Cholinergic Innervation of the Autotransplanted Canine Kidney

By Howard A. Weitsen, B.A., and John E. Norvell, Ph.D.

ABSTRACT

The degeneration of acetylcholinesterase-positive nerve fibers in 11 canine renal autotransplants was investigated with the thiocholine method of Karnovsky and Roots as modified for cryostat sections. Seven renal hila were also examined by silver impregnation and thiocholine techniques. Total denervation was demonstrated in only 3 of 11 autotransplants; the other 8 showed variable degrees of degeneration, from almost total to very little. Acetylcholinesterase-positive nerve fibers accompanied the renal, interlobar, arcuate, and interlobular arteries and the afferent arterioles and vasa recta. Acetylcholinesterase activity was apparent in glomeruli but was greatly diminished or abolished by renal perfusion with normal saline before histologic procedures were begun. Discrete autonomic ganglia were seen in the renal hila. We believe these acetylcholinesterase-positive ganglion cells (also found scattered along hilar nerve bundles) are the source of the nerve fibers which remain intact and viable following vascular division during transplantation.

ADDITIONAL KEY WORDS
acracylcholinesterase
histochemistry
interlobular artery
renal denervation
ganglion
interlobar artery
arcuate artery
vasa recta
afferent arteriole
glomerulus

Knowledge regarding the intrinsic innervation of the kidney was based for years on experiments using various standard nerve-staining techniques, particularly silver impregnation and methylene blue (1-4). Recently, there have been several reports on the application of newer histochemical methods to the study of renal innervation.

Using the fluorescent technique of Falck and Owman (5), the adrenergic innervation of the rat and rabbit kidney has been investigated (6), and also that of the dog (7-9). Norvell et al. (10) found total degeneration of all adrenergic fibers following autotransplantation, in direct agreement with the results of McKenna and Angelakos (9) in dogs whose nerves were stripped from the renal vessels.

The cholinergic innervation of the mammalian kidney has not been studied extensively until recent years (11-17). Most investigators agree that the cholinergic nerve fibers are associated with the renal arterial vessels. Williams (14) notes that cholinesterase-positive juxtaglomerular structures and also medullary filaments accompany the arteriolae rectae in cats. Coupland (11) mentions fibers that appear to penetrate the glomeruli and even a few that are intertubular. Gosling (16) detected occasional fibers extending a short distance along efferent arterioles, and cholinesterase-positive granules in glomeruli, although he did not identify this activity as neural in origin; he also found a few nerve filaments between tubules. Ballantyne (13) described nerves passing into, and within, the glomerulus and also intertubular fibers. McKenna and Angelakos (15) found no fibers entering the glomeruli or in association with the efferent arterioles, veins, or tubules.

In most degeneration studies the renal vessels were stripped to disrupt innervation. However, as early as 1916, Quinby (18)
pointed out the hazards of the stripping method: "nerve filaments supplying the organ not only lie in a network closely applied to the renal vessels, but also run partly within the walls of these vessels." He stated that chemical means, such as painting with carbolic acid, have been suggested, but these techniques are uncertain and one can never be sure all nerves are removed. He concluded that to produce an organ entirely outside the sphere of all nervous influences for a time at least, if not permanently, it is necessary to sever its vascular connections completely, remove the organ, and then to reanastomose the vessels (i.e., autotransplantation).

In the present study, we decided to investigate the cholinergic innervation of the autotransplanted canine kidney to determine (1) if total degeneration of the cholinergic nerves does indeed occur, (2) the presence or absence of renal hilar ganglia, and (3) if the acetylcholinesterase activity in the glomerulus is due to nervous elements.

Materials and Methods

In each of 11 female mongrel dogs anesthetized with sodium pentobarbital, 30 mg/kg iv, the right kidney was transplanted to the ipsilateral iliac fossa. The renal vessels were anastomosed, end to end, with the common iliac artery and common iliac vein. The ureter was sutured to a new site in the urinary bladder. One operation was performed in which identical surgical procedures were followed, except for the transplant. This sham operation and the left kidney in each dog served as controls.

Eighteen studies were carried out on graft material, 7 by wedge biopsy, and 11 by total graft nephrectomy. Of the 11 whole grafts, the final 7 were perfused with 500 ml normal saline before being prepared for histologic study. Four samples were taken 1 week after transplant, four at 2 weeks, two at 1 month, two at 2 months, one at 2½ months, one at 4 months, two at 4½ months, one at 5 months, and one at 11 months.

