic effect of angiotensin, presumably central in origin, was abolished in the immunized animals, and therefore led the authors to conclude that sympathetic effects of angiotensin that could conceivably contribute to renal hypertension should also be abolished by immunization. It will have to be determined whether other adrenergic interactions brought about by angiotensin are also amenable to blockade by the immunizing procedure, since it is conceivable that sites with great affinity for the angiotensin molecule may still be effected even when antibodies are present.

Other evidence has been provided which is at variance with the work cited above. Christlieb et al reported that active immunization against angiotensin in renal hypertensive rats did result in lowering of the arterial pressure (58). In rats made hypertensive by unilateral renal artery occlusion alone or including contralateral nephrectomy there was a sustained hypotensive response of 30 mm Hg or more when the antibody titers achieved a certain level. No depressor effect was found in mock immunized hypertensives. Work by Worcel et al (59) and Bing & Poulsen (60) employing the injection of antiangiotensin II rabbit plasma into normal (59, 60) and renal hypertensive rats (60) did reveal a *transient* blood pressure lowering effect lasting about 5–30 min. This depressor effect of anti-angiotensin II is indicative of a specific antagonism of the effect of endogenous angiotensin for the following reasons: (*a*) injection of a similar volume of plasma from normal rabbits or from those with a low antibody titer had no consistent effect on blood pressure, (*b*) injection of antiangiotensin into nephrectomized rats when renin levels were negligible produced no effect, and (*c*) a second injection produced no effect on blood pressure unless more renin or angiotensin was given prior to its administration. Based on the results of these two studies it appears that angiotensin is involved in the maintenance of normal and elevated blood pressure due to renal artery occlusion, but after blocking its effect compensatory mechanisms, e.g. baroreceptor resetting, come into play to restore the initial pressure. It is intriguing to consider that a similar event occurred in the studies previously alluded to in which no influence of active or passive immunization was detected on the renal hypertensive's blood pressure.

More work must be done to determine how the renin-angiotensin system is involved in initiating hypertension of renal origin and at what stage influence of the sympathetic nervous system comes into play. As suggested by the results of the experiments involving angiotensin II immunization, possibly other factors besides angiotensin II are involved or can become

involved in this process more rapidly than had previously been envisioned. The possibility that angiotensin I is itself partially responsible for initiating renal hypertension or can take over this role when the action of angiotensin II is interfered with should be more seriously considered.

Cardiovascular Effects of Clonidine



The discovery of the potent hypotensive action of clonidine (2—[2,6-chlorophenylamino]2-imidazoline hydrochloride) in animals by Hoefke & Kobinger (61) represents a unique contribution because this is the first among a new class of compounds that lower blood pressure probably entirely by a central mechanism. Numerous publications have appeared within the past five years describing the cardiovascular pharmacology of this agent. Generally, the cardiovascular effects brought about by administration of clonidine in μg/kg doses are: (*a*) A transient elevation in systemic arterial pressure followed by (*b*) a prolonged period of hypotension which is accompanied by (*c*) cardiac slowing and a decrease in cardiac output.

In the various species studied (rats, cats, dogs, and monkeys) intravenous injection of clonidine produced a rise in systemic arterial blood pressure lasting for only several minutes (61–64). The magnitude and duration of this response varies depending on the dose and species employed, and both the pressor and depressor effects appear to be shorter-lasting in the unanesthetized animal (65). A direct vasoconstrictor action of the compound that appears to be related to alpha receptor stimulation (62, 66) accounts for the transient hypertension. Constantine & McShane (62) have found, in addition, that clonidine is more potent than epinephrine in providing alpha receptor protection in the rabbit aortic strip, and it produces vasoconstriction in the dog's forelimb in a dose as low as 0.25 μg.

