Prior Receptor Occupancy as a Determinant of the Pressor Activity of Infused Angiotensin II in the Rat

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ABSTRACT

The pressor responsiveness to angiotensin II and norepinephrine was examined in rats before and during blockade of converting enzyme activity with the nonapeptide SQ 20881. Responses to angiotensin II were impaired by sodium deprivation but enhanced by sodium loading or bilateral nephrectomy. During the period of converting enzyme blockade, a twofold increase in the angiotensin II pressor response was observed in the salt-restricted rats, whereas only a small change occurred in the salt-loaded rats. Infusion of the inhibitor produced a profound fall in the blood pressure of the salt-depleted rats with a relatively minor fall in the sodium-loaded rats. Norepinephrine pressor responses were slightly potentiated in the salt-restricted rats after administration of SQ 20881, but no change occurred in the salt-loaded or the nephrectomized rats. These observations support the view that the decrease in angiotensin II pressor activity during salt deprivation is the result of a prior occupancy of receptor sites by endogenous hormone. Therefore, a change in the number or the affinity of receptors consequent to changes in sodium balance need not be postulated to explain the phenomenon.

KEY WORDS

pressor response  blood pressure  norepinephrine
sodium depletion and loading  converting enzyme blockade  bilateral nephrectomy

Most studies of the renin-angiotensin system depend on measurements of the circulating levels of renin and angiotensin II. A reciprocal relationship between plasma renin activity and sodium balance has been well documented (1, 2). Thus, sodium depletion stimulates renin secretion, and sodium loading suppresses renin release. However, under circumstances of body fluid volume depletion, during which increased renin levels are found, pressor reactivity to exogenous angiotensin becomes markedly impaired (3–7). Conversely, when sodium is given in excess, especially if a mineralocorticoid is also administered, the pressor response to angiotensin infusion is enhanced (6). Thus, angiotensin pressor reactivity appears to correlate directly with sodium balance but inversely with plasma renin concentration. Some investigators believe that high endogenous levels of circulating angiotensin II dampen the receptor response to infused hormone (5). Others (8) have suggested that changes in the vascular response to and the avidity for angiotensin result from a more direct action of the sodium ion on the receptor per se. However, if the latter explanation obtains, vascular receptor affinity will be decreased in a situation in which the vasoconstrictor action of angiotensin II comes into play. Such a decrease might either nullify or counterbalance the homeostatic mechanism for blood pressure regulation.

Renin, angiotensinogen (renin substrate), and angiotensin I have no physiological actions of their own. Angiotensin I, the less active decapptide, must be hydrolyzed by a converting enzyme (9) to the octapeptide angiotensin II, a potent vasoconstrictor substance. Recently, a synthetic nonapeptide converting enzyme inhibitor SQ 20881 (Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro) (Squibb Laboratories) has been developed from the venom of Bothrops jararaca (10). This inhibitor, which produces prolonged blockade (half life > 50 minutes) of angiotensin I conversion in the rat (11), affords an opportunity to

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separate and quantify the individual roles of the sodium ion and angiotensin II on vascular receptor reactivity. The purpose of this investigation was to determine whether the decrease in pressor response observed during sodium restriction is the result of competition between endogenous and infused angiotensin.

**Methods**

Male Wistar rats weighing 250-350 g were used throughout this investigation. The experimental groups comprised seven rats each. Group 1 rats were salt depleted; they were given a low-salt diet (12) and tap water ad libitum for 21 days. Group 2 rats were salt loaded; they received normal Purina rat chow and 1% saline to drink for 10 days. Group 3 rats were bilaterally nephrectomized. They were fed Purina rat chow and tap water before surgery and were studied 24 hours after bilateral nephrectomy, having been deprived of food but not water in the postoperative period.