To determine the presence or absence of cholinergic nervous elements, the thiocholine method of Kamovsky and Roots (19), as modified by El-Badawi and Schenk (20), was used. To prepare the tissue for cryostat sectioning, slices of renal tissue 3 mm thick containing both cortex and medulla were placed on a chuck previously covered with a liberal amount of Tissue-Tek O.C.T. embedding compound. The tissue was then covered with more O.C.T. compound and plunged rapidly into a Dewar flask containing isopentane (2-methylbutane) cooled by dry ice to —85°C. The renal samples were placed in a cryostat kept at —25°C and sectioned at 14μ.

The incubation medium used for the staining of acetylcholinesterase activity contained a concentration of 8 X 10⁻⁶ M iso-OMPA (tetraisopropylpyrophosphoramide) to ensure complete inhibition of nonspecific cholinesterase. A complete description of the staining technique is given by El-Badawi and Schenk (20). Because of the relatively low activity of acetylcholinesterase in the canine renal nerves, the slides were allowed to incubate for 5 to 7 hours as contrasted to 30 to 120 minutes as previously described (20). After incubation the slides were lightly counterstained with Harris hematoxylin. The slides were examined by bright- and dark-field microscopy according to the technique of Norvell and Harris (unpublished data).

To investigate the renal hilum for ganglia, 10μ paraffin-embedded serial sections of four normal canine renal hila were stained by Holmes's silver impregnation method (21). To gain further information regarding the hilar ganglia, three additional normal hila were prepared according to the thiocholine technique and the resulting tissue samples were sectioned at a thickness of 14μ to 42μ, incubated as previously described, and examined by bright- and dark-field microscopy. The common iliac arteries from three normal dogs were investigated with the thiocholine technique to determine the distribution of cholinergic fibers in this area.

Results

The canine kidney has fewer cholinergic than adrenergic nerves. These acetylcholinesterase-positive nerves form a fine but dense plexus around the media of the renal, interlobar, arcuate, and interlobular arteries. Because the fibers decrease in number as they approach the arterial distribution toward the glomeruli, only a few can be seen coursing along afferent arterioles (Fig. 1A). Not only were the nerves seen surrounding the media (Fig. 1C), but large bundles were detected in the adventitia and to a great degree in the hilar connective tissue (Fig. 1D). The same distribution was seen one week following the sham operation.

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a small, intensely stained acetylcholinesterase-positive ganglion was discovered (Fig. 3C, D) in one. It was also seen in several adjacent sections cut at 42μ.

Cholinergic fibers were found in large nerve bundles in the adventitia of the common iliac artery but they were extremely few. The extensive plexus which surrounds the media of the renal artery was not seen in this site, although the vasa vasora did receive fibers.

**Discussion**

Couch et al. (3) found morphologic evidence of neural degeneration by Bodian's silver method 1 week after autotransplantation of the canine kidney. After removal of the aortorenal ganglion in eight dogs, Dolezel (8), also using silver impregnation, observed wrinkled Schwann cell nuclei with remnants of degenerated axons in the vicinity of the renal arterial walls. In the same study, Dolezel also used the fluorescent method of Falek and found degeneration of adrenergic fibers.

Disappearance of fluorescing adrenergic fibers has been demonstrated (9 and Weitsen, unpublished data) in denervated kidneys, and this was accompanied by a drop of catecholamine levels to negligible amounts and controls remained normal. Pharmacologic agents such as reserpine could be responsible for depleting the catecholamines in these adrenergic neurons (9). However, it is our hypothesis that disappearance of monoamine fluorescence in transplanted kidneys is indeed indicative of physiologic neuronal degeneration. Likewise, the absence of staining for acetylcholinesterase in nerve fibers after trans-
plantation would seem to indicate a degeneration of these nerves.

No acetylcholinesterase-containing fibers were seen between tubules, although they have been reported in studies using various techniques (4, 13, 16, 24-31 and Norvell, unpublished data). The use of silver impregnation is questionable because non-nervous elements may be stained, most notably reticular fibers. The peritubular capillary networks may provide the answer if they remain filled with positively stained erythrocytes. No adrenergic fibers have been described between cortical tubules (6-10).

