Renin Secretion: An Anatomical Basis for Tubular Control

Abstract. *By means of electron microscopy of serial sections and three-dimensional reconstruction of the juxtaglomerular apparatus a quantitative study has been made of the distribution of the areas of contact between the tubular and vascular components. Of the two arterioles the efferent is the only one consistently in contact with the distal tubule. The extraglomerular mesangium is also in contact with the distal tubule in all the apparatuses examined. Two morphologically distinct types of contact are described; one is thought to be permanent and the other reversible. An analysis of the cells of the juxtaglomerular apparatus for their position, granularity, and contact has revealed that the majority of granular cells are not in contact with the distal tubule. The anatomical findings are integrated in a model of the control of renin secretion based on variations in contact between the elements of the juxtaglomerular apparatus.*

An anatomical description of the special relationship between the distal tubule and the glomerular arteriole preceded good physiological evidence of its function by almost half a century (*1*, *2*). In recent years good evidence has been obtained that the granular cells (JG cells) are actually the source of renin (*3*) and that this region, the juxtaglomerular apparatus, is the site of a feedback between the distal tubule and the arterioles which controls the retention of sodium by the kidney (*4*, *5*). Some confusion, however, still remains over the precise structure of the apparatus because (i) the basement membranes and cell processes involved in the contact areas are beyond the resolution of the light microscope and (ii) the anatomical complexity of the JG apparatus is such that the most logical approach to studying the relationship between its components is by three-dimensional reconstruction. Since interpretation of current physiological data rests heavily on the anatomical evidence of contact between the granular cells of the vascular component and the distal tubule, a three-dimensional study of the JG apparatus including the areas of contact between the tubular and vascular components has been made by means of electron microscopy of serial sections. This note deals mainly with the physiological implications of the findings reported in an extensively illustrated paper on the three-dimensional reconstruction of the JG apparatus (*2*) and is supplemented with more recent information on the cell distribution by granularity and distal tubular contact.

