Tachyphylaxis to Alpha- and Beta-Angiotensin in Dogs Perfused with Ringer's Solution or Blood

By William J. Louie, M.D., and George Jerums, M.B., B.S.

ABSTRACT
Repeated injections of α-asparaginyl-angiotensin in the hindquarters of dogs perfused either with blood or with Ringer's solution did not lead to tachyphylaxis with doses of less than 0.5 μg. Above this dose tachyphylaxis developed in preparations perfused with Ringer's solution. Tachyphylaxis occurred in blood-perfused preparations at doses of 2 μg and above. Tachyphylaxis to β-aspartyl-angiotensin appeared with doses above 0.5 μg irrespective of the perfusing medium used. It is concluded that plasma angiotensinase activity plays some part in limiting the development of tachyphylaxis only to α-asparaginyl-angiotensin; also, that removal of angiotensin from the biophase is dependent in part on the angiotensin being removed by perfusion. A dynamic model for the action of angiotensin on its receptor is proposed.

ADDITIONAL KEY WORDS: enzyme activity or diffusion, removal from biophase, receptor complex

Prior to 1961 the literature on angiotensin tachyphylaxis is somewhat confusing. Page and Bumpus (1) supposed that earlier reports of tachyphylaxis to angiotensin were due to protein impurities and stated that tachyphylaxis to the synthetic octapeptide only developed during renin tachyphylaxis (2, 3). Tachyphylaxis to the octapeptide angiotensin was conclusively demonstrated by Bock and Gross (4) in the dog. This work has since been confirmed in man (5), the rat (6, 7), and the dog (8).

More recently Khairallah et al. (9), in an elegant series of experiments using spirally cut arterial and venous strips from cats, dogs, sheep, and rats, studied the development of angiotensin tachyphylaxis and its reversal. They concluded that tachyphylaxis was due to receptor occupation (10, 11) and that it could be reversed by plasma fractions rich in angiotensinase A. Furthermore, tachyphylaxis did not occur in rabbit and guinea pig arteries, perhaps because of the high concentration of tissue angiotensinase activity (9).

It does not necessarily follow that peripheral vascular beds will behave in the same manner as the aortic strip. Thus Bean (12) could not produce tachyphylaxis to angiotensin in the toad and snake, although in these species angiotensin destruction by enzymes was probably inhibited by low body temperatures (12). If plasma angiotensinase is important in preventing the development of angiotensin tachyphylaxis, it is difficult to correlate the observation that β-aspartyl-angiotensin is inactivated at a much slower rate than α-asparaginyl-angiotensin when incubated with plasma or blood with the fact that in vivo the duration and magnitude of the pressor responses of equal doses of α-asparaginyl- and β-aspartyl-angiotensin II are identical and that the two analogues exhibit cross tachyphylaxis (9, 13, 14).

This paper reports a study of the development of tachyphylaxis to α-asparaginyl- and β-aspartyl-angiotensin in the hindquarters of dogs perfused either with blood or with Ringer's solution.

