PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 164, 556–560 (1980)

Effect of Ganglionic Blockade on Pressor Responses to Angiotensin II during Converting Enzyme Inhibition (40916)[1]

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Abstract. Dose–response curves to angiotensin II were consecutively performed in rats after anesthesia (pentobarbital 60 mg/kg ip), converting enzyme inhibition (SQ14225 1 mg/kg iv), and ganglionic blockade (pentolinium 10 mg/kg sc) to explore the participation of the sympathetic nervous system in the immediate pressor action of the peptide. Experiments were conducted after the animals were maintained on a sodium-deficient diet for 2 weeks. Converting enzyme inhibition was maintained during ganglionic blockade to avoid changes in the circulating level of endogenous angiotensin II which might be caused by the latter maneuver. SQ14225 caused a 35 ± 4 mm Hg ($P < 0.001$) fall in systolic pressure and a significant shift to the left of the dose–response curve to angiotensin II compared to that obtained after anesthesia alone. In contrast, pentolinium did not further modify responses to angiotensin II despite significantly lowering systolic blood pressure 49 ± 5 mm Hg ($P < 0.001$). Our data confirm that a major determinant for the response to injected angiotensin II is the prevailing circulating level of the peptide and suggest that a direct effect of angiotensin II on the vascular bed is the most likely mechanism of action in the whole animal, since an intact sympathetic tone is not required for the peptide to evoke its full pressor response.

A possible participation of the sympathetic nervous system in the pressor response to injected angiotensin II has been explored previously because of the well-known stimulating effects of the peptide on adrenergic mechanisms (1–5). Results have been inconclusive with regard to the effect of angiotensin II in the intact animal (6–8).

A major role for the circulating level of the endogenously generated peptide on responses produced by exogenously administered angiotensin II has also been described (9). Hence, an ideal experiment to explore the effect of sympathetic nervous system blockade on pressor responses to angiotensin II should avoid changes in the circulating level of the peptide simultaneously produced by the blocking maneuver.

Our present experiments were designed to study the pressor response to angiotensin II in the salt-depleted anesthetized rat before and after ganglionic blockade in the presence of converting enzyme inhibition, in order to suppress the generation of endogenous angiotensin II. The effect of converting enzyme inhibition itself produced by injection of SQ14225 on pressor responses to the peptide were also analyzed in this study.

Methods. Eleven female Sprague–Dawley rats with an initial weight of approximately 200 g were used for these experiments. They were kept on a sodium-deficient diet (Teklad 170950) and given tap water to drink (sodium concentration 0.125 meq/liter) for 2 weeks before an infusion study was performed (see below). Low-salt diets were employed in order to stimulate the renin angiotensin system and maximize the effect of inhibition of the converting enzyme with captopril. This allowed comparison of dose–response curves to angiotensin II when circulating angiotensin II is high (precaptopril) and low (postcaptopril) as described below. No sodium was detected in a sample of the diet which was evaluated by flame photometry (I.L.) after dissolution in concentrated sulfuric acid. During the first 9 days of the study the rats were housed in groups of three to four in regular cages. On days 10–14, animals were kept in individual metabolic cages for collection of urine samples over each 24-hr period. The mean sodium excretion was 6 ± 2 μeq/24 hr (range 0–18).

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[1] This study was in part supported by USPHS Grant HLB 13595 and the Hypertension Division Fund.

0037-9727/80/080556-05$01.00/0

On the 15th day rats were anesthetized with pentobarbital 60 mg/kg ip, given atropine 0.4 mg/kg sc, and tracheotomized. A carotid artery was cannulated with a PE 50 (Clay Adams) catheter for measurement of arterial pressure by means of a Statham P23Db transducer and a Physiograph DMP recording system. Two PE 10 catheters were placed in one or both jugular veins for injection of drugs. Once the blood pressure stabilized, angiotensin II (Hypertensin, CIBA) was injected at doses of 50, 100, and 200 ng/kg. Each dose was tested in duplicate, and was administered in microliter amounts by means of a Gilmont syringe. This dose–response curve to angiotensin II was repeated after 10 min of converting enzyme inhibition with SQ14225 (1 mg/kg iv) and after the subsequent injection of pentolinium tartrate (Ansolysen, 10 mg/kg sc), while the effect of the former was still present. The volume of the injections of SQ14225 and pentolinium was less than 0.23 ml. The chosen dose of SQ14225 is 67 times the ID_{50} for the anesthetized rat (10). In preliminary studies we found an almost complete inhibition of the converting enzyme from 10 to 60 min after the injection of this dose of SQ14225, by means of repetitive injections of angiotensin I (310 ng/kg iv).

In order to assess the significance of the shifts observed in the dose–response curves, the dose required to produce an elevation of 20 mm Hg in systolic pressure was used (PD_{20}) as derived from the semilog plot of each dose–response curve. Student's test for paired and unpaired data and the calculation of correlation coefficients were performed according to the usual techniques. Significance was considered to be present if $P < 0.05$.

