tation antigens (*9*). At face value, the resemblance between Lp(a) lipoprotein of human serum and murine cellular histocompatibility antigen appears to be even greater than the similarity between transplantation antigens of man and mouse.

Within the limitations of the methods, I conclude that the results of the analyses support the concept (*1*) that a relation exists between lipoproteins in the serum and histocompatibility antigens on cell membranes.

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Angiotensin II: Rapid Localization in Nuclei of Smooth and Cardiac Muscle

Abstract. *Five to ten nanograms of labeled angiotensin II rapidly injected in the left ventricle of adult rats was found to induce significant ultrastructural endothelial changes, resulting in net increases in number and size of pinocytotic vesicles as well as widening of intercellular spaces. This effect was followed by preferential localization of the compound in the nuclear zone of vascular and cardiac muscle cells. The selective cellular localization of angiotensin II suggests that this vasoactive agent or some of its metabolic fragments may have specific effects on nuclear function.*

The exact mechanism of action of the octapeptide angiotensin II at vascular cell level is not known. One of the current hypotheses is that this potent vasoactive agent binds to specific receptor sites on the muscle cell membranes (*1*), altering membrane permeability to several ions, thus leading to a physiological response (*2*). As part of studies on the effects of this and other vasoactive agents on vascular permeability, as well as on the initiation of atherosclerosis, we have investigated the short-term distribution of angiotensin II on endothelial and muscle components of the arterial wall and cardiac muscle.

Fasting adult Wistar rats weighing between 200 and 220 g were anesthetized with ether, and a midline thoracotomy was made without delay. The heart was exposed and an 18-gauge needle was rapidly inserted into the left ventricle, avoiding the interventricular septum. A bolus of either 5 to 10 ng of cold angiotensin II [Hypertensin (Ciba)], or [isoleucyl-^{14}C]angiotensin II (*3*), or [tyrosyl-^{3}H]angiotensin II (*4*), diluted in 1 ml of Ringer's solution, was injected within 8 to 12 seconds through a three-way stopcock, followed by 1 to 2 ml of Ringer's solution and immediately thereafter by injection of 1 to 5 ml of a 1 percent solution of phosphate-buffered glutaraldehyde until induction of cardiac arrest. Average total perfusion times were 32.5 seconds (24 to 48 seconds). Slight tachycardia usually developed after injection of angiotensin II, and when approximately half the volume of fixative solution was injected, left ventricular contraction ceased, while the right ventricle continued contracting for another 30 to 40 seconds. Similar injection techniques were used for control studies with 50 μc of L-[^{3}H]tyrosine or 1-[^{3}H]isoleucine.

Immediately following this in vivo fixation, matching specimens (approximately 1 mm^3 in size) of the left and right ventricles, as well as full thickness specimens of the right and left coronary arteries and ascending portions of the arch of the aorta and thoracic aorta, were further fixed in 1 percent phosphate-buffered glutaraldehyde at 4°C for 30 minutes. One half of these samples was then processed for light microscopy–autoradiography following embedding in paraffin, and the other half was postfixed in buffered osmium tetroxide at 4°C for another 30 minutes, dehydrated in ascending concentrations of ethanol, and embedded in Epon resin for electron microscopy. For light microscopy–autoradiography, sections 6-μm thick were coated with diluted L-4 emulsion (Ilford) in a 0.01 percent solution of sodium lauryl sulfate and exposed in the dark at 4°C for 15 to 30 days. Sections 1 μm thick of Epon-embedded samples were stained with toluidine blue for orientation, and selected blocks were cut at a thickness of 300 to 600 Å in an LKB ultratome and stained with uranyl acetate–lead citrate. Sections mounted in Formvar-coated grids were also coated with L-4 emulsion and exposed in the dark at 4°C for periods of 60 to 140 days.

The injection of angiotensin II at the above concentrations failed to show any cytological changes by routine light microscopy. Electron microscopy, however, showed considerable increase in the number of pinocytotic vesicles in endothelial cells of both aorta and coronary arteries (Fig. 1a) with widening of intercellular gaps and occasional separation of desmosomes. Constantinides and Robinson (*5*) have previously reported that angiotensin II widens the interendothelial junctions by causing contraction of endothelial cells in superficial femoral arteries. However, in their study they used a much higher concentration of the peptide (66 μg/ml). Furthermore, in our studies, autoradiography consistently showed presence of radioactivity in the nuclear zone of endothelial cells, as well as in nuclei of smooth muscle cells of the aortic media and mitochondria of cardiac muscle. In contrast, little or no radioactivity could be shown in other cell organelles. In cardiac muscle, the label was not present in all nuclei and seemed to predominate in some muscle bundles more than in others, with higher radioactivity usually localized closer to blood vessels (Fig. 1b). Endothelial nuclei of intraparenchymal coronary branches, as well as surrounding pericapillary connective tissue, were often labeled. In order to demonstrate whether the intact peptide or individual amino

acids metabolically derived from angiotensin localized in the nuclear zone, 50 μc of either tritiated L-tyrosine or isoleucine was injected. Autoradiography failed to show, in the short time of the injections, evidence of the passage of these amino acids beyond the endothelial layers of aorta or coronary arteries or in intact cardiac muscle.