Methods
Without previous medication, anesthesia was...
induced in mongrel dogs by an intravenous injection of a mixture of thiopentone (0.4 g) and sodium pentobarbitone (0.24 g) made up in 20 ml of distilled water. The dogs were intubated and connected to an automatic respirator; anesthesia was maintained with a nitrous oxide-oxygen mixture, and if necessary further small doses of the same anesthetic solution were given intravenously. The surgical technique was similar to that used by Zimmerman (15). The aorta was exposed via a left flank incision and the lower lumbar arteries tied. In some experiments blood was led from a cannula in the proximal aorta through a peristaltic pump to a cannula in the aorta proximal to the bifurcation. Heparin, 400 IU/kg, was used to prevent clotting, and the hindquarters were perfused with blood at a constant flow through the distal cannula. Because of difficulties in getting a steady baseline and to abolish the central action of angiotensin (16, 17) these animals were first treated with hexamethonium bromide, 5 mg/kg, intravenously. In other experiments, the dogs were heparinized and then rapidly exsanguinated through the cannula in the proximal aorta, the blood being collected in heparin. The animals were killed by opening the chest, and the inferior vena cava was cannulated. In some of these experiments the hindquarters were perfused at a constant flow with heparinized Ringer's solution (18) at 37°C, oxygenated with 95% oxygen and 5% carbon dioxide. The Ringer's solution was not recirculated, the effluent from the vena cava being allowed to drain into a bucket. In similar experiments in the exsanguinated dead dog, the hindquarters were perfused at a constant flow with the animal's own blood. In these experiments the venous effluent was collected into a container, oxygenated with 95% oxygen and 5% carbon dioxide and recirculated via the proximal aorta. The reservoir was supplemented if necessary with donor blood. The pH of the perfusing blood was measured at intervals throughout the course of the experiment and did not vary more than .05 pH units throughout any one experiment. The range of pH in all the blood-perfused experiments was between 7.25 and 7.35 pH units. It has previously been reported that angiotensinase activity is maximal between pH 6.5 and 8.8 (19, 20). The pump was calibrated and found to maintain a constant outflow against inflow pressures of 15—150 mm Hg. When blood was perfused, the perfusion pressure varied from animal to animal (range, 40 to 100 mm Hg). The level was approximately constant in any one experiment. When Ringer's solution was used, the perfusion pressure was much less (15 to 50 mm Hg), but this could not be appreciably increased by increasing the rate of perfusion. This is similar to the experience of Doyle (21).

The changes in peripheral resistance produced by successive injections of α-asparaginyl-angiotensin are compared at each dose level. The ratio of the height of the response to the third injection of angiotensin as compared to the height of the response to the first injection (Fig. 2) is expressed as a percentage; 100% = no change, 30% = a 70% diminution in response. Solid vertical line with cross bars = animals perfused with Ringer's solution. Dotted vertical lines = dead animals perfused with their own blood. Solid black circles in vertical line = live perfused animals. Each point represents the mean plus the standard error of the mean of seven individual experiments.
Systemic arterial pressures and perfusion pressures were recorded with a strain gauge transducer (Model P.D. 750, Statham Instruments Inc.) with a Sanborn 2-channel recorder (Model 299). Any change in perfusion pressure represented a corresponding change in vascular resistance, since blood flow through the bed was constant. Once perfusion had been established, it was maintained for 15 minutes before the first dose of drug was given. Injections of drugs were made into the rubber tubing attached to the cannula in the distal aorta. All drugs were dissolved in M/6 saline, and angiotensin infusions were given with a Harvard infusion pump. The drugs were α-asparaglinyl-angiotensin (val-n-hypertension II-asp-β-amide: Hypertensin, Ciba); tyramine hydrochloride (B.D.H.); β-norepinephrine (Levophed, Winthrop); β-aspartyl-valyl-angiotensin II (33'902 Ba, Ciba Pharmaceutical Co., Basle) and hexamethonium bromide (Vegolyse, May & Baker).

**Results**

**ALPHA-ASPARAGINYL-ANGIOTENSIN**

The results of these experiments are sum-
FIGURE 3

The responses to α-asparaginyl-angiotensin (A) before and after tachyphylaxis was induced with a dose of 5 µg. There was a gradual but not complete recovery in response over the next 45 minutes. All doses are in micrograms.

marized in Figure 1, where the change in peripheral resistance produced by the first and third injections of each dose of α-asparaginyl-angiotensin is expressed as a percentage. Part of one experiment is shown in Figure 2. Tachyphylaxis to α-asparaginyl-angiotensin did not occur with doses below 1.0 µg intra-arterially in either blood or Ringer's perfused preparations. In the two types of blood-perfused preparations, tachyphylaxis appeared at 2.0 µg and was obvious with injections of 5.0 µg. This dose level of α-asparaginyl-angiotensin was larger than that at which tachyphylaxis appeared in the Ringer's perfused preparations (Fig. 1). Thus blood perfusion appears to diminish the rate at which tachyphylaxis to α-asparaginyl-angiotensin occurs.

The tachyphylaxis which occurred with Ringer's perfused preparations was unlike that previously reported in the aortic strip, for when doses of 0.5 µg or less were used, no tachyphylaxis could be demonstrated, even after as many as 20 injections of angiotensin. Moreover, when tachyphylaxis was induced by a single large injection of angiotensin (Fig. 3), the response to angiotensin was gradually but not completely restored over the next 45 minutes.

