Angiotensin I, II, and III Tachyphylaxis in the Mesenteric Vascular Circuit of the Rat

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SUMMARY Angiotensin tachyphylaxis is rapidly induced in the mesenteric vascular circuit of the rat perfused with a saline solution. There is crossed tachyphylaxis among angiotensins (AI, AII, and AIII). The angiotensin antagonist Sar 1-Ileu 8-AII is ineffective when the vascular preparation is previously rendered tachyphylactic to AII, showing that the AII receptors are not available during tachyphylaxis. This finding supports the theory that angiotensin tachyphylaxis is caused by receptor occupancy by the agonist. By perfusing the vascular preparation with AII solutions that were too diluted to produce vasoconstriction, tachyphylaxis to AII was induced. Therefore, AII receptors can be slowly saturated without producing vasoconstriction.

The recoveries of the vasoconstrictor effect of AII and AIII at 30 and 60 minutes after tachyphylaxis are similar; thus, the dissociation constants of the AII- and AIII-receptor complexes should be alike.

After three bolus injections of AII, the vascular preparation is completely refractory to AII, AII, and AIII. When the conversion of AII to AIII is inhibited with captopril, AII no longer induces tachyphylaxis to AII and AIII. Thus, tachyphylaxis from AII and AIII induced by AII seems to be due not to the occupancy of AII receptors by AII but to the AII formation from AII in situ. (Hypertension 3 (suppl II): H-166-H-170, 1981)

KEY WORDS • vascular smooth muscle • angiotensin receptors • captopril • angiotensin I converting enzyme • Sar 1-Ileu 8-AII

TACHYPHYLAXIS is the reduction or the complete lack of response of an effector after the first effective dose of an agonist. Tachyphylaxis is restricted to the agonist considered or to a closely related group of substances, while others retain their full effect.

Tachyphylaxis to the pressor effect of angiotensin was first described by Page and Helmer1 in 1940. Isolated preparations of vascular and nonvascular smooth muscle are easily rendered tachyphylactic to angiotensin, a fact that facilitates the study of its production and mechanism.

Two theories have been proposed to explain angiotensin tachyphylaxis. One, first advanced by Page and Bumpus2 in 1961, postulates that tachyphylaxis is due to the long-lasting occupancy of the angiotensin receptors by the agonist. This theory, with modifications, has been adopted by several investigators.5 The other theory explains tachyphylaxis as a consequence of the exhaustion of a hypothetical secondary messenger of angiotensin,6,9 catecholamines and prostaglandins have been proposed as the substances involved.

The isolated mesenteric vascular circuit of the rat perfused with a Ringer solution, according to the method of McGregor,10 develops rapid and complete tachyphylaxis to angiotensin; after a period of time, full recovery of the vasoconstrictor response is observed. This pattern is very reproducible, so the preparation is suitable for the study of angiotensin tachyphylaxis and its mechanism.

In a previous study,11 we were unable to support the theory of the exhaustion of a messenger. In this report we present evidence that supports the interpretation that angiotensin tachyphylaxis is caused by occupancy of the receptors.

Material and Methods

Perfusion

The vascular circuit of the superior mesenteric artery of the rat was prepared according to McGregor10 and perfused with a saline solution; it was used in all the experiments. The composition of the perfusing fluid was as follows (in mEq/liter): Na+ 134; K+ 5; Ca++ 2.2; Cl− 137; H2PO4− and HPO42− = 4; glucose 5.5 mM/liter. The osmolarity was 285 mOsm/liter and pH 7.45. A peristaltic pump of constant out-
put was used for perfusion. The output was set between 3-4 ml to maintain a basal perfusion pressure of 17-20 mm Hg. The perfusion pressure was recorded with a Statham pressure transducer (P-23AC) and a one-channel electronic recorder. After perfusion of the vessels began, 60 minutes were allowed for stabilization. The perfusing solution was previously gassed by bubbling pure oxygen for 15 minutes at 38°C. The temperature of the perfusing fluid was maintained at 38°C by immersing the plastic tube leading to the mesenteric artery in water from a circulating thermostatic bath.

Drugs

Angiotensin I (AI) (Schwarz); Angiotensin II amide (AII) (Ciba); Angiotensin III (AIII) (supplied by Dr. J.C. Romero), norepinephrine bitartrate (NA) (Sterling-Winthrop); Sar 1-Ileu 8-angiotensin II (Sar 1-Ileu 8-AII) (supplied by Dr. J.C. Romero); captopril (SQ 14, 225) (Squibb).

