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AN ANALYSIS OF THE INNERVATION OF THE KIDNEY IN THE RAT

by

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A DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of
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ABSTRACT OF DISSERTATION
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The fluorescent dye and horseradish peroxidase (HRP) techniques were used to analyze the innervation of the rat kidney. In the first series of experiments, albino rats were anesthetized and their left or right kidney was surgically exposed. An injection of 2% true blue was placed in the parenchyma of the kidney and after appropriate survival times the animal was perfused transcardially. The celiac ganglion and dorsal root ganglia (DRG), splanchnic nerves, sympathetic trunk, spinal cord and brainstem were removed and viewed under fluorescence microscopy with a Leitz filter A cube system. In the DRG, labeled cells were located from T6 through L2 spinal cord levels with the greatest concentration of cells found at T13 after left kidney injections and at T11 after right kidney injections. The efferent neuronal supply was concentrated in the celiac ganglion where the majority of labeled cells was observed, and cells were also labeled in the ipsilateral sympathetic trunk.

The central projections of the renal afferent neurons were then studied using anterograde transport of HRP in young rats. After processing, labeled fibers were identified in the medial and lateral portions of Lissauer's tract. The medial group of fibers appeared to terminate in the intermediomedial cell column, and the lateral group terminated in the vicinity of the intermediolateral

nucleus. In order to assess the central connections of the renal afferents in adult rats, the fluorescent dye double labeling technique was employed. With this technique, approximately 8% of the renal afferent neurons were observed to project monosynaptically to the medulla.

Finally, the sensory innervation of the urinary system (bladder, ureter and kidney) and the adrenal gland was examined. In each experimental animal, the kidney was injected with 5% nuclear yellow and the other organs with 3% fast blue. After processing, no double labeled cells were observed in the DRG, thus indicating no collateral innervation between the kidneys and these organs. Following injections into the ureter or kidney, thoracic and lumbar segments of the ipsilateral DRG were labeled; however, adrenal injections resulted in mainly thoracic DRG labeling, and bladder injections labeled DRG cell bodies in sacral and lower lumbar segments. Thus the pattern of innervation is such that more caudal organs are innervated by more caudal DRG neurons. The size of afferent cell bodies also differentiates the separate populations.

Abstract Approved by: Committee Chairman _____

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Date 12-2-83 Dean of Graduate School _____

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LIST OF ABBREVIATIONS

Ach	acetylcholine
AChE	acetylcholinesterase
AV3V	anterioventral third ventricle region
CNS	central nervous system
DRG	dorsal root ganglia
H_2O_2	Hydrogen Peroxidase
HRP	Horseradish Peroxidase
IMLC	Intermediolateral cell column
IMMC	Intermediomedial cell column
LT	Lissauer's Tract
L	Lumbar
PM	Nucleus Posteriomarginalis
SG	Substantia gelatinosa
T	Thoracic
TMB	Tetramethylbenzidine

I. INTRODUCTION

Recent advances in anatomical tract tracing methods have renewed interest in the study of the course and termination of the neural supply of the kidneys. While these studies are in most cases in their early descriptive stages, it is important to reassess the current status of these and past studies in order to appreciate the advances that have recently been made and those that will develop in the near future as the newer anatomical techniques are exploited.

A. Sympathetic Innervation

Relative to its size the kidney receives a more profuse and widespread nerve supply than any other visceral organ except the adrenal gland (Mitchell, 1950). The major efferent nervous control of the kidney is via the sympathetic nervous system (Bradford, 1889). Early descriptions of the spinal cord assumed that the preganglionic sympathetic nerves did not extend beyond the limits of the lateral horn of the thoracic and upper lumbar segments (Laruelle, 1937). More recent studies in cat, which utilized the retrograde degeneration technique following sympathectomy (Cummings, 1969; Petras and Cummings, 1972) demonstrated that the preganglionic supply of the sympathetic nervous system originated in at least four separate groups of neuronal cell bodies in the spinal cord. These cell groups are located in the intermediolateral cell column, the lateral funiculus, the intercalated cell column, and the region dorsal to the central canal. In addition to these locations, Petras and Cummings (1972)

reported that some sympathetic, preganglionic cell bodies were located in the lateral funiculus contralateral to the side of sympathectomy. Chung et al., (1975) using the more reliable HRP technique (LaVail and LaVail, 1972), observed labeled cells only on the ipsilateral side. Of these cells, 82% were in the intermediolateral cell column, 12% were in the lateral funiculus, and 6% were in the intercalated nucleus and central autonomic area. The sympathetic preganglionic neurons supplying the kidney are presumably located in parts of these described areas. The majority of preganglionic sympathetic neurons which innervate abdominal visceral structures enter the abdomen via the splanchnic nerves (Kuntz, 1946).

In 1664, Willis illustrated a direct connection between the splanchnic nerves and the aorticorenal ganglia (see Mitchell, 1950). Later studies indicated that the axons comprising these nerves were virtually all preganglionic sympathetic fibers originating from cells in the spinal cord (Kuntz, 1946); however, other studies continued to emphasize that at least a small proportion of these fibers are postganglionic with cell bodies situated in the paravertebral ganglia (Langley, 1896; Ranson and Billingsley, 1918). Recently, the organization of these postganglionic splanchnic neurons in the sympathetic chain was reinvestigated in the cat using horseradish peroxidase (HRP) as the tracer of choice (Kuo and Krauthamer, 1981). The results demonstrate that the postganglionic labeled cell bodies are located in all of the sympathetic ganglia associated with the spinal cord levels from which the splanchnic nerve originates (T_6-L_2), with the greatest concentration of cells located at the level of T13.

Nerve fibers enter the kidney as a plexus around the renal artery. Early gross dissections in humans demonstrated that this plexus arises mainly from the

celiac ganglion or its aorticorenal subdivisions (Mitchell, 1950). Other renal fibers have their origin in the superior mesenteric ganglion (Mitchell, 1950) or from the superior hypogastric plexus (Mitchell, 1935, 1938). Upon entering the renal parenchyma the renal plexus divides into interlobar, arcuate, and interlobular nerves corresponding to the divisions of the artery. Both myelinated and unmyelinated fibers have been observed in all areas (DeMuylder, 1952; Abraham, 1969; AsFouri, 1971; Barajas, 1978). A close association often exists between the nerves and smooth muscle cells of the arterial walls. Other axonal contacts are established with the granular cells of the afferent and efferent arterioles, in the region of the juxtaglomerular apparatus and, less frequently, with tubular cells (Barajas, 1978). At the contact sites, the basement membranes of the nerve and arteriole or tubule fuse, and a neuroeffecter junction is established (Barajas, 1978).

B. Direct Innervation of the Tubules

Whereas the postganglionic sympathetic innervation of the renal arterioles (Bradford, 1889) is widely accepted, the possibility of direct innervation of the renal tubules continues to be controversial. In the 19th century, it was assumed that the nervous system influenced the function of the kidney only through the innervation of the blood vessels (Bradford, 1889), and suggestions of direct tubular innervation were largely refuted or ignored. During this century, anatomical evidence has indicated that nerve fibers reach the renal tubules in all mammalian species examined including humans (Kauffman and Gottlieb, 1931; Harman and Davies, 1948; Mitchell, 1965). The first combined electron microscopic and histochemical demonstration of the direct innervation of the tubular cells was reported by Barajas and Muller (1972) in monkey and rat

kidneys. They found that adrenergic nerve terminals were separated from the proximal and distal tubular cells only by basement membrane material. In his studies on the rat kidney, Dietrich (1974) was able to confirm renal blood vessel innervation, but unable to document tubular innervation. Recently, DiBona (1977) has supported Muller and Barajas' (1972) finding by demonstrating an adrenergic tubular innervation in the cortex of the dog kidney. The renal nerves terminate on the tubules as free endings, beaded filaments, or fusiform or branched endings, all of which are situated either between the epithelial cells or on the basement membrane (Muller and Barajas, 1972). In addition to the above patterns of innervation it has been demonstrated that the renal nerves innervate the glomerulus and juxtaglomerular structures (Mitchell, 1965; Barajas et al., 1973).

Physiological evidence supporting a direct functional effect of renal sympathetic nerves on the tubules remains controversial. It has been known for over a century that sectioning of the splanchnic nerves causes an increased urinary volume (Bradford, 1889), an effect attributed to vasomotor influences. Second, Pearce and Carter (1915) failed to confirm their earlier findings of direct secretory fibers. Third, although Maluf (1943) reported that the renal nerve supply does not effect renal tubular secretion, at least with regards to H_2O, Cl, and phenol-red, several lines of evidence indicate that tubular secretion is effected by renal nerve input. Direct secretory activity of renal nerves affects uric acid secretion (Grabfield et al., 1937) and sodium excretion and reabsorption (Marshall and Kolls, 1919; Blake, 1955; Gill, 1969; Schrier and DeWardener, 1971), and the findings of Szalay, Benscath and colleagues (1972, 1974, 1976, 1977) and DiBona et al., (1977) support the direct sympathetic control of sodium reabsorption. These authors demonstrated that a decreased

proximal reabsorption of salt and water occurred without a change in single nephron glomerular filtration rate following acute renal denervation. Thus, at present the current anatomical and physiological data suggest that some direct innervation of the renal tubules does exist.

