THE CELLULAR SITE OF ACTION OF ANGIOTENSIN

JOHN B. RICHARDSON and AURÈLE BEAULNES

From the Departments of Pharmacology and Therapeutics and Pathology, McGill University, Montreal, Quebec, Canada

ABSTRACT

The site of action and the distribution of angiotensin II have been studied in the mouse. A comparison of the ratios of angiotensin-14C and inulin-3H at the time of the pressor effect reveals an extracellular pattern of distribution. Morphological studies were made using angiotensin coupled to exogenous enzymes which can be demonstrated histochemically. Coupling of angiotensin to horseradish peroxidase or cytochrome c, with glutaraldehyde or difluorodinitrodiphenylsulfone (FNPS) as the coupling agent, does not alter the pattern of its vasopressor response or that of its inactivation; nor are differences present between angiotensin and the angiotensin-enzyme complexes in the stimulation of in vitro tissue preparations. Dissociation of the complexes was shown not to occur in vitro, but the possibility of a serum factor splitting the complexes immediately after intravenous injection cannot be excluded. Since these complexes are localized on the endothelium and not on the smooth muscle at the time of maximum hypertension, the endothelium is proposed as the site of action for angiotensin.

INTRODUCTION

The vasopressor action of angiotensin is rapid in onset and short-lived. The greater part of the pressor response is thought to be due to a direct action of the polypeptide on smooth muscle cells of small arterioles (1) although actions on peripheral neuronal stores with release of catechol amines (2) and actions on the central nervous system (3) have been shown.

Angiotensin II results from the enzymatic removal of leucine and histidine from the decapeptide angiotensin I. This conversion occurs largely in the lungs (4) and possibly in the kidney (5). On circulation through peripheral vascular beds other than the lung, angiotensin II loses the greater part of its biological activity (6). Circulation through the lungs removes little if any of this activity even though bradykinin, another vasoactive polypeptide, is inactivated on passage through this organ (7). 35S-labeled angiotensin II is very rapidly cleared from the circulation, and metabolites are found in the first few minutes in both the blood and the urine (8). The speed with which angiotensin II is removed has been interpreted as indicating tissue binding or uptake rather than enzymatic degradation (8, 9). Thus, while the conversion of angiotensin I to II involves an enzymatic action, the initial biological inactivation of angiotensin II may not be enzymatic. This paper reports on the localization of angiotensin II at the onset and the termination of its action on vascular smooth muscle and in organs where inactivation has been shown to occur, using a new technique which allows for the ultrastructural localization of angiotensin.

MATERIALS AND METHODS

Isotope Studies

1-Aspartic-5-isoleucine-angiotensin II-14C with a specific activity of 236 mCi/mnmole (New England
Nuclear Corp., Boston, Mass.) and methoxy-inulin-\(^3\)H with a specific activity of 128 mCi/g (New England Nuclear Corp.) as a marker of the extracellular space, were used. A saline solution of these substances was prepared so that the specific activity of the inulin-\(^3\)H was five times that of the angiotensin-\(^14\)C. Male Swiss mice weighing 20–30 g each were given intravenous doses of angiotensin 10 \(\mu g\)/kg and inulin 100 \(\mu g\)/kg in a volume of 0.25–0.35 ml via the lateral tail vein. Three groups of five mice each were killed by decapitation 0.5, 5, and 15 min after the injection. Samples of the liver, kidney, brain, lung, heart, and thymus were collected and were placed on a Sephadex G 100 column 2.5 X 30 cm with 0.1\( \times \)Difluorodinitrophenylsulfone (FNPS) was also used as a coupling agent to make complexes. To couple angiotensin to enzymes with FNPS, 10 mg of horseradish peroxidase or 20 mg of cytochrome \(\epsilon\) were dissolved in 1 ml of 0.1\( \times \)phosphate buffer at pH 7.3.