Substances were dissolved in the fluid used for perfusion and 50 μl was injected with a microsyringe proximal to the vessels. Injection time was about 1 second. AII in some experiments and captopril in all cases were added directly to the flask containing the perfusion fluid; AII in some other experiments was infused at a constant rate with a Harvard pump.

Analysis of variance and test of multiple comparisons were used, according to Scheffe.12

Results

Induction of Tachyphylaxis

The bolus injection of 50 μl (of a 20 μg/ml solution) of AII gave rise to an increase of the perfusion pressure of 10-20 mm Hg which lasted from 2 to 3 minutes. An equal dose, injected immediately after the effect of the first one had disappeared, produced a much smaller effect, while a third dose was either totally ineffective or the vasoconstrictor effect was minimal (fig. 1, middle tracing). The vascular preparation is then completely refractory to angiotensin since 10 times more concentrated AII solution (200 μg/ml) is also without effect (fig. 2 upper).

A1 and AIII solutions (20 μg/ml) had vasoconstrictor effects similar in magnitude to AII, also followed by rapid tachyphylaxis. There was crossed tachyphylaxis among the three angiotensins (fig. 1). Rapid tachyphylaxis could also be induced with more diluted angiotensin solutions. An AII solution containing 2 μg/ml (1/20 of the concentration formerly used), induced complete tachyphylaxis after two or three bolus injections. The blockade was only partial, however, since the injection of a more concentrated AII solution (200 μg/ml) was followed by a small vasoconstrictor effect (fig. 2 lower).

More diluted AII solutions (0.2 μg/ml) were devoid of vasoconstrictor effect and did not induce tachyphylaxis, even after several bolus injections. Even more diluted solutions (1-5 μg/ml), although devoid of vasoconstrictor effect induced tachyphylaxis when infused for 20-40 minutes.

To assess the reactivity of the preparation, injections of a norepinephrine (NE) solution containing 20 μg/ml were used throughout the experiments. NE did not produce tachyphylaxis; at the same concentration as AII (20 μg/ml), the vasoconstrictor effect of NE is 2 to 3 times greater than that of AII.

Recovery from Tachyphylaxis

Vascular reactivity to the angiotensins recovered with time. Figure 3 shows the recovery of the vasoconstrictor effect of AII and AIII at 30, 60, and 120
Mechanism of Angiotensin Tachyphylaxis

At present there is no direct evidence to support the theory that tachyphylaxis is due to the long lasting occupancy of the receptors by angiotensin. To investigate this possibility, the effect of a potent angiotensin antagonist, Sar 1-Ileu 8-AII, on the vascular preparation was studied. This substance showed very small agonist effect in these experiments and, by remaining attached to angiotensin receptors, prevented the vasoconstrictor effect of angiotensin. We reasoned that if angiotensin tachyphylaxis is caused by the occupancy of receptors by the agonist, the effect of Sar 1-Ileu 8-AII must be prevented if the receptors are already occupied.

Figure 2. All tachyphylaxis induced with injections of 50 μl of angiotensin solutions of different concentrations. Upper Tracing: Complete tachyphylaxis after one bolus injection of the 20 μg/ml All solution (1 μg). A 10 times more concentrated All solution (200 μg/ml, 10 μg) was also ineffective. Lower Tracing: Three injections of an All solution with 0.2 μg/ml (0.01 μg) were without vasoconstrictor effect and did not induce tachyphylaxis to a 2 μg/ml All solution (0.1 μg). After two injections of the latter solution, the third one was without effect. The blockade, however, was not complete since the 200 μg/ml solution (10 μg) still produced a small effect.

Figure 3. Recovery of the vasoconstrictor effect of All and AIII after tachyphylaxis. Complete tachyphylaxis was induced by three bolus injections of 50 μl of the 20 μg/ml All solution, except in the experiment of the graphic at lower left, in which tachyphylaxis was produced by infusing 0.5 ml of this angiotensin solution in 1 minute. The vasoconstrictor effect of the All solution was assayed 30, 60 and 120 minutes after tachyphylaxis. Recovery (R) is expressed as the ratio effect at t time/initial effect.
Two bolus injections of a 20 μg/ml solution of Sar 1-Ileu 8-AII completely blocked the effect of a subsequent injection of 20 μg/ml solution of All. The vasoconstrictor effect of All recovered slowly: 0.13 ± 0.03 of the previous pressor effect (considered as 1) at 1 hour after the injection of the antagonist, and 0.33 ± 0.04 after 2 hours (n = 8) (fig. 4, middle bars). The recovery of the effect after complete tachyphylaxis to All was more rapid; in this series of experiments 0.83 ± 0.06 of the pressor effect recovered 1 hour after and 1.09 ± 0.23 2 hours after complete tachyphylaxis (n = 8) (fig. 4, right bars).