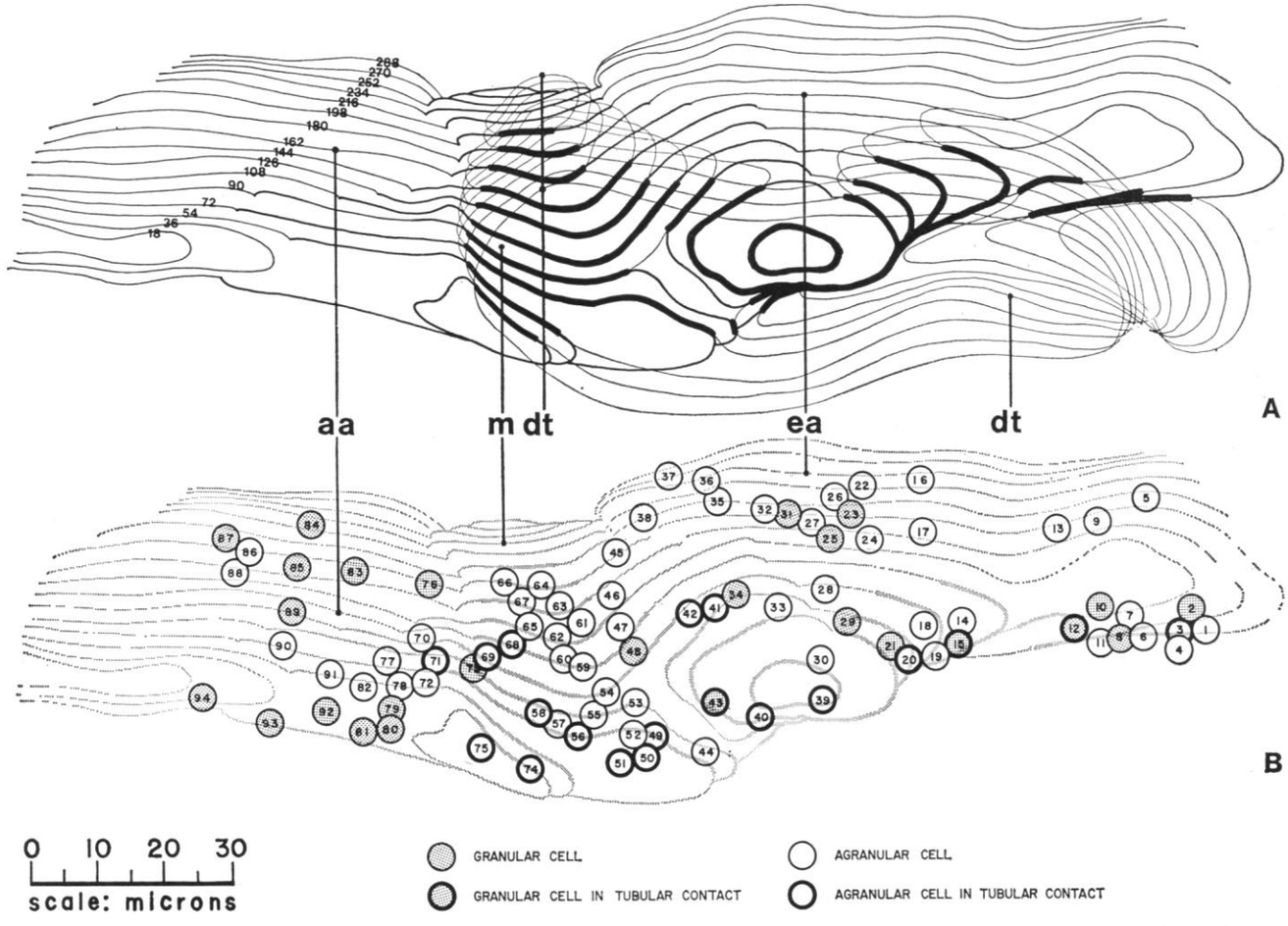

Fig. 1. (A) A three-dimensional reconstruction of the JG 2 section series constructed by superimposing schematic drawings of every 18th section. The lines are numbered according to their corresponding section. The areas in which contact occurs are represented by thick lines; the different components of the juxtaglomerular apparatus are outlined in thin lines. Distal tubule (*dt*), mesangial region (*m*), afferent arteriole (*aa*), and efferent arteriole (*ea*). (B) Topographic distribution of cells along the different parts of the vascular component. The circles mark the approximate position of the nucleus. Also indicated are whether the cell is granular and whether it is in contact with the distal tubule (Table 1).

Table 1. Juxtaglomerular cell distribution by granularity and distal tubular contact.

Cell location	Agranular cell (No.)		Granular cell (No.)	
	Contact	No contact	Contact	No contact
	Juxtaglomerular apparatus 1			
Mesangium	6	8	0	3
Efferent arteriole	5	13	7	6
Afferent arteriole	0	5	1	10
	Juxtaglomerular apparatus 2			
Mesangium	15	17	1	1
Efferent arteriole	2	25	2	9
Afferent arteriole	0	9	0	13
	Juxtaglomerular apparatus 3			
Mesangium	15	19	0	3
Efferent arteriole	2	10	0	0
Afferent arteriole	0	4	0	11
	Juxtaglomerular apparatus 4			
Mesangium	17	17	0	5
Efferent arteriole	8	10	0	1
Afferent arteriole	0	21	1	14

Rat kidney was fixed either by the dripping on of and immersion in osmium tetroxide or by perfusion with 1 percent glutaraldehyde followed by immersion in osmium tetroxide. The tissue was embedded in Vestopal, and series of 500 ultrathin sections were cut. Preparatory methods and methods of three-dimensional reconstruction have been discussed in detail (*2*). Cells on the montage of each section were given a number which was located in a circle placed approximately at the center of the nucleus of each cell. The numbered cells were then classified by location (efferent arteriole, mesangial, or afferent arteriole) on the basis of the position of their nuclei. The numbered cells were then transferred to plastic film and superimposed, offset, and oriented as in the previous three-dimensional reconstructions of the vascular and tubular components of the apparatus. The three-dimensional representation was then matched up with the representation of the other components to provide a more comprehensive view of the distribution of cells in the JG apparatus (Fig. 1, A and B).

The vascular component of the JG apparatus includes the afferent and efferent arterioles and the extraglomerular mesangial region. For quantitative purposes the vascular component was defined as including (i) those portions of the afferent and efferent arterioles from the point where the granular cells away from the glomerulus first make their appearance to the mesangial region, and (ii) the extraglomerular mesangial and arteriolar areas in contact with the distal tubule. The tubular component consists of that portion of the distal tubule in contact with the elements of the vascular component. The distribution of the surface of contact between the distal tubule and the different parts of the vascular component was measured in four juxtaglomerular apparatuses (*2*). The data showed that, of the two arterioles, only the efferent was consistently in contact with the distal tubule, a finding which goes against the still prevalent misconception that contact exists exclusively between the distal tubule and the afferent arteriole.