**Enzyme Coupling**

To study the localization of angiotensin, synthetic valyl-5-angiotensin II amide (Hypertensin, Ciba Limited, Dorval, Quebec, Canada) was coupled to either horseradish peroxidase (Sigma type II, mol wt 40,000) or cytochrome \(\epsilon\) (Sigma type VI, mol wt 12,000), using a technique similar to that used for the coupling of antibodies to enzymes (10). Angiotensin was used without removal of the excipients, mannitol and thimerosal, as they did not interfere with the reaction. 2.5 mg of angiotensin plus 10 mg of horseradish peroxidase or 30 mg of cytochrome \(\epsilon\) were dissolved in 1 ml of 0.1\( \times \)phosphate buffer at pH 7.3. 50 \(\mu l\) of aqueous glutaraldehyde 1\% (Ladd Research Industries, Inc., Burlington, Vt.) were then added to the angiotensin-peroxidase solution and 100 \(\mu l\) to the angiotensin-cytochrome \(\epsilon\) solution. The glutaraldehyde was added while the flask containing the enzyme and the angiotensin was agitated. The mixture was allowed to react at room temperature for 2 hr with continuous agitation. After 2 hr the final volume was increased to 2 ml by the addition of further buffer and was dialyzed for 48 hr against a total of 12 liters of 0.001 M Tris buffered saline at pH 7.4. Difluorodinitrophenylsulfone (FNPS) was also used as a coupling agent to make complexes. To couple angiotensin to enzymes with FNPS, 10 mg of horseradish peroxidase or 20 mg of cytochrome \(\epsilon\) were dissolved in 1 ml of 0.5\( \times \)carbonate buffer at pH 10 and 200 \(\mu l\) of freshly prepared 0.5% FNPS in acetone were added. The reaction was allowed to continue overnight at 4°C and the reaction mixture was dialyzed for 40 hr as above. Control experiments were done with angiotensin and enzyme mixed without the addition of the coupling agents to assure that dialysis would separate the two.

Following the dialysis the vasopressor activity of the dialysate and of the angiotensin-enzyme complex was tested in the rat. To test the completeness of the separation by dialysis, assay tissues were surrounded by a dialysis sac in an organ bath and the complexes were added to the bath solution outside the sac. If free angiotensin is present outside the sac it will reach the assay tissue and produce a response because angiotensin, with a molecular weight of 1000, can pass freely through the sac.

Glutaraldehyde has been shown to be polymeric (11) and large complexes might form between the enzyme and the angiotensin. To determine the approximate molecular weight of the complexes, samples were placed on a Sephadex G 100 column 2.5 X 30 cm with 0.1\( \times \)phosphate buffer at pH 7.4. The protein peaks were lyophilized, reconstituted, and tested for biological activity. Similar studies were done with the FNPS coupled complexes.

To learn how many molecules of angiotensin were attached to each molecule of enzyme, a known amount of angiotensin-\(^14\)C was added to the angiotensin-enzyme solution before the addition of glutaraldehyde. A sample of this mixture was then taken for counting and a similar sample taken following the coupling reaction. Protein estimations were done using the Biuret technique (12). The efficiency of the counting was found by adding an internal standard of leucine-\(^14\)C. These experiments were done twice with both enzymes.

**Biological Activity**

Several systems were employed to test the biological activity of the complexes. The rat blood pressure assay was performed using male Sprague-Dawley rats, weighing 250–300 g, anesthetized with sodium pentobarbital 30 mg/kg intraperitoneally. Pressure was measured from a carotid artery and recorded on a Grass polygraph. All injections were made intravenously via the jugular or the femoral vein. In the early experiments every preparation of the enzyme-angiotensin complex was tested using the rat blood pressure assay, but after it was found that the activity was constant, this testing and equating with known angiotensin concentrations for each experiment was discontinued. Isolated rabbit aortic strips in 10 ml baths containing Krebs-Henseleit solution at 37°C, through which bubbled a mixture of 95% \(O_2\) and 5% \(CO_2\), were also used; contractile responses to angiotensin were isotonically recorded on a smoked drum after a 2 hr relaxation period (13). Ten different sets of complexes of both enzymes were tested on a total of 20 aortic strips. In ten experiments on aortic strips, the bath solution was replaced with mineral oil at 37°C to study the inactivation of the complexes and of angiotensin by angiotensinases (14). Three other sets of complexes were tested in three
TABLE I

DPM Ratios of Angiotensin-14C to Inulin-3H in Mouse Groups of Perfused and Superfused Organs. To test the degree of inactivation by tissues, rabbit lungs and kidneys were perfused with Krebs-Henseleit solution and the angiotensin activity was measured by superfusing an isolated rat ascending colon with the perfusate (15).

