Sympathetic Innervation of the Juxtaglomerular Cells of the Kidney
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ABSTRACT
Combination of a histochemical fluorescence method for biogenic monoamines and staining of juxtaglomerular cell granules demonstrated sympathetic nerve terminals in the rat kidney in the walls of the parts of the juxtaglomerular arterioles that contain granulated cells. This forms a morphological basis for a direct influence of sympathetic nervous activity on the liberation of renin. The juxtaglomerular granules were nonfluorescent, an indication that, in the rat, the granulated juxtaglomerular cells are not equivalent to mast cells.

ADDITIONAL KEY WORDS
renin release monoamines
mast cells adrenergic mechanisms biogenic amines rats

There is considerable evidence that the production and release of renin is intimately linked to the granulated juxtaglomerular cells of the kidney (1-3). The mechanisms which regulate this function of the cells are obscure, but recent physiological data indicate that sympathetic nervous stimulation may be one important factor (4, 5).

A major question is whether the influence of sympathetic nervous activity on the liberation of renin is a result of a direct influence of sympathetic nerves on the granulated juxtaglomerular cells, or is indirect and secondary to changes in intravascular pressure or tubule fluid composition. In an attempt to establish whether there is a morphological basis for a sympathetic nervous influence directly on the granulated juxtaglomerular cells, we examined rat kidney sections with the histochemical fluorescence method, which is highly specific for biogenic monoamines (6, 7), followed by staining of the same sections for juxtaglomerular cell granules.

Materials and Methods
Twenty female Sprague-Dawley rats weighing between 150 and 180 g were used. The animals were killed by rapid bleeding or decapitation during light chloroform anesthesia. The kidneys were immediately removed. Three transverse blocks were cut from one kidney in each animal. The blocks were about 2 mm thick and included both cortex and medulla from the renal capsule to the papillary tip.

The blocks were rapidly frozen in liquid propane cooled by liquid nitrogen. After freeze-drying at -30°C the blocks were treated in jars containing formaldehyde powder (water content 0.6%) for 1 hour at 80°C. They were then embedded in paraffin. From each paraffin block five to eight sections, 2 to 3 μ thick, were cut at different levels and mounted in xylol for fluorescence microscopy. (For further details see references 6 and 7.)

The sections were examined in a fluorescence microscope, and photomicrographs were taken of each area in which a yellowish green fluorescence was observed in the vicinity of a glomerulus. The photomicrographs also included glomeruli that were devoid of any such fluorescence in their vicinity.

After the sections had been examined and photographed in the fluorescence microscope, they were placed overnight in a 2.5% sodium bichromate solution. The sections then were stained with a slightly modified Bowie stain for juxtaglomerular cell granules (8) and examined in the light microscope. The areas photographed in the fluorescence microscope were identified in the Bowie stained sections, and the posi-
FIGURE 1

Left: Fluorescence photomicrograph showing nerve terminals with their characteristic varicosities (arrows). These varicosities are grouped in two separate open circles. The fluorescent compound shows a slight diffusion. There is autofluorescence of the renal tubule cells. Bottom right, a large nonfluorescent area. × 1100.

Right: Same section, Bowie stained. Two cross-sectioned segments of a juxtaglomerular arteriole correspond to the spaces encircled by the fluorescent nerve terminals. The segment to the left (large arrow) contains no granulated cells in the wall, whereas the segment to the right contains granulated cells (small arrows). A glomerular tuft corresponds to the nonfluorescent area in bottom right part of the fluorescence photomicrograph. × 1100.

Results

Under the fluorescence microscope the glomeruli were nonfluorescent and appeared as dark areas, whereas the tubules showed a varying degree of yellow autofluorescence which was sometimes quite strong (Figs. 1, left, and 2, left). In the immediate vicinity of most glomeruli there were fibers that exhibited the yellowish green fluorescence specific for monoamines (6, 7). These fibers showed a number of varicosities and were either grouped in open circles (Fig. 1, left) or appeared as elongated chains (Fig. 2, left). These were regular features in successfully prepared specimens from the various animals. Examination of the Bowie stained sections showed that the varicose fibers were distributed immediately outside the media of the juxtaglomerular parts of the arterioles. Where the varicose fibers formed open circles the arterioles were cross-sectioned (Fig. 1, right); where they appeared as chains the arterioles were longitudinally sectioned (Fig. 2, right).
Some glomeruli were devoid of any varicose fibers in their vicinity when seen in the fluorescence microscope. The plane of section through these glomeruli, as seen in the Bowie stained sections, included the tuft but not the juxtaglomerular parts of the arterioles.

Varicose fibers occurred both in the parts of the arterioles that contained granulated juxtaglomerular cells (Figs. 1, right, and 2, right) and in the parts that were devoid of any granulated cell (Fig. 1, right). The juxtaglomerular granules themselves were non-fluorescent. No fluorescent varicose fibers could be seen in the groups of lacis cells or in close relation to the macula densa segments, also parts of the juxtaglomerular apparatus of the kidney.

**Discussion**

The method used in the present study has been thoroughly analyzed with chemical and pharmacological methods and shown to be specific for biogenic monoamines (6). It has been used in the way presented here in a number of other studies in this laboratory (for references, see reference 7).

The fluorescent varicose fibers demonstrated in the present investigation have the same morphological characteristics as typical norepinephrine-containing nerve terminals of

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*Figure 2*

Left: Fluorescence photomicrograph showing two varicose nerve terminals which extend from bottom left corner towards the center (arrows). Further towards top right there is a non-fluorescent area. Autofluorescence of the tubule cells. X 600.

Right: Same section, Bowie stained. Corresponding to the area enclosed by the nerve terminals in the fluorescence photomicrograph is a longitudinally sectioned arteriole which runs to its glomerulus. A granulated cell (arrow) lies in the wall of the arteriole near the glomerulus. Higher magnification of this cell in inset, bottom right. The glomerulus corresponds to the nonfluorescent area in the fluorescence photomicrograph. X 600. Inset X 2400.
the autonomic ground plexus (7). These nerve terminals, then, are present not only in the intrarenal arterioles in general, as reported earlier by others (9, 10), but also in the walls of the parts of the arterioles that contain granulated juxtaglomerular cells. It is probable that these nerve terminals correspond to those which have been shown in the electron microscope to be in close contact with the epithelioid cells in the walls of the juxtaglomerular parts of intrarenal arterioles (11) and have been assumed to be adrenergic in type (12).

The functional role of the granulated juxtaglomerular cells is better understood than that of the lacis cells and the macula densa to the extent that there is good evidence that the granulated cells constitute the source of renin in the kidney (1-3, 13). It is believed that the production and release of renin are governed by the intraluminal pressure and blood volume in the segments of the vessels where granulated cells are located (14), or by the composition of the fluid in the macula densa segment of the distal tubule (15). The recently demonstrated influence of sympathetic nervous activity on the liberation of renin (4, 5) could be mediated by either of these two proposed mechanisms or by action directly on the granulated juxtaglomerular cells. The results of the present investigation demonstrate a morphological basis for a sympathetic nervous influence directly on the granulated juxtaglomerular cells.

It has been claimed that the granulated juxtaglomerular cells are functionally and morphologically equivalent to tissue mast cells (16), but this has been denied by others (17). Formaldehyde vapor fixation renders rat mast cells fluorescent, probably due to their contents of 5-hydroxytryptamine (18). The lack of fluorescence of the granulated juxtaglomerular cells in the present investigation indicates that, in the rat, these cells are not equivalent to mast cells.

References

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