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Unusual Venoconstrictor Effects of Angiotensin II[1,2] (38884)

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Contractile effects of angiotensin II (10^{-10}–10^{-5} *M*) on isolated veins have been demonstrated in rat, rabbit, dog, and sheep portal veins (1–7). When tested, specific antagonists of norepinephrine, acetylcholine, and histamine, i.e., phentolamine, atropine, and mepyramine, did not alter these responses (5, 6). In contrast, similar concentrations of angiotensin have been reported to elicit only small and variable contractions in canine saphenous veins (2, 7), even though this tissue responds substantially to a wide variety of other vasoactive agents including norepinephrine, tyramine, serotonin, histamine, acetylcholine, and isoproterenol (8), as well as bradykinin (9). In view of these differences, we decided to study the effects of a wider range of angiotensin concentrations on isolated canine saphenous vein.

Methods. Mongrel dogs of either sex weighing 18–25 kg were anesthetized with pentobarbital (30 mg/kg iv). Both lateral saphenous veins were excised and used immediately or stored overnight at 4° in modified Tyrode's solution. The composition of this solution (m*M*) was NaCl (125), KCl (2.7), $CaCl_2$ (1.8), $MgCl_2$ (0.5), glucose (11), Tris (23.8). The pH was adjusted to 7.4 with 6 *N* HCl. Each segment of vein was trimmed of excess connective tissue and cut into two helical strips (2.0 × 0.3 cm) according to the method of Furchgott and Bhadrakom (10). Strips were suspended in 10-ml muscle baths containing modified Tyrode's solution. The solution was bubbled with 100% O_2 and maintained at 37°. One end of each strip was attached to a force-displacement transducer for recording isometric changes in force on a polygraph. Strips were allowed to stabilize at a load of 4 g during a 1-hr equilibration period. This resting load was maintained throughout the day.

After equilibration, reference contractions were obtained by replacing the modified Tyrode's solution with an isosmotic Tyrode's solution containing 27 m*M* K^+. After a 1-hr recovery period during which strips remained undisturbed in modified Tyrode's solution, partial or complete cumulative dose-response curves (11) for angiotensin were determined. When contractile response was expressed as g/cm² or g/strip, the dose-response curve for angiotensin was skewed by large responses at high concentrations in several experiments. Normalizing the data in terms of percent (± SEM) reference contraction to 27 m*M* K^+ more accurately reflected the average response as seen in individual strips. In some experiments dose-response curves for angiotensin were repeated 2 hr after the initial exposure.

Angiotensin II (5-valine-angiotensin II amide (Hypertensin-Ciba), or 5-isoleucine-angiotensin II (Beckman, or Schwarz-Mann)) was dissolved in distilled water to make a 10^{-3} *M* (1 mg/ml) stock solution. From this, serial dilutions were prepared daily in Tyrode's solution for administration to the venous strips.

Results. Both fresh and cold-stored saphenous vein strips responded to angiotensin with great sensitivity (Figs. 1B, 4A, 4B). When compared, mean increases in tension in response to angiotensin (10^{-7} *M*) was approximately half the maximal response to norepinephrine (500 g/cm² vs 250 g/cm²). A composite of responses at all angiotensin concentrations is shown in Fig. 2. This complex curve shows the average responses of 52 strips each of which responded with

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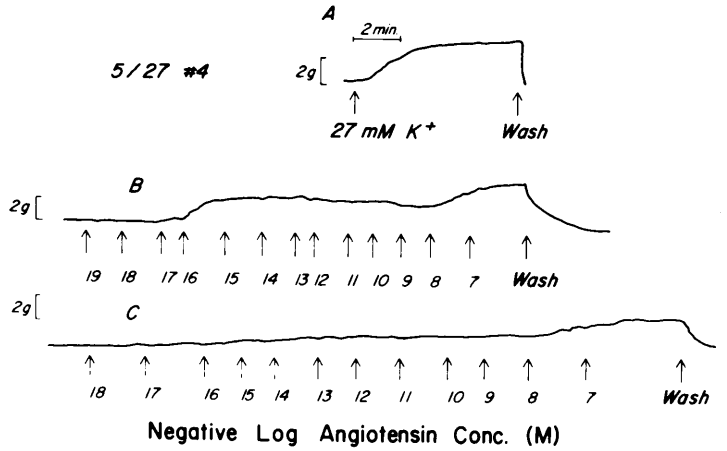

FIG. 1. Cumulative dose-response curves of a single canine lateral saphenous vein strip to angiotensin II. A. Reference contraction elicited by replacing modified Tyrode's solution (2.7 mM K^+) with 27 mM K^+ Tyrode's solution. B. 1st cumulative dose-response curve for angiotensin II. C. Repeated dose-response curve.