### Histochemistry

To localize the angiotensin-enzyme complex, male Swiss mice weighing 20-30 g were injected with the complex in a volume not greater than 0.6 ml, so that they received 2-5 mg of horseradish peroxidase or 10-15 mg of cytochrome c and a pressor dose equivalent to 2-20 µg/kg of angiotensin. After the injection, the mice were killed by decapitation at intervals of 0-1, 2, and 5 min. Two mice for each of the enzymes were used at each interval, giving a total of 12 mice. Samples from the kidney, liver, lung, diaphragm, adrenal, and choroid plexus were fixed in 3% 0.1 M phosphate-buffered glutaraldehyde at pH 7.3 for 3 hr and then washed overnight in the same buffer. Sections of the fixed tissues were cut at 50 µm on a Smith-Farquhar microtome and the sections were placed in a Tris-buffer solution of 3,3'-diaminobenzidine (Sigma Chemical Co., St. Louis, Mo.) for a 20 min preincubation before being placed in the final reaction medium of 3,3'-diaminobenzidine and hydrogen peroxide according to the method of Graham and Karnovsky (16). Following the reaction, the tissues were postfixed with 2% osmium tetroxide for 1 hr, then dehydrated in alcohol and embedded in Epon. A similar procedure was used for cytochrome c except that the tissues were incubated in a citrate buffer at pH 3.9 according to the method of Karnovsky and Rice (17). Tissues used in the in vitro system either were fixed and treated as above for electron microscopy or were frozen, sectioned and then fixed, and the histochemical reactions were carried out on the slide. Sections of the embedded material were cut with a Dupont diamond knife and examined in a Philips EM 300 microscope, first in an unstained condition and then, if the reaction was adequate, the sections were stained with lead citrate and uranyl acetate and reexamined.

### Results

#### Isotope Studies

The ratios of angiotensin to inulin as deduced from their DPMs are shown in Table I. In the early interval, up until 1 min after the injection, the ratios for all tissues are 1:1. The liver then accumulates more angiotensin, or its metabolites, than inulin so that at 5 min the ratio is 25.2 ± 4.05 and at 15 min it is 27.9 ± 7.53, whereas in most other tissues it is still around 1. At 5 min the brain has increased its ratio from 0.9 ± 0.15 to 2.3 ± 0.5 (p < 0.05) and at 15 min the ratio reaches 3.6 ± 0.65 (p < 0.01). At 15 min, ratios for the kidney and the lung are significantly lowered and the low ratio persists for the lung at 15 min.

#### Enzyme Coupling and Biological Activity

A typical assay of the activity of the enzyme-angiotensin complexes is seen in Fig. 1 where two different amounts of horseradish peroxidase have been coupled to the same amount of angiotensin. The ratio of angiotensin and the enzyme in the complexes could be altered by changing the proportions of the two constituents before the addition of glutaraldehyde. Thus, a balance could be achieved where there was sufficient enzyme to be histochemically demonstrable and the dose of...
angiotensin could be kept low. The onset in the elevation of the blood pressure and the duration of the response produced by the complexes are identical to those obtained with angiotensin alone. The FNPS coupled complexes were nearly as active as those produced by glutaraldehyde. In the experiments in which no coupling agent was added to the mixture of angiotensin and enzyme, the contents of the dialysis sac after dialysis had no biological activity. Fig. 2 shows the absence of free angiotensin in the complexes following dialysis for 48 hr.

The cytochrome c-angiotensin complexes coupled with glutaraldehyde came off the Sephadex column as a single peak partially with the void volume. The FNPS coupled angiotensin-cytochrome c complexes came off as a peak in a position similar to ribonuclease (mol wt 13,700). The angiotensin-horseradish peroxidase complexes made with either glutaraldehyde or FNPS came off the column in a position behind bovine albumin (mol wt 69,000). The complexes retained their biological activity after lyophilization.

Results typical of the aortic strip assay are shown in Fig. 3. Equipotent amounts of angiotensin and cytochrome c-angiotensin complex produce responses characterized by a similar onset of action. The aortic strip contracted by free angiotensin relaxes rapidly when the bath is washed out or the bath solution is substituted by oil. Such a relaxation is not seen when the aortic strip is contracted by either of the angiotensin-enzyme complexes and oil is substituted for the bath solution. If, after being in oil for 15 min the strips are washed, they relax slowly but if left in oil for 1 hr or more they do not relax when they are washed.