The above findings indicate that transport of angiotensin II across the endothelial "barrier" and into smooth muscle cells of the media of arteries seems to be extremely rapid and that, once in the vascular wall itself or in the extracapillary space in the case of the cardiac muscle, radioactivity is localized predominately in the nuclear area of these cells. This selective localization suggests that either angiotensin II or some of the rapidly generated metabolic fragments may have an action on nuclear function. Although the exact nature of the peptide localized on the nucleus is not known, the rapidity of the effect most likely excludes extensive degradation. This does not necessarily mean, however, that a hepta- or hexapeptide fragment could not be the labeled material localized in the nuclear area. Since angiotensin II is known to have many actions other than inducing smooth muscle contraction (*6*), it is proposed that one of the indirect cellular effects of this peptide may be that of altering nuclear function through synthesis of nucleotides. This in turn, particularly through synthesis of messenger RNA, could affect other biosynthetic cell mechanisms, especially those related to protein synthesis. Preliminary results in our laboratory indicate that angiotensin II stimulates both DNA and RNA synthesis in isolated rat auricles and that when the peptide is injected into rats that had received prior treatment with [^{3}H]uridine there is an increased specific activity of RNA isolated from spleen, heart, kidney, brain, and liver. These findings further emphasize the rather unusual characteristics of angiotensin II not only as a vasoactive agent but also as a factor regulating vascular transport and metabolism at the cellular level.

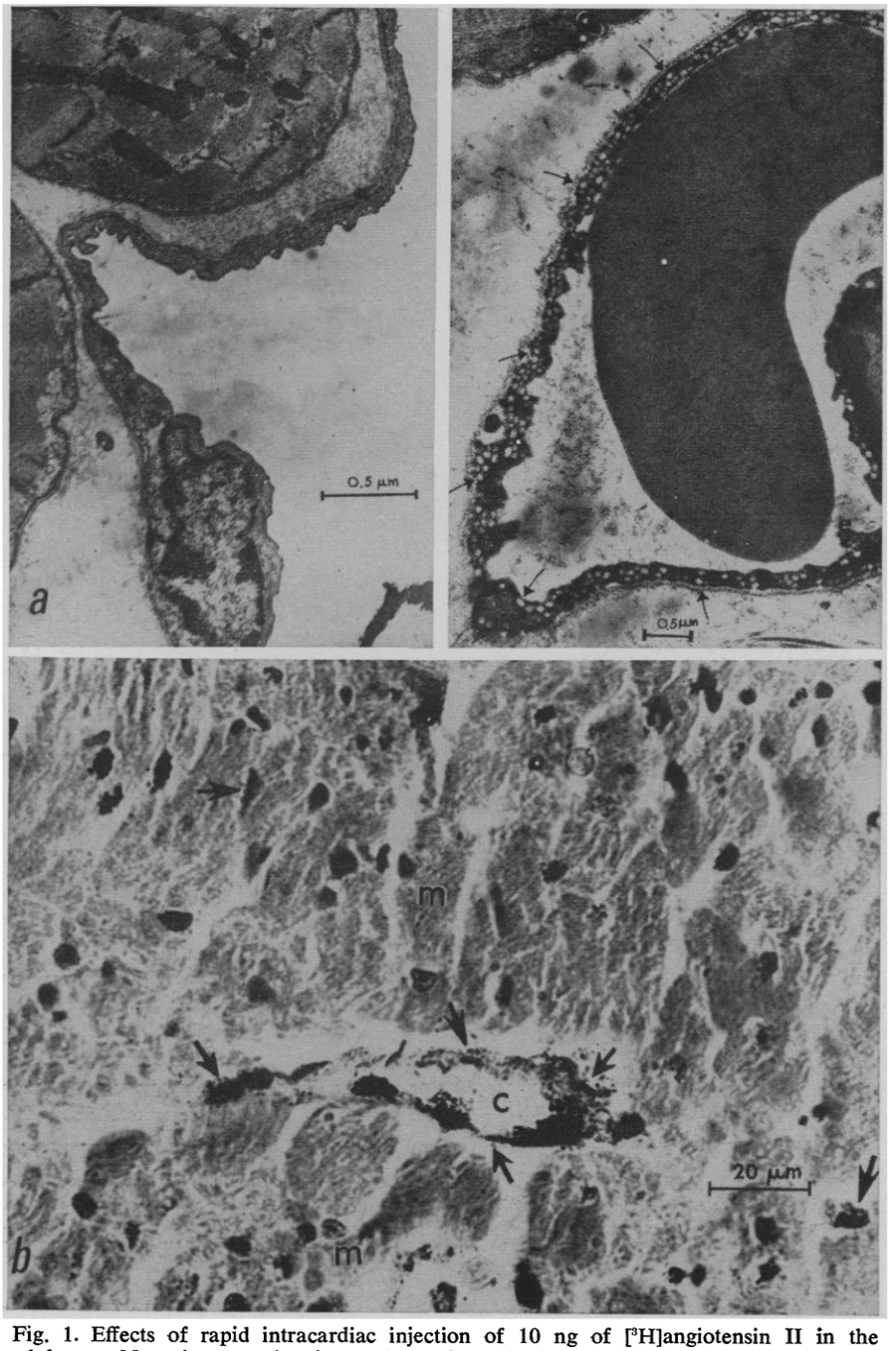

Fig. 1. Effects of rapid intracardiac injection of 10 ng of [^{3}H]angiotensin II in the adult rat. Note increase in size and number of pinocytotic vesicles (arrows). (a) Electron micrograph of myocardial capillary vessels. (Left) Control injected with Ringer's solution; (right) 31 seconds after injection of angiotensin II. Uranyl acetate–lead citrate staining. (b) Autoradiograph of papillary muscle of the heart left ventricle showing localization of labeled angiotensin II in nuclei and cytoplasm of endothelial cells of myocardial capillary (*c*), as well as in nuclei of surrounding cardiac muscle fibers (*m*).

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