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Angiotensin II Responses of Rabbit Aortic Strips Compared with Fast and Slow Epinephrine Responses[1] (39837)

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Epinephrine produces a distinct biphasic contraction of a number of arteries, including rabbit aorta (1, 2). The two phases of the epinephrine contraction of isolated strips of rabbit aorta have been described as (i) an early, fast component (F), lasting 30–45 sec, and (ii) a further slow component (S), lasting up to 5–10 min (2). There is evidence that F depends on release of ionized calcium from intracellular binding sites, and S on transport of calcium into the cell from the surrounding medium (1, 3, 4).

In contrast, angiotensin II (angiotensin) produces predominantly a single-phase contraction of rabbit aorta, intermediate in rate of development between the F and S epinephrine components (1). Bohr (1) has noted a resemblance between the behavior of the epinephrine F response and that of the angiotensin response of rabbit aorta in the presence of desoxycorticosterone acetate, or of increased medium calcium levels. This similarity has not been explored further, yet is of importance, because if it were more firmly established it would suggest that the angiotensin response of this tissue depends principally on an intracellular rather than an extracellular source of calcium ions.

Our present purpose was therefore to seek definitive evidence of an analogy between angiotensin and epinephrine F responses of rabbit aortic strips by comparing their behavior during a variety of interventions which are known to have dissociated effects on the epinephrine F and S responses.

Materials and methods. Male or female New Zealand white rabbits of 2–3.5 kg weight given standard laboratory chow and water *ad libitum* were used. They were killed by a blow to the neck, and the abdominal aorta was promptly removed and placed in physiological salt solution (PSS). Aortae were used immediately or were refrigerated and used in 1–3 days.

Spirally cut 10 × 1-mm strips of aortae suspended in a bath at 37° were employed according to the technique of Furchgott and Bhadrakom (5). Strips were placed under a resting tension of 800 mg. They were attached to Grass force displacement transducers, connected in turn to a Grass polygraph. The PSS used for the bath contained 140 m*M* Na^+, 5 m*M* K^+, 1.2 m*M* Mg^{2+}, 1.6 m*M* Ca^{2+}, 150 m*M* Cl^-, and 5 m*M* dextrose. It was buffered with 5.0 m*M* Tris [(tris(hydroxymethyl)aminomethane] at a pH of 7.35, and constantly bubbled with oxygen. Strips were equilibrated for 2 h prior to experiments. Only one pressor drug was used with one batch of strips, except in two experiments in Group C (below) in one of which angiotensin was used before epinephrine, and in the other epinephrine before angiotensin, the sequence not affecting the results.

In each experiment, two to three control contractions at 20-min intervals were first obtained to either L-epinephrine or Val_5-angiotensin II amide (Hypertensin, Ciba). A dose of 10^{-6} *M* angiotensin was used, at which level the problem of diminishing aortic responses to this drug (6) was not encountered. The epinephrine dose was adjusted for each experiment to provide a tension development comparable to that obtained for angiotensin, the doses of epinephrine in fact ranging from 10^{-8} to 10^{-6} *M*. After control contractions, the following interventions were employed.

Group A. The PSS was changed for a solution of similar composition but calcium was omitted. The tissue bath was rinsed several times over a 20-min period with the calcium-free medium prior to further drug

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additions. Twelve strips were treated with epinephrine and eight with angiotensin.

Group B. The PSS was changed for a similar calcium-free solution, but containing 0.4 m*M* $BaCl_2$. As in Group A, a 20-min period with several rinses with the new medium was used prior to further drug additions. Eleven aortic strips were treated with each drug.

Group C. Plasma and blood potentiate the action of vasoconstrictors in rabbits by an unknown mechanism (7). In preliminary studies, we found rabbit serum also had this effect, together with dissociated actions on epinephrine F and S responses which made it a useful intervention in the present comparative study. Therefore, in Group C, after control studies, rabbit serum (1% by volume, 0.2 ml in 20-ml bath) was added; then a further drug response was elicited 30–50 min later. After rinsing the bath, the same serum in the same quantity was again added, and a further drug response was obtained 20–30 min later. Twelve aortic strips were treated with each drug. Three different rabbit sera were used. They were derived free of hemolysis from carotid arterial blood, refrigerated, and used within a week of collection.

Group D. Ouabain is also known to have dissociated effects on epinephrine responses, augmenting the S but not the F component (8, 9), making it useful for the present purpose. Therefore, in Group D, 15 min after control responses were obtained, ouabain was added to the 20-ml tissue bath in a volume of 0.2 ml, to yield a bath ouabain concentration of 10^{-6} *M*. Thirty seconds later, one response to epinephrine or to angiotensin was obtained. Twelve aortic strips were treated with each drug.

