Adrenergic Innervation of the Canine Kidney
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ABSTRACT
The adrenergic innervation of the canine kidney was studied with the fluorescence histochemical method of Falck and Hillarp in conjunction with chemical assays for catecholamines in specific portions of the kidney. Adrenergic nerve fibers were seen traveling along the interlobar, arcuate, and interlobular arteries and along the afferent arterioles. The vasa recta of the outer medulla also received adrenergic innervation. Adrenergic nerve fibers were never seen in association with the glomerulus, the efferent arteriole, or the tubules. The distribution of the nerve fibers agreed with the distribution of norepinephrine. After administration of reserpine or denervation of the kidney, no adrenergic nerve fibers were seen, and the norepinephrine level in all portions of the kidney fell to negligible levels. Accumulation of norepinephrine in the nerve fibers was observed after kidney slices had been exposed to solutions of norepinephrine. On the basis of these experiments, it was concluded that the fibers visualized with the fluorescence histochemical method were adrenergic nerve fibers. The possible physiologic role of the adrenergic innervation of the canine kidney is discussed.

ADDITIONAL KEY WORDS
histochemistry fluorescence arcuate artery interlobular artery catecholamines vasa recta interlobar artery norepinephrine afferent arteriole

Stimulation of the renal nerves, either direct or reflex, produces a decrease in renal blood flow (1). Initially, only the blood flow in the outer cortex is reduced. However, if the stimulation is of great intensity and duration, a reduction in blood flow occurs progressively from the peripheral cortex toward the medulla (2). A similar change in the distribution of renal blood flow has been demonstrated during progressive hemorrhagic hypotension (3). It is likely that during hemorrhage the renal nerves are at least partly responsible for the alterations in the distribution of blood flow in the kidney.

The distribution of the nerves within the kidney has been the subject of several extensive histologic studies (4-8). Although authors generally agree that the nerves follow the arterial supply into the cortex, they disagree regarding innervation of the efferent arteriole, the glomerulus, cortical tubule cells, and structures in the outer medulla. In an effort to resolve these conflicting results, we investigated the adrenergic innervation of the canine kidney with the specific histochemical fluorescence method for catecholamines of Falck and Hillarp (9-12). In addition, we made chemical analyses of catecholamines in specific portions of the kidney to compare the distribution and concentration of norepinephrine with the distribution and density of nerve fibers visualized with the histochemical method. To confirm that the fluorescent fibers seen with the histochemical method were adrenergic nerve fibers, the effects of kidney denervation, reserpine treatment, and exposure to norepinephrine were studied.

Methods
Dogs were killed by an intravenous injection of sodium pentobarbital (60 mg/kg) and both...
kidneys were immediately removed. For the histochemical studies, small portions of each kidney were quickly frozen on a Freon plate in a cryostat kept at −25°C. Frozen sections 10 to 20 μ thick were cut in the cryostat, and the individual sections were freeze-dried in an Edwards High Vacuum Freeze Drier at −40°C and 10⁻⁴ mm Hg for 2 hours. The sections were subsequently exposed to paraformaldehyde fumes in a closed vessel at 80°C for 40 minutes. To study the histochemical reactivity of the nerve fibers in various portions of the kidney, different sections were exposed to paraformaldehyde gas that had been equilibrated at several relative humidities (13). After exposure to formaldehyde gas, the sections were mounted with paraffin oil and examined in the fluorescence microscope using the Schott BC 12 and Kodak No. 15 filters or the 405 μm interference and Kodak No. 2E filters, as previously described (14).

Immediately after placing pieces of the kidney in the cryostat, slices of the remainder of each kidney were dissected into the five areas shown in Figure 1. These areas were easily distinguished from one another by differences in color and texture in the gross specimen. The subcapsular cortex was identified as the thin outermost layer of the cortex, which is lighter in color and less granular because of the absence of glomeruli. The remainder of the cortex was equally divided into two portions, the outer of which represented the midcortex and the inner, the juxtamedullary cortex. The radially striped area adjacent to the juxtamedullary cortex was identified as the outer medulla. The innermost gray area represented the inner medulla.