When tachyphylaxis was induced with a large intra-arterial infusion of angiotensin (Fig. 4), the response to subsequent injections of angiotensin was abolished, but the responses to tyramine and norepinephrine were increased.

FIGURE 4

Responses to single injections of α-asparaginyl-angiotensin before and after tachyphylaxis was induced by an infusion of α-asparaginyl-angiotensin. T = tyramine; N = norepinephrine. Note that the responses to tyramine and to norepinephrine are not abolished during α-asparaginyl-angiotensin tachyphylaxis, and if anything are slightly enhanced. All doses are in micrograms except the dose of tyramine, which is in milligrams.

β-ASPARTYL-ANGIOTENSIN

The results of these experiments are summarized in Figure 5, where the change in peripheral resistance produced by the first and third injections of each dose of β-aspartyl-angiotensin is expressed as a percentage. As with α-asparaginyl-angiotensin, tachyphylaxis did not occur with doses below 1.0 µg intra-arterially in either type of preparation. In contrast to the results with α-asparaginyl-angiotensin, tachyphylaxis to β-aspartyl-angiotensin occurred at the same dose in blood and Ringer's perfused preparations, and the rate of development of tachyphylaxis in both types of preparation was also the same. The dose of β-aspartyl-angiotensin required to produce tachyphylaxis in both blood and Ringer's perfused preparations was similar to the dose of α-asparaginyl-angiotensin required to produce tachyphylaxis in preparations perfused with Ringer's solution.

In experiments in which tachyphylaxis was induced by a single large injection of β-aspartyl-angiotensin, the response to β-angio-
ANGIOTENSINASE AND ANGIOTENSIN TACHYPHYLAXIS

The changes in peripheral resistance produced by successive injections of β-aspartyl angiotensin are compared at each dose level (see Fig. 1). Dashed vertical line = animals perfused with their own blood. Other symbols are the same as in Figure 1.

The results obtained are similar to those obtained with α-asparaginyl-angiotensin (Fig. 3).

Discussion

The results presented here demonstrate that tachyphylaxis to angiotensin is a local phenomenon (4, 8, 9) probably due to receptor occupation (10, 11). The fact that during angiotensin tachyphylaxis the responses to norepinephrine and to tyramine do not diminish suggests that tachyphylaxis to angiotensin is specific and that it does not involve substantial depletion of stores of norepinephrine that can be released by tyramine. These results are not consistent with those of Distler et al. (22), but they do support the view of Khairallah et al. (9) that angiotensin tachyphylaxis cannot be reversed by adding norepinephrine to muscle baths containing aortic strips made tachyphylactic to angiotensin. Similar results with tyramine injections have previously been reported when angiotensin tachyphylaxis is produced in the intact dog (8, 23). This data suggests that angiotensin has an action not mediated via α-receptors.

The mechanism of the supersensitivity remains to be determined. Palaic and Khairallah (24) have proposed that angiotensin has a cocaine-like action that blocks the uptake of norepinephrine at the level of the membrane pump. This mechanism, however, does not explain the supersensitivity to tyramine, for cocaine also blocks the uptake of tyramine into the nerve ending, and in contrast to angiotensin, inhibits its pressor response (25). Thus far the studies demonstrating that angiotensin blocks the uptake of norepinephrine have been carried out in tissue slices in the presence of very high concentrations of angiotensin (24). If angiotensin can be shown to have the same effect at physiological concentrations in vivo, then it would seem that, unlike cocaine, angiotensin can selectively block the uptake of norepinephrine and not tyramine. Alternatively, angiotensin may have...
multiple actions at the nerve ending receptor complex.