Group E. Since complete removal of calcium, especially tissue calcium, was not achieved in Group A or B studies, in Group E much more thorough calcium removal was attempted by incubation of the strips for 10 min in calcium-free medium containing 3 m*M* EGTA [1,2-di(2-aminoethoxy)ethane tetraacetic acid]. Following this, calcium-free medium without EGTA was restored, and epinephrine or angiotensin was added. This process was repeated with a further two to three 5-min exposures to EGTA-containing medium until virtually no response to pressor drug occurred. The medium was then replaced with one containing 0.4 m*M* $BaCl_2$ in place of $CaCl_2$, and after a 20-min incubation in this, a further drug response was elicited. Twelve aortic strips were used for each drug.

In all experiments, after restoration of the original PSS to the bath, one or two "recovery" contractions with the pressor drug in use were obtained. All epinephrine S responses were measured only after contraction plateaued.

Standard statistical methods were used (10); the SEM is included in the results.

Results. Control responses to both pressor agents were very reproducible. The coefficient of variation of duplicate control responses to epinephrine was 6.5% ($n = 25$), and to angiotensin 4.4% ($n = 25$). There was no change in contractions produced by these drugs used repeatedly over the time period of the experiments if no intervention was used. Control tension development for each drug did not differ significantly within any group. Average control tension development for all experiments was: epinephrine, 1191 ± 85 mg; angiotensin, 1113 ± 105 mg. Results obtained were as follows.

Group A. At the first stimulation after addition of calcium-free medium, the F response to epinephrine was reasonably well preserved at 58 ± 11% of control, but the S response had fallen steeply to 22 ± 4% of control (Fig. 1A). These results differed significantly from each other ($P < 0.01$) and represented significant reductions from their mean control values ($P < 0.005$ and < 0.001, respectively). The first angiotensin stimulus after addition of calcium-free medium produced 73 ± 2% of control response, which was also significantly reduced ($P < 0.001$). This value did not differ significantly from the epinephrine F response at this time, but was greater than the S response ($P < 0.001$).

In the second and third stimulations after addition of calcium-free medium with both drugs, epinephrine F and S components and the angiotensin response declined. All second and third responses during this intervention were significantly below their mean control values ($P < 0.001$) and did not differ significantly from each other.

Thirty minutes after restoration of the

normal calcium medium, the "recovery" F epinephrine and angiotensin responses did not differ significantly from their control values. The epinephrine S component remained a little depressed at 87 ± 11% of its control value ($P < 0.005$).

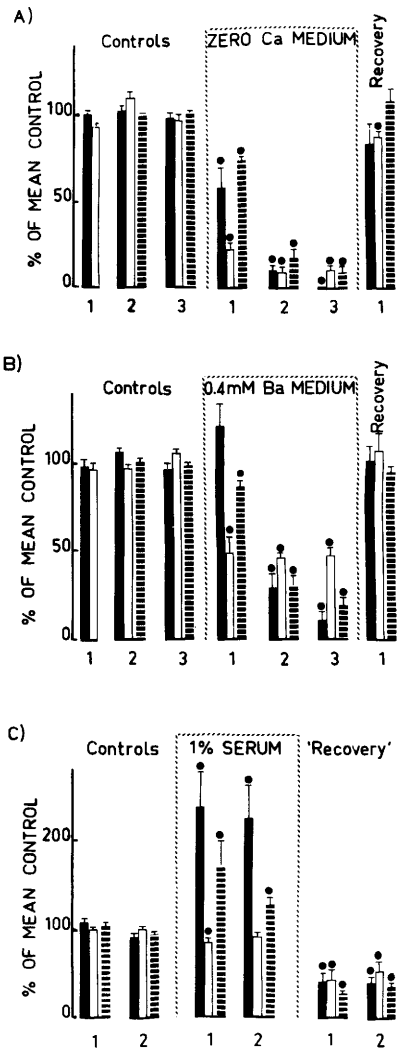

FIG. 1. Mean results in Groups A–C. The degree of response at each drug stimulus is expressed as a percentage of its relevant mean control value, thin vertical lines above each block representing the SEM. Epinephrine F responses are shown as solid black blocks, epinephrine S responses as clear blocks, and angiotensin responses as cross-hatched blocks. Responses in control periods, during the intervention indicated, and subsequently in the "recovery" phase are shown, and are numbered on the axis; a time scale is not implied. A significant change from mean control value (P at least < 0.05) is indicated by a black dot above a result. The ordinate scale for Group C is half that in Groups A and B because of the extent of the changes seen.