The perfused rabbit lungs and kidneys and the superfused rat ascending colon experiments show no detectable differences in the pattern of response to angiotensin and the angiotensin-enzyme complexes when equipotent doses are used. The kidneys remove about 50% of the biological activity of both angiotensin and the angiotensin-enzyme complexes and no detectable loss of activity occurs when either angiotensin or the complexes are perfused through the lungs (Fig. 4).

The results of the addition of angiotensin-\textsuperscript{11}C to the angiotensin-enzyme-glutaraldehyde complexes show that, for each molecule of horseradish...
Figure 2 Contractions of a portion of rat ascending colon in a 40 ml organ bath. The assay tissue is surrounded by a dialysis sac. The upper tracing shows the effect of 1 ml of cytochrome-angiotensin complex (CYTO-A), made with FNPS, equipotent to 1 ml of 10^{-6} g/ml of angiotensin added to the bath outside the sac and later when 0.5 ml is added inside the sac. The lower tracing shows the effects of 1 ml of 10^{-6} g/ml of angiotensin (ANGIO) added outside.

Figure 3 Kymograph tracings of rabbit aortic strips contracted by angiotensin, cytochrome c-angiotensin complex (CYTO-A), and horseradish peroxidase-angiotensin complex (HRP-A). The bath solution was changed to oil where indicated (oil).

peroxidase, there are one to two molecules of angiotensin and one molecule of angiotensin for each molecule of cytochrome c with some cytochrome not coupled with angiotensin.

Histochemistry

Aorta: The histochemical studies on the aortic strips exposed to the angiotensin-enzyme complexes show the reaction product at the periphery of the tissue. The reaction product is present in the endothelial region, in scattered areas at the margins, and in a few macrophages in the adventitia. Electron microscope studies of the aortic strips show the reaction product in small vesicles in the endothelial cells as well as adhering to the cell surface. Loose collections of the reaction product are also found in the subendothelial area in those strips left in oil for 1 hr or longer. In some areas where the structure of the strip shows disruption due to the dissection of the strip, no endothelium is seen but collections of the reaction product are seen within the ground substance and adhering to the elastica (Fig. 5). No reaction product is seen in any of the smooth muscle cells of the aorta, and in only a few areas is the reaction product seen in contact with the smooth muscle cell surfaces at the margin of the strips.

The histochemical findings of the in vivo studies are given for each of the organs or tissues examined over the 5 min interval.

Kidney: In the 2 min interval after the injection of either of the enzyme-angiotensin complexes, reaction product is prominent in the glomeruli where it passes through with no diffi-

Figure 4 Tracings showing the contraction of the superfused rat ascending colon. Addition of angiotensin (ANGIO) or either of the two enzyme-angiotensin complexes to the superfusate is indicated by B. Passage of angiotensin or the enzyme complexes through the kidney is indicated by K, and through the lung by L. CYTO-A, cytochrome c-angiotensin complex; HRP-A, horseradish peroxidase-angiotensin complex.
FIGURE 5  5a, A frozen section of an aortic strip contracted by cytochrome c-angiotensin complex with the histochemical reaction performed on the slide. Reaction product only on endothelium. $\times$ 250. 5b, An aortic strip contracted by angiotensin and treated similarly to that of 5a. No reaction product is seen. $\times$ 250. 5c, An electron micrograph of an aortic strip contracted by cytochrome c-angiotensin complex. Reaction product is seen beneath the endothelium and on the endothelial surface. $\times$ 14,000. 5d, a different area of the same strip showing reaction product adhering to elastic tissue. $\times$ 11,000.
culity. As early as 30 sec after the injection, the reaction product is clearly seen in the tubules, both adhering to the microvilli and within small vesicles. A marked interstitial deposition of the product is also seen and there is extensive permeation of the basal infoldings of many of the tubule cells (Fig. 6).

LIVER: Large quantities of the reaction product are found in the liver as early as 1 min after the injection of the complexes. The localization of this product is in vesicles of the sinusoidal lining cells and within the space of Disse. No intracellular reaction product is found in the hepatocytes up to 5 min after the injection.

ADRENALS: As early as 30 sec, both enzyme complexes penetrate into the interstitial spaces of the adrenal cortex (Fig. 7). The reaction product is still present 5 min after the injection and appears in small spaces between adjacent cells.

DIAPHRAGM: In the half-minute interval after the injection of the complexes there is no visible reaction product at the basal lamina of the basal lamina of the blood vessels (Fig. 8), but at 5 min after the injection there is abundant reaction product in the extracellular space (Fig. 9). Many of the blood vessels show the reaction product in endothelial vesicles both at the early intervals and later.