Each of these five portions was weighed, and placed in 10% trichloroacetic acid (3 ml/g) and 10% EDTA (0.3 ml/g). After homogenization and filtration, each filtrate was titrated to pH 8.3, passed over an alumina column and eluted with 0.5 N acetic acid. Norepinephrine and epinephrine were analyzed fluorimetrically by oxidation with ferricyanide according to the method of von Euler and Lishajko (15). Measurements were made on a Turner Fluorimeter using two sets of filters, 405/495 μm and 436/535 μm (Turner nos. 405, 65A, 2A-476, and 16).

Further studies were made on the effects of renal denervation, treatment with reserpine, and exposure of slices of the kidney to norepinephrine. Chronic kidney denervations were performed on five dogs. After sodium pentobarbital anesthesia, a left lateral rectus incision was made and the left kidney exposed. The nerves traveling with the renal vessels of the left kidney and the adventitia of the renal artery were carefully stripped, beginning at the hilus and extending toward the aorta. The right kidney served as a...
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FIGURE 2

Interlobar artery running between the renal pyramids. Adrenergic nerve fibers (anf) are between the media and the adventitia. The connective tissue (ct), seen on either side of the artery and the internal elastic membrane (iem), are naturally fluorescent structures.

control. After 2 weeks the dogs were killed and both kidneys were removed. Histochemical and chemical studies were performed on right and left kidneys as in the control studies.

The effect of reserpine on the norepinephrine content and the innervation of the kidney was studied in three dogs. Reserpine (0.25 mg/kg ip) was injected every 24 hours for 2 days. Twenty-four hours after the second injection, the dogs were killed and both kidneys were removed. Histochemical and chemical studies were performed on the six kidneys.

In addition, 0.5-mm slices from control and denervated kidneys were made with a Stadie-Riggs microtome and placed in Locke's solution containing 10 μg/ml norepinephrine bitartrate for 40 minutes at room temperature. They were then washed in three changes of Locke's solution for 20 minutes each and placed in the cryostat, and histochemical studies were performed.

Results

In five untreated dogs, fluorometric analyses showed that no significant amount of epinephrine was present. The mean norepinephrine concentration, expressed in milligrams per gram of each of the five renal areas, is shown in Figure 1; there was no significant difference in norepinephrine concentration between right and left kidneys. A statistical analysis of variance and Tukey's test showed that all portions differed significantly ($P < 0.05$) from one another except for the juxtamedullary cortex and outer medulla. The inner medulla contained very little norepinephrine.

In the histochemical studies, adrenergic nerve fibers were seen only in association with blood vessels. They entered the kidney with the renal arteries and traveled with the arterial supply as it divided. They followed the interlobar arteries between the pyramids to the arcuate arteries at the cortico-medullary junction (Figs. 2 and 3). In addition to the nerve fibers lying adjacent to
the smooth muscle of the arcuate and interlobar arteries, nerve fibers were also scattered throughout the connective tissue sheaths surrounding the interlobar and arcuate vessels (Fig. 4). As the arcuate arteries gave rise to the interlobular arteries, nerve fibers followed the latter into the cortex (Fig. 5). Adrenergic nerves branched from the interlobular arteries and followed the afferent arteriole as far as the glomerulus (Fig. 6). No fibers were seen within the glomerulus or in association with the efferent arteriole.

Adrenergic fibers could also be seen traveling with the vasa recta in the outer medulla (Fig. 7), usually originating from nerve fibers in the connective tissue sheath surrounding the arcuate vessels (Fig. 4). Occasionally they appeared to come from the juxtamedullary area. These fibers traveled with the vasa recta through the outer medulla, decreasing in number as they reached the inner medulla.