Group B. The first epinephrine stimulus in barium-containing medium (Fig. 1B) was associated with an average increase in the F component above the mean control level (121 ± 14%), but not to a significant degree. At this time, the angiotensin response was 87 ± 3% of its mean control level, significantly reduced ($P < 0.005$) and significantly less than the value for F ($P < 0.05$). However, it did not fall nearly as much as the epinephrine S response, which was then only 49 ± 10% of control, a significant reduction ($P < 0.005$), and also a value significantly less than either the epinephrine F ($P < 0.001$) or angiotensin ($P < 0.005$) responses.

At the second drug stimulus in the presence of barium, the epinephrine F response was 29 ± 8% and the angiotensin response 30 ± 6% of control values, and at the third stimulus these reached 11 ± 5% and 20 ± 4% of their respective control levels, all these reductions being significant (all $P < 0.001$), but at neither time were they significantly different from each other. On the other hand, epinephrine S responses on the second and third stimuli in barium were 46 ± 4% and 47 ± 5% of mean control value, respectively, the latter value being significantly greater than that for F or angiotensin at the third stimulus (both $P < 0.001$). In the "recovery" phase, all responses did not differ significantly from their control values.

Group C. Addition of rabbit serum itself caused some contraction of the strips, but the mean tension development on its first addition to the bath in the epinephrine-treated group was the same (80 mg; range 0–400 mg) as that in the angiotensin-treated group (80 mg; range 0–480 mg). This contractile effect of serum diminished on its subsequent additions to the bath.

Serum markedly augmented the epinephrine F and angiotensin responses (Fig. 1C). At the first drug addition in the presence of serum, the F response reached 237 ± 40% of mean control value ($P < 0.01$), and the angiotensin response reached 172 ± 26% of its mean control value ($P < 0.05$). These results did not differ significantly from each other. In contrast, the epinephrine S re-

sponse fell significantly to 84 ± 5% of control ($P < 0.005$) at this time, a value significantly less than the result for F ($P < 0.001$) or angiotensin ($P < 0.01$).

At the second drug stimulus in the presence of serum, the epinephrine F and angiotensin responses were still significantly elevated ($P < 0.01$ and < 0.05, respectively), while the epinephrine S contraction was still depressed, although not then significantly.

In the absence of serum in the "recovery" periods, all drug responses were markedly depressed to 40–52% of control levels ($P < 0.005$–< 0.001).

These patterns of drug response in the presence of serum were the same for both drugs used whether serum and aortic strips were derived from the same or different rabbits.

Group D. Addition of ouabain did not itself cause contraction of the strips. It also caused no significant change in the epinephrine F or angiotensin responses (Fig. 2D), but the epinephrine S response rose significantly to 126 ± 7% of its mean control value ($P < 0.005$), a result which differed significantly from both F ($P < 0.001$) and angiotensin ($P < 0.005$) responses. The latter two responses did not differ significantly from each other.

In the "recovery" phase, epinephrine S responses remained elevated, significantly so (113 ± 6% of control) in the second stimulus ($P < 0.05$). By the second "recovery" stimulus, epinephrine F and angiotensin responses were not significantly different from their control values.

Group E. The epinephrine F and angiotensin responses were obliterated by EGTA treatment prior to addition of barium-containing medium, but a small epinephrine S component (12% of control) persisted (Fig. 2E). Notably, no epinephrine F component could be seen during subsequent stimulation in barium-containing medium, but 90 ± 10% of the S response was preserved, a result not significantly below the control value. The angiotensin response in barium-containing medium was 30 ± 3% of control, a significant reduction ($P < 0.001$), and a value significantly less than the epinephrine S ($P < 0.001$) and greater than the then absent epinephrine F response ($P < 0.001$). "Recovery" contractions to both drugs did not differ significantly from their corresponding control values.

Discussion. During the first four interventions employed in this study, the alterations induced in angiotensin responses of rabbit aortic strips clearly resembled those induced in the epinephrine F component, and contrasted with the behavior of the epinephrine S response. Even in the fifth intervention (Group E), depression of angiotensin responses to 30% of control value in a barium-containing medium was more closely allied to the total depression of epinephrine F responses than to the good preservation (90%) of S responses. An analogy between angiotensin and epinephrine F responses has therefore been firmly established.

It is widely agreed that the source of calcium ions for the F component of the epinephrine response is intracellular (1, 3, 4, 11, 12). In view of this, the resemblance between angiotensin and epinephrine F responses of rabbit aorta takes on the added significance that angiotensin-induced contraction of this artery may also depend on an intracellular calcium source.