On the day of the study, each rat was anesthetized with sodium pentobarbital (5 mg/100 g body weight, ip). The carotid artery was cannulated with a PE50 polyethylene catheter, and two PE10 catheters were placed in the jugular vein. Arterial blood pressure was monitored using a Sanborn pressure transducer, and the mean blood pressure was recorded. The pressor responses to angiotensin II (1-Asp-5-Val-angiotensin II) (Hypertensin, Ciba) and norepinephrine were determined by observing the peak responses to 6.25-, 12.5-, 25-, and 50-ng doses of angiotensin II and 50-, 100-, and 150-ng doses of norepinephrine. The pressor action of 50 ng of intravenously injected angiotensin I (1-Asp-5-Ile-angiotensin I) (Schwartz-Mann) was then tested.

After blood pressure had returned to the baseline level, the nonapeptide converting enzyme inhibitor SQ 208811 (100 /xg/100 g body weight) was injected intravenously over about 5-10 seconds. Preliminary studies in the nephrectomized rat (N = 3) had shown that this dose of inhibitor reduced the angiotensin I pressor response initially to 17%; after 60 minutes the response was still only 36% of the pretreatment value. When blood pressure had stabilized (5-10 minutes), the responses to angiotensin II, norepinephrine, and 50 ng of angiotensin I were again determined.

All injections were given via the jugular catheters. Angiotensin II, angiotensin I, norepinephrine and SQ 20881 were dissolved in 5% dextrose. The responses to angiotensin II and norepinephrine before and during peptide blockade were compared using a paired Student’s t-test. All results are expressed as means ± SE.

**Results**

There was no significant difference between the initial mean blood pressures of the salt-restricted and the salt-loaded rats; they were 116.0 ± 4.2 mm Hg and 120.6 ± 3.3 mm Hg, respectively (P > 0.1). The nephrectomized rats had a significantly lower mean blood pressure than did either of the other two groups of rats (P < 0.01).

Infusion of SQ 20881 resulted in a profound acute fall in the blood pressure of the salt-depleted rats with only a minor decrease in the salt-loaded group (Table 1). Thus, the volume component of blood pressure was

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1 Generously supplied by Dr. R. A. Vukovich of the Squibb Institute.
AN Gi OTENSIN PRESSOR RESPONSE TO SODIUM BALANCE

115

a. 20Onen

Before Sq

Alter Sq

H.S.

NX.

Pressor responses (means ± SE) in low-salt (L.S.), high-salt (H.S.), and nephrectomized (N.X.) rats before and after the administration of the converting enzyme inhibitor SQ 20881 (Sq).

Clearly exposed, since there was a 30-mm Hg pressure difference between the low-salt and the high-salt rats after converting enzyme blockade (Table 1). Virtually no change was observed in the nephrectomized rats (Table 1).

Pressor responses to angiotensin II are shown in Figure 1. Prior to SQ 20881 injection, there was a significant difference between the angiotensin II pressor response in the salt-restricted rats and the other two groups of rats (P < 0.01). During converting enzyme blockade, angiotensin pressor responses were nearly identical in all three groups of rats. Norepinephrine pressor action was slightly impaired by sodium deprivation (Fig. 2). After administration of SQ 20881, the norepinephrine responses were unchanged in the high-salt and the nephrectomized rats with a small but just significant increase in the low-salt rats. Angiotensin I pressor action was also impaired by sodium deprivation. However, during converting enzyme blockade, the response to angiotensin I decreased to the same low level in all three groups (Fig. 3). The pressor response to 50 ng of angiotensin I in the untreated nephrectomized group was equivalent to the generation of 40 ng of angiotensin II, whereas after SQ 20881 administration the response to angiotensin I was only equivalent to the generation of 3.5 ng of angiotensin II. Thus, almost a 90% reduction in conversion of angiotensin I had occurred.