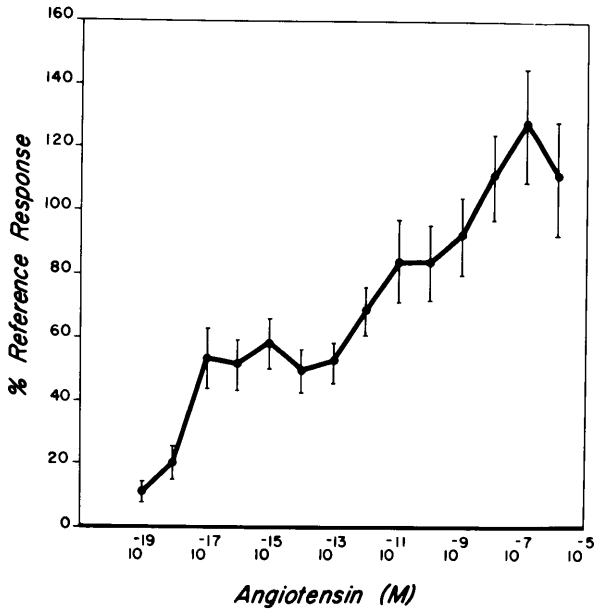

FIG. 2. Composite dose-response curve of canine lateral saphenous vein to angiotensin II. Partial and complete cumulative dose-response curves were determined in 52 helical strips from 15 dogs. Results are expressed as percentage ± SEM reference contraction elicited by 27 mM K^+.

slightly different sensitivity to angiotensin. This difference between strips can be seen in the responses to low concentrations of angiotensin shown in Figs. 1B, 4A, and 4B. Angiotensin failed to alter tone in 16 strips. These results were not included in Fig. 2. Mean reference contractions of these latter strips were less than mean reference contractions of the other strips (Table I). In strips from 14 other dogs a reference contraction to 10^{-6} M norepinephrine was obtained prior to determination of angiotensin responses. These veins did not contract during exposure to low concentrations of angiotensin. Strips from 10 of these dogs did not respond at all.

TABLE I. COMPARISON OF REFERENCE RESPONSES[a] FOR STRIPS SHOWING POOR AND GOOD RESPONSES TO ANGIOTENSIN II.

Number of strips	Reference response ($g \pm$ SEM)	Range (g)	Response to 10^{-7} M angiotensin II ($g \pm$ SEM)
16[b]	2.1 ± 0.3	0.5-4.5	0.21 ± 0.1
52[c]	4.5 ± 0.3	0.8-10.5	5.7 ± 0.4

[a] Contraction elicited by 27 mM K^+ Tyrode's solution.
[b] From five dogs.
[c] From 15 dogs.

The dose-response curve (Fig. 2) can be divided into three components. Initial large contractions were observed at concentrations of 10^{-18}–10^{-17} M. In each strip, this contraction peaked within 1–2 min, as compared to the reference response which took 4–6 min to reach a steady state (Figs. 1 and 4). After the initial rapid response, further increases in angiotensin concentration from 10^{-17}–10^{-12} M did not increase tone. During the third component of the dose-response curve, tone increased gradually over concentrations of 10^{-12}–10^{-7} M.

Contractions elicited by angiotensin at all concentrations were not well maintained. This is shown in tracing 4A in which 11 min were allowed to elapse before addition of higher concentrations of the peptide and 5 min elapsed between the peak response and washout. As shown in tracing 1B and 4A, however, more frequent addition of increasing concentrations of angiotensin (10^{-17}–10^{-12} M) while not increasing isometric force seemed to prevent the rapid desensitization.

After a 2-hr interval, as shown in Fig. 1, a second dose-response curve was characterized by a decrease in force generation at all angiotensin concentrations. In 16 strips from six dogs, response at 10^{-7} M angiotensin changed from 103 ± 11% to 66 ± 12% ($P < 0.05$).