C. Parasympathetics

Despite the demonstration that the vagus communicates directly with the celiac plexus, the existence of a direct parasympathetic innervation of the kidney remains questionable. Most investigators have regarded these parasympathetic connections as indirect, however some investigators have reported that vagal fibers pass directly into the renal plexus (McCrea, 1924; Kuntz, 1946). In contrast to the earlier assumptions, Mitchell (1935, 1938) suggested, on anatomical and embryological grounds, that a parasympathetic supply to the ureter, renal pelvis, and possibly the renal collecting tubules was derived through sacral (pelvic splanchnic) rather than cranial (vagal) outflow.

Within the parasympathetic nervous system, postganglionic neuronal cell bodies are usually located in close proximity to the organ innervated. Conversely, sympathetic postganglionic neuronal somata tend to lie at some distance from the organ. Several studies have demonstrated that some neuronal cell bodies lie within the kidney. In the amphibian (frog) the primary kidney (mesonephros), which functions in the adult animal, contains numerous ganglion cells (Shvalev, 1965). Clusters of nerve cells are also present in various parts of the reptilian kidney (metanephros). The majority of these cells lie along the efferent ducts; however, some are positioned between the tubules. In avians (duck and chicken), small nerve ganglia have been observed along the course of the vessels, efferent ducts, and tubules (Shvalev, 1965).

Conflicting reports concerning the presence of nerve cells in the rat kidney parenchyma have arisen over the past 20 years. Several light microscopic studies have demonstrated small groups of neurons in the renal cortex, with ganglion cells positioned next to the uriniferous tubules. Also nerve cell bodies have been demonstrated in the smooth muscle tissue of the renal vasculature and in the connective tissue of the hilum of the kidney (Shvalev, 1965); however, no ganglion cells have been observed in the rodent kidney at the EM level (Dietrich, 1974; Barajas and Wang, 1975). This inability to visualize ganglion cells could be due to technical difficulties, to the paucity of ganglion cell bodies in the rodent kidney, or their total absence.

In canines, small ganglion cells have been observed along the renal nerve deep in the renal sinus, and along the capsule of the kidney (Shvalev, 1965). Examination of kidney preparations from the macaque and human demonstrated a concentration of ganglion cells in the region of the renal sinus (Shvalev, 1965). Taken together these findings indicate the possibility of a phylogenetic shift of the renal ganglion cells from the renal parenchyma towards the renal sinus in successively higher forms. Whether these ganglion cells are postganglionic parasympathetic neurons or displaced postganglionic sympathetic neurons remains equivocal.

Studies suggesting a renal cholinergic (ACh) input have provided further support for a parasympathetic innervation of the kidney. Using the thiocholine method for localization of acetylcholinesterase (AChE) (Koelle and Friedenwald, 1949) many investigators have demonstrated at the light microscopic level, the labeling of nerves in the parenchyma of the kidney (Williams, 1949; McKenna and Angelakos, 1968; Gosling, 1969, Norvell, 1969). However, a study using both light and electron microscopic techniques with thiocholine and fluorescent

histochemistry demonstrated that a majority of the acetylcholinesterase positive fibers observed in the light microscope corresponded to adrenergic nerves seen with fluorescent histochemistry (Barajas et al., 1974). Further, Barajas et al. (1974) demonstrated the adrenergisity of AChE stained nerves associated with the blood vessels and tubules. Therefore, the classification of the AChE positive nerve fibers present in the kidney remains ambiguous. These fibers could be postganglionic (but AChE positive) or preganglionic (with displaced postganglionic neurons) sympathetic fibers; postganglionic or preganglionic parasympathetic fibers, or a combination of these types.

Physiological studies have also failed to support the existence of a renal parasympathetic innervation. In this regard, the effects of acetylcholine, atropine (ACh receptor blocker), and physiostigmine (acetylcholinesterase inhibitor) on renal function in the dog has been examined (Vander, 1964). Arterial infusions of acetylcholine resulted in variable changes in renal function, and these effects could be altered by atropine; however, systemic injections of either atropine or physiostigmine alone failed to produce a change in renal function. This would contradict the assumption that there is a renal parasympathetic innervation. Finally, no manipulation of the vagus has produced vasodilation in the renal arteries suggesting the absence of parasympathetic innervation to the kidney (Bradford, 1889; Smith, 1951; Takeuchi et al., 1965).

Thus, previous anatomical, biochemical and physiological data did not provide consistent support for a parasympathetic innervation of the kidney; however, in a recent electrophysiological study by Stella et al. (1982), the renal nerves of the cat were activated by stimulation of preganglionic axons present in the cervical vagus suggesting that vagal efferent fibers play a role in the regulation of renal function. Also reports of a direct projection from the brain

stem, parasympathetic nuclei (nucleus of the vagus or nucleus of the solitary tract) (Ciriello et al., 1982; Lappe et al., 1982) have renewed interest in this possibility.

D. Sensory (Afferent) Innervation

The existence of renal sensory fibers coursing along the sympathetic system has been demonstrated physiologically (Astrom and Crafoord, 1967). Clinically, a common phenomenon in humans is severe pain associated with distension of the renal pelvis, and in extreme cases, this pain has been alleviated by sympathectomies performed on the affected kidney (Papin and Ambard, 1924; Stone, 1934). The exact origin of this pain is still in question. Although early investigators indicated that the pain receptors were located only in the renal pelvis and capsule (Papin and Ambard, 1924; Earlam and Brown, 1931), subsequent studies have demonstrated several types of sensory receptors within the kidney.

Afferent impulses have been recorded from the ramus communicans VI–VII of the frog's sympathetic trunks when mechanical stimuli were applied to the kidney (Towers, 1933). In the dog, an increase in arterial pressure is correlated with a concurrent increase in afferent discharge in the renal nerve (Takeuchi et al., 1965), thus supporting the view that mechanoreceptors present around the arterial walls in the kidney relay information to the CNS (Astrom and Crafoord, 1967, 1968; Beacham and Kunze, 1969; Ueda et al., 1967; Uchida et al., 1971; Nijima, 1971, 1972, 1975; Kady and Nashat, 1975). These afferent discharges are increased after either stimulation of the sympathetic fibers in the renal nerves or increases in systemic noradrenaline (Nijima, 1972).

Other renal receptors have the characteristics of chemoreceptors and are distinctly different from the known mechanoreceptors (Recordati et al., 1980). These receptors are active during conditions of impaired renal blood flow such as are produced by clamping of the renal artery, severe hypotension, prolonged renal venous occlusion, or systemic asphyxia not due to changes in arterial, urethral, or renal venous pressures. Further support for renal chemoreceptors is the observation that an increase in afferent impulses follows an intrapelvic perfusion of solutions of selected chemical composition, independent of intrapelvic pressures (Astrom and Crafoord, 1967). An increase in afferent discharge in the renal nerves of rats occurs when renal pressure is elevated or when an i.v. infusion of saline is given. Detailed information of the sensitivity of the chemo receptors to specific effector substances awaits further study.

Histological data have also supported the existence of both baroreceptors and mechanoreceptors in the walls of the renal arteries (Abraham, 1953, 1969). In these studies, dendritic terminal ramifications, glomerular formations and terminal structures similar to the Pacinian Corpuscles were observed under light microscopy in the adventitia of the renal arteries in humans and cattle.

The central connections of these sensory receptors remain to be elucidated. In cats, changes in the electrical activity in the hypothalamus have been recorded after stimulation of the renal afferent nerves. Most of the responsive units were located in regions of the lateral preoptic nucleus, lateral hypothalamus, and the paraventricular nucleus (Ciriello and Calaresu, 1980). In addition, Brody and Johnson (1980) found that stimulation of the renal afferent nerves in rats resulted in alterations in resistence in hindlimb, mesenteric, and renal vascular beds, and that this response was abolished by lesioning the periventricular tissue surrounding the anterioventral third ventricle (AV3V

region). These results indicate that renal afferent impulses provide information to hypothalmaic structures known to be involved in visceral control including regulation of arterial pressure and fluid balance. Supporting the role of renal afferents in cardiovascular regulation is the finding that electrical stimulation of afferent renal nerves in the cat consistently elicited an increase in arterial pressure and heart rate over a wide range of parameters (Calaresu et al., 1976). More recent studies have indicated that renal afferents may also be important in the pathogenesis of experimental hypertension (Katholi et al., 1982).