All the nerve fibers seen in the kidney exhibited the same properties as adrenergic fibers in other tissues. In cross section the nerves could be seen lying in the adventitia of the arteries adjacent to the smooth muscle but never entering the media (Figs. 2-4). The internal elastic membrane and adventitia seen in these figures are naturally fluorescent structures. The nerve fibers acquire a specific fluorescence only after the sections are exposed to formaldehyde gas. When viewed in longitudinal section they exhibit the varicosities characteristic of adrenergic fibers (Figs. 5-7). The fluorescence properties of the nerve fibers in the kidney agreed with those of adrenergic fibers in other tissues. With the Schott BG 12 filter the fibers appeared green-yellow and with the 405 mμ.
interference filter they appeared sky blue (14).

When histologic sections of the kidney were exposed to formaldehyde equilibrated at different relative humidities, it was found that the reaction conditions for optimal fluorescence were different for the cortex and medulla. The fibers in the cortex developed a weak fluorescence when the relative humidity of the formaldehyde was as low as 45% and developed optimal fluorescence at 50% or greater. The fibers in the outer medulla required formaldehyde equilibrated at 70% relative humidity or greater for optimal fluorescence. When both the cortex and medulla were exposed to formaldehyde at 70% relative humidity the nerve fibers in both these areas developed fluorescence of the same intensity.

In general, the distribution and concentration of norepinephrine in the five areas of the kidney agreed with the distribution and density of adrenergic nerve fibers. However, no fluorescent fibers were seen in the inner medulla and only rarely in the subcapsular cortex. The norepinephrine of these two areas may therefore represent contamination from adjacent areas. If all the norepinephrine found in the subcapsular cortex and the inner medulla was due entirely to contamination, the amounts present there could be added to those found in the adjacent regions. When recalculated on this basis, the norepinephrine concentrations for the midcortex and outer medulla were 0.40 and 0.43 µg/g, respectively. These values are not significantly different from those found in the juxtamedullary cortex (0.42 µg/g). However, on rare occasions adrenergic fibers were observed histochemically in association with interlobular arteries in the subcapsular cortex; these might account for at least part of the norepinephrine found in this area.
The studies of chronic denervation confirmed that the fluorescent fibers seen with the histochemical method were nerve fibers. In none of the sections taken from the five denervated kidneys were any fluorescent fibers seen in any area of the kidneys. The norepinephrine levels also fell in all five areas to less than 0.02 μg/g. In contrast, histochemical and chemical studies of the control right kidneys showed the same adrenergic innervation and distribution of norepinephrine as normal kidneys.

Reserpine treatment produced similar results in the three dogs studied. The norepinephrine concentration of all five areas of the kidney fell to less than 0.02 μg/g and no nerves could be visualized in any of the six kidneys studied.

Histochemical studies of slices from normal kidneys exposed to norepinephrine showed an increase in formaldehyde-induced fluorescence of the nerve fibers. The increase in intensity of fluorescence appeared to be proportionately the same in all fibers in all areas. No fluorescent fibers were seen in sections from slices of chronically denervated kidneys exposed to norepinephrine.

**Discussion**

The results of this study of the adrenergic innervation of the dog kidney indicate that the interlobar, arcuate, and interlobular arteries, the afferent arterioles, and the vasa recta are innervated by adrenergic nerves. Similar observations on the kidney have been reported in other studies using the fluorescent
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FIGURE 6

Adrenergic innervation of an afferent arteriole in the midcortex. The nerve fibers (anf) travel with the afferent arteriole and end abruptly at the glomerulus (glm). Naturally fluorescent tubules (tub) are seen in the surrounding area.

histochemical method (16, 17). In the present studies, confirmation of the histochemical observations was provided by chemical analyses of catecholamines and by studies on the effects of kidney denervation, of reserpine treatment, and of exposure of kidney slices to norepinephrine. The presence or absence of fluorescent fibers always correlated with the presence or absence of norepinephrine. The disappearance of fluorescent fibers and the fall in norepinephrine concentration after kidney denervation or reserpine treatment indicates that the fluorescent fibers were nerve fibers containing norepinephrine. Further evidence was provided by studies demonstrating the accumulation of norepinephrine. In control kidneys, the norepinephrine was apparently taken up by nerve fibers, since slices from denervated kidneys exposed to norepinephrine failed to show any formaldehyde-induced fluorescence.