The unusual shape of dose-response curves, spontaneous decay of angiotensin contractions, rapidly developing tachyphylaxis, and postulated interactions between angiotensin and the autonomic nervous system (12–15) suggested that angiotensin might be acting at low concentrations to release catecholamines from nerve endings within the venous strips. Two types of experiments were performed to evaluate whether these contractions could be due to norepinephrine release. Initially, strips were exposed to angiotensin until the low threshold contraction had reached a "steady state." Then, prior to addition of higher angiotensin concentrations, 10^{-8} M phentolamine was added to the bath. Since this concentration of phentolamine will inhibit the response to norepinephrine (Fig. 3) and does not alter responses to acetylcholine, observed effects were assumed to be due to α-receptor blockade. As seen in Fig. 4B, phentolamine (10^{-8} M) reduced the contraction produced by angiotensin in this strip 100%. The failure of angiotensin contractions to maintain a steady state during the interval required for phentolamine to act (Fig. 4A) made this type of experiment difficult to interpret. However, the reduction in force after addition of phentolamine was more rapid in onset and more complete than the spontaneous decay of angiotensin responses.

In another series of experiments, two strips from each of four dogs were incubated for 20 min in the presence of phentolamine

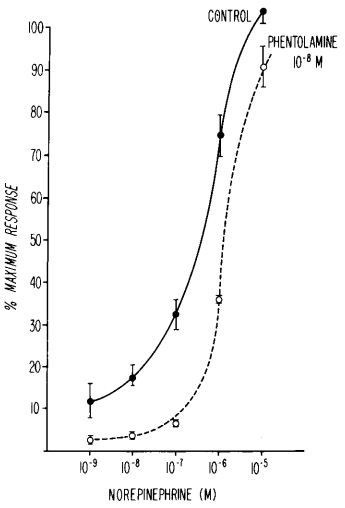

FIG. 3. Effect of phentolamine (10^{-8} M) on cumulative dose-response curve for norepinephrine. Results are expressed as percentage maximal response to norepinephrine.

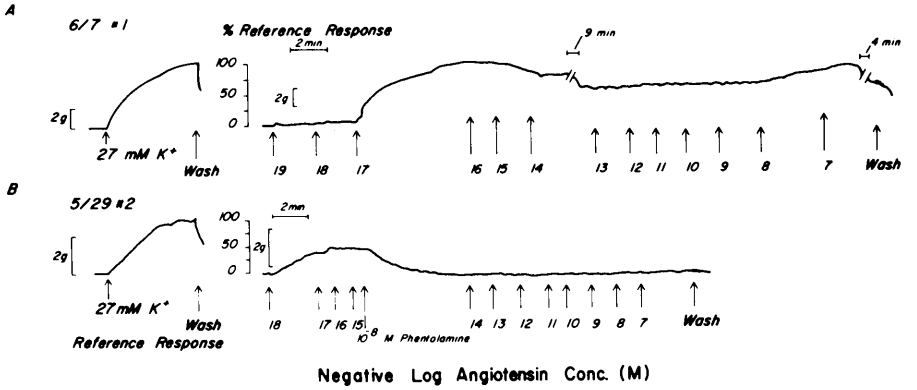

FIG. 4. Effect of 10^{-8} *M* phentolamine on contraction produced by low concentrations of angiotensin. A. Reference response to 27 m*M* K^+ followed by angiotensin II cumulative dose-response curve for that strip. After response to low concentrations of angiotinsin had stabilized, natural decline in tone was observed prior to continuation of cumulative dose-response curve. B. Reference response to 27 m*M* K^+ followed by angiotensin II cumulative dose-response curve. When no further increase in tone was elicited at low concentrations of angiotensin, 10^{-8} *M* phentolamine was added to the bath. After the evoked relaxation had reached a steady state the cumulative dose-response curve was completed.

(10^{-8} *M*). Without washout, dose-response curves for angiotensin were determined in these strips and in strips from the same dogs which had not been pretreated. Responses at each concentration of angiotensin were compared using a paired *t* test. As shown in Fig. 5, responses at all except the lowest angiotensin concentrations were significantly depressed in the presence of phentolamine. Two hours after washout, although responses of strips treated previously with phentolamine still seemed to be less than responses of control strips, these differences were not statistically significant (Fig. 6). Comparison of the responses of control strips in Figs. 5 and 6 demonstrates tachyphylaxis to repeated determinations of angiotensin dose-response curves. Responses at 10^{-16}–10^{-10} *M* were about 120% of reference in first dose-response curves and were about 60% of reference in second dose-response curves for these strips. Phentolamine-treated strips did not show tachyphylaxis.

