Studies on the Mechanism of the Angiotensin Pressor Effect and of its Inhibition by Neotetrazolium[1] (36352)

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Ditetrazolium salts exhibit antihypertensive effects on rats with experimental renal hypertension (1, 2). Exploration of various possible mechanisms of action revealed that neotetrazolium impairs sympathetic ganglionic transmission (3) and causes blockade of alpha-adrenergic receptors (4). Preliminary investigations regarding possible influence on the renin–angiotensin system have shown that neotetrazolium also reduces the pressor response to angiotensin (4).

Growing evidence suggests that the mechanism of the vasoconstrictor effect of angiotensin is bimodal, involving a direct myotropic action as well as an indirect effect implicating adrenergic components (5–10). In view of the possibility that norepinephrine mediated vasoconstriction may contribute to the overall pressor response to angiotensin, it was hypothesized that inhibition of angiotensin by neotetrazolium might be due to the adrenolytic effect of the latter. Interference with the direct myotropic action of angiotensin was considered a possible alternative or additional mechanism (11).

It is apparent that exploration of these possibilities and a meaningful interpretation of the results requires adequate knowledge of the mechanism underlying the pressor response to angiotensin.

The direct effect of angiotensin on vascular smooth muscle is generally accepted. There is considerable doubt and controversy, however, regarding the site(s) and relative significance of the indirect pressor activity (5, 12). The spectrum of the reported indirect vasoconstrictor effects of angiotensin includes central stimulation of the sympathetic nervous system (13–16), release of norepinephrine from nerve endings (17, 18), potentiation of responses to agents or procedures which release endogenous norepinephrine (19–21), enhancement of responses to exogenous norepinephrine (22, 23), release of catecholamines from the adrenals (24–27) and inhibition of re-uptake of norepinephrine (28) (Fig. 1). Participation of adrenergic components in the mechanism of the overall pressor response to angiotensin is suggested by the observed reduction of this response by ganglionic blockade (10), presynaptic adrenergic blockade (9, 29), sympathectomy (8), and blockade of alpha-adrenergic receptors (7, 13, 29, 30). Other authors, however, question any major contribution of the sympathetic nervous system to the vascular response to angiotensin and present data contradicting some of the above-mentioned findings. It is claimed, for example, that the vasoconstrictor effect of angiotensin is not influenced by ganglionic blockade, denervation, depletion of norepinephrine (31), cocaine (32), presynaptic adrenergic blockade (33), or alpha-adrenergic blockade (12, 34, 35). Many of these apparently conflicting results, however, reflect differences in species, dose and other experimental conditions.

Considering these variations and in view of the relatively meager literature on the mechanism of the vasopressor effect of angiotensin in the rat, it seemed necessary first to explore the mode of action of angiotensin under our experimental conditions and then determine the mechansim of the inhibitory effect of neotetrazolium.

Materials and Methods. CFN female rats weighing approximately 250 g and main-

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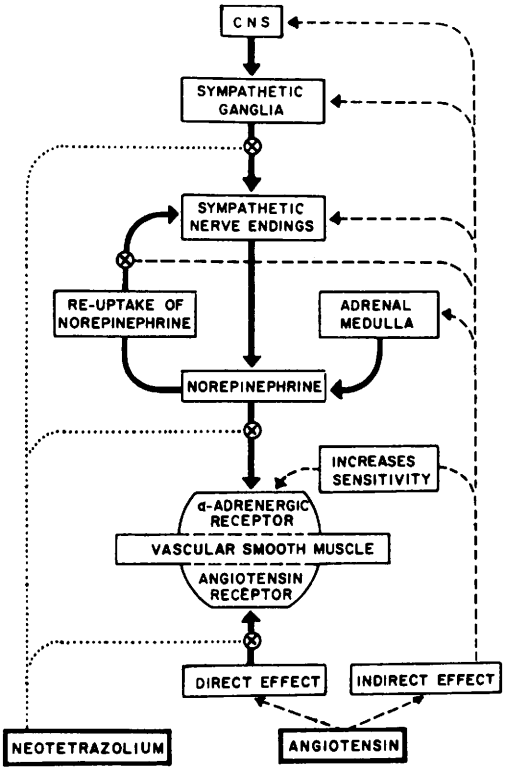

FIG. 1. The vasoconstrictor effect of angiotensin and its inhibition by neotetrazolium. Schema of the mechanisms involved. Dashed lines: indirect effects of angiotensin (based on ref. 13–28). Dotted lines: neotetrazolium effects. Encircled ×: blocking action.

tained on Wayne Lab-Blox and water *ad libitum* were used in these studies. All animals were vagotomized and treated with pentolinium (4 mg/kg) prior to each experiment.