E. Central Connections

The renal afferent nerves effect many areas of the CNS which are implicated in the regulation of renal efferent activity. The hypothalamus exerts an important regulating influence on the autonomic nervous system (Bard, 1928; Hess, 1957). Early physiological studies indicated that there are descending hypothalamic pathways which reach the intermediolateral cell column (Kabat et al., 1935; Ranson et al., 1935). Whereas studies based on axonal degenerative techniques suggested that the input from the hypothalamus to the IMLC was relayed through the brainstem (Beattie et al., 1930; Smith, 1965; Enoch and Kerr, 1967; Nauta and Haymaker, 1969; Loewy et al., 1973; Calaresu et al., 1975), other studies utilizing the retrograde transport of HRP have demonstrated that a direct projection from the hypothalamus to the spinal cord does exist (Kuypers and Maisky, 1975; Saper et al., 1976). Saper et al. (1976), using both retrograde HRP and anterograde autoradiographic methods, demonstrated that several areas of the hypothalamus monosynaptically project to the spinal cord. These include the paraventricular nucleus, the lateral and dorsal hypothalamic areas, the lateral part of the dorsomedial nucleus, and the

posterior hypothalamic area. Using fluorescent dye retrograde transport technique, Swanson and Kuypers (1980) described projections from the ventromedial hypothalamic nucleus and retrochiasmatic area to the spinal cord.

Several of these hypothalamic nuclei have afferent (McKellar and Loewy, 1981) and efferent (Swanson et al., 1980) projections to brainstem regions which in turn project to the spinal cord (Henry and Calaresu, 1974; Amendt et al., 1978; Loewy and Burton, 1978; Hokfelt et al., 1979; Holstege et al., 1979; Loewy et al., 1979; Wiklund and Bjorklund, 1980; Loewy and McKellar, 1981; Blessing et al., 1981; Bowker et al., 1981; Ross et al., 1981). Descending projections to the thoracic spinal cord have been identified in the raphe nuclear group (Bowker et al., 1981), the ventrolateral reticular formation (Hokfelt et al., 1979), a group of neurons on the ventrolateral surface of the hindbrain (Ross et al., 1981) and the nucleus tractus solitarius (Loewy and Burton, 1978). In addition, catecholamine containing neurons which project to the spinal cord were identified by Blessing et al. (1981), and these cells were located in the A_1, A_4, A_5, A_7, subcoerulus and the locus coerulus. Descending pathways directly to the intermediolateral cell column have been described to originate in the A_1, A_2 (Henry and Calaresu, 1974), A_5 (Loewy et al., 1979), the ventral medulla (B_1 and B_3 groups), the raphe nuclear group (Amendt et al., 1978), the ventrolateral reticular formation (Holstege et al., 1979) and the nucleus tractus solitarius (Amendt et al., 1978). Finally, Loewy and McKellar (1981) identified the projections of the ventral medulla as being serotenergic.

II. SPECIFIC AIMS

The purpose of this study was to elucidate further the organization of the afferent and the efferent innervation of the kidney in the rat. Specifically this research was designed:

(A) to demonstrate anatomically the location of renal sensory neurons in the dorsal root ganglia (DRG) of the rat;

(B) to identify the course of the central projection of the renal afferents;

(C) to determine whether any renal afferents project to the brainstem;

(D) to identify the location of afferent neuronal cell bodies which project to the urinary system (bladder, ureter and kidney) and the adrenal gland;

(E) to localize the renal efferents in the celiac plexus and the sympathetic trunk.

III. MATERIALS AND METHODS

A. Experimental Animals and their Care

In these experiments male and female Sprague Dawley albino rats (Charles River) were used. The animals were housed in the central animal care facility at the University of Alabama in Birmingham and maintained on a 14/10 light/dark cycle. The care of all animals and the research involving them conformed to the principles outlined in "Public Health Service Policy on Humane Care and use of Animals" (U.S. Government Printing Office 19790-281-217/3195).

During all surgical procedures the rats were deeply anesthetized with either sodium pentobarbital (Nembutal) (35 mg/kg) injected i.p. or ether. All surgery was performed under aseptic conditions and the post-operative recovery of the animals was monitored. Prior to sacrifice the animals were deeply reanesthetized with sodium pentobarbital (40 mg/kg).

B. General Surgical Procedure

In order to identify the origin and the course of the renal innervation various tracer substances were applied to the renal nerves using one of two general approaches. The first involved a dorsal incision through the skin and musculature overlying the kidney and subsequent exposure of the kidney through the incision. The renal capsule was then removed and the tracer substance was either injected into the kidney parenchyma with a 30 gauge needle attached to

a 5 ul Hamilton syringe over a 2–10 minute period or a small pellet of solid tracer was pushed into the kidney and the opening was sealed with cyanoacrylate. After placement of the tracer, there was a 5-minute delay prior to reinsertion of the kidney and closing the incision. This procedure prevented leakage of the substance into the surrounding peritoneum and organs. The second method was a ventral approach, which permitted greater visibility of the renal plexus which was observed with the aid of an aus Jena surgery microscope (Model 212). Following the abdominal incision, the renal nerve was stripped from the renal artery with fine forceps and transected near the hilus of the kidney. The central cut end of the nerve was then placed on a small strip of Parafilm^{R} and the tracer substance was applied on the surface. After 30 minutes the nerve and tracer were enclosed in a polyethylene tube (2–3 mm; P.E. 100) and sealed with cyanoacrylate to prevent leakage into surrounding areas.

An appropriate survival time was determined for each experiment since the time needed for maximal cell labeling, the rate of accumulation of the substance in cells and the rate of degradation and exocytosis of the substance all vary according to the tracer used (Kuypers et al., 1980; LaVail and LaVail, 1975). After appropriate survival times the animals were reanesthetized and perfused transcardially with the necessary solutions. Appropriate segments of the brain, spinal cord, dorsal root ganglia, sympathetic ganglia, celiac plexus, nodose ganglia, vagus nerves and kidneys were removed immediately and processed according to one of the protocols listed below.

C. The Horseradish Peroxidase (HRP) Technique (General Methodology)

Horseradish peroxidase (HRP) histochemistry was introduced by Straus (1957) and applied to the peripheral system in 1971 by Kristensson et al. At the

present time, it is one of the most frequently used methods for neuronal tract tracing. The stages of HRP neurohistochemistry consist of injection of the substance, neuronal uptake, axoplasmic transport and histochemical visualization of the enzyme. Injected HRP in the extracellular space is taken up by the presynaptic membrane and axonal and somal membranes by endocytosis. Some recent studies have suggested that pinocytosis of HRP by undamaged axons is a rare phenomenom (LaVail and LaVail, 1974, 1975; Turner and Harris, 1974), but traumatized axons are known to take up large amounts of HRP (Kristensson and Olsson, 1974, 1975, 1976; Adams and Warr, 1976; Wakefield and Shonnard, 1979). Although mechanical trauma has most often been used in these studies, osmotic shock or drastic chemical alterations may equally traumatize the axonal membrane. After uptake, HRP is transported bidirectionally to the cell body and axon terminals. Once in the perikaryon, HRP fuses with lysozymes and is destroyed and cleared from the perikaryon in 4-8 days (Turner and Harris, 1974; LaVail and LaVail, 1975; Price and Fisher, 1978). Thus appropriate survival time depends not only on the rate of accumulation of HRP but also its removal by lysozymal inactivation. Histochemical visualization of the HRP is achieved by incubating the glutaraldehyde-paraformaldehyde fixed tissue in a medium containing hydrogen peroxide and chromogenic aromatic amine. The chromogens polymerize and become more intense in color when oxidized. Therefore, at the sites of HRP activity, the HRP-H_2O_2 complex oxidizes the chromagen, resulting in precipitation of a colored reaction product which acts as a marker for the presence of HRP.

In this study, the animals were anesthetized with Nembutal and the kidneys were exposed. HRP (Sigma) was applied to the kidney in one of three ways 1) placement of 3 ul 50% HRP/H_2O solution on the central cut end of the

renal nerve and after 30 minutes sealing in a polyethylene tube (see surgical procedures), 2) injection of 3 ul 50% HRP/H_2O solution directly into the parenchyma of the kidney or 3) making a small incision into the kidney and pushing in a small pellet of HRP-gel foam with a pair of sharp forceps after which the kidney was sealed with cyanoacrylate. The pellet was prepared by placing gel foam particles into a tube containing 20 ul of a 6% HRP solution and centrifuging under vacuum for 30 minutes to evaporate the water. When dry, the gel "plugs" were separated into 4 small pieces (approximately .25 mg HRP/plug) for surgical use. Twenty-four hours later the animals were reanesthetized and perfused transcardially with 0.05 M phosphate buffer (pH 7.4) followed by 1.25% glutaraldehyde-1% paraformaldehyde in 0.1 M phosphate buffer (350 ml). After 30 minutes of perfusion, the HRP fixative was flushed out of the rat with 10% sucrose (200 ml in 20 minutes) in 0.1 M phosphate buffer, pH 7.4 at 4°C. Tissues were removed. Each DRG was placed into a separate section of a compartmentalized tray with wilk screen bottom for processing. The spinal cord was divided into segmental groups using the dorsal roots as a reference. These segments were cut in 40 um sections either longitudinally or transversely and placed into the tray.