The demonstration of total degeneration in acetylcholinesterase-containing nerve fibers in 3 of 11 transplants supports previous findings in studies using renal artery stripping methods (15). In the eight autografts in which these nerves were seen after transplantation, the incidence and intensity of nerve staining was remarkably variable. Although there was always a reduction in the number of fibers, two autografts (Fig. 1B) were strikingly similar to controls (Fig. 1A).

The nature of the acetylcholinesterase-positive granules in the glomeruli was studied by initial perfusion of the kidney with 500 ml normal saline. In every case, the glomerular acetylcholinesterase was either abolished completely or drastically reduced and that in neural elements was not affected. It appears, therefore, that the acetylcholinesterase activity demonstrated by erythrocytes is partially responsible, if not totally, for the staining seen in glomeruli.
normal saline before freezing. Pale-staining fibers were seen entering the medullary parenchyma along the vasa recta; however, capillaries filled with stained erythrocytes occasionally mimicked the appearance of nerves.

No fibers were seen between cortical tubules, in association with cortical veins, or with efferent arterioles, which supports recent studies (15). No acetylcholinesterase-positive juxtaglomerular structures such as Williams found in the cat (14) were seen. No intrarenal nerve cells (intrarenal microganglia) were observed as previously reported (22, 23). No fibers were detected in the renal capsule.

Of the 11 autotransplants, only three demonstrated total degeneration of acetylcholinesterase-positive nerves. The remaining eight showed varying degrees of degeneration, from practically complete to almost none (Figs. 1B and 2). The fibers seen in the transplants conformed to the normal distribution. In the three dogs that had total degeneration of nerves, acetylcholinesterase-positive granules could be seen in scattered glomeruli and also in the vasa recta of the medulla.

One of the four serially sectioned renal hila stained by silver impregnation revealed a large, well-encapsulated autonomic ganglion (Fig. 3A). The extremely large nerve bundles found in this hilum also contained scattered groups of nerve cell bodies (Fig. 3B). Using the thiocholine technique on three other hila,
To explain the perseverance of acetylcholinesterase-positive fibers following transplantation, the study of normal renal hila for the presence of ganglia was undertaken. Holmes’s silver impregnation method was used initially on four hila to facilitate serial sectioning and therefore to achieve a complete picture of this area. Although McKenna and Angelakos found cells scattered along nerve bundles (15), they never found them aggregated into ganglia. However, not only were scattered nerve cells encountered several times in our study (Fig. 3B), but in one case a large ganglion was discovered associated with the renal artery deep within the renal hilum (Fig. 3A).

The thiocholine method was used to investigate three other normal hila in an attempt to find if these ganglia were acetylcholinesterase positive. Because serial sections were extremely difficult to achieve on frozen tissue, representative sections, 42μ thick, were used. The discovery of an acetylcholinesterase-containing ganglion with associated acetylcholinesterase-positive fibers (Fig. 3C, D) lends support to the hypothesis that these cells, being close to the kidney, would remain distal to the line of vascular division during transplantation. They would be viable, although decentralized, and no longer under the direct influence of the central nervous system via preganglionic nerve fibers, but possibly affected by circulating factors such as catecholamines. The anatomical position of these acetylcholinesterase-positive cells also explains the presence of fibers in the kidney following transplantation. Ganglia in such a location would most likely be missed in the stripping of nerves from the renal vessels and ureter.

Keeping in mind the classical thinking regarding pre- and postganglionic neurons in the autonomic nervous system, the results here are understandable. Transplantation totally disrupts the adrenergic innervation of the kidney because the postganglionic fibers are severed. However, the short parasympathetic postganglionic neuron would be found near or in the organ innervated, as in this case. But the ganglia stained by the thiocholine technique vary in position along the renal artery and if found far enough away from the kidney, may be proximal to the line of vascular division. This situation is most probably the reason for the several total denervations seen and also the variable number of fibers in the other autotransplants. The discovery of acetylcholinesterase-positive ganglia in the renal hilum supports the belief that the kidney does indeed receive some cholinergic fibers, which are probably postganglionic parasympathetics.

Acknowledgment
The authors wish to express their thanks to Mr. J. J. Dwyer, Department of Surgical Research, for his assistance in performing the renal transplants.

References
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doi: 10.1161/01.RES.25.5.535

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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