Mean carotid blood pressures were measured directly under Amytal-Na anesthesia (100 mg/kg body wt) by means of a Statham transducer coupled to a Grass polygraph. The average of at least two blood pressure responses was computed for each dose of a pressor substance administered to an animal. Angiotensin dose-response curves for a group of animals were made by plotting the mean of the responses of the individual animals of that group. Repeated dose-response curves in control animals have shown that there is no diminution of the responses to angiotensin with time.

All test substances, unless otherwise indicated, were injected into the right external jugular vein (in a volume of 0.1 ml) by means of a cannula and flushed with 0.15 ml of heparinized normal saline (20 units/ml).

Alpha-adrenergic blockade. Alpha-adrenergic blockade was instituted by an intravenous (4 mg/kg) and a subcutaneous (6 mg/kg) injection of phentolamine. The blockade was maintained, when necessary, by additional (2 mg/kg) subcutaneous injections of phentolamine. Absence or reversal of the pressor response to 4 μg/kg of norepinephrine indicated effective alpha-adrenergic blockade. It should be noted that the 4 μg/kg dose of norepinephrine elicited pressor responses of greater magnitude than those produced by the highest angiotensin dose used in these experiments. The above treatment with phentolamine did not alter the base line blood pressure.

Norepinephrine depletion and adrenalectomy. Norepinephrine depletion was accom-

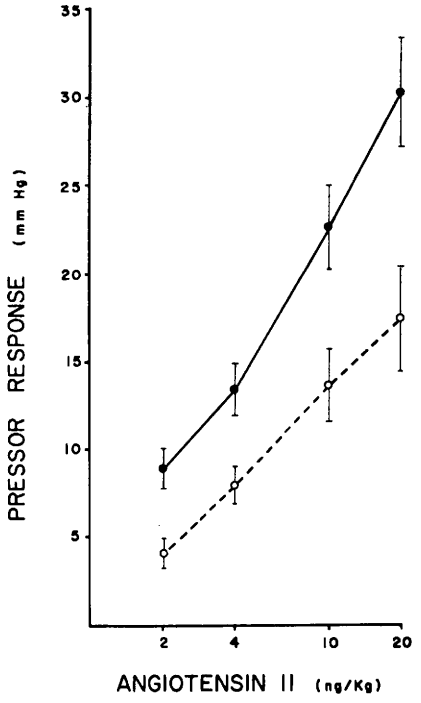

FIG. 2. Inhibition of pressor response to angiotensin II by phentolamine. Angiotensin dose-response curves before (closed circles) and after alpha-adrenergic blockade (open circles). Means ± SEM are shown.

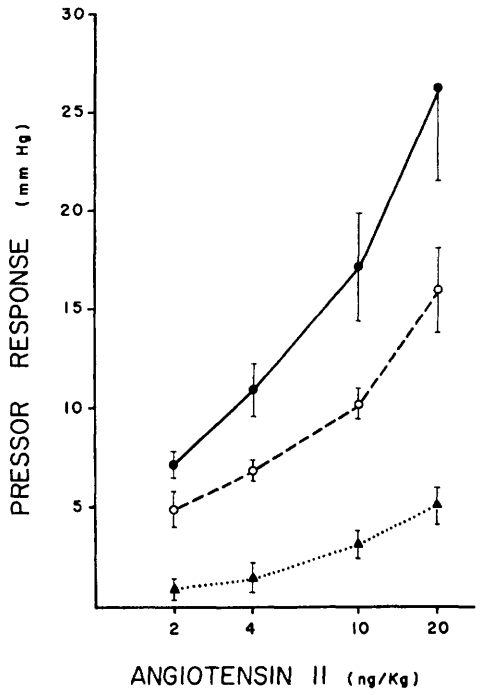

FIG. 3. Inhibition of pressor responses to angiotensin II by neotetrazolium. Angiotensin dose-response curves before (closed cirlces) and after 1 mg/kg (open circles) and 2 mg/kg (closed triangles) of neotetrazolium. Means ± SEM are shown.

plished by intraperitoneal injection of 5 mg/kg reserpine administered 20–24 hr prior to the experiment. These norepinephrine-depleted animals were also bilaterally adrenalectomized 2 hr before injection of the test substances. Lack of response to tyramine (100 μg/kg) was used as evidence of satisfactory norepinephrine depletion.

