Development of Tachyphylaxis to Aspartyl$^1$ and Not to Asparginy1$^1$ Angiotensin II in the Rat

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ABSTRACT

Pressor responses to single increasing doses (0.001 to 10 μg) and constant infusions (0.01 to 5 mg/kg per hr) of synthetic aspartyl$^1$ angiotensin II (Asp$^1$) and asparginy1$^1$ angiotensin II (Aspg$^1$, Hypertensin, CUBA) were measured in normal and nephrectomized rats. Mean blood pressure was recorded directly using strain gauges. Angiotensin tachyphylaxis (decline of maximal response to increasing doses, or return of blood pressure to control levels on constant infusion) was consistently seen with greater than minimal doses of Asp$^1$ while the phenomenon was not observed with Aspg$^1$ at any dose level. The results indicate that in the rat the two octapeptide analogues of angiotensin are not identical in biologic activity as has been previously held, and that the N-terminal amino acid plays an important part in determining the pressor responsiveness especially to higher doses of angiotensin peptides. The pattern of response and development of tachyphylaxis to Asp$^1$ closely resembles that of well known tachyphylaxis to renin. These results lend further support to our earlier findings that Asp$^1$ represents the natural form of angiotensin released in vivo. Renin tachyphylaxis may in its entirety be explained to depend upon the actions of Asp$^1$ released by renin in plasma. Tachyphylaxis to endogenous angiotensin in pathologic as well as certain physiologic conditions may be expected to occur at lower dose levels than is indicated by the available experimental work with Aspg$^1$.

ADDITIONAL KEY WORDS

renin tachyphylaxis  N-terminal amino acid of angiotensin
endogenous angiotensin

Development of tachyphylaxis to renin was described in the original report by Tigerstedt and Bergman (1). Subsequent workers (2-9) have confirmed it. It is recognized now that renin tachyphylaxis is due to unresponsiveness to its end product, angiotensin, and not to any factors limiting the enzymatic reaction of renin upon its substrate (4, 5, 6). Tachyphylaxis to angiotensin, however, has not been demonstrated to occur with the same speed of onset and to the same degree of completeness as to renin. Braun-Menéndez et al. (7) using crude preparations of natural angiotensin doubted if tachyphylaxis ever developed in response to angiotensin. Page and associates (8, 9) showed that infusion of purified preparations of synthetic and natural angiotensin increased and maintained blood pressure in a dog without evidence of tachyphylaxis though the latter was readily demonstrable in the same animal when crude renin was infused instead. Langford (5) proposed the mediation of an unknown factor X that might be released by renin in addition to angiotensin. Bock and Gross (6) refuted this proposal but did not account for their findings showing that continued infusions of renin in greater than minimal doses caused the blood pressure of the dog to return to the preinfusion basal level, although infusions of angiotensin always maintained some increase.
in blood pressure for the duration of the infusion. The question of whether or not the consistent and full blown tachyphylaxis that develops in response to large and repeated doses of renin is entirely due to tachyphylaxis developing to angiotensin that is liberated by enzymatic action of injected renin has never been satisfactorily answered. The present studies provide a possible explanation for this difference between the pressor effects of renin and angiotensin.

Methods

RECORDING OF PRESSOR ACTIVITY IN RATS

Wistar male rats, both intact and nephrectomized, weighing 100 to 150 g were anesthetized with sodium pentobarbital, 4 to 6 mg/100 g body weight. Following the initial dose, the nephrectomized rats required no further administration of anesthetic during the procedure up to 6 to 8 hr. In normal rats with intact kidneys the anesthetic effect diminished within a couple of hours. Repetition of the anesthetic was avoided during the procedure as long as the decreasing level of anesthesia did not result in any obvious discomfort to the animals. To eliminate a possible role in altering the pattern of pressor response to angiotensin, of inconstant level of anesthesia in certain normal rats, parallel studies were carried out in rats that had undergone bilateral ureteral ligation with intact kidneys. In these animals the effect of anesthesia was maintained as in the nephrectomized rats.

The trachea was cannulated. Both external jugular veins were cannulated with polyethylene tubing (Clay Adams PE-20) with an inner diameter of 0.015 inch; the carotid artery was cannulated with a tube (PE-50) having an inner diameter of 0.023 inch. Injections and infusions were made into the jugular veins using a microburette syringe that was capable of delivering 0.1 μliter accurately. For single injections the volume injected varied from 0.001 to 0.08 ml. Constant infusions were made with the aid of Bristol Synchronous Motors, with speeds of 1/15 to 1/10 rpm, that were attached to the microsyringe. Carotid arterial blood pressure was measured through a strain gauge pressure transducer (Sanborn 267 AC) and the electrically integrated mean was recorded on a Sanborn Recorder. The system was calibrated so that a change of 1 mm Hg pressure caused a 1-mm shift of the stylus on the recording paper.

