Recent observations have shown, that besides alpha-adrenergic mediators, carbachol, a cholinomimetic agent can, at low concentrations activate the kallikrein system of rat whole blood in vivo or in vitro. These observations strengthen the view that kinins may be accessories in the control of vascular tone.

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FURTHER EVIDENCE ON THE SUBCELLULAR SITES OF KININASE II (ANGIOTENSIN CONVERTING ENZYME)

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There is abundant indirect evidence indicating that bradykinin and angiotensin I are metabolized by enzymes situated along the luminal surface of pulmonary endothelial cells. Recently, it has become evident that a single lung enzyme (kininase II or angiotensin converting enzyme) is capable of metabolizing bradykinin and angiotensin I to yield metabolic products like those produced by intact lungs. The enzyme was purified to homogeneity and antibodies were prepared against the enzyme. The antibodies, purified by $(NH_4)_2\ SO_4$ precipitation and chromatography on DEAE-cellulose, were conjugated to microperoxidase (11-MP, a heme-undecapeptide of cytochrome c) or to its lower homolog, 8-MP, using glutaraldehyde or a bifunctional active ester. The antibody coniugates were further purified by chromatography on Bio-Gel P-300 and on DEAE-cellulose. The latter step was particularly efficacious for the separation of the free antibody from conjugated antibody. Further purification was obtainable by immunoabsorption using kininase II covalently bound to glass beads. The purified antibody conjugates retained both immunoreactivity and enzymic activity: The conjugates inhibited the hydrolysis of Hip-His-Leu by kininase II and were capable of oxidizing

diaminobenzidine in the presence of H_2O_2. Blocks of fixed lung tissue incubated with the antibody-marker conjugates and then reacted with H_2O_2 and 3, 3'-diaminobenzidine showed deposition of oxidized diaminobenzidine along the luminal surface of endothelial cells, especially those of capillaries and venules. These results indicate that an enzyme capable of inactivating bradykinin and of converting angiotensin I to angiotensin II exists on the luminal surface of pulmonary endothelial cells. As suggested previously, the ability of a single enzyme, thus situated, to eliminate a hypotensive agent while forming a hypertensive agent may have implications for blood pressure homeostasis. (Supported by the U.S.P.H.S. (HL 15691, HL 16407, contract NO1 HR3-3015), the John A. Hartford Foundation, The Council for Tobacco Research-U.S.A., Inc., the Veterans Administration (Project no. 7963-01), and by an Established Investigatorship award to Dr. Una S. Ryan from the American Heart Association).

KININASE II (ANGIOTENSIN CONVERTING ENZYME) AND ENDOTHELIAL CELLS IN CULTURE

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In addition to our studies of the subcellular sites of kininase II using lung tissue, we have examined pulmonary endothelial cells grown in culture. Endothelial cells of the pulmonary artery were harvested by two methods: Either a pure monolayer of endothelium was collected on cellulose acetate paper or endothelial cells were washed from the artery with 0.2% collagenase. Using either method, the cells divide and can be maintained in culture for at least 21 days. The action of collagenase was stopped by addition of fetal calf serum and by repeated washings. Prior to assay, cultures were washed 3x with medium containing no fetal calf serum, a possible source of kininase activity. In the EM the cells are characterized by the same features which obtain in pulmonary artery endothelium **in situ**, e.g. caveolae, tight junctions, projections, filaments and lipid droplets.
Weibel-Palade bodies occur but are not numerous. The cells have also been investigated by freeze-fracturing to reveal properties of the plasma membranes and junctions which cannot be ascertained by other techniques. In these respects, the cells resemble pulmonary endothelial cells **in situ**. Kininase II occurs in abundance: Culture flasks containing 10^4 to 10^5 cells degraded 125I-Tyr8-bradykinin at a rate of approx. 1%/min. The initial reaction product was 125I-Tyr-Arg, the product produced by purified angiotensin converting