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Action of Tonin on the Response of Rat on Mesenteric Vessels to Norepinephrine[1] (39746)

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A recently described specific enzyme (1) called tonin forms angiotensin II directly from a natural protein, the renin tetradecapeptide substrate and also from angiotensin I. On the basis of the high specificity (2) of the enzyme and the presence in plasma of a strong protein inhibitor, it has been suggested that tonin may play an important role in the local generation of angiotensin II in tissue. The physiological role of this enzyme is still unknown. Angiotensins I and II potentiate the vasoconstrictor response to norepinephrine in some vascular beds (3-8). We report here the effect of highly purified tonin (9), as well as of angiotensins I and II, on responses of the perfused rat mesenteric beds to norepinephrine. Tonin also has vascular actions which are similar to those of angiotensin II but are not abolished by the Sar^1,Ala^8-analog of angiotensin II, an antagonist of angiotensin II.

Materials and methods. Materials used included: angiotensins I and II (Beckman, lots 33771 and 337777); Sar^1,Ala^8-angiotensin II (saralasin); nonapeptide converting-enzyme inhibitor (pGlu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro-OH) (Beckman, lot 337720); renin (hog kidney, 1 Goldblatt unit/mg, Grand Island Biological Company, lot A942458).

Isolated preparations of the superior mesenteric vascular bed from male, 200-g, Sprague-Dawley rats were prepared (10-12). The dissected mesenteric vascular bed was perfused with Krebs-bicarbonate buffer (pH 7.4) at 30° under 95% oxygen-5% carbon dioxide. The flow rate was adjusted to give a steady perfusion pressure within the range of 25-30 mm Hg, and this rate of 3-4 ml/min was then left constant for the remainder of the experiment. At intervals of 5 min, 10 ng of norepinephrine (L-arterenol bitartrate; Sigma) in 0.1 ml of the same buffer was injected into the rat mesenteric artery. This amount of norepinephrine gave about 50% of the maximal pressor response to norepinephrine. Once three pressor responses of constant amplitude (±5%) to injected norepinephrine had been obtained, the experiments were started, results being expressed as percentages of the mean initial response. The tested drugs were added to the perfusate.

Results. Angiotensins I and II in concentrations up to 1 ng/ml and 200 pg/ml, respectively, had no effect on the baseline perfusion pressure, although an increase was observed at higher concentrations. A significant potentiation of the response to norepinephrine was observed with much lower concentrations of angiotensins, the response being linear with doses from 0.32 pg to 5 ng/ml for angiotensin II and 0.32 pg to 25 ng/ml for angiotensin I (Fig. 1). Simultaneous administration of Sar^1,Ala^8-angiotensin II at 0.75 μg/ml, or the nonapeptide converting-enzyme inhibitor at 10 μg/ml, prevented the increase in the norepinephrine responses produced by angiotensins II and I, respectively (Fig. 1). Neither inhibitor showed any intrinsic effect.

Renin, which generates angiotensin I from the renin substrate, had no effect on rat mesenteric arteries in concentrations of 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, and 1 GU/ml. In contrast to renin, tonin significantly potentiated the response to norepinephrine and produced constriction of the mesen-

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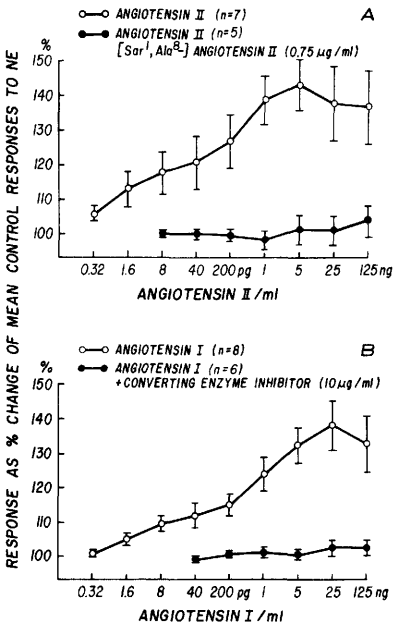

FIG. 1. Effect of angiotensin II on the pressor responses to norepinephrine. Closed circles indicate the effect of angiotensin II in the presence of ¹Sar, ⁸ Ala-angiotensin II. (B) The effect of angiotensin I on the pressor responses of the preparation to norepinephrine. Closed circles indicate the effect of angiotensin I in the presence of the converting-enzyme inhibitor. Results are expressed as percentages of the mean pressor amplitude of three control responses to norepinephrine obtained prior to the addition of these drugs. Each point represents the mean ± SEM; *n* indicates the number of experiments.