Results. The average level of systolic pressure observed during all phases of the experiment in all animals is represented in Fig. 1. Initial systolic blood pressure was 136 ± 6 mm Hg and there was no significant change in this baseline value during or after the first dose–response curve to angiotensin II. Injection of SQ14225 caused a rapid (1–2 min) and significant fall in systolic blood pressure of 35 ± 4 mm Hg, followed by a slow (5–10 min) and small but also significant recovery of 8 ± 2 mm Hg. Again, the level of systolic blood pressure attained (110 ± 5 mm Hg) was not significantly altered during the performance of the second dose–response curve to angiotensin II. The injection of pentolinium significantly reduced systolic blood pressure 49 ± 5 mm Hg to a level of 65 ± 4 mm Hg which was stable while the third dose–response curve to angiotensin II was performed.

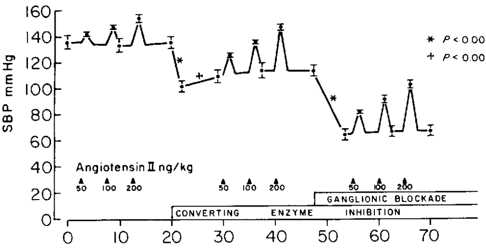

FIG. 1. Systolic blood pressure (mean ± SEM) observed during the different phases of the experimental procedure. Injections of angiotensin II and periods of converting enzyme inhibition and ganglionic blockade are indicated. The three significant changes in the baseline blood pressure are shown.

Figure 2 shows the dose–response curves to angiotensin II for the three conditions of the experiment. As can be seen, inhibition of converting enzyme provoked a markedly significant enhancement of responses at all three dose levels of angiotensin II, in comparison to the ones obtained after anesthesia only. The result was a par-

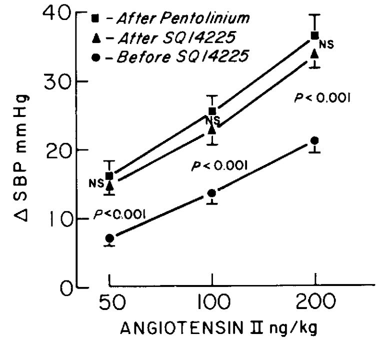

FIG. 2. Dose–response curves to angiotensin II in the salt-depleted normal rat, before and after converting enzyme inhibition, and after ganglionic blockade. Results expressed as mean ± SEM. P values for the paired comparisons are given. NS = not significant.

allel shift of the curve to the left. In contrast, responses to angiotensin II, measured after pentolinium injection in the converting enzyme-inhibited rats, were not significantly different from that obtained after converting enzyme inhibition alone.

The dose of angiotensin II required to produce an elevation of 20 mm Hg in systolic blood pressure (PD_{20}) for each of the three conditions of the experiment is shown in Fig. 3. The marked reduction of PD_{20} caused by inhibition of the converting enzyme by SQ14225 is highly significant. The addition of ganglionic blockade produced a minimal reduction in PD_{20}, which was not statistically significant.

Discussion. There is extensive evidence indicating that the sympathetic nervous system is stimulated by angiotensin II at several levels. The peptide: (a) augments the synthesis of catecholamines (1), (b) acts on presynaptic receptors of sympathetic nervous terminals to facilitate the release of norepinephrine provoked by sympathetic nervous stimulation (2, 11), (c) elicits catecholamine release from the adrenal medulla (3), (d) stimulates sympathetic ganglia (4), and (e) modifies the central nervous control of sympathetic function through stimulating actions upon central adrenergic pathways (5, 12, 13).

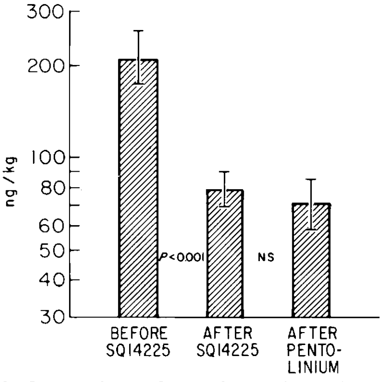

FIG. 3. Comparison of PD_{20} for angiotensin II before converting enzyme inhibition (left bar), after converting enzyme inhibition (middle bar), and after ganglionic blockade during continued converting enzyme inhibition (right bar). The *P* values for paired comparisons between adjacent bars are given. NS = not significant.

It is still unclear whether the actions of angiotensin II upon sympathetic function contribute to its immediate pressor effect. The reduction of the vasoconstrictor effect of angiotensin II, produced by surgical sympathectomy in the hind quarter of the dog, seems to support this suggestion (14). On the contrary, experiments in the whole animal have failed to show a diminished pressor response to angiotensin II when the sympathetic nervous system is blocked by administration of reserpine (6), phentolamine (7), or 6-OH-dopamine (8).