DOSE-RESPONSE CURVES

The following angiotensin peptides were used:

1. α-Asp¹-Val⁸-angiotensin octapeptide (Aspartyl angiotensin II) (Asp¹-Arg²-Val³-Tyr⁴-Val⁵-His⁶-Pro⁷-Phe⁸)
2. α-Asp¹-β-Amide-Val⁸-angiotensin octapeptide

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FIGURE 2

Log dose response curves with asparginy1\textsuperscript{1} angiotensin octapeptide in 1 normal (squares) and 2 nephrectomized rats (circles). In some animals the response at higher doses levels off; it does not decline.

FIGURE 3

Continuous intravenous infusion of asparginy1\textsuperscript{1} angiotensin octapeptide at two dose levels in a normal rat. The ordinate shows the increments in mean blood pressure in millimeters of mercury and the abscissa shows the duration of the infusion in hours. The duration of the two infusion periods is indicated by the arrows. The infusion was stopped during the intervening period and the resulting drop in blood pressure is shown by the dotted line. The decrease after the initial peak probably represents reflex cardiovascular adjustments at the start of the infusion.

(Asparginy1\textsuperscript{1} angiotensin II) (Asp-NH\textsubscript{2}-Arg\textsuperscript{2}-Val\textsuperscript{3}-Tyr\textsuperscript{4}-Val\textsuperscript{5}-His\textsuperscript{6}-Pro\textsuperscript{7}-Phe\textsuperscript{8}). Peptide no. 1 represents the naturally occurring form of angiotensin. Peptide no. 2 is the commercially available synthetic analogue (Hypertensin) that has been used, since its availability, in almost all of the pharmacologic and biochemical investigative work on angiotensin.

The peptides were dissolved and injected in 20\% ethanol in appropriate concentrations. Single sequential injections of increasing doses of the angiotensin analogues were given intravenously and the absolute rise of mean blood pressure in millimeters of mercury was recorded in response to each injection. The blood pressure always returned completely to the original baseline before the next injection. The response to each angiotensin analogue was recorded in 10 different rats. Each dose was injected at least twice. Blood pressure responses to repeated doses were remarkably reproducible in the same rat, rarely varying more than 1 mm Hg. Blood pressure response to similar doses in different rats, however, varied widely. For this reason the dose response curves presented were chosen to show representative examples in individual rats. All experiments in normal rats.
were repeated on nephrectomized rats to discount any possible role of endogenously produced renin and angiotensin. These animals were studied 16 to 18 hr postnephrectomy.

**Results**

**DOSE-RESPONSE CURVES**

Pressor responses to single injections of aspartyl and the asparginyl octapeptides of angiotensin, over a dose range of 0.001 to 10 μg, were recorded in 10 normal rats. In each animal dose-response curves were obtained for both octapeptides (aspartyl after asparginyl). The remaining rats received one or the other analogue only. The shape of the dose-response curve for one octapeptide was consistently different from that of the other in each animal tested. This was also true of 4 additional nephrectomized rats and 2 rats that had undergone bilateral ureteral ligation. Figures 1 and 2 show typical dose response curves obtained.

The maximal pressor response to the asparginyl analogue varied from 23 to 42 mm Hg in the normal and 25 to 32 mm Hg in the nephrectomized animals. The corresponding range for the aspartyl octapeptide was 18 to 22 mm Hg for the normal and 15 to 32 mm Hg for the nephrectomized rats. The doses eliciting the maximal response in different animals varied from 50 to 500 ng of aspartyl, and from 1 to 10 μg of asparginyl angiotensin. The two octapeptides were essentially equipressor in the lower dose range when tested in the same animal. At higher doses the response to the asparginyl analogue in 5 out of 7 rats reached a plateau but never declined; as a contrast, the response to the aspartyl octapeptide consistently declined well below the maximal level. Following large doses of aspartyl angiotensin, the response to injected doses of either analogue was markedly reduced, yet the response to injections of norepinephrine (0.5 to 2.5 μg) was unchanged.

**CONTINUOUS INFUSIONS**

Constant infusion of asparginyl analogue at all pressor dose levels maintained a stable response for the duration of the infusion in 5 rats tested up to 6 hr. Figure 3 diagrammatically shows changes in mean blood pressure at two dose levels. On stopping the infusion, blood pressure returned to the preinfusion level. Immediately following the infusion, pressor responses to single injections of either octapeptide (25 to 1000 ng) were moderately reduced in comparison with responses to similar doses before the infusion.