The substances used were: Phentolamine (Regitine mesylate, Ciba), reserpine (serpivite, The Vitarine Co.), 3,3′(4,4′biphenylene)-bis(2,5 diphenyl tetrazolium chloride) (Neotetrazolium chloride, Nutritional Biochemicals Corp.), Val 5-angiotensin II (Angiotensin Research Standard A, Division of Biological Standards, National Institute for Medical Research, London), Ileu 5-angiotensin II (Schwarz Bio-Research), tyramine-HCl (K & K Laboratories, Inc.), norepinephrine bitartrate (Levophed Winthrop), vasopressin (Pitressin, Parke, Davis & Co.), pentolinium (Ansolysen, Wyeth Laboratories, Inc.).

Results. Influence of alpha-adrenergic blockade on responses to angiotensin. Pressor responses to 2–20 ng/kg angiotensin obtained in eight animals before and after phentolamine administration revealed that alpha-adrenergic blockade results in a shift of the angiotensin dose response curve to the right (Fig. 2). Phentolamine did not alter the baseline blood pressure. The decrease of the mean response to the various doses of angiotensin ranged between 40–53%. The ratio of the angiotensin doses which produced equal responses before and after phentolamine administration was 1:2 or smaller. Production of a pressor response of 13.3 mm Hg, for example, required an angiotensin dose of 4 ng/kg before phentolamine and 10 ng/kg after phentolamine administration. The inhibitory effect of alpha-adrenergic blockade on the pressor responses to angiotensin is statistically significant at high levels of confidence ($p < .001$, t test for paired observations).

Effect of neotetrazolium on responses to angiotensin and vasopressin in control rats. A dose response curve to angiotensin (2–20 ng/kg) was obtained in five control rats before and after administration of 1 and 2 mg/kg neotetrazolium. Figure 3 shows that neotetrazolium resulted in a shift of the angiotensin dose-response curve to the right. The degree of inhibition was directly related to the dose of tetrazolium ($p < .02$) and was significant with both doses of the compound ($p < .05$). The high neotetrazolium dose produced 87% reduction of the mean response to 2 ng/kg angiotensin. In two animals the response was completely abolished. The effect of neotetrazolium lasted for 2 to 3 hr.

In another group of eleven rats neotetrazolium (2 mg/kg) did not influence responses to 0.001–0.04 units/kg vasopressin, which were equivalent to those produced by 2–20 ng/kg angiotensin. Three animals of this group received both vasopressin and angiotensin in random order before and after neotetrazolium administration. Responses to vasopressin remained unaltered while those to angiotensin were markedly inhibited.

Effect of neotetrazolium on angiotensin responses in rats with alpha-adrenergic blockade. Dose response curves to angiotensin