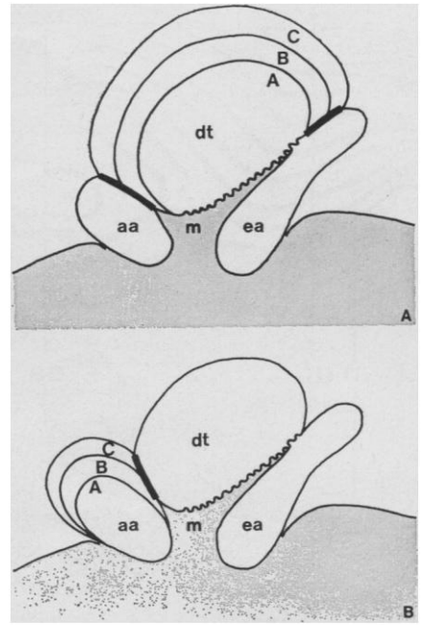

Fig. 2. A simplified schematic representation of the proposed functional model of the juxtaglomerular apparatus. The contact between the distal tubule (*dt*) and the mesangial region (*m*) and the hilar efferent arteriole (*ea*) which is interpreted as permanent is represented by wavy lines, whereas the reversible type of contact is represented by heavy lines. (A) As the distal tubule expands (lines *B* and *C*) the area of "reversible" contact with the vascular components increases. (B) Representation of the changes in contact between the distal tubule and the afferent arterioles resulting from changes in the volume of the afferent arteriole.

A central structural feature in the relationship between the tubular and vascular components appears to be an extensive area of contact between the distal tubule, the extraglomerular mesangial region, and the efferent arteriole (*2*). Two types of contact can be distinguished morphologically: Type 1 is characterized by the presence of cytoplasmic projections from the bases of the cells of the distal tubule and the formation of a network by the basement membranes of the tubular and vascular components; type 2 is a simple adjacency between the basement membranes. Contact of type 1 is thought to be permanent, the distal tubule anchored at the mesangial and hilar efferent arteriole, whereas contact of type 2 is thought to be reversible, occurring with the arteriolar region away from the glomerular hilus (where granular cells are often located). The space separating the plasma membranes in both types of contact may be as narrow as 1500 ± 500 Å along extensive areas, thus perhaps facilitating the transfer of active substances.

The distribution of the cells by granularity and distal tubular contact is shown in Table 1. A new and interesting finding made possible by the use of serial sections is that the majority of the granular cells are not in contact with the distal tubule; in fact, in one of the JG apparatuses perfused with glutaraldehyde there is no contact between any of the granular cells and the distal tubule. In the three remaining JG apparatuses, contact between the granular cells and the distal tubule varies in extent and location (*2*); it is usually of the "reversible" type 2. Although this variation may be due to the differing responses of individual nephrons to the preparatory techniques, it may also reflect variations in the functional states of the nephrons and as such serve as the basis for a model for JG function proposed below.

Current theories on the intrarenal control of renin secretion have implicated either changes in sodium concentration at the distal tubule (macula densa theory) (*4, 5*) or changes in the volume and stretch of the afferent arteriole (stretch receptor theory) (*6*) as the factors which control renin secretion. The anatomical findings pre-

sented here and previously (*2*) have led to the concept that variations in contact between the elements of the JG apparatus may be responsible for the control of renin secretion and that supporting evidence for both theories may be unified by this model.

With regard to the macula densa theory, many physiologists interpret their data as indicating that lowered sodium transport through the distal tubule increases renin secretion (*5*). A smaller sodium load in the distal tubule would probably be accompanied by a decrease in its volume and therefore decreased contact with granular cells (Fig. 2A). Consistent with this line of reasoning we can propose that less contact leads to an increase while more contact would decrease renin secretion. This mechanism also fits the stretch receptor theory which is based on the fact that lowering the blood volume passing through the arterioles increases renin secretion and vice versa. The same mechanism involving reversible contact as a key factor could be involved; a larger arteriolar volume would increase the contact and decrease the renin secretion, whereas a smaller volume would decrease the contact and increase renin secretion (Fig. 2B).

