POTENTIATION OF THE CONTRACTILE RESPONSE OF ISOLATED AORTAE TO TRANSMURAL STIMULATION BY ANGIOTENSIN

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Abstract—Electrical transmural stimulation (0.3 msec duration at frequencies of 5, 20 and 100/sec) was given to spirally-cut strips of the ascending aorta from rabbits. Angiotensin ($5 \times 10^{-11}$ to $10^{-9}$ M) significantly potentiated the contractile response to transmural stimulation but did not influence the response to exogenously-applied noradrenaline. In response to angiotensin, percent increase in the duration of contraction induced by transmural stimulation was approx. proportional to that in the developed tension, whereas a prolongation of the duration by cocaine greatly exceeded the increase in the tension. The contractile response to transmural stimulation was suppressed by $5 \times 10^{-6}$ M bretylium. The inhibitory effect of bretylium was not antagonized by angiotensin when administered prior to bretylium and after the bretylium-induced inhibition had been established. Cocaine reversed the inhibition by bretylium to a potentiation. The Mg$^{++}$-induced inhibition of the response to transmural stimulation was not reversed by angiotensin but by excess Ca$^{++}$. It appears that the angiotensin-induced potentiation of the contractile response to transmural neural stimulation is due mainly to a facilitation of the release of noradrenaline and that angiotensin does not participate in Ca$^{++}$-Mg$^{++}$ interactions relating to the release of sympathetic transmitters.

Numerous studies have demonstrated that angiotensin potentiates responses to stimulation of postganglionic sympathetic nerves. This potentiation has been suggested as deriving either from an increased amount of noradrenaline released during nerve stimulation (1–3) or from an inhibition of the re-uptake of noradrenaline by adrenergic nerves (4–6). Electrical stimulation applied transmurally to arterial strips causes contraction and a sharp rise in the overflow of noradrenaline (7). Both these responses are suppressed by tetrodotoxin and bretylium. The contractile response of strips of the pulmonary artery and aorta from the rabbit to transmural stimulation is potentiated by cocaine, desipramine and pyrogallol that inhibit noradrenaline inactivation: a prolongation of the duration of contraction greatly exceeds an increase in the developed tension (8). The aim of the present study was to quantify the effect of angiotensin on the tension developed and the duration of contraction induced by electrical transmural stimulation, to compare the potentiating effect of angiotensin and cocaine on the contractile response and to investigate interactions between angiotensin and agents that inhibit the release of noradrenaline from adrenergic nerves in isolated ascending aortae from the rabbit.
MATERIALS AND METHODS

Fifty-three albino rabbits of both sexes, weighing 1.8 to 2.3 kg, were used. Under ether anesthesia the animals were sacrificed by exsanguination from common carotid arteries. The ascending aorta was isolated and spirally cut into a strip of approx. 25 x 4 mm. The specimen was fixed vertically between hooks under a resting tension of 2 g in a muscle bath (20 ml capacity) containing the nutrient solution. Hooks anchoring the upper end were connected to the lever arm of a force-displacement transducer (Nihonkoden Kogyo Co., Tokyo). The solution was maintained at 37 ± 0.5°C and aerated with a mixture of 95% O₂ and 5% CO₂. The composition of the nutrient solution was as follows (mM): Na⁺, 162.1; K⁺, 5.4; Ca²⁺, 2.2; Cl⁻, 157.0; HCO₃⁻, 14.9; dextrose, 5.6. The pH of the solution was 7.2 to 7.4. Osmotic adjustment was not made when Ca²⁺ and Mg²⁺ were added. Preparations were equilibrated for 120 to 150 min in control solutions before measurements were taken. During the equilibration period, the solution was exchanged every 20 min.

The aortic strips were placed between a pair of stimulating electrodes of platinum plate (5 x 15 mm). Gaps between the electrodes and the strip were wide enough to allow undisturbed contraction and yet sufficiently narrow to permit an effective stimulation of intramural nerve terminals (8). The preparations were stimulated by a train of 0.3 msec square pulses of supramaximal intensity (approx. 80 volts; Toda et al., 9), applied at frequencies of 5, 20 and 100/sec for periods of 40, 10 and 2 sec, respectively. Thus, the number of pulses applied was kept constant (200 pulses). Electrical stimuli were provided by an electronic stimulator (type WSE-3R, Nihonkoden Kogyo Co.).

Contractile responses to transmural neural stimulation and noradrenaline were recorded on a two-channel penwriter (8511–8–33, Sanei Sokki Co., Tokyo). From contractions induced by transmural stimulation, two parameters were measured: the maximum tension developed and the duration of contraction at the level of the half maximum tension, which will be termed "duration" in this report. Values of the parameters obtained when preparations were stimulated for 10 sec at a frequency of 20/sec were taken as control (100%) and relative values to the control are presented in the figures and the text. Noradrenaline was added directly to the nutrient solution of the muscle bath in cumulative concentrations. The tension developed by 5 x 10⁻⁶ M noradrenaline in control media was taken as 100%. After a 10 min-exposure of preparations to test solutions, the contractile response to transmural stimulation and the dose-response relationship of noradrenaline were obtained. Transmural stimulation was applied repeatedly at frequencies of 5, 20 and 100/sec until steady responses at the frequencies were attained. The results shown in the text and figures are expressed as mean values ± standard errors of the means. Statistical analyses were made using the Student's t test.