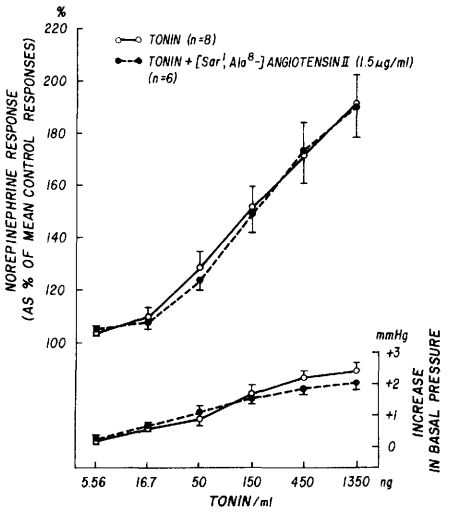

FIG. 2. Effects of tonin on the pressor responses to norepinephrine (upper) and on the basal pressure (lower). Closed circles indicate the effects of tonin in the presence of ¹Sar, ⁸Ala-angiotensin II. The pressor responses to norepinephrine are expressed as percentages of the mean pressor amplitude of three control responses obtained prior to the addition of these drugs. Each point represents the mean ± SEM; *n* indicates the number of experiments.

teric arteries, as shown by an increase in the baseline pressure (Fig. 2). In concentrations ranging from 16.7 to 1350 ng/ml the relation between the logarithm of tonin concentration and the percentage of potentiation to norepinephrine was linear.

Discussion. The abolition of angiotensin I action by a converting-enzyme inhibitor strongly suggests that the physiological activity of angiotensin I is dependent on its conversion to angiotensin II. It has been reported that converting enzyme is present in vascular tissues (13, 14) and that the concentration of angiotensin I in plasma is rather high (15). The percentage of conversion rate of angiotensin I into angiotensin II in the perfused rat mesenteric arteries, which was calculated according to the method reported by Di Salvo *et al.* (16), was between 3.3 and 14.7%. This percentage of conversion rate was in accordance with that reported in other vascular tissues (16–19). Our results confirmed the observations of Malik and Jasjletti (8) that both angiotensins I and II potentiated the vasoconstrictor response of isolated perfused rat mesenteric arteries to injected norepinephrine. However, in our experiments concentrations as low as 0.32 pg/ml of angiotensin II and 1.6 pg/ml of angiotensin I significantly potentiated the response to norepinephrine ($P < 0.05$, by Student's unpaired t test). Panisset and Bourdois (4) have also reported that, in cat mesenteric blood vessels, 1 pg/ml of angiotensin II potentiated the vasoconstrictor response to norepinephrine. These results suggest that a very slight change in concentrations of angiotensins I and II in plasma, even within the range of physiological concentrations (20), could potentiate the vasoconstrictor response to norepinephrine in some vascular beds and induce an increase in systemic blood pressure.

The converting-enzyme inhibitor, at a

concentration of 10 μg/ml, did not dispel the effects of tonin. Surprisingly, Sar[1],Ala[8]-angiotensin II also failed to abolish these effects of tonin on the mesenteric arteries. The concentration of Sar[1],Ala[8]-angiotensin II used in this experiment was 1.5 μg/ml, namely, twice the concentration that inhibits the effect of exogenous angiotensin II. Even higher concentrations of Sar[1],Ala[8]-angiotensin II (up to 24 μg/ml) did not inhibit these effects of tonin (Fig. 2).

This strongly suggests that the tonin acts on the protein substrate in endothelial cell membranes and endogenously generates angiotensin II at a site inaccessible to Sar[1],Ala[8]-angiotensin II, that is, if one assumes that the effect of tonin is in fact mediated through angiotensin II formation. It is possible that tonin itself has vasoactive effects similar to those of angiotensin II or that it generates some other vasoactive substance. The latter possibility seems unlikely for the enzyme appears highly specific.

Since the mesenteric blood vessels are consistently constricted in hypertensive animals (21), our findings suggest that tonin may play an important role in hypertension, either through the mediation of angiotensin II or independently.

Summary. Infusion into the rat mesenteric artery of tonin, an enzyme which forms angiotensin II directly from a natural protein plus the tetradecapeptide renin substrate, significantly potentiated the vasoconstrictor response to norepinephrine. This effect was not inhibited by the simultaneous administration of an inhibitor of angiotensin I-converting enzyme or by Sar[1],Ala[8]-angiotensin II. These substances block the pressor effect of the angiotensins I and II, respectively.

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