The tetramethyl benzidine (TMB) method of Mesulam (1978) as modified by Dr. Barry Davis (personal communication) was used for processing the tissues. This included a 20 minute soaking of the sections in the TMB incubation medium (500 mg nitroprusside (Sigma) and 2.5 gm gelatin (Sigma) in 487.5 ml of .01 M acetate buffer (pH 3.3) added to 25 mg TMB in 12.5 ml of 100% EtOH) cooled to 20°C. After 20 minutes, the tissue

was removed from the medium and 2-4 ml of fresh 0.3% hydrogen peroxide per 100 ml incubation medium was added. The tissue was reintroduced and slowly

agitated in the solution for 15 minutes. To stop the reaction the tissue was rinsed in 0.1M acetate buffer five times. Finally tissues were mounted from the acetate buffer onto gelatin coated slides, air dried, defatted, and coverslipped. The sections were examined for anterograde and retrograde transport, by both light and darkfield microscopy.

D. The Fluorescent Dye Technique (General Procedure)

Despite the wide use of the HRP technique and extensive efforts to improve it, there are a number of limitations on its usefulness, most importantly is its lack of sensitivity which can result in false negatives (LaVail, 1978). For this reason the more sensitive fluorescent dye technique (Kuypers et al, 1979) was employed in these studies. An effective, fluorescent dye technique for retrograde labeling was developed by Kuypers et al in 1979. This method is based on the physiological properties of the neuron, whereby substances are transported from distal portions of the neuron to the cell body and are concentrated in various cell compartments (e.g., either cytoplasmic or nuclear). Of these substances several have different emission spectra and consolidate in different regions of the cell body. In this study, the markers used were the blue dyes (true blue, fast blue and granular blue) and the yellow dyes (nuclear yellow and bisbenzimide). The blue dyes specifically collect within the cytoplasm of the cell body whereas the yellow dyes become localized within the nucleus. These tracers can be used individually to demonstrate neuronal pathways or can be combined (blue and yellow) to demonstrate divergent axon collaterals. When used in combination, different dyes are injected into unique terminal fields and those neuronal somata connected to each field are labeled with the appropriate marker. If the neuron has collateral projections to both areas then the cell body

is double labeled (e.g., contains blue dye in the cytoplasm and yellow dye in the nucleus).

One potential difficulty with this technique, especially with the yellow dyes is that the substances tend to be transported transcellularly to glial cells located in regions near labeled neuronal somata (Kuypers et al., 1980). For this reason, varied survival periods after injections were used in order to determine the optimal survival time for each dye, i.e., the time which resulted in the densest labeling of the neuron with little or no glial labeling around the neuronal cell body.

In this series of experiments, adult albino rats were anesthetized and one of the kidneys was injected with 2-3 ul of either true blue (5% in distilled H_2O, Illing, FDR) or fast blue (3% in distilled H_2O, Illing, FDR). In studies which involved injections into the kidney and other organs nuclear yellow (5% in distilled H_2O, Loewy, FDR) was also used. Since the nuclear yellow is transported much more rapidly than the blue dyes, animals were sacrificed within 24 hours of injection of nuclear yellow. Survival times after injections of the blue dyes ranged from 4-7 days. The animals were sacrificed and processed according to the histological procedure of Kuypers et al. (1979) as modified by Swanson and Kuypers (1980). This involved a perfusion with 0.9% saline (100 ml) followed by 10% formalin in a 0.1 M phosphate buffer ph 7.4 (300 ml). The tissues were removed and the ganglia were placed directly on clean uncoated slides. The medulla, spinal cord and kidney were soaked overnight in 14% buffered sucrose solution. Frozen sections were cut at 30 um thickness and mounted on clean, uncoated slides. All sections were examined using a fluorescence microscope with the Leitz Filter A cube system.

IV. LOCALIZATION OF RENAL SENSORY NEURONS

Action potentials initiated by the activation of sensory nerve endings in the kidney have been recorded from the renal nerves of rats (Astrom and Crafoord, 1967; Recordati et al., 1980), cats (Pines, 1960; Astrom and Crafoord, 1968), dogs (Uchida et al., 1971) and rabbits (Nijima, 1971). However, there are few anatomical data concerning the location of these renal sensory neurons. In this study, the fluorescent dye technique of Kuypers et al. (1979) was used to demonstrate the location of renal sensory neurons in the dorsal root ganglia (DRG) of the rat.

A. Experimental Protocol

A total of 26 adult albino (275-350 gm) Sprague Dawley rats (50% male/50% female) were each anesthetized with ether. In the experimental cases either the left (10 animals) or right (10 animals) kidney was surgically exposed through a dorsal incision. The kidney was injected with 2 ul of either 5% true blue (in DH_2O) or 3% fast blue (in DH_2O). The position of the injections varied but typically a 1 ul injection was placed in both the dorsal and ventral regions of the kidney cortex. The animals were reanesthetized and perfused transcardially as previously described (Fluorescent Dye Technique). Immediately following perfusion, the kidneys, dorsal root ganglia (DRG), nodose ganglia, and the brainstem were removed and processed for viewing under fluorescence microscopy.

Three control experiments were performed. To determine the extent which blood borne dye labels DRG neurons, the tail vein of 2 rats was injected with 5 ul of 5% true blue. To determine whether a non-neural transport route was involved in the labeling, in another 2 animals, 2 ul of 5% true blue were injected into the left kidney which had been previously denervated by stripping off advential and neural elements. Finally to analyze the results of inadvertant leakage of dye from the renal injections, the retroperitoneal space surrounding the left kidney was injected with 2 ul of true blue in 2 rats. All control animals were processed according to the above protocol.

B. Results

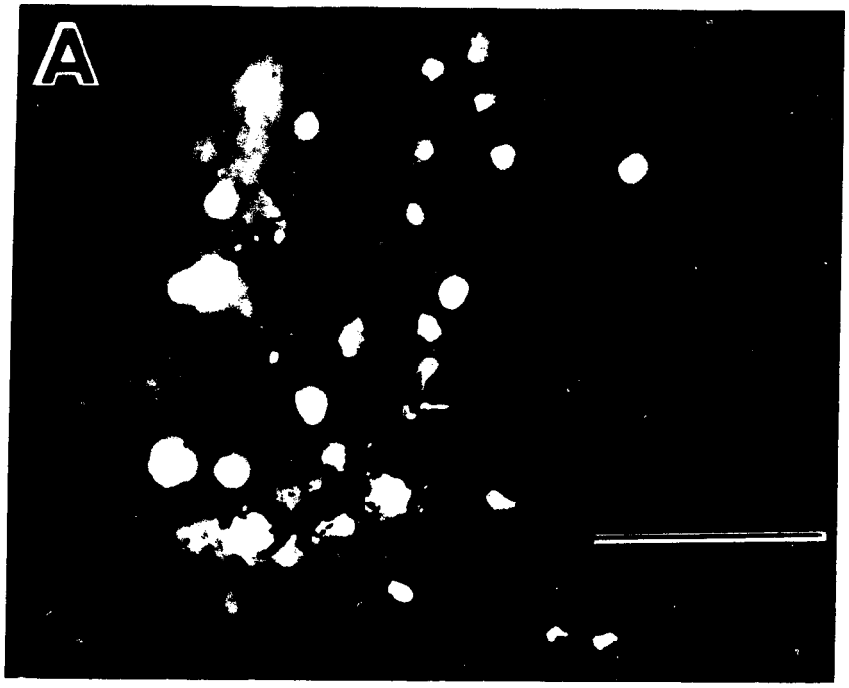
The kidney injections were generally confined to the renal cortex, but in a few cases the superficial portion of the medulla of the kidney was also involved. No injections involved deeper portions of the kidney and no label was observed in the renal pelvis of any animal. The size of the injection was typically 1.0 mm in diameter, with one injection of this size in the dorsal cortex and one in the ventral cortex. All injections resulted in the accumulation of the injected dye in the perikaryon of the labeled neurons (Fig. 1). The nuclei of these cells were unlabeled. Both dyes fluoresce blue under Leitz filter A system, however, fast blue is more granulated and brighter in appearance than true blue.