The high specificity of the fluorescence histochemical method used in these studies has been established by numerous investigations (10-12). Various specificity tests have been established to identify catecholamine-containing fibers (9, 11, 12, 18, 19). These tests were used in the present studies. In all cases the fluorescent fibers of the kidney were found to meet the specificity criteria for catecholamines.

Contrary to some previous conclusions from results obtained with classical histologic techniques such as silver impregnation or methylene blue staining (5-8), we have never seen adrenergic nerve fibers in association
with the glomerulus, the efferent arteriole, or the tubules. This discrepancy could be explained by the fact that the previously available methods demonstrated not only adrenergic nerve fibers but also cholinergic and sensory fibers, or alternatively, by the non-specific nature of the metal stains and dyes used in previous studies. Mitchell (7) has noted that some nerve stains demonstrate “reticular fibers, endothelial, 'Rouget' or other cells, intercellular substance, or cellular inclusions.”

In the kidney, only the nerves of the cortical arterioles have been studied with the electron microscope (20, 21). Nerves containing granulated vesicles were seen in association with the arterioles in both these studies. These nerves are thought to be adrenergic. Evidence for this interpretation has been derived from studies in which granulated vesicles were isolated from tissues with adrenergic innervation and norepinephrine found to be concentrated in the same fraction as the vesicles (22, 23). In addition, tritiated norepinephrine has been located in these vesicles by electron microscopic autoradiography (24). If this interpretation is correct, then nerves containing granulated vesicles visualized with the electron microscope are equivalent to those seen with the fluorescence histochemical method.

Since the adrenergic innervation is associated only with vessels in the dog kidney, it would appear that the adrenergic nerves play a role in regulating blood flow within the kidney. Many studies have demonstrated that cortical blood flow is reduced during hemorrhage or during stimulation of the

**FIGURE 7**

Adrenergic nerve fibers (anf) traveling with the vasa recta between nonfluorescent columns of tubules (tub) in the outer medulla.

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splanchnic or renal nerves. In the past this change in cortical blood flow has been demonstrated by ink or dye injection immediately before removal of the kidneys (2, 25-29). More recently, using autoradiography and the $^{85}$Kr method for measuring the distribution of blood flow in the kidney, Carriere et al. (3) have described a progressive decrease in blood flow from the peripheral cortex to the outer medulla during hemorrhagic hypotension.

While the evidence for a vasoconstrictor role for the nerves in the cortex is consistent, the evidence for a vasomotor function of the outer medullary fibers is conflicting. Some investigators have reported no change in outer medullary blood flow during nerve stimulation or hemorrhage (3, 25, 29); some have reported both ischemia and engorgement of the medulla (26, 27), while others report that total ischemia of the cortex and medulla can occur (2). Goodwin et al. (2) found a decrease in outer medullary blood flow only when the stimulus was of long duration and great intensity.

There is also a question whether the vasa recta of the outer medulla have the smooth muscle necessary to change the diameter of these vessels. In an extensive study of several species, Bensley (30) found a layer of cells closely applied to the endothelium of the arteriolae rectae of the medulla. These pericytes resembled smooth muscle cells but did not appear to be fully organized. Longley (31) has reported a similar structure in association with the arteriolae rectae of the rat. To determine whether these pericytes are actually smooth muscle cells, it is apparent that an electron microscopic study of this region of the dog kidney must be made.

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