The reduction in blood pressure that ensues after the pressor effect lasts for an hour or so depending on the dose employed. Because the hypotensive action was lacking in pithed (63) or spinal transected animals (67) or in animals treated with adrenergic blocking agents (62) it was apparent that clonidine interfered with sympathetic tone. Differences appear to exist in the degree to which sympathetic tone is interfered with, depending on the region studied. In an investigation performed on unanesthetized rabbits changes in cardiac output, hind limb, and skin blood flow were monitored before and after clonidine administration (20 μg/kg followed by infusion of 0.5 μg/kg/min) (65). Muscle blood flow was taken as the difference between hind limb and skin flow. At the time of the hypotensive effect of the drug, cutaneous and muscle vascular resistances were decreased, but the latter rose 27% during the last half hour of giving clonidine. Heart rate and

cardiac output were also reduced by the drug and tended to return to control even though the blood pressure was still depressed. Since muscle vascular resistance, heart rate, and cardiac output remained depressed by clonidine in debuffered animals these restorative effects were due to compensatory reflexes overriding the effect of clonidine on the heart and skeletal muscle vessels, but not in the skin.

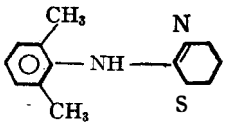
A wide variety of studies have clearly indicated that the sympathetic blocking effect of clonidine is exerted primarily by a central action. The central site of action was originally suggested by experiments done by Kobinger (68) in which the intra-cisternal injection of small doses of clonidine lowered blood pressure in vagotomized cats. Later, Constantine & McShane found that intravertebral arterial injection of 16 μg of clonidine decreased blood pressure and vascular resistance of the perfused forelimb in the dog (62). When given intravenously this dose had no significant effect. H. Schmitt & Mme. H. Schmitt (67) localized the effect of clonidine in the medullary centers by transection experiments. After an intercollicular or subpontine transection, clonidine was still capable of exerting its typical effects on blood pressure, heart rate, and discharge of the splanchnic sympathetic nerve. Sectioning below the medulla, however, abolished all these actions. Experiments in which the effect of clonidine has been examined on nerve recording most clearly demonstrate its central sympathetic blockade. Both pre- and post-ganglionic sympathetic nerve activity were recorded before and after administration of the drug (69). Spontaneous activity in the splanchnic and inferior cardiac nerves was consistently diminished during the clonidine (30 μg/kg) induced hypotension in dogs and cats. Because pre- as well as post-ganglionic discharges were reduced, a central rather than ganglionic blocking effect was indicated. Bolme & Fuxe (70) presented indirect evidence suggesting that the central sympathetic blocking effect of clonidine is accounted for by the stimulation of central adrenergic receptors. Haloperidol, 1 mg/kg, and phenoxybenzamine, 5–10 mg/kg, both blocked the hypotensive action of clonidine in cats but did not attenuate the initial pressor effect. Because in the dose employed, haloperidol is considered to block central adrenergic receptors they felt that both agents were blocking the central site of action of clonidine. This conclusion is, however, at variance with results reported earlier by H. Schmitt & Mme. H. Schmitt (71), but was supported by a later report from the same laboratory (72). In the first study they found that in cats or dogs given either phentolamine or phenoxybenzamine, there was no impairment in the ability of clonidine to diminish the splanchnic nerve discharge whereas in their later work intravenously or intracisternal administration of piperoxan, another alpha receptor blocking agent, antagonized the cardiovascular effects of clonidine and its depressant action on splanchnic nerve discharges. They concluded that clonidine stimulates alpha receptors in the medulla as well as in the periphery and postulate that the vasomotor center in the medulla represents a noradrenergic synapse and also the third neurone in the chain of three cells beginning with the stretch receptor in the baroreceptor regions. If their hy-

pothesis proves correct, that the action of clonidine simulates the response due to a prolonged stimulation of the baroreceptors, this would indeed prove interesting.