No difference in the renal innervation between male and female rats was observed. All injections of either kidney resulted in labeling of the cells in the ipsilateral DRG only. Labeled cells were consistently located from thoracic level 8 (T8) through lumbar level 1 (L1) (Fig. 2 and 3), with the greatest concentration of cells observed at T12-T13 levels after left kidney injections,

or at T10–T11 after the right kidney was injected. Very few cells were observed at spinal root ganglia above T8 or below L1. The drop off in the number of labeled cells was more precipitous caudal to T13 than rostral with no more than two cells labeled at L2 levels in any animal and no cells labeled below this. Above the T13 segment the number of labeled cells decreased more gradually at each consecutively higher level. Following left kidney injections, no more than 3 labeled cells were observed in the T8 DRG in any case. In contrast, right kidney injections resulted in more labeling at T8 but little or no labeling at T7 and T6. The labeled cell bodies were not segregated to any particular part of the ganglia, but rather were scattered throughout all regions (Fig. 1A and 4). The somata of the labeled neurons were round with centrally placed nuclei (Fig. 1B), and ranged from 18 um to 56 um in diameter, thus they appear to be typical DRG cells in both of these parameters (Hatai, 1902 and our own measurements in Nissl stained sections).

In addition to the labeled afferent cell bodies associated with the sympathetic system, somata associated with the parasympathetic nervous system were also labeled by the kidney injections. Labeled somata were observed in the nodose ganglia of the vagus as well as throughout the region of the dorsal motor nucleus of the vagus and nucleus of the solitary tract (see also Lappe et al., 1982). Such labeling might indicate that a direct brainstem innervation of the kidney is present; however, the presence of a similar number of labeled neurons in these same locations after injections of the tail vein or the denervated kidney, indicate that part or all of this label is nonspecific. Neither the control tail vein injections nor the injections of the denervated kidney resulted in the consistent labeling of DRG neurons in the T6–L2 ganglia. Thus the label observed after intact renal injections is specific to the renal

Figure 1. Two photomicrographs to demonstrate the appearance of fluorescence dye labeled neurons in the twelfth thoracic dorsal root ganglion of animal TB 63. Scale: A = 200um; B = 50um.


A

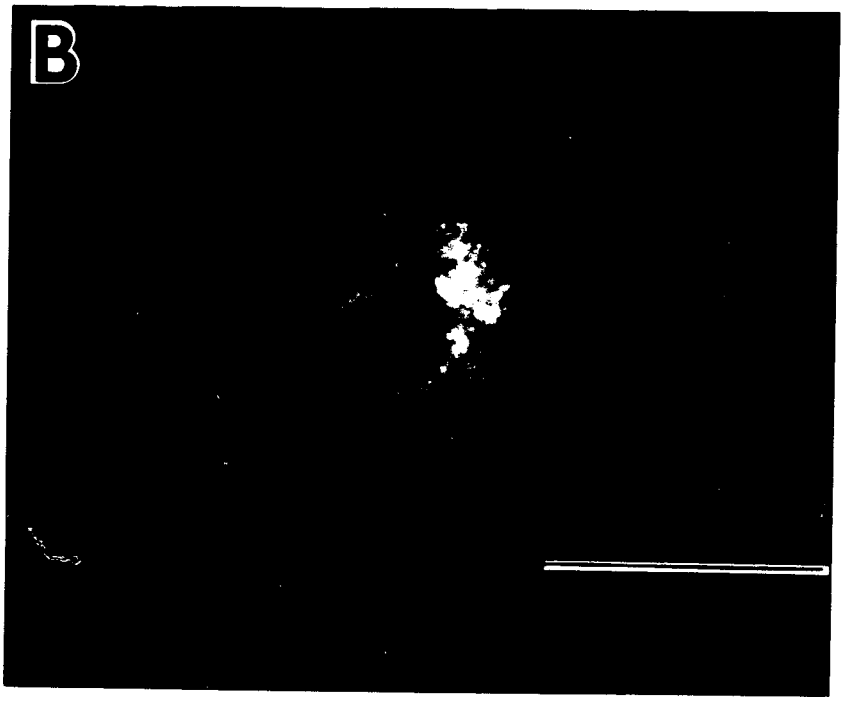

B

Figure 2. A histogram to display the number of labeled cells in each of the left dorsal root ganglia (Thoracic (T) 7 - Lumbar (L) 5) following an injection of 2ul of true blue into the left kidney of animal TB 20.

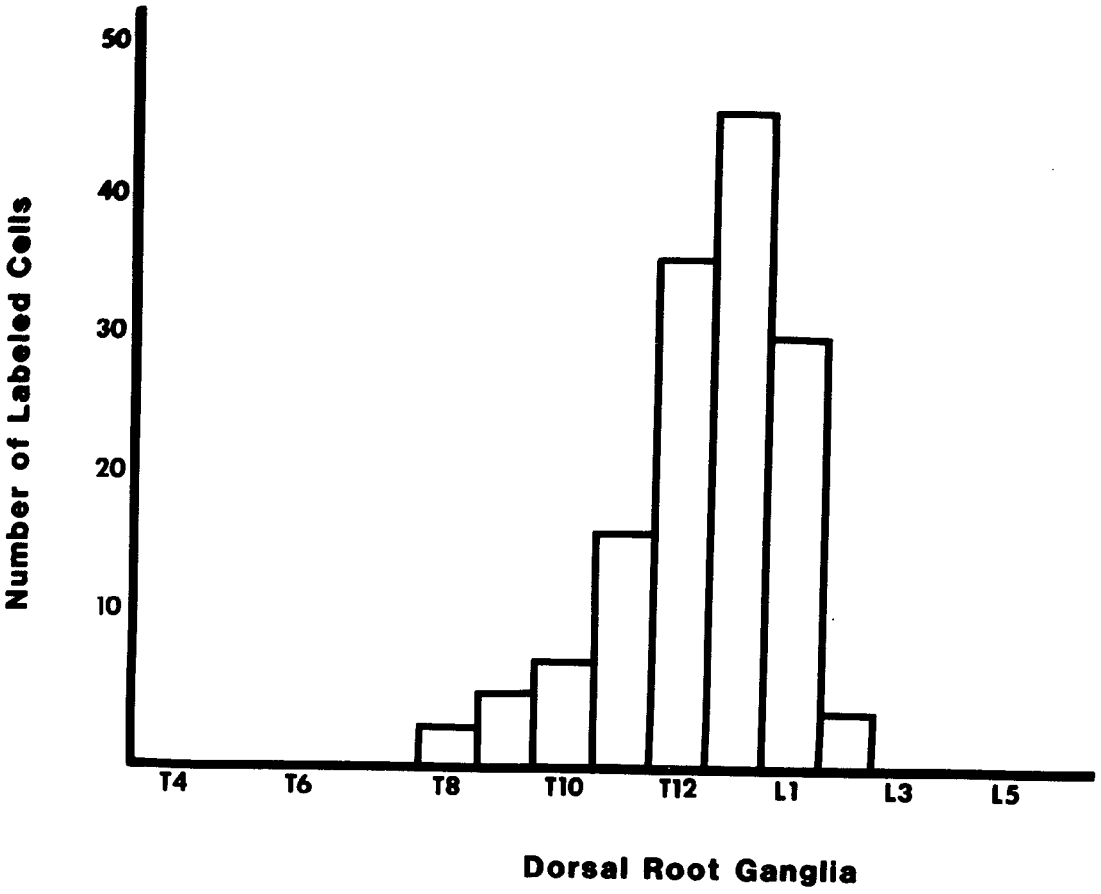

Number of Labeled Cells
50
40
30
20
10
T4
T6
T8
T10
T12
L1
L3
L5
Dorsal Root Ganglia

Figure 3. A histogram to display the number of labeled cells present in each of the right dorsal root ganglia (T_6-L_5) after a right kidney injection of true blue in animal TB 63.

