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

enzyme. The reaction rates were consistent with that expected of intact lungs: Human lungs have been estimated to contain more than 10^{11} capillary endothelial cells. Kininase II was localized along the plasma membrane of the endothelial cells in culture by immunocytochemistry and immunofluorescence using both direct and indirect techniques. The cells, reacted with unconjugated antibody (goat) to kininase II and then reacted with fluorescent conjugates of rabbit anti-goat ± globulin, became fluorescent. In the EM, reaction product (oxidized diaminobenzidine) was found along the plasma membrane and caveola membranes. In view of the apparent localization of kininase II along the plasma membrane and the large molecular size of kininase II, it may become feasible to visualize the enzyme itself in situ by freeze-etching. (Supported by the U.S.P.H.S. (HL 15691, HL 16407, contract NO1 HR3-3015), the John A. Hartford Foundation, and The Council for Tobacco Research-U.S.A., Inc.).

CARDIOVASCULAR AND RESPIRATORY REFLEXES ELICITED BY BRADYKININ ACTING ON RECEPTOR SITES (K & P) IN THE MUSCULAR CIRCULATORY AREA

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The injection of microdoses of bradykinin into the femoral artery of conscious rabbits causes two types of reflex effects displayed in succession. The first group (first-type effects) appeared earlier and were prevalently of inhibitory nature, represented by a fall in arterial pressure, peripheral vasodilatation, bradycardia and hypertachypnea; the second group (second-type effects) appeared later, were of excitatory nature typical of the "alarm reaction" and consisted of a rise in arterial pressure, peripheral vasoconstriction, tachycardia, hypertachypnea, and behavioural excitation. The first type effects were obtained with the minimal amounts of substance; increasing the doses caused the appearance of the second-type effects. Hypertonic solutions (sodium, chloride, glucose) and acid solutions (pH 6) determine only second-type responses. Sectioning the somatic nerves of the limb (femoral and sciatic) abolishes any type of response. General anesthesia eliminates the second-type responses and potentiates first-type responses. Both type of responses are not at all affected by removing the skin of the limb. The reflex manifestations elicited by the injection of bradykinin into the femoral artery seem to be due to the activation of two distinct groups of chemosensitive receptors. Those