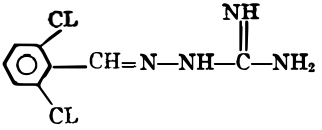
Some degree of peripheral adrenergic neuronal blockade may contribute to the sympathetic blocking action of clonidine. Heart rate responses to low, but not high frequency nerve stimulation were reduced after administration of clonidine (68, 73).

As indicated above, cardiac slowing and a reduction in cardiac output also accompany the fall in blood pressure caused by clonidine. There has been some dispute as to the mechanisms responsible for the bradycardia; however, it is probable that several actions of the drug account for this effect. Schmitt et al, in the study alluded to previously (69), found a marked decrease in the sympathetic discharge registered from the inferior cardiac nerve, which points to a decrease in cardiac sympathetic tone. This, as well as a sensitizing effect to the baroreceptor reflex (74, 75), probably accounts for the cardiac actions of clonidine.

Effects of Drugs Related to Clonidine

II  Bayer 1470

Schmitt et al studied the pharmacological actions of Bayer 1470, 2(2,6-dimethylphenylamino)4-H,5,6 dihydro-1,3-thiazin, an agent structurally similar to clonidine, and found its effects to resemble those of clonidine (76). In doses (0.25–1 mg/kg) somewhat larger than the usual dose of clonidine, it caused the typical biphasic effect on blood pressure in dogs, and the hypotensive period was also accompanied by cardiac slowing. The bradycardia was very marked in some animals, the rate decreasing to as low as 30–40 beats/min. The spontaneous sympathetic discharge recorded from the splanchnic and inferior cardiac nerves was depressed after administration of this compound in dogs. The effect on cardiac nerve activity appeared to be greater and to last longer than that on the splanchnic nerve. Evidence of some peripheral sympathetic blocking activity was also obtained, in that the pressor responses to low intensity and low frequency stimulation of the entire sympathetic outflow in rats were reduced after administration of Bayer 1470.

III  Wy-8678

Baum and co-workers reported on the cardiovascular effects of another similar compound known as Wy-8678 (2,6-dichlorobenzylidene aminoguanidine) (77). Systemic arterial blood pressure of anesthetized dogs was ini-

tially elevated and then fell 5–10 min later after 0.01–0.1 mg/kg. The highest dose employed, 1 mg/kg, produced a larger blood pressure increase but hypotension subsequently in only half of the animals. The initial pressor response produced by this compound was not antagonized by alpha receptor blocking agents as that of clonidine, although the direct vasoconstrictor response in the dog's perfused hindquarter was reduced following administration of an alpha blocker. Evidence of adrenergic neuronal blockade produced by this agent was obtained with doses of 32 μg/kg and larger. Responses to low frequency sympathetic stimulation were more profoundly affected than responses to higher frequencies, which is similar to the results obtained with clonidine. Baum & Shropshire concluded that Wy-8678 also acted centrally to reduce sympathetic tone based on results of a study in which nerve activity was recorded from cats' inferior cardiac and external carotid nerves (78). Doses of from 0.032–320 μg/kg reduced spontaneous activity, with the blockade increasing progressively with the higher doses. Preganglionic nerve discharge recorded from the cervical sympathetic trunk was found to be similarly reduced, indicating that the effect was a central one and not due to ganglionic blockade.