Discussion

The results clearly demonstrate the reciprocal relationship between the renin-angiotensin system and sodium balance in the maintenance of normal blood pressure. When salt depletion occurs, the renin vasoconstrictor mechanism assumes a major role as evidenced by the marked fall in blood pressure following blockade of angiotensin I conversion. At the other extreme, sodium loading suppresses renin release, and the expanded body fluid volumes become sufficient to sustain a normal blood pressure without a major contribution from the renin-angiotensin mechanism. These observations complement those made in the single-kidney Goldblatt hypertensive rat, using the antagonist 1-Sar-8-Ala-angiotensin II, since renin dependency can also be exposed or abolished by sodium depletion or loading, respectively, in this model (13). This important relationship and role for the renin-angiotensin system in the regulation of arterial blood pressure has also been demonstrated in dogs with ligation of the thoracic vena cava (14) and in salt-depleted and adrenalectomized dogs (15). However, paradoxically, a reduction in pressor responsiveness to angiotensin during sodium deprivation is well documented (3-7). Such a change in vascular reactivity tends to counteract this homeostatic mechanism for blood pressure preservation. Since similar changes in reactivity to angiotensin II have
been demonstrated in fresh in vitro preparations of aortic strips from sodium-depleted rabbits (16), neither a reduction in plasma volume and cardiac output nor the presence of high levels of circulating angiotensin II provides an explanation for this phenomenon.

Our studies indicate that blocking the renin-angiotensin system in salt-depleted rats by administration of converting enzyme inhibitor restores pressor reactivity to values observed in salt-loaded or nephrectomized rats. The norepinephrine response was studied to check the specificity of angiotensin II responsiveness. Potentiation of the norepinephrine response in the sodium-depleted rats during converting enzyme blockade was unexpected but appears to be an indirect action, since no change occurred in the nephrectomized group. The effect might represent a greater potential contractility of the blood vessels when they are in a more relaxed state after inhibition of angiotensin II generation. Alternately, a change in interaction between the renin-angiotensin system and the autonomic nervous system might explain this observation. The marked potentiation of angiotensin II responsiveness cannot be attributed to the fall in blood pressure, since only a small increase in the response to norepinephrine was observed. Equally, since no change in sodium balance was possible during the acute study, a direct action of the sodium ion on vascular receptors seems unlikely. These observations support the view that observed differences in pressor responsiveness to exogenous angiotensin II in the three groups of rats are due to competition between endogenous and infused hormone for vascular receptor sites. However, since differences in reactivity to angiotensin II have also been demonstrated in in vitro preparations (16) only a partial role can be ascribed to circulating angiotensin II.

Recently, a new hypothesis has been advanced which suggests that renin accumulates in peripheral arteriolar walls, leading to local generation of angiotensin II (17, 18). Reninlike activity has been detected in isolated arterial walls (19) and appears to vary quantitatively with sodium balance in exactly the same manner as does plasma renin activity (20, 21). This hypothesis is strengthened by the proven contiguity of converting enzyme activity within the mesenteric (22) and hind-limb (23) vasculature. Therefore, according to this hypothesis, in the presence of raised renin levels (group 1), large quantities of angiotensin II will be generated both in the plasma and in the arteriolar wall and will occupy many vascular angiotensin receptors. Thus, few receptor sites will be available to respond to infused angiotensin, and the pressor action will be impaired. When renin is suppressed or absent (groups 2 and 3), there will be little or no angiotensin II generated, leaving the majority of receptors free to react with exogenous hormone. Converting enzyme inhibitor blocks the generation of angiotensin II in both the lung (24) and the peripheral vessels (22, 23); therefore, during the period of blockade many receptor sites will be free to respond to infused angiotensin II. The equality of the small residual pressor response to angiotensin I in the three groups after converting enzyme blockade also supports this view.

On the basis of the studies described in the present paper, we suggest that the decreased pressor response to exogenous angiotensin II observed during sodium restriction results from increased competition for receptor sites from endogenous angiotensin II generated both in the plasma and also locally within peripheral blood vessel walls.

References

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Prior receptor occupancy as a determinant of the pressor activity of infused angiotensin II in the rat.

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