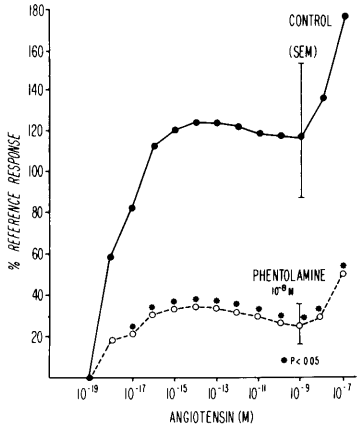

FIG. 5. Effects of phentolamine (10^{-8} *M*) on cumulative dose-response curves for angiotensin (*N* = four dogs). Control (●), phentolamine (○).

Discussion. Results of the present study show that angiotensin is capable of eliciting contractions in isolated strips of canine saphenous vein. Responses to angiotensin were variable. Not all strips from all dogs responded similarly. Contractions produced by angiotensin relaxed spontaneously. And repeated dose-response curves showed markedly reduced responses. Nevertheless, concentrations of angiotensin which produced contractions in the first portion of the dose-response curve are a factor of 100,000 less than those previously reported for other venous smooth muscle preparations (1–7), and for arterial smooth muscle preparations (7, 12, 13, 16, 17). These concentrations are also less than canine *in vivo* basal blood levels of angiotensin (about 5×10^{-10} *M*) (18).

Under the conditions used, the responses

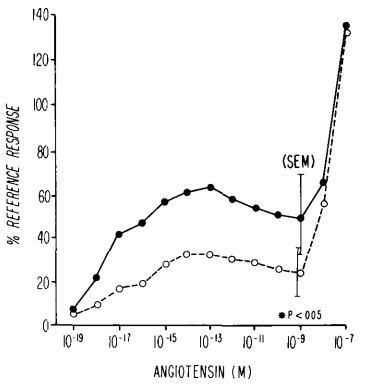

FIG. 6. Responses to angiotensin 2 hr after washout in control strips (●) and strips previously treated with phentolamine (10^{-8} *M*) (○). (*N* = four dogs).

to angiotensin showed variability both within and between dogs. This variability was manifest both qualitatively and quantitatively. Many strips did not respond (16/68) to angiotensin and increases in isometric tension at 10^{-7} *M* ranged from 86 to 545 g/cm² (mean 248 ± 30) for those strips which did respond. Differences between present responses to angiotensin and those reported by others for canine saphenous vein may be due to differences in experimental conditions and protocols (7) and methods of measurement of contractility changes (2). Additionally, these investigators examined responses in veins from a more limited sampling of the canine population than sampled in the present study.

In other isolated veins, single concentrations of angiotensin elicited dose-related contraction over a broad range of concentrations (10^{-10}–10^{-6} *M*) (5, 6). In the present study, using cumulative-dose techniques, only responses at high concentrations show a dose relationship similar to that of other veins. Responses at low concentrations, as seen in individual tracings, occur over a narrow concentration range in an all-or-none manner and attain a steady state which is maintained until higher concentrations are reached. The failure of intermediate angiotensin concentrations to increase tone may suggest that the observed steady state represents an on-going desensitization. However, comparison of cumulative responses with single exposures over the same concentration range (10^{-17}–10^{-12} *M*) showed no significant differences, implying that the observed steady-state represents a plateau in the dose-response curve. The composite dose-response curve could, therefore, be predicted if contractions elicited by high and low concentrations were additive.

Attenuation of contractions elicited by angiotensin in the presence of low concentrations of phentolamine strongly suggests an adrenergic component to the action of angiotensin on isolated canine saphenous vein. We used concentrations of the alpha blocker which caused only a 2-fold increase in ED_{50} for norepinephrine and had no effect on responses to acetylcholine. This specificity in effects on responses to other agonists supports our proposal that inhibition of the venoconstrictor response observed at low concentrations of angiotensin is due to blockade of alpha receptors. The depression, rather than the shift, of the dose-response curve which followed phentolamine suggests that a relatively constant amount of norepinephrine is being released and that continued release does not overcome the competitive blockade (11) produced by this concentration of phentolamine (Fig. 3). Phentolamine (10^{-6}–10^{-4} *M*) has also been shown to enhance norepinephrine overflow from isolated rabbit heart and dog spleen during nervous stimulation (19, 20). In the present experiments, while transmitter uptake and overflow were not determined, enhanced release would have resulted in an increased effect of angiotensin, not inhibition. An enhanced response should have been apparent, considering the minimal inhibition of responses to norepinephrine that were obtained. The studies of Greenberg *et al.* (7), made in superfused saphenous and mesenteric venous strips from reserpinized dogs, also suggest that angiotensin-induced venoconstriction may depend on intact adrenergic nerve terminals. These authors, however, did not study the extremely low concentrations of angiotensin used in the present study.