Although the basic mechanisms are still unknown, it is probable that whatever chemical influence the distal tubule exerts on the granular cells will be altered by changes in contact. We can further speculate that sodium itself might be the critical link in controlling renin secretion. Teleologically this makes good sense since the renin-angiotensin-aldosterone system acts to conserve sodium. Larger areas of contact would increase the sodium delivered by the distal tubule to the granular cells, and the greater sodium transport or concentration would then act to decrease renin secretion. Conversely, decreased contact would produce the opposite effects.

LUCIANO BARAJAS
Department of Zoology, University of California, Los Angeles 90024

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Dopamine: Stimulation-Induced Release from Central Neurons

Abstract. *Dopamine, synthesized in rat brain slices from labeled* L-*tyrosine or* L-*dopa, can be released by electrical stimulation of a type known to induce neuronal depolarization. Pretreatment of the animals with 6-hydroxydopamine, which destroys central catecholamine-containing nerve terminals, substantially reduced the release of dopamine synthesized from [^{14}C]tyrosine or from a low concentration of [^{3}H]dopa, whereas the release of dopamine formed from a high concentration of [^{3}H]dopa remained essentially unchanged. The observations that at high concentrations* L-*dopa may enter noncatecholaminergic cells, undergo decarboxylation to dopamine, and subsequently be liberated in response to depolarization suggest that dopamine may act as a substitute central transmitter, possibly in serotonergic neurons. This mechanism may contribute to* L-*dopa's clinical effects in parkinsonian patients.*

Considerable evidence suggests that dopamine may serve as a neurotransmitter in certain neural pathways within the mammalian central nervous system (*1*). Dopamine occurs in high concentrations within the terminals of some neurons together with enzymes for its synthesis and degradation (*2*), and its microiontophoretic application influences the firing patterns of specific neurons (*3*). Furthermore, indirect evidence of cerebral dopamine release following stimulation has been obtained from various in vivo experiments (*4*). We now report that dopamine formed in cerebral tissues in vitro from labeled tyrosine or L-dopa can be released by procedures which induce nerve cell depolarization. At relatively high L-dopa concentrations, dopamine release appears to derive from noncatecholaminergic as well as catecholamine-containing neurons.

Observations both in vitro and in vivo indicate that exogenous catecholamines may be actively taken up by cerebral tissues, where they mix with endogenous amine stores (*5*). It has previously been observed that labeled dopamine accumulated by brain slices may be liberated by electrical field stimulation (*6*). Although most exogenous catecholamines are taken up specifically, it appears that some uptake may also occur in noncatecholaminergic cells, especially at high amine concentrations (*7*). In an attempt to examine this possibility further, as well as to approximate the situation in patients treated with high doses of L-dopa, we have studied the stimulation-induced release of dopamine formed from labeled precursors in brain slices obtained from normal rats and from rats in which most catecholaminergic neurons had been destroyed by pretreatment with 6-hydroxydopamine.

Striatal slices weighing approximately 10 mg prepared from the brains of adult, male Sprague-Dawley rats were used in all experiments. After incubation for 30 minutes at 37°C in a supplemented Krebs-Ringer medium saturated with 5 percent CO_2 in O_2 and containing [^{14}C]tyrosine (0.4 curie/mmole; $2 \times 10^{-6} M$) or L-[^{3}H]-dopa (23.5 curie/mmole; $1 \times 10^{-7} M$ or $1 \times 10^{-4} M$), slices were placed between platinum coil electrodes in individual superfusion chambers (*8*). After 40 minutes of superfusion with fresh, oxygenated medium (150 μl/min), when the spontaneous efflux of radioactivity had fallen to a nearly steady level, rectangular, d-c, pulsatile stimulation (10 ma, 100 hertz, 4.0 msec) was applied for 1 minute, with a Grass S-4 stimulator and CCU-IA constant current control unit. Serial fractions of effluent medium were collected immediately before and during electrical stimulation in chilled vials containing 0.001 percent tyrosine, dopa, and dopamine in 2*N* HCl. Assays for labeled dopamine and its deaminated, *O*-methylated metabolites in the effluent superfusate or in tissue homogenates were carried out by adsorption chromatography through alumina (*9*) and Dowex-50 (Na^+ form) (*10*).