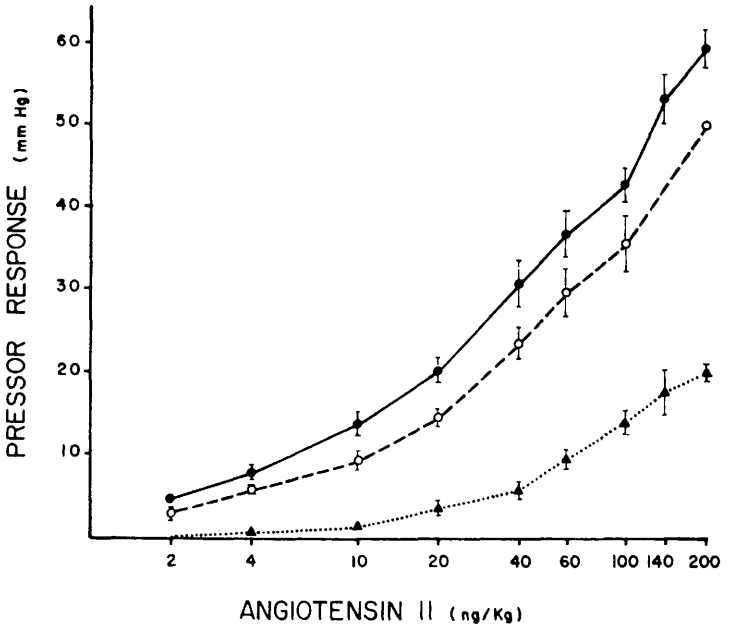

FIG. 4. Inhibition of pressor response to angiotensin II by neotetrazolium in alpha-blocked animals. Angiotensin dose-response curves before (closed circles) and after 1 mg/kg (open circles) and 2 mg/kg (closed triangles) of neotetrazolium. Means ± SEM are shown.

(2–200 ng+kg) were obtained before and after administration of 1 and 2 mg/kg neotetrazolium in ten rats with alpha-adrenergic blockade. Both neotetrazolium doses produced a significant shift of the angiotensin dose-response curves to the right (Fig. 4). The degree of inhibition was directly related to the dose of neotetrazolium ($p < .001$). With high neotetrazolium dose the suppression of responses to angiotensin was striking and highly significant ($p < .001$). The dose of angiotensin had to be increased at least tenfold in order to produce the same magnitude of responses as before neotetrazolium administration. The type of inhibition seemed to be competitive since the inhibitory effect was surmountable and on reciprocal plotting the dose-response curves had the same intercept (Fig. 5).

Effect of neotetrazolium on angiotensin responses in adrenalectomized and norepinephrine depleted rats. Responses to angiotensin (2–20 ng/kg) were obtained in five norepinephrine-depleted and adrenalectomized rats before and after neotetrazolium (1 and 2 mg/kg) administration. Neotetrazolium exhibited a significant ($p < .001$) dose related inhibitory effect of approximately the same magnitude as that observed in animals with alpha-adrenergic blockade.

Discussion. The first aim of this investigation was to explore the mechanism of the pressor response to angiotensin in the rat, at least as far as it would have relevance to the study of the inhibitory effect of neotetrazolium. More specifically, we were interested to determine whether sympathetically mediated vasoconstriction contributes significantly to the overall pressor response to angiotensin. Our approach to the problem assumed that if adrenergic components are implicated, then alpha-adrenergic blockade should modify the pressor effect of angiotensin regardless of which particular adrenergic component is involved.

The results demonstrate that blood pressure responses to angiotensin are significantly reduced following adrenergic blockade by phentolamine. Blood pressure changes as a factor influencing responses to angiotensin can be ruled out since phentolamine did not alter the baseline blood pressure. Inhibition

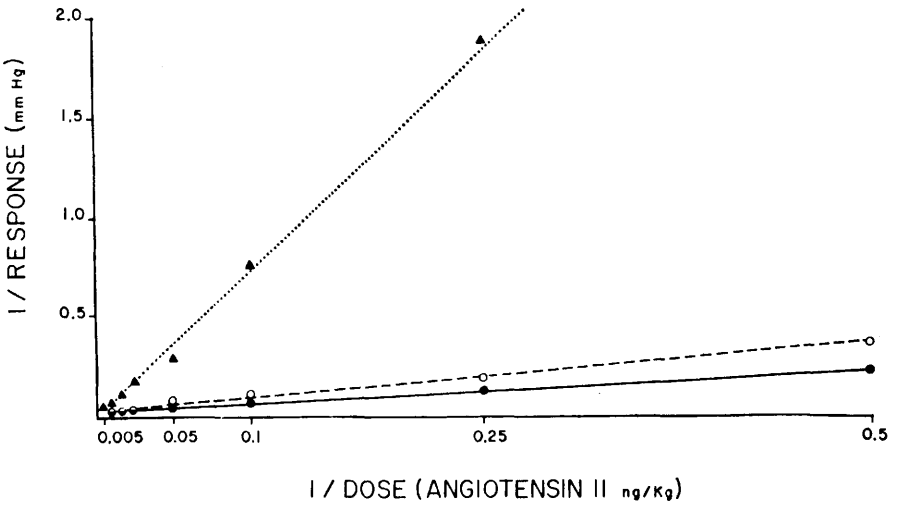

FIG. 5. Reciprocal plotting of the data in Fig. 4. Common intercept of the angiotensin dose-response curves suggesting competitive inhibition.