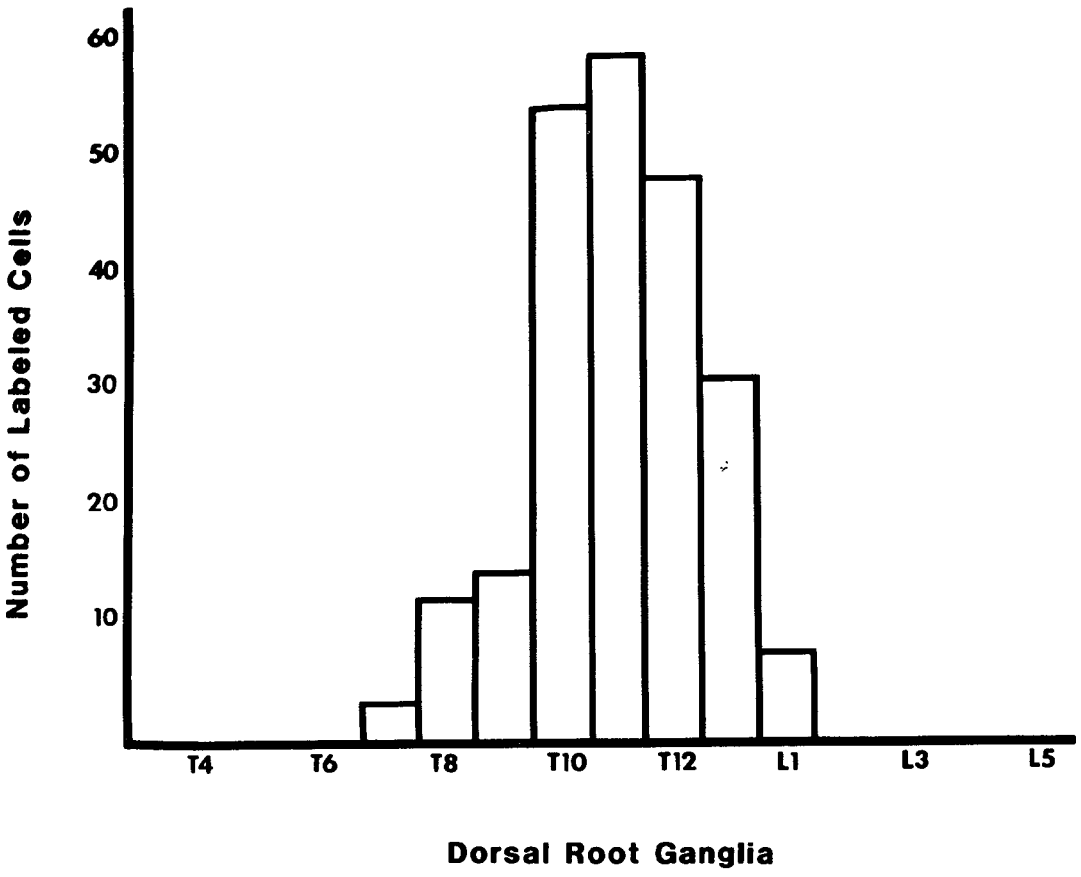
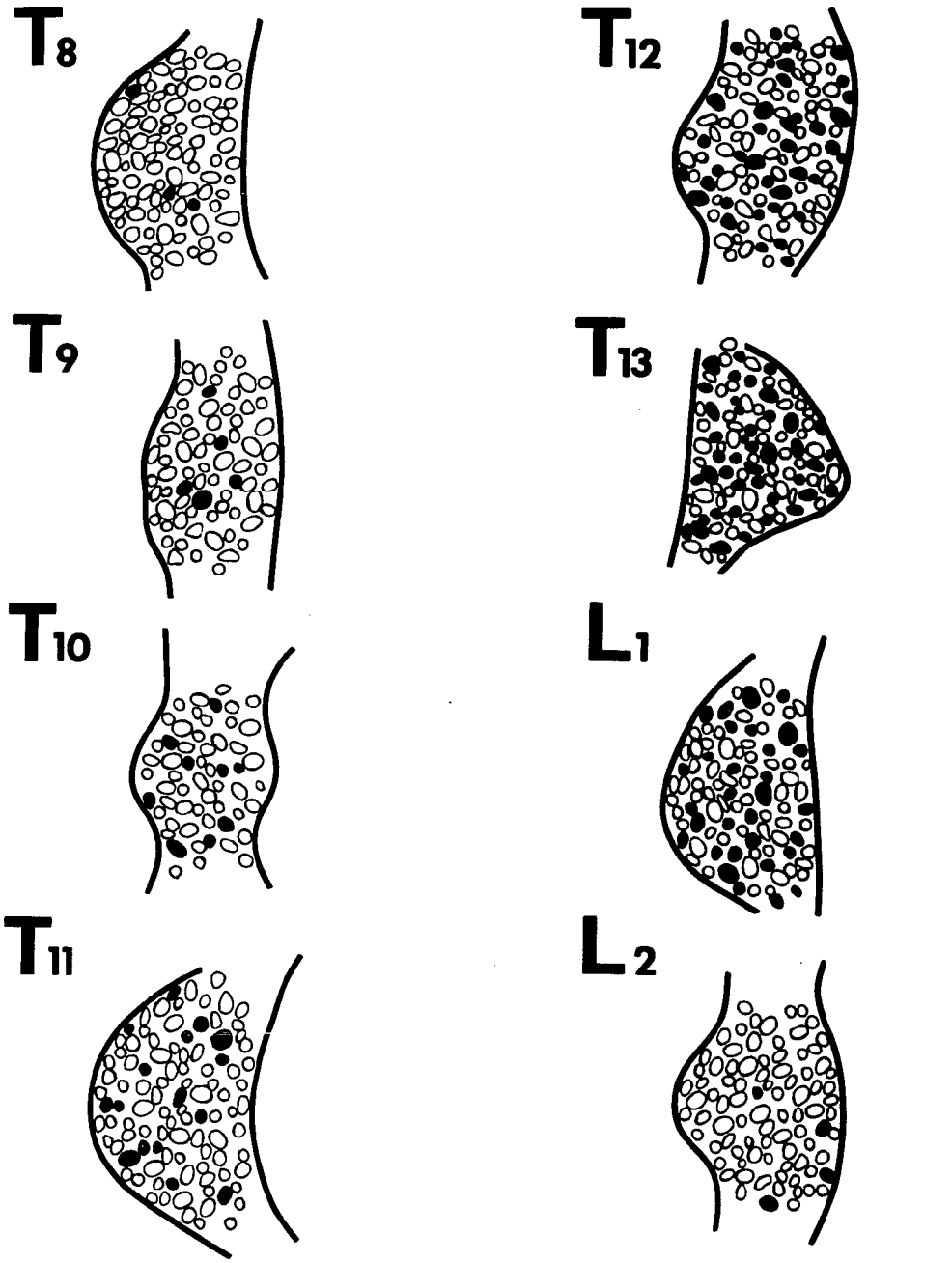

Number of Labeled Cells
60
50
40
30
20
10
T4
T6
T8
T10
T12
L1
L3
L5
Dorsal Root Ganglia

Figure 4. These diagrams demonstrate the location of labeled cells (filled circles) versus unlabeled cells (open circles) in each of the ipsilateral dorsal root ganglia (T_8-L_2) following an injection of true blue into the left kidney in animal TB 20. Scale = 300um.


T8
T9
T10
T11
T12
T13
L1
L2

afferents. Finally, injections of the retroperitoneal space were undertaken, since leakage from the renal injection site to this area might confound the interpretation of the results. Following these injections, labeled cells were present bilaterally in all the DRG from T1-L5. Since the injections restricted to the kidney resulted in labeled cells only in the ipsilateral T6-L2 segments, leakage into the peritoneum does not compromise the interpretation of these present data.

C. Discussion

As demonstrated in many physiological studies, afferents enter the kidney via the renal plexus (Astrom and Crafoord, 1967, 1968; Recordati et al., 1980). The renal plexus is mainly composed of postganglionic sympathetic fibers which arise from cells in the celiac ganglia (Mitchell, 1950). The majority of preganglionic sympathetic fibers enter the abdomen in the splanchnic nerves and it has been assumed that the afferent fibers travel with this system. The present experiments, which employed the fluorescent dye technique, support this hypothesis, by demonstrating that renal sensory neurons are located in the DRG at lower thoracic and upper lumbar spinal cord levels, corresponding to the origin of the splanchnic nerves. In the rat, the right kidney lies more cephalad than the left kidney (Green, 1955) and thus the greatest concentration of right renal sensory neurons is at slightly higher DRG levels (T11-T12) than the left (T12-T13) renal sensory neurons.

The fact that the labeled DRG cells were of various sizes is consistent with the physiological observations that more than one type of receptor is involved in transmission of renal sensory impulses. Thus far, possible functions of these renal sensory neurons include mechano- and chemoreception. The

mechanoreceptors have been demonstrated to respond to increases in renal pressure produced by clamping the renal vein (Astrom and Crafoord, 1967; Beacham and Kunze, 1969), increases in subcapsular pressure (Astrom and Crafoord, 1967), and increases in ureteral pressure (Beacham and Kunze, 1969). Two groups of renal chemoreceptors have been identified. The first group consists of afferent fibers which are activated by renal ischemia and do not respond to back-flow of urine into the renal pelvis (Recordati et al., 1980). The second group of fibers has a resting discharge rate which is higher in non-diuretic than in diuretic conditions. These fibers are activated by renal ischemia and respond markedly to back-flow of urine into the renal pelvis. The latter response was found to be dependent on the chemical composition of the fluid to which sensory endings were exposed and not to mechanical distension of the pelvis or changes in intrapelvic pressure (Recordati et al., 1980). Assessment of the influence of these receptors on overall functioning of the kidney awaits further study.