This conclusion is supported by other evidence. Angiotensin-induced contractions of rabbit aorta are at least initially resistant to inhibition by SKF 525 (13), by high concentrations of verapamil (14), and by 2 mM lanthanum (15), all of which are inhibitors of calcium movement into the cell, indicating this hormone's ability to use an intracellular calcium pool.

Notwithstanding all this evidence, the present data also show some quantitative differences between the behavior of the epinephrine F component and the angiotensin response which suggest that the dependence of the latter on an intracellular calcium source may not be complete. In this regard, in Groups B, C, and E, whilst the angiotensin responses qualitatively followed the pattern of epinephrine F responses during the interventions, quantitatively they lay between F and S (Figs. 1 and 2). These differences might imply that some part of the angiotensin response may depend on extracellular calcium.

The following comments should be made regarding the interventions used. In Group A, depression of epinephrine F and S components 20 min after bath substitution with

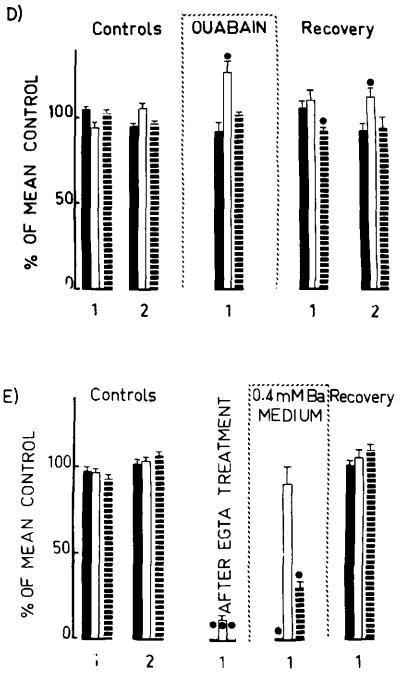

FIG. 2. Results for Groups D and E expressed with the conventions used in Fig. 1. In Group E, epinephrine and angiotensin responses were largely abolished after calcium removal by EGTA treatment prior to use of barium-containing medium.

calcium-free medium was in keeping with previous findings in rabbit aorta (1). Residual, and presumably declining tissue calcium was the probable source of support for the residual, and also declining drug responses seen while the strips remained in this medium.

In Group B, similarly declining angiotensin and epinephrine F responses during the intervention probably also reflected the falling tissue and bath calcium content. However, barium itself may have helped maintain the S epinephrine response at about half of the control levels. This concept is strengthened by results in Group E, where medium calcium was virtually absent and so unavailable to support the slow concentration with epinephrine, yet the S component stayed at 90% of control value. Therefore, barium may, in fact, have mediated a slow contraction of the rabbit aorta to both drugs.

Augmentation of epinephrine F and angiotensin responess by serum in Group C may have been due to 5-hydroxytryptamine (5HT), because (i) 5HT is abundant in serum; (ii) 5HT potentiates epinephrine (16) and angiotensin (17) responses of arteries; and (iii) depression of epinephrine responses occurs after 5HT treatment of arteries (16), as it did in the "recovery" phase of Group C. However, a role of other substances in serum cannot be excluded.

Augmentation of the epinephrine S response by ouabain in Group D was in keeping with previous findings (8, 9). This effect has been ascribed to inhibition by ouabain of sodium extrusion by smooth muscle cells in exchange for potassium, with consequent accentuation of sodium extrusion in exchange for calcium, the resultant increase in cell calcium augmenting contraction (8). Data on the effect of ouabain on angiotensin-induced contractions of rabbit aorta strips were not previously available.

We conclude from these studies that the nature of the angiotensin response of rabbit aorta bears a close resemblance to that of the epinephrine F response of this tissue, and contrasts with that of the epinephrine S response. This suggests that the principle source of activator calcium for angiotensin is intracellular in this artery. However, some quantitative differences between angiotensin and epinephrine F responses suggest that extracellular calcium may make a small contribution to the angiotensin response.

Summary. Epinephrine causes a biphasic contraction of rabbit aorta, consisting of (i) a fast component, F, ascribed to release of intracellular calcium, and (ii) a slow component, S, ascribed to calcium movement into the muscle cell. Angiotensin produces a single-phase contraction of uncertain calcium source. Insight into the latter might be gained if angiotensin contractions consistently resembled the F or S epinephrine response. Contractions of rabbit aorta strips produced by both drugs were therefore compared during five interventions which had dissociated effects on F or S epinephrine responses. These interventions consistently altered the angiotensin responses in the same way as they affected epinephrine F responses, and differently from their effects on epinephrine S responses. These results are compatible with the view that the likely major source of activator calcium for angiotensin-induced contractions of rabbit aorta

is intracellular, although a small extracellular calcium contribution could not be excluded.

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