Val-angiotensin II-amide (hypertensin, Ciba-Geigy Pharmaceutical Co., Summit, N.J.), cocaine hydrochloride, bretylium tosylate and dl-noradrenaline hydrochloride were used.
RESULTS

Effects of angiotensin and cocaine on the contractile response to transmural stimulation

Electrical stimulation applied transmurally at frequencies of 5, 20 and 100/sec caused a transient, frequency-dependent increase in the tension of spirally-cut strips of the ascending aorta. Treatment of preparations with angiotensin in concentrations of \(5 \times 10^{-11}\), \(2 \times 10^{-10}\) and \(10^{-9}\) M caused an increase in the resting tension by \(0.13 \pm 0.05\) g (\(N=8\)), \(0.28 \pm 0.06\) g (\(N=14\)) and \(0.79 \pm 0.11\) g (\(N=13\)), respectively, and produced a potentiation of the contractile response to transmural stimulation in a dose-dependent manner (Fig. 1). Percent increase in the duration of contractions (11 to 20%) at a frequency of 20/sec was approx. proportional to that in the developed tension (14 to 30% at 20/sec). In contrast, a prolongation by cocaine (\(3 \times 10^{-6}\) M) of the duration greatly exceeded an increase in

![Figure 1](attachment:fig1.png)

**Fig. 1.** Modification by angiotensin and cocaine of the contractile response to transmural stimulation. • control (\(N=14\)), ○ \(5 \times 10^{-11}\) M angiotensin (\(N=8\)), × \(2 \times 10^{-10}\) M angiotensin (\(N=14\)), △ \(10^{-9}\) M angiotensin (\(N=13\)), ★ \(3 \times 10^{-6}\) M cocaine (\(N=6\)). Values obtained by transmural stimulation for 10 sec at a frequency of 20/sec were taken as 100%. Mean values of the tension and the duration of contractions at 20/sec in control media were \(0.88 \pm 0.10\) g (\(N=14\)) and \(1.00 \pm 0.04\) min (\(N=14\)), respectively. Vertical bars represent standard errors of means.
the tension (Fig. 1). Typical patterns of the potentiation by angiotensin and cocaine of the contractile response to transmural stimulation are demonstrated in Fig. 2. Further potentiation of the response was produced following the application of angiotensin in preparations where the contractile response was increased by cocaine. The dose-response curve of noradrenaline in aortic strips was not significantly altered by treatment with angiotensin ($2 \times 10^{-10}$ to $5 \times 10^{-9}$ M) (Fig. 3).

Interaction between angiotensin and agents that inhibit the release of adrenergic transmitters

Bretylium and Mg$^{++}$ were used as agents to inhibit the release of noradrenaline from adrenergic nerves which innervate the aortic wall.

Treatment with $5 \times 10^{-8}$ M bretylium did not significantly influence the resting tension but markedly inhibited the contractile response to transmural stimulation at all frequencies.
FIG. 4. Effects of angiotensin on the contractile response of strips treated with bretylium to transmural stimulation. Angiotensin was added after the bretylium-induced inhibition had been established. Mean values of the tension and the duration of contractions induced by transmural stimulation at 20/sec in control media were $0.77 \pm 0.14 \text{ g (N}=9) \text{ and } 1.12 \pm 0.12 \text{ min (N} = 9)$, respectively.

FIG. 5. Modification by bretylium and cocaine of the contractile response to transmural stimulation in angiotensin-treated preparations. Cocaine was added after the bretylium-induced inhibition had been established. Mean values of the tension and the duration of contractions induced by transmural stimulation at 20/sec in solutions containing $10^{-9} \text{ M angiotensin were } 1.13 \pm 0.14 \text{ g (N}=5) \text{ and } 1.07 \pm 0.10 \text{ min (N} = 5)$, respectively.
applied. Angiotensin in a concentration (10^{-9} \text{ M}) sufficient to induce a potentiation of the response to the stimulation in control preparations did not restore the response (Fig. 4). Further, prior treatment with angiotensin did not prevent the effect of bretylium (Fig. 5). The inhibition induced by bretylium was reversed to a potentiation by 3 \times 10^{-8} \text{ M} cocaine.