V. CENTRAL PROCESSES OF RENAL AFFERENT NEURONS

Afferent information from the kidney may play an important regulatory role in renal and cardiovascular function. Renal receptors have been identified (Astrom and Crafoord, 1968; Recordati et al., 1980) and the physiological characteristics of renal reflexes mediated by spinal and supraspinal pathways have been studied (Calaresu et al., 1978; Recordati et al., 1980; Colindres et al., 1980). Our previous study of the localization of renal sensory neurons in the rat has demonstrated that renal sensory neuronal cell bodies reside in the DRG from T6–L2 spinal cord levels, however, until recently virtually nothing was known about the central distribution of the renal afferents (see Kuo et al., 1983). In the following study the course and putative termination of the renal afferent projections in the spinal cord of the rat have been investigated using the technique of transganglionic transport of horseradish peroxidase.

A. Experimental Protocol

Ten adult (60–90 days old) and fifteen young (3–25 days old) rats were used in this study. The rats were anesthetized and an incision was made through which the left kidney was exposed. The HRP was applied to the kidney (see Horseradish Peroxidase Technique) and 24 hours after surgery the animals were reanesthetized and perfused as described previously (HRP Technique). The spinal cord and the thoracic and lumbar DRG were removed and then processed according to the HRP technique. Reacted tissues were mounted on gelatinized

slides, dried, defatted through xylene and coverslipped. The tissues were viewed with a Leitz microscope under both light- and darkfield microscopy.

B. Results

Ipsilateral to the side of HRP injection, labeled DRG cell bodies were observed under brightfield light microscopy. These cells contained dense cytoplasmic granular reaction product clearly distinguished from the background level of nonspecific labeling. Labeled cells were located in the DRG from T8–L2 with the greatest concentration of cells at T13. These cells were distributed in all parts of the ganglia with no noticeable topographic location.

The central processes of the renal afferents were labeled in the young rats. In adult animals, transport of HRP into the central processes of the renal afferent fibers was not observed. The segmental distribution and relative frequency of renal afferent collaterals in the spinal cord of the young rats paralleled the segmental distribution and relative frequency of the renal afferent neurons in the ganglia. That is, the central afferent labeling was strongest from T10 through L1. The intensity diminished in T8, T9 and L2 and was not apparent at other spinal cord levels. In longitudinally cut sections, the afferents containing HRP reaction product were identified as entering the spinal cord in discrete bundles at the dorsal surface. In transverse sections, afferent projections were distributed around Lissauer's tract from which collateral projections could be traced along the medial (Fig. 5A) and the lateral (Fig. 5B) margins of the dorsal horn. The medial fiber group extended along the dorsal horn to Lamina 7 of Rexed (1952) and appeared to terminate in the intermediomedial cell column. The lateral bundle of labeled fibers appeared heaviest near the visceromotor region of the intermediolateral cell column (Fig.

5B). The pathways taken by the central projection are diagrammed in Figure 6. Renal afferent fibers entering the spinal cord at one level (T13) contain some collateral fibers which terminate at the level of entry. Other collaterals extend either rostrally or caudally before synapsing. HRP labeling is present in the spinal cord 2 segments caudal and several segments rostral to the entry but no label is apparent in more rostral (T6 and above) levels of the spinal cord.

C. Discussion

The pattern of renal afferent innervation observed in this HRP study was similar to that seen in the previous fluorescent dye study localizing renal afferent neurons in the DRG (Donovan et al., 1983). The actual number of labeled cells in these studies was less, which may be due to the sensitivity of the two techniques. The fluorescent dye technique is purported to be more sensitive (Swanson et al., 1981) and, in that study, the blue dyes labeled over 60% more neurons than HRP.

Although HRP labeled cells were observed in both the adult and young rats, only in the young animals were renal afferent central processes labeled. This is consistent with the results of Kuo et al. (1983) in which the segmental and central distributions of renal nerve afferents were studied in adult cats and kittens using HRP. In the latter study, HRP labeled renal afferents were observed in the T11 to L6 spinal cord levels of the kittens, but no labeled central processes were demonstrated in the adult cats. One possible explanation for this result is that adult and young animals may have different axonal transport characteristics related to their stages of development. Whereas anterograde transport provides the axon with the necessary proteins for maintenance and growth, which only the cell soma has the ability to synthesize,

Figure 5. Darkfield photomicrographs of HRP-labeled renal afferent central projections in a transverse section of the spinal cord at T13. (A) Shows a group of labeled fibers extending along the medial margin of the dorsal horn. (B) Demonstrates lateral fibers at the same spinal cord level which appear to terminate in the intermediolateral cell column. Scale bar = 300 um.


A

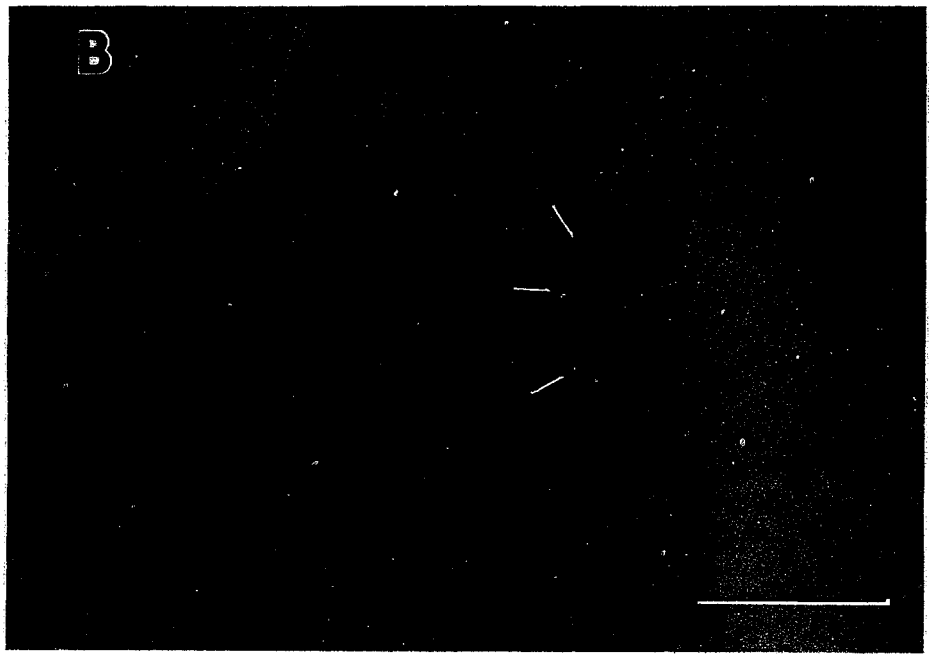
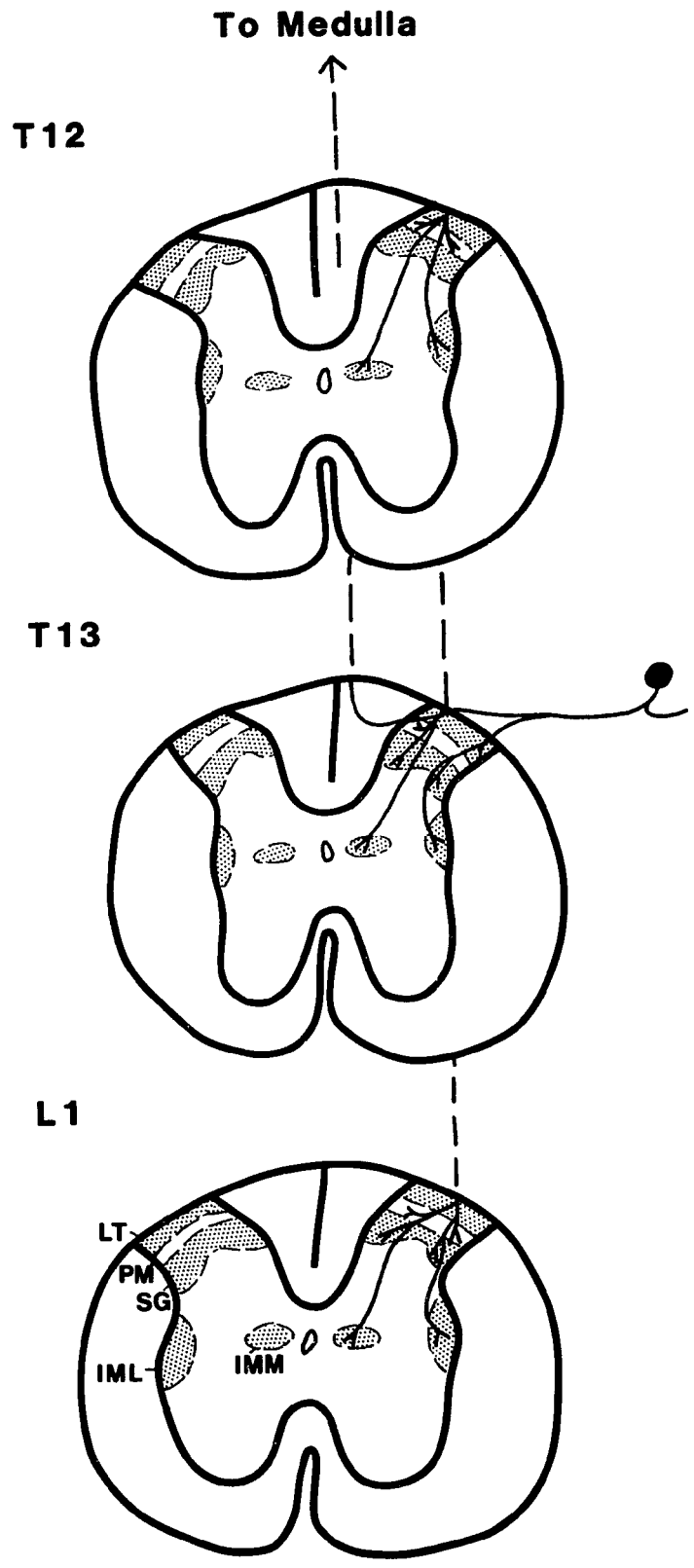