A possible explanation for such conflicting data is that components of the action of angiotensin II which are mediated by the sympathetic nervous system are restricted to some vascular beds and not sufficiently widespread as to affect the overall pressor action of the peptide in the intact animal. Another explanation may be found in the more recent evidence that the circulating level of angiotensin II is one of the major determinants for the pressor response evoked by the exogenously administered peptide (9). It is well known that the lower the circulating level of angiotensin II (secondary to the level of circulating renin) the greater the response to the injection of the peptide. Renal denervation and most antiadrenergic drugs block the release of renin from the kidney (15, 16) consequently decreasing the circulating level of angiotensin II. Therefore, in experiments utilizing surgical or pharmacological sympathectomy, responses to angiotensin II would be increased by this mechanism. If a sympathetic component in the pressor action of the peptide (which these experiments intend to explore) does in fact exist, and was simultaneously blocked, the consequent decrease in response to angiotensin II that would be expected may well have been present but concealed by the formerly described action. The final effect of the sympathetic blocking agent on the pressor response to angiotensin II could merely represent the algebraic sum of two mechanisms operating in opposite directions. The predominance of one or the other in a particular experiment could account for the extreme variation in the results obtained.

Our present experiment was designed to

study the role played by the sympathetic nervous system in the immediate response to angiotensin II, by means of comparison of the responses before and after ganglionic blockade. To avoid any effect of this maneuver on the level of circulating angiotensin II, our experiments were conducted in rats pretreated with the converting enzyme inhibitor SQ14225.

Administration of SQ14225 produced a highly significant reduction in blood pressure consistent with the salt depleted state of our animals. The pattern of the hypotensive action of SQ14225 was similar to the one previously described when salt depleted rats were given SQ20881 intravenously (17), showing a rapid initial fall in blood pressure followed by a smaller recovery to a stable plateau. This biphasic pattern of arterial pressure response to intravenous converting enzyme inhibition remains unexplained.

It has been shown that barbiturate anesthesia does not modify the response to angiotensin II obtained in the unanesthetized rat (18). Thus, our dose–response curve to angiotensin II obtained after pentobarbital anesthesia alone was considered a meaningful baseline. Compared with this curve, the one obtained after SQ14225 was shifted to the left in a parallel manner. The enhancement of responses to angiotensin II caused by inhibition of the converting enzyme was highly significant by comparing either the absolute increases in blood pressure or the change in PD_{20}, before and after SQ14225. This finding is a confirmation of results previously obtained with the peptide inhibitor of the converting enzyme, SQ20881. In that publication the enhancement of response to angiotensin II after converting enzyme inhibition was attributed to a diminished occupancy of receptors secondary to the reduction in circulating levels of the peptide (9).

The subsequent administration of pentolinium tartrate did not cause any significant change in the pressor responses to angiotensin II. Increases in blood pressure were similar before and after ganglionic blockade and as a result, neither the dose–response curve was shifted nor the PD_{20} changed. It has previously been reported that pentolinium enhances the response to injected angiotensin II in rats with an intact renin axis. Results were attributed to an effect of the reduction of the blood pressure itself on responses to angiotensin II (18). Our data clearly show that even when pentolinium exerted its known hypotensive action, responses to angiotensin II were not modified, making this explanation highly unlikely. The interruption of the renin axis in our rats by means of converting enzyme inhibition is the main difference between our experimental protocol and the one previously mentioned. Thus, it is possible to explain the differences in results by postulating that the enhancement of pressor responses to the peptide provoked by pentolinium in animals with an intact renin axis is due to a reduction in the circulating levels of renin and angiotensin II. The lack of effect of pentolinium upon angiotensin II dose–response curve in the animals pretreated with SQ14225 tends to exclude a requirement for sympathetic tone in the immediate pressor action of angiotensin II. However, a role played by catecholamines released by the peptide from the adrenal medulla cannot be completely ruled out since pentolinium does not block this action of angiotensin II (19).

In conclusion our data: (a) confirm that a major factor modulating the immediate pressor response to injected angiotensin II in the whole animal is the prevailing level of the circulating peptide itself, giving some support to the receptor occupancy theory, (b) demonstrate that the immediate pressor effect of angiotensin II is more likely a direct effect, independent of its actions on the sympathetic nervous system, and (c) suggest that previous conflicting reports concerning the effects of several maneuvers blocking the sympathetic nervous system on responses to angiotensin II are probably better explained by effects of those maneuvers on the circulating levels of angiotensin II, rather than on a hypothetical sympathetic component in the pressor action of the peptide.

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Received October 25, 1979. P.S.E.B.M. 1980. Vol. 164.