CHOROID PLEXUS: Vesicles containing the reaction product are seen in the cells of the choroid plexus 5 min after the injection, and some of these vesicles are seen near the villous border but none are seen to be empty into the ventricular side. The reaction product is also seen in the extracellular space surrounding the blood vessels.

LUNGS: In the interval up to 5 min after the injection, the reaction product is seen in the vessels. There is also some uptake into the endothelial vesicles and passage through the endothelial, but not the epithelial, junctions.

DISCUSSION

Intravenous inulin rapidly moves from the plasma into the extracellular space where it reaches a peak concentration 5–10 min after the injection (18), and it has been shown to be extracellular in such tissues as the cat cervical ganglion 2 min after an injection (19). If a substance has a 1:1 ratio to inulin, it may be interpreted as meaning that it has a tissue distribution similar to that of inulin. The reduction of the ratio in the kidney at 5 min is most likely due to the high clearance of inulin in relation to the amount of angiotensin present. The statistically significant low ratios in the lung at 5–15 min are consistent with the known inability of the lung to remove angiotensin on passage through its vascular bed.

From these isotope studies several conclusions can be made. In the early stages of its action, when the pressor effect is maximal and at the time period when it is being inactivated or removed from the circulation, angiotensin is extracellular and at the cell surface. This indicates that the site of the pressor action at least, and of the inactivation or removal of angiotensin, is at the plasma membrane of either the endothelial or the smooth muscle cells. Since inulin has not been shown to pass into the extracellular space of the brain at this early stage, our findings for the 1 min interval indicate that angiotensin is still outside the blood brain barrier but, at a later time, it does gain access to the brain substance. The gradual increase in the ratio may indicate passage either by way of the blood vessels or by the choroid plexus.

The angiotensin-enzyme complexes have been shown to be strongly linked by glutaraldehyde and FNPS, and that, at the most, two molecules of angiotensin are coupled to each molecule of enzyme. The coupling procedures reduce the vasopressor response to angiotensin but do not alter the pattern of this response in terms of either time of onset or duration of action. The rate of inactivation of the complex by perfusion through the kidney and the lung is identical to that of angiotensin alone.

The substitution of oil for the aqueous phase of an organ bath removes all of the pharmacological substance from the bath except that amount which is in, or on, the assay tissue and prevents this latter amount from leaving the tissue (14). If the tissue can inactivate the pharmacological substance by metabolic means, or if the substance can diffuse into the tissue and thus leave the site of its action, it will cease to stimulate the tissue (14). This has been illustrated by the action of noradrenaline on aortic strips. If a strip is first contracted by noradrenaline in an aqueous bath and the aqueous solution is then replaced with oil, the contracted strip will relax because the noradrenaline is either metabolized or diffuses away from its site of action. The situation is similar for angiotensin. An aortic strip contracted by the action of angiotensin will relax when the aqueous solution is replaced by oil (14), because free angiotensin can diffuse into the
FIGURE 6  Kidney cortex 30 sec after an injection of HRP-A complex. Reaction product is seen in the interstitium (1) and within the basal infoldings (2). × 17,160.
Figure 7  Adrenal cortex 30 sec after an injection of HRP-A complex. There is reaction product in the interstitium and just starting to penetrate between the cells (arrow). $\times$ 19,000.
Figure 8  A capillary in the diaphragm 30 sec after an injection of HRP-A complex. The reaction product is present in the lumen, within luminal endothelial vesicles (1), and just starting to penetrate the endothelial junction (2). A similar distribution is found in arterioles and arteries. $\times$ 27,000.
Figure 9 A capillary in the diaphragm 5 min after an injection of HRP-A complex. There is reaction product in the lumen, in endothelial vesicles, and in the interstitium (arrow). × 34,000.
tissue. The interpretation of the sustained contraction found when the aortic strips are stimulated by the angiotensin-enzyme complexes and the aqueous solution is replaced by oil is as follows: the complexes are too large to diffuse into the tissue and thus they remain at their site of action and continue to stimulate. If free angiotensin was present, or if the tissue was dissociating the complexes and producing free angiotensin, the strips would be expected to relax as the free angiotensin was either metabolized or diffused away from its site of action. The sustained contraction of the aortic strip contracted by the angiotensin-enzyme complexes demonstrates that free angiotensin is not present, nor is it produced by an action of the tissue on the complexes.