The pressor response to constant infusions of aspartyl octapeptide, on the other hand, was not maintained except at the minimal pressor dose (0.01 mg/kg per hr). Higher doses (0.02 to 1 mg/kg per hr) failed to maintain a pressor response in 6 normal and
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4 nephrectomized rats. Figure 4 illustrates the pattern of response of aspartyl\textsuperscript{1} octapeptide at the same dose levels shown in Figure 3 for asparginyln\textsuperscript{1} octapeptide. Following the initial rise upon start of the infusion, the mean blood pressure steadily declined. Within the first hour it returned to the preinfusion level and stayed at this level despite continued infusion (up to 4 hr). Stopping the infusion during this period usually caused no change in blood pressure, except that a slight drop (<5 mm Hg) was sometimes seen after prolonged infusions. The response to single injected doses of either octapeptide (25 to 1000 ng) was virtually absent and that to norepinephrine (0.5 to 2.5 \textmu g) was fully intact. Sensitivity to angiotensin analogues usually returned within 30 to 60 min if the animal was left alone. Upon resumption of the infusion at double dose: (a) the initial pressor response was the same or even somewhat lower, and (b) the return to the baseline was faster than before.

Discussion

The experiments clearly establish that tachyphylaxis in the rat develops rapidly and completely to greater than minimal doses of aspartyl\textsuperscript{1} angiotensin and that the same phenomenon is not seen with asparginyln\textsuperscript{1} angiotensin at any dose level tested. Comparable studies with the aspartyl\textsuperscript{1} octapeptide in other species have not been reported. Brown et al. (10) who studied the rabbit, and Louis and Doyle (11) who studied the dog and man have reported that continuous infusions of large doses of the asparginyln\textsuperscript{1} analogue lead to either a drop in maximal pressure or sometimes a complete return to baseline. In these studies, however, except in a single experiment in a conscious man, discontinuance of the infusion of larger doses of Hypertensin was followed by moderate to profound hypotension, thus altering the validity of "complete return of blood pressure to baseline." In our studies on rats baseline blood pressure before and after the infusion was unchanged.

Differences in experimental design, in dose-body weight relationships, in pattern of cardiovascular responses to maximal vasoconstriction, or a true species variation may all partly account for the results in the studies cited above. Nevertheless, only the highest doses of asparginyln\textsuperscript{1} angiotensin used produced a state resembling tachyphylaxis. Renin injections or infusions, on the other hand, except in minimal doses always give rise to tachyphylaxis in all species studied (1-9), a circumstance exactly comparable to our findings with aspartyl\textsuperscript{1} angiotensin in the rat.

These findings strongly suggest that renin tachyphylaxis can be accounted for by the action of aspartyl\textsuperscript{1} angiotensin released by the interaction of renin and its substrate in the plasma. These considerations strengthen our recent findings (high voltage paper electrophoretic separation of angiotensin peptides from plasma incubates—unpublished observations) that the natural form of angiotensin released by human renin is indeed the aspartyl form and so is the same as the peptides first isolated from bovine (12), equine (13), and porcine (14) plasma. Furthermore, the endogenous production of angiotensin when considered to be the aspartyl\textsuperscript{1} form, may certainly be sufficient, at much lower doses than estimated or indicated by infusion experiments using the asparginyln\textsuperscript{1} analogue, to account for development of general vascular or local renal vascular (11) tachyphylaxis in clinical and experimental states accompanying secondary aldosteronism (15) and renal ischemia (16).

In the early studies on angiotensin tachyphylaxis, the natural as well as synthetic peptides used were of varying degree of purity, the range of dosage used was narrowly limited, and the index of response commonly monitored was systolic blood pressure alone. The latter variable is subjected, much more than diastolic, to the self-limiting influence of cardiovascular reflexes especially when higher levels of dose and response are studied. Since the availability of synthetic angiotensin and of improved recording procedures, a wider range of doses and responses to only the asparginyln\textsuperscript{1} analogue has been studied. These considerations and our results make it desirable that the actions of the aspartyl\textsuperscript{1}
octapeptide should be systematically studied in other species, not only on the cardiovascular system but also on the renal tubules and the adrenal cortex. The N-terminal amino acid by virtue of its different charge in aspartyl$^1$ vs. asparaginyl$^1$ may play an important, hitherto unrecognized, part in the actions of angiotensin upon its target organs.

References
7. BRAUN-MENÈDEZ et al.: op. cit., p. 147.
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