The application of 3 mM Mg^{++} decreased the resting tension by 0.20±0.03 g (N=12) and markedly reduced the angiotensin-induced contracture: mean values of an increase in the resting tension by 2 \times 10^{-10} and 10^{-9} \text{ M} angiotensin were 0.04±0.01 g (N=6) and 0.12±0.03 g (N=6), respectively, in Mg^{++}-added solutions, whereas those in control media were 0.28±0.06 g (N=14) and 0.79±0.11 g (N=13), respectively. The contractile response to transmural stimulation was inhibited by Mg^{++} (Fig. 6). The inhibition related inversely to the frequency of stimulation. Angiotensin (5 \times 10^{-11} to 10^{-9} \text{ M}) did not re-
verse the inhibitory effect of Mg++. Calcium ions (3 mM) increased the resting tension by 0.06±0.02 g (N=6) in solutions containing 3 mM Mg++. In a previous study performed under the same experimental conditions, it was observed that the application of 4.4 mM Ca++ to control media did not significantly influence the response to transmural stimulation (10). The Mg++-induced inhibition of the response however was reversed by 3 mM Ca++ (Fig. 7). Antagonism of Ca++ to Mg++ action was the greatest when preparations were stimulated transmurally at 5/sec.

Following treatment with 3 mM Mg++ the contractile response to noradrenaline was reduced: mean values of contractions induced by 10^{-7}, 5×10^{-7} and 2.5×10^{-6} M noradrenaline were 0.18±0.05 g (28% control), 1.35±0.19 g (76% control) and 2.98±0.32 g (105% control) (N=10), respectively, in the presence of 3 mM Mg++, whereas those were 0.65±0.11 g, 1.78±0.22 g and 2.85±0.28 g (N=10), respectively, in control media. This

![Fig. 7. Antagonism of Ca++ to the effect of Mg++ on the contractile response to transmural stimulation. Calcium ions were added after the Mg++-induced inhibition of the response had been established. Mean values of the tension and the duration of contractions induced by transmural stimulation at 20/sec in control media were 0.87±0.10 g (N=6) and 0.95±0.10 min (N=6), respectively.](image)
reduction was not reversed by 3 mM Ca++: mean values of contractions by $10^{-6}$, $5 \times 10^{-7}$ and $2.5 \times 10^{-6}$ M noradrenaline were $0.15 \pm 0.06$ g, $1.06 \pm 0.31$ g and $2.52 \pm 0.33$ g (N = 4), respectively, in preparations exposed to Mg** (3 mM)- and Ca+++ (3 mM)-added solutions.

**DISCUSSION**

The contractile response of aortic strips to electrical transmural stimulation was significantly potentiated by angiotensin. It has been suggested that contractions induced by transmural stimulation under conditions used in the present study result from noradrenaline released by excitation of adrenergic nerves (9). Similar potentiation of responses to sympathetic nerve stimulation by angiotensin has been observed in isolated rabbit hearts (11), isolated rabbit portal veins and coeliac arteries (3), cat mesenteric blood vessels (5) and dog saphenous veins (12). It has been suggested that the angiotensin-induced potentiation is due either to a facilitation of the release of sympathetic transmitter (1-3) or to an inhibition of the re-uptake of noradrenaline by adrenergic nerves (4-6). In the present study the patterns of potentiation by angiotensin and cocaine of the contractile response to transmural stimulation differed considerably. When the response was potentiated by angiotensin, percent increase in the duration of contractions was approx. proportional to that in the developed tension. Similar findings have been observed when aortic preparations were subjected to a longer period (15 sec at 20/sec) of transmural stimulation and have been postulated to result from an increased amount of released noradrenaline (8). In contrast, a prolongation of the duration by cocaine, desipramine and pyrogallol that inhibit processes of inactivating noradrenaline greatly exceeds an increase in the tension (8). Further, angiotensin did not potentiate the response of aortic strips to exogenously-applied noradrenaline but to sympathetic nerve stimulation. These findings support the assumption that angiotensin-induced potentiation of responses to the nerve stimulation is due to a facilitation of the release of noradrenaline when the nerve is stimulated, rather than an inhibition of inactivation of the amine.

Bretylium has been shown to depress peripheral adrenergic nerve function by preventing the release of noradrenaline (13). The inhibitory effect of bretylium on the contractile response to transmural stimulation was neither prevented nor reversed by angiotensin or excess Ca++ (14) but rather by cocaine and desipramine (8). Unlike cocaine, angiotensin shows no antagonism to bretylium.

It is suggested that Mg++ depresses responses to stimulation of postganglionic sympathetic nerves by interfering with the release of noradrenaline in the isolated rat vas deferens (15), rabbit ileum (16) and rabbit ear artery (17). Magnesium-induced inhibition of the responses to nerve stimulation is reversed by excess Ca++ (17). Findings in the present study on isolated rabbit ascending aortae confirm the above. Yet Mg++-induced inhibition of the response of aortic strips to noradrenaline was not reversed by excess Ca++. The hypothesis that Mg++ interferes with the release of noradrenaline by transmural neural stimulation, and Ca++ antagonizes this Mg++ action is thus supported. Inhibition by Mg++ of the response to transmural stimulation, however, was not antagonized by treat-
ment with angiotensin. Angiotensin does not appear to participate in Ca\textsuperscript{2+}-Mg\textsuperscript{2+} interaction relating to the release of sympathetic transmitters.

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