In a third group of experiments, angiotensin tachyphylaxis was induced as described above and, immediately afterward, two injections of Sar 1-Ileu 8-AII (same doses as above) were given. The vasoconstrictor effect of All was assayed 60 and 120 minutes after the injection of the antagonist. The recovery of the All effect was 0.74 ± 0.08 after 1 hour and 0.94 ± 0.16 after 2 hours (n = 10). These recoveries do not differ significantly from those observed after total tachyphylaxis, but the difference with the recoveries of All effect after the antagonist is highly significant (fig. 4, the left bars compared with middle bars).

These results show that the blocking effect of Sar 1-Ileu 8-AII is prevented if the mesenteric vascular preparation is previously rendered tachyphylactic to All. This is probably because angiotensin receptors, being "protected" with All, are not available for the antagonist.

**Al Conversion to All and Tachyphylaxis**

If angiotensin tachyphylaxis results from the occupancy of the receptors, crossed tachyphylaxis among Al, All, and AIII would be an indication that the three angiotensins share the same receptors. But since Al, to be active on the vascular preparation, must be converted "in situ" to All, it is possible that tachyphylaxis to All produced by Al would be due not to the occupancy of All receptors by Al but to the formation of All from Al.

To investigate this point, the conversion of Al was inhibited by the converting enzyme inhibitor, captopril. Complete inhibition of the vascular effect of Al was usually achieved after 1 hour of infusion with 200 mg of captopril/liter of infusing fluid.

In five experiments, a bolus injection of Al (20 μg/ml solution) produced an increase of 14 ± 2.5 mm Hg in the perfusion pressure. Complete tachyphylaxis to Al and All followed after three such Al injections.

One hour after the start of the captopril infusion, Al was devoid of any vasoconstrictor effect. After three injections of the Al solution and no vasoconstrictor effect, All was injected as usual; an average rise of the perfusion pressure of 9.7 ± 1.8 mm Hg was produced. Thus, when the converting enzyme was inhibited by captopril, Al was no longer able to induce tachyphylaxis to All (fig. 5).
Discussion

The hypothesis that angiotensin tachyphylaxis is due to the exhaustion of a secondary messenger has received support from some investigators but not by others. We were also unable to find involvement of catecholamines, serotonin, or prostaglandins in angiotensin tachyphylaxis induced in the mesenteric preparation.

The theory of receptor occupancy has up to now received only indirect evidence. Palaic and Lemorvan, using tritiated All, tried to find out whether the receptor sites for All were reduced during tachyphylaxis. Curiously enough, they concluded that receptor sites increased during tachyphylaxis.

The results presented here, showing that angiotensin receptors are not available for the angiotensin antagonist Sar 1-Ileu 8-AII when the mesenteric preparation is made tachyphylatic to angiotensin, gives strong support to the theory of receptor occupancy. By perfusing the mesenteric preparation with diluted angiotensin solutions, we were able to induce tachyphylaxis without any vascular effect of the agonist. It is difficult to think of the exhaustion of any mediator when the agonist is totally ineffective.

In the receptor occupancy theory, the time of recovery from tachyphylaxis would depend upon the dissociation constant of the angiotensin-receptor complex. AI and All recovered, in 30 minutes, some 50% of their initial vasoconstrictor effect, an indication that 50% of the receptors are again available for All. The recoveries of the All and AIII effects 60 minutes after tachyphylaxis do not differ significantly. The dissociation constant of the receptor-angiotensin complex appears to be of similar magnitude for both angiotensins.

The experiments with captopril show that crossed tachyphylaxis between AI and All occurs only when AI can be converted to All. This means that AI is unable by itself to block All receptors. One explanation would be that AI may have specific receptors and will not attach to All receptors. Another possibility is that both angiotensins I and II attach to a common receptor, but the complex AI-common receptor dissociates rapidly, being then unable to induce tachyphylaxis to All.

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