The fact that tachyphylaxis to α-asparaginyl-angiotensin occurs at a lower dose level in preparations perfused with Ringer’s solution than in blood-perfused preparations, whereas tachyphylaxis to β-aspartyl-angiotensin is not influenced by the perfusing medium, indicates that aminopeptidases (angiotensinase A) play a role in limiting the onset of tachyphylaxis (14) to the α-asparaginyl form. These results therefore support in part those of Khairallah et al. (9), in which they demonstrated that tachyphylaxis to α-asparaginyl but not to β-aspartyl-angiotensin could be reversed by plasma fractions rich in angiotensinase A in aortic and carotid strips. If the strips were bathed in Ringer’s solution, they did not respond to more than one dose of α-asparaginyl-angiotensin, unless exposed to angiotensinases. By contrast, the results presented here demonstrate that blood vessels perfused with Ringer’s solution develop tachyphylaxis only to relatively large intra-arterial injections of α-asparaginyl-angiotensin, and even then the tachyphylaxis is incomplete.

The different results in the two systems are probably due to the fact that the isolated strip is a static system, whereas the perfused blood vessel is in a more dynamic equilibrium with the perfusing medium. In either case, α-asparaginyl-angiotensin trapped in the biophase would be removed either by enzymatic destruction or by diffusion, the latter process probably being a very slow one in the aortic strip. In the perfused blood vessel, diffusion or washing out might be expected to occur much more rapidly, so that enzymatic destruction might play a comparatively minor role in such a system except at high concentrations of α-asparaginyl-angiotensin. When an isomer relatively resistant to plasma and tissue angiotensinases is infused, the dominant mechanism for clearing the biophase appears to be perfusion. This accounts for the reported difficulty in reversing tachyphylaxis to β-aspartyl-angiotensin in the aortic strip (9).

The relative importance of enzyme activity and perfusion in clearing the biophase of free angiotensin in physiological situations is uncertain. Recently Sambhi and Barrett (26) demonstrated that, in the rat, tachyphylaxis to α-aspartyl-angiotensin, which may be the common natural form (26-30), occurs at lower doses than does tachyphylaxis to the α-asparaginyl form. Their inability to demonstrate a more clear-cut tachyphylaxis with the asparaginyl form probably reflects a failure to infuse a large enough dose (4, 8). The more rapid development of tachyphylaxis to the aspartyl form is consistent with the observation that both plasma and tissue angiotensinases have been shown to inactivate α-aspartyl-angiotensin more slowly than the synthetic amide (31), thus allowing more prolonged receptor occupation. It might be expected that tachyphylaxis to β-aspartyl angiotensin would occur with even smaller doses, as this isomer of angiotensin is even more resistant to degradation by plasma and tissue enzymes (14, 19, 31). When tachyphylaxis does occur, however, it appears to be due to occupation of a common receptor, for complete cross tachyphylaxis between the various isomers of angiotensin has been clearly demonstrated (9, 14, 26).

These data make it possible to propose the following dynamic model for the action of angiotensin on its receptor (32). The model shows the postulated sequence of reactions between angiotensin, free receptors, angiotensinase, angiotensin receptor complex, and the perfusion effect. The relative importance of each of these factors depends on the isomer of angiotensin being investigated and the condition of the study.

It has previously been suggested (8, 9, 33) that angiotensin tachyphylaxis is due to saturation of a specific receptor for angiotensin (10, 11) and that occupation of such a receptor, with a slowly dissociating angiotensin receptor complex, is likely. It appears probable that the
rate of dissociation of this complex is governed by an equilibrium between the angiotensin receptor complex and the angiotensin in the biophase and the unoccupied receptors. Factors which tend to accelerate the removal of angiotensin from the biophase, such as angiotensinase activity, perfusion, or nonspecific binding would also diminish the amount of angiotensin bound to the receptor. It seems clear that in any particular situation the development of tachyphylaxis depends on the form of angiotensin used, its rate of formation, its level in blood and tissues, and its susceptibility to enzymatic degradation.

Acknowledgments

We are indebted to Professors A. E. Doyle, R. R. H. Lovell, and M. J. Rand for their advice and encouragement, and for allowing us access to apparatus. We are also indebted to Dr. Sydney Spector for reading the manuscript.

References

Tachyphylaxis to Alpha- and Beta-Angiotensin in Dogs Perfused with Ringer's Solution or Blood

WILLIAM J. LOUIS and GEORGE JERUMS

doi: 10.1161/01.RES.22.1.75

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1968 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://circres.ahajournals.org/content/22/1/75

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation Research* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation Research* is online at:
http://circres.ahajournals.org/subscriptions/