of angiotensin effects by adrenolytic agents has already been reported by several investigators, both in intact animals (7, 13, 29), and in isolated vessels (30). The influence of adrenergic blockade observed in our experiments strongly suggests adrenergic participation in the mechanism of the overall response to angiotensin. The same conclusion was reached in similar studies on dogs (13), cats (7, 10), and man (29). Regarding the rat, it has been reported that the vasoconstrictor action of angiotensin is direct, unaffected by sympathetic blockade or by local denervation (31). These findings, however, do not necessarily exclude adrenergic involvement since the treatment used as not sufficient to block all possible pathways through which angiotensin could produce norepinephrine mediated vasoconstriction. To our knowledge there are no studies reported in which adrenolytic agents were used to explore the mechanism of the pressor effect of angiotensin in the rat.

Although the present experiments do not indicate the precise site(s) of the indirect action of angiotensin, they do suggest that the norepinephrine mediated vasoconstriction probably involves a mechanism other than or in addition to centrally induced sympathetic discharge. This conclusion is based on the fact that in our animals ganglionic transmission was blocked and any possible central sympathetic stimulation could not have significantly contributed to the pressor effect of the polypeptide.

The other aim of this study was to determine the mechanism involved in the inhibition of angiotensin by neotetrazolium. In view of the herein reported observation that blockade of alpha-adrenergic receptors by phentolamine reduces the pressor response to angiotensin and considering the fact that neotetrazolium is an alpha blocker, it seemed reasonable that alpha-adrenergic blockade is responsible, at least in part, for the inhibitory effect of neotetrazolium. The entire inhibitory effect, however, could not be attributed to adrenergic blockade since the suppression of responses to angiotensin by neotetrazolium was of greater magnitude than that produced by doses of phentolamine which effectively blocked alpha-adrenergic receptors. In fact, complete inhibition of responses to angiotensin, which neotetrazolium exhibited in some animals, was never observed with phentolamine.

Since these findings indicate that alpha-adrenergic blockade alone cannot account for the inhibitory effect of neotetrazolium, the possibility of interference with the direct myotropic action of angiotensin was explored. The adrenergic component of the vasocon-

strictor effect of angiotensin was eliminated in one group of animals by ganglionic blockade (pentolinium), norepinephrine depletion (reserpine) and adrenalectomy, and in another group by blockade of ganglia (pentolinium) and of alpha-adrenergic receptors (phentolamine). Lack of response to tyramine and to norepinephrine indicated effective norepinephrine depletion and alpha-adrenergic blockade respectively. Consequently, it was assumed that in these animals pressor responses to angiotensin would be caused primarily, if not exclusively, by the direct myotropic action of the polypeptide. In both these groups neotetrazolium produced marked suppression of the angiotensin responses indicating inhibition of the direct effect of the polypeptide.

The antagonism of angiotensin exhibited by neotetrazolium is not a novel pharmacologic effect since several compounds have already been reported to inhibit angiotensin (36–38). Certain properties of neotetrazolium, however, distinguish it from other angiotensin inhibitors. It has been stated, for example, that no agent has been shown to exhibit truly specific angiotensin antagonism (5). The data obtained in the present study indicate that neotetrazolium, which blocks angiotensin responses, does not inhibit vasopressin *in vivo*. These results, although not sufficient to establish specificity, are consistent with this possibility. Furthermore, neotetrazolium exhibits antihypertensive activity (1, 2) and sympathetic blocking action (3, 4) which have not been claimed for other angiotensin inhibitors.

The ability of neotetrazolium to impair ganglionic transmission, to block alpha-adrenergic receptors and to inhibit the pressor effect of angiotensin (Fig. 1) makes it an interesting, if not unique, experimental antihypertensive compound since it combines pharmacologic actions against several factors which are believed to play a role in certain types of hypertension.

Conclusions.

1. Angiotensin exerts its pressor effect in the rat by both a direct myotropic action and an indirect mechanism involving alpha-adrenergic receptors.

2. Neotetrazolium inhibits both the indirect as well as the direct effects of angiotensin.

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