Prior exposure of strips to norepinephrine seems to alter sensitivity to angiotensin. Concentrations of norepinephrine used (10^{-6} *M*) produced a 50% maximal con-

traction which was readily terminated by washout. It has been reported that norepinephrine may, in part, produce contraction in canine saphenous vein by mobilizing intracellular Ca^{2+} (21). It may be that exposure to exogenous norepinephrine alters intracellular Ca^{2+} distribution at least temporarily, both pre- and postjunctionally, so that an angiotensin-responsive pool is depleted. The tachyphylaxis observed with angiotensin may likewise be due to depletion of Ca^{2+} from this same pool by angiotensin. Other explanations for the observed tachyphylaxis include relatively permanent binding of angiotensin to its receptor (16) and depletion of an angiotensin-sensitive pool of neurotransmitter (12, 13). Another possibility is that angiotensin stimulates production of an inhibitory substance which acts either pre- or postjunctionally to attenuate responses to angiotensin. Likely candidates for this substance are the PGE's which depress vasoconstrictor responses to angiotensin in canine hindlimbs (22) and which are released from certain organ beds by angiotensin (23). The decreased responses are not, however, due to decreases in contractile activity of the venous strip. Responses to norepinephrine, acetylcholine, and bradykinin in the same vessels, and during the same time in the bath, either do not change or increase (Goldberg, unpublished). Further experiments are needed to elucidate the mechanism of angiotensin tachyphylaxis in saphenous vein.

Prior studies of angiotensin activity at the vascular smooth muscle neuroeffector junction indicated that the peptide can act to facilitate transmitter release from the stimulated adrenergic nerve terminal. Enhanced adrenergic responses have been shown in the intact dog in the kidney (15), hindpaw (15), and saphenous vein (24) perfused at constant flow. Hughes and Roth (4) demonstrated similar effects on responses to transmural stimulation in isolated rabbit portal vein. Present results were, however, obtained in venous strips which were not being stimulated at the time of angiotensin administration, suggesting that angiotensin is acting to initiate transmitter release from nerve endings in isolated canine saphenous vein. Differences between the present findings and previous studies are shown by the marked tachyphylaxis which occurred. This is in contrast to interactions of angiotensin with stimulated nerves where tachyphylaxis to enhanced transmitter release was not observed, at the same time tachyphylaxis to direct effects was present (25, 26).

Angiotensin has been reported to release catecholamines from cat adrenal medulla and superior cervical ganglion (14). Tachyphylaxis to angiotensin in rat and rabbit aortas could be partially overcome by incubating strips in high concentrations of norepinephrine prior to retesting with angiotensin (12, 13). These studies suggested that angiotensin acted by releasing norepinephrine from sympathetic nerve endings. Contractions elicited by angiotensin in rabbit portal vein were accompanied by a small increase in ^{3}H-norepinephrine efflux, and were potentiated by veratrine and attenuated by tetrodotoxin as was the response to transmural stimulation (14). The latter is known to produce contraction in isolated veins by causing transmitter release from nerve terminals (3, 27). Our results suggest that, in canine lateral saphenous vein strips, extremely low concentrations of angiotensin may cause venoconstriction by releasing catecholamines from adrenergic nerve terminals. An action of angiotensin at such low concentrations has not been previously described.

Summary. Cumulative dose-response curves to angiotensin II were performed on helical strips from canine lateral saphenous vein. Threshold concentrations were in the range of 10^{-18}–10^{-17} *M*. Increases in angiotensin from 10^{-17}–10^{-12} *M* failed to elicit further increases in tension. Subsequent increases in angiotensin concentration from 10^{-11}–10^{-7} *M* again produced dose-related increases in tension. Repeated dose-response curves in the same strips showed reduced maximal response. Responses to low concentrations of angiotensin were attenuated by low concentrations of phentolamine. These results suggest that, at extremely low concentrations angiotensin produced marked contractions in canine saphenous vein

strips by releasing endogenous norepinephrine from sympathetic nerve terminals.

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