The aortic strip experiments, in conjunction with the experiments in which the assay tissue was protected by a dialysis sac, show that free angiotensin is neither present in the complexes nor produced by the tissue acting on the complexes. It is possible, however, that upon intravenous injection of the complexes a serum factor splits the complex and the vasopressor response seen is due to free angiotensin. If this was the case, complete dissociation would have to occur within 5 sec after the intravenous injection, since this is the average period, including circulation through the lungs, between injection and initiation of the vasopressor response. The dissociation must be complete because equipressor amounts of the complexes are also equal in the stimulation of in vitro assay systems, and dissociation can be shown not to occur in these systems. Furthermore, no difference in the onset or in the pattern of the vasopressor response between the complexes and angiotensin was found. A difference would be expected if the complexes had to be dissociated before they could act. From these considerations, dissociation of the complexes is thought to be unlikely.

The distribution of horseradish peroxidase after an intravenous injection has been studied in the mouse (16, 20-25). These studies have been done at varying intervals and have included nearly all organs. Karnovsky has shown that the endothelial junctions of the continuous type endothelium most likely represent the small pore system which has been proposed from physiological studies (20). This system applies to lipid insoluble substances with a low molecular weight and a radius less than 40-45 Å. Horseradish peroxidase has a radius of 25 Å, its molecular weight is 40,000, and it would be expected to just fit a pore system (20). In vessels where there are zonulae occludentes such as in the brain, no passage of the enzyme is seen between the endothelial cells (24). In Karnovsky's studies on cardiac and skeletal muscle the capillaries showed the histochemical reaction product of horseradish peroxidase in the lumen of the vessels, in endothelial vesicles, and at the beginning of the intercellular junctions, 1-5 min after the injection of the peroxidase (20). After 5 min there was reaction product at the basal lamina, and some penetration into the T system (20). In skeletal muscle the penetration to the basal lamina was thought to be slower than in the cardiac muscle capillaries (20). Although, in theory, transport across the endothelium of a marker such as horseradish peroxidase could occur in less than 1 sec, this has not been morphologically demonstrated (26, 27). Retention of injected proteins such as horseradish peroxidase does not occur in organs which have fenestrated capillaries. In these organs there is a rapid passage of the enzyme into the extracellular space. In the adrenal and the kidney the reaction product is seen throughout the extracellular space 30 sec after the injection. In the pancreatic islets there is an accumulation 40 sec after an injection (22), and a similar rapid accumulation is seen in the intestine (25).

The angiotensin-enzyme complexes of both horseradish peroxidase and cytochrome c were found to have a distribution like that of the pure horseradish peroxidase at the times studied and there did not appear to be any alteration in the vessel permeability to the complexes, although elevations in blood pressure do produce changes in vascular permeability (28). The similarity in distribution of the cytochrome c and the horseradish peroxidase complexes is likely due to the large molecular weight of the former produced by polymerization with glutaraldehyde.

The detailed anatomy of the microvasculature, small arterioles, precapillary sphincters, arteriole capillaries, and venules has been recently described using a new technique which permits the examination of the vessels in longitudinal section before selection of smaller sites for detailed examination (29, 30). It was possible to demonstrate the detailed anatomy of these vessels at points where their gross structure changes. Several findings of these studies are relevant to this discussion. The small arterioles (less than 50 µ) and the precapillary...
sphincters are shown to have numerous myo-endothelial connections (Fig. 10) of a tight junction type (29). Similar connections also exist between endothelial cells and pericytes (31), as well as in larger arterioles and arteries where they penetrate the elastic interna (32). Rhodin (29) has suggested that the endothelial cells act as receptors for humoral substances. He suggests that they may allow these substances to pass through to the smooth muscle cells or may transmit some signal by means of the myoendothelial connections to the smooth muscle cell. Capillary endothelium has also been shown to be the site of action for other vasoactive substances where endothelial contraction has been demonstrated (33).

The localization of the angiotensin-enzyme complexes on the endothelium and not on the smooth muscle during the vasopressor response is compatible with the hypothesis that receptor sites are localized on the surface of endothelial cells. This hypothesis is also supported by the observation that aortic strips contract when exposed to the angiotensin-enzyme complexes in vitro and that the histochemical reaction product of these complexes is found in the region of the endothelium and not on the smooth muscle cells.

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Richardson and Beaulnes The Cellular Site of Action of Angiotensin 431
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