Adrenergic Neuronal Blocking Agents

Results of several recent investigations suggest a number of different mechanisms of action of adrenergic neuronal blockers. A technique for eliciting antidromic firing of postganglionic sympathetic nerves in the perfused cat heart and spleen by injection of acetylcholine and KCl was employed by Hauseler et al to study the effect of bretylium on the adrenergic nerve endings (79). Bretylium in concentrations of 10^{-6} to 10^{-5} M in the heart perfusate produced a parallel reduction in antidromic firing of the inferior cardiac nerve produced by KCl and in the positive inotropic and chronotropic response to electrical stimulation of the cardiac nerves. The blocking action of bretylium on antidromic firing increased with time, suggesting accumulation in the nerve terminals, and was attributed to a local anesthetic action at that site. It was estimated that bretylium was concentrated approximately 1000–3000 fold in the adrenergic nerve endings based on the relative quantities of bretylium necessary to block antidromic adrenergic discharges and the firing of the sensory fibers in the perfused carotid sinus. Blockade of transmitter release by bretylium would, therefore, appear to be related to a membrane stabilizing action (local anesthesia) on the adrenergic nerve endings. Although bretylium is concentrated in the adrenergic neuron, it is not believed to be bound in the vesicles (80), but appears to act elsewhere in the neuron. Interestingly, it has been reported by Krauss et al that bretylium actually enhances the release of false transmitters e.g., octopamine, alpha-methyloctopamine, and metaraminol (but not catecholamines) from adrenergic nerves in the rat heart (81). The authors believe that this effect may reflect an interaction of the blocker with calcium at the neuronal membrane. It may, however, merely represent a common storage site for bretylium and false transmitters. The results of a study by Abbs & Robertson

led to the conclusion that a selective norepinephrine depleting effect was responsible for the blocking action of bretylium (82). In cats treated with 10 mg/kg of bretylium a substantial decrease (50–75%) was achieved in the norepinephrine content of the supernatant fraction of splenic homogenates. A lesser but significant decrease in the total norepinephrine content of the homogenate was also obtained, which has not been consistently demonstrated in other tissues by previous workers. Because administration of amphetamine prevented or reversed the adrenergic neuronal blockade, the store depleted by bretylium was considered essential for normal functioning of the adrenergic neuron.

Two recent reports have suggested that β-TM 10 possesses the property of inhibiting the enzyme monoamine oxidase (83, 84). Suggestion of a monoamine oxidase inhibitory effect of debrisoquin also came from a study by Medina et al involving its disposition in the rat (85). Uptake of m-octopamine by rat heart slices was enhanced by 1–2 $\times$ 10^{-6}M debrisoquin, and the effect was abolished by preincubation with the monoamine oxidase inhibitor, pheniprazine. Increased uptake was presumably related to decreased metabolism by monoamine oxidase. Since in the same series of experiments the rat's pressor response to physostigmine (thought to be indicative of a central mediated sympathetic response) was diminished at 1 but not 1.5 hr after debrisoquin even though its depressor effect is long-lasting, it was suggested that the hypotensive effect of debrisoquin was not due to adrenergic neuronal blockade. If in fact, monoamine oxidase inhibition does account for, or contribute to the hypotensive action of this agent, it is puzzling why sympathetic function would not be interfered with, during the period of hypotension.

Kroneberg et al reported on the adrenergic neuronal blocking effect of guanacline (N—[2-guanidinoethyl]—4 methyl-1,2,3,6-tetrahydropyridine) a compound having a chemical configuration resembling guanethidine (86). Because this agent when administered to hypertensive patients produced postural hypotension that appeared to persist some months after termination of treatment (87), Burnstock et al considered the possibility that the drug or its metabolites might damage the adrenergic nerves (88). They compared in rats the effects of guanacline and guanethidine given once a day (5 mg/kg) for 18 weeks on systolic blood pressure, norepinephrine levels in the heart and vas deferens, and microscopic appearance of the sympathetic ganglion cells. The systolic blood pressure and norepinephrine levels were decreased to a similar extent during treatment with these agents. Following cessation of drug administration, the catecholamine levels were restored to approximately the same degree within 5 weeks. Blood pressure appeared to recover to the control level more quickly after guanethidine than after guanacline. An accumulation of a lipoprotein material was observed in the sympathetic ganglion cells 4–18 days after treatment with guanacline and persisted for 12 weeks or more. This was not found in the guanethidine treated animals. Since guanacline was itself reported previously to accumulate in the ganglion cells (87), it may bring about destruction at this site which results in deposition of this material.

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