B

Figure 6. A diagram demonstrating the possible collateral projections of a single renal afferent neuron located in the T13 DRG.


To Medulla
T12
T13
L1
LT
PM
SG
IML
IMM

retrograde transport provides the cell body with information concerning the status of the axon and synaptic membrane. In young animals with rapidly growing axons, it would appear necessary for such information to be relayed more rapidly and more often than in the adults which possess a more stable axonal network (Jacobson, 1978).

Labeled renal afferent central processes were observed to enter the mediolateral extent of Lissauer's tract. Recent studies in rat (Chung et al., 1979) and cat (Chung and Coggeshall, 1979) have demonstrated that a large number of axons in Lissauer's tract are small caliber primary afferents, distributed over the entire mediolateral extent of the tract. Furthermore, Morgan et al. (1981) observed that large numbers of afferent fibers from the pelvic nerve project into Lissauer's tract in the lower lumbar, sacral and coccygeal spinal cord. This study suggests that Lissauer's tract may be an integral part of the central autonomic pathways. Our results support Morgan's view in that labeled central processes of the renal afferent neurons were located in the medial and lateral portions of Lissauer's tract from the T8 through L2 segments, indicating Lissauer's tract as an important renal afferent pathway.

Excitation of renal afferents by increasing renal venous pressure induces a reflex discharge in renal efferents and a fall in renal vascular resistance and systemic arterial pressure (Beachum and Kunze, 1969; Ueda et al., 1967; Aars and Akre, 1970). Our data demonstrate a close relationship between a portion of the renal afferent fibers and sympathetic preganglionic neurons since HRP labeled fibers were observed in the intermediolateral nucleus after kidney injections. Whether this is a monosynaptic connection is unknown. Early studies (Szentagothai, 1948; Petras and Cummings, 1972) using fiber degeneration

techniques failed to reveal primary afferents projecting to autonomic nuclei. However more recently electrophysiological evidence of monosynaptic connections between primary afferents and sympathetic preganglionic neurons has been presented by LeBedev et al. (1976). The precise nature of these connections requires further investigation.

VI. DEMONSTRATION OF A DIRECT PROJECTION OF RENAL SENSORY NEURONS TO THE POSTERIOR MEDULLA

The previous study demonstrated that renal sensory fibers do enter the spinal cord and appear to synapse on preganglionic sympathetic areas near their entry. The known cardiovascular control role played by the renal afferents may be affected by these terminals onto spinal cord neurons which in turn project to higher centers (Kuo et al., 1983); however, recent physiological data suggest that the higher cardiovascular centers are affected by direct renal sensory projections to the brainstem (Fernandez et al., 1974; Calaresu and Ciriello, 1981; Winternitz and Oparil, 1982). In this present study, the fluorescent dye technique (Kuypers et al., 1979) was used to evaluate the possibility of a monosynaptic projection from the renal nerves to the lower brainstem.

A. Experimental Protocol

Twenty adult male albino rats were used in this study. Each rat was anesthetized as previously described and the left kidney exposed via a dorsal incision. An injection of 3.0 ul of 3% fast blue was placed into the cortex of the kidney. Four days later, and 24 hours prior to sacrifice, a separate injection of 0.5 ul of 4% nuclear yellow was made either into the posterior brainstem or the lower lumbar spinal cord. In two cases the dyes were reversed (i.e., fast blue in the brainstem and nuclear yellow in the kidney). Following the appropriate survival times, the animals were deeply reanesthetized and perfused

accordingly. The brainstem, spinal cord and the dorsal root ganglia were removed. The brainstem and spinal cord were cut transversely in 30 um sections. One in every five sections was placed on slides with adjacent sections stained with thionine (0.25% in distilled water) to provide a cytoarchitectonic reference for labeled neurons in these regions..

B. Results

Most kidney injections were confined to the renal cortex, although four injections also labeled the superficial portion of the renal medulla. No injection involved deeper portions of the kidney and dye was never observed in the renal pelvis or extra-axonally within the renal nerve. The size of injection was typically 1.5 mm in diameter. Each injection resulted in the accumulation of the injected dye in the cytoplasm of 200-300 (mean = 240) DRG neuronal cell bodies. Injections into the spinal cord or brainstem resulted in the labeling of a consistent number of cell bodies in the DRG.

Following combined kidney and control brainstem injections, three populations of labeled cell bodies could be identified within the DRG. Most of the labeled cell bodies had a yellow fluorescent nucleus, indicating a central projection to the caudal brainstem, and unlabeled cytoplasm, indicating that these cells do not provide a renal afferent innervation. The number of labeled cell bodies in the DRG averaged approximately 200 in each ganglion. The absolute number was greater in the cervical DRG than in the thoracic DRG, however, the percentage (30%) of labeled (yellow) cell bodies remained relatively constant. In cases of bilateral injections, equal numbers of these labeled cells were present in the DRG on both sides. The second class of neurons labeled by these injections consisted of those with blue cytoplasmic

labeling but no nuclear labeling. These cells project to the kidney but do not project directly to the brainstem.

The third class of labeled cell bodies consisted of those which contained both blue cytoplasm and yellow nuclear labeling (Fig. 7). These accounted for approximately 8% of all renal afferent (blue labeled) cell bodies (16–24 somata) (Fig. 8). All renal afferent cell bodies were located in the ipsilateral T8–L2 ganglia. The peak number of singly labeled cell bodies was present in the T12 or T13 DRG, but the relative distribution of double labeled somata was different. Typically, the peak number of double labeled cell bodies was located in the T11 ganglion and there was a gradual drop off in the number of double labeled somata in the adjacent ganglia, i.e. from T10 to T8 and from T12 to L1. This contrasts with the pattern of distribution of total renal afferent somata with its steep caudal and more gradual rostral drop off in number of labeled DRG cell bodies (Fig. 8).

Two control experiments were carried out in order to validate the above data. First, injections of nuclear yellow into the pons did not result in the labeling of DRG cell bodies, demonstrating that the renal afferents appear to terminate in more caudal areas of the brainstem. Second, in two animals, an injection of nuclear yellow was placed into the lower lumbar spinal cord (the left, dorso-lateral quadrant of the L4–L5 spinal cord segment) and a 2 ul fast blue injection was placed in the renal cortex. These injections resulted in fast blue labeled cells in the ipsilateral T8–L2 DRG according to the pattern shown in figure 8. The lumber spinal cord injection resulted in labeled neuronal cell bodies in the L2–coccygeal DRG. This demonstrates that the central projections of the lower thoracic and upper lumbar DRG cell bodies do not course caudally through more than two or three segments of the rat spinal cord. In contrast

Figure 7. (Top) A photomicrograph of 3 fluorescently labeled DRG neurons. Cells B and C were cytoplasmically labeled by transport of dye from an injection of the kidney. Cell A was labeled by both the kidney injection and a brain stem injection. (Scale bar = 50um).

(Bottom) A photomicrograph of a double labeled DRG neuron after a fast blue injection of the kidney and a nuclear yellow injection of the brain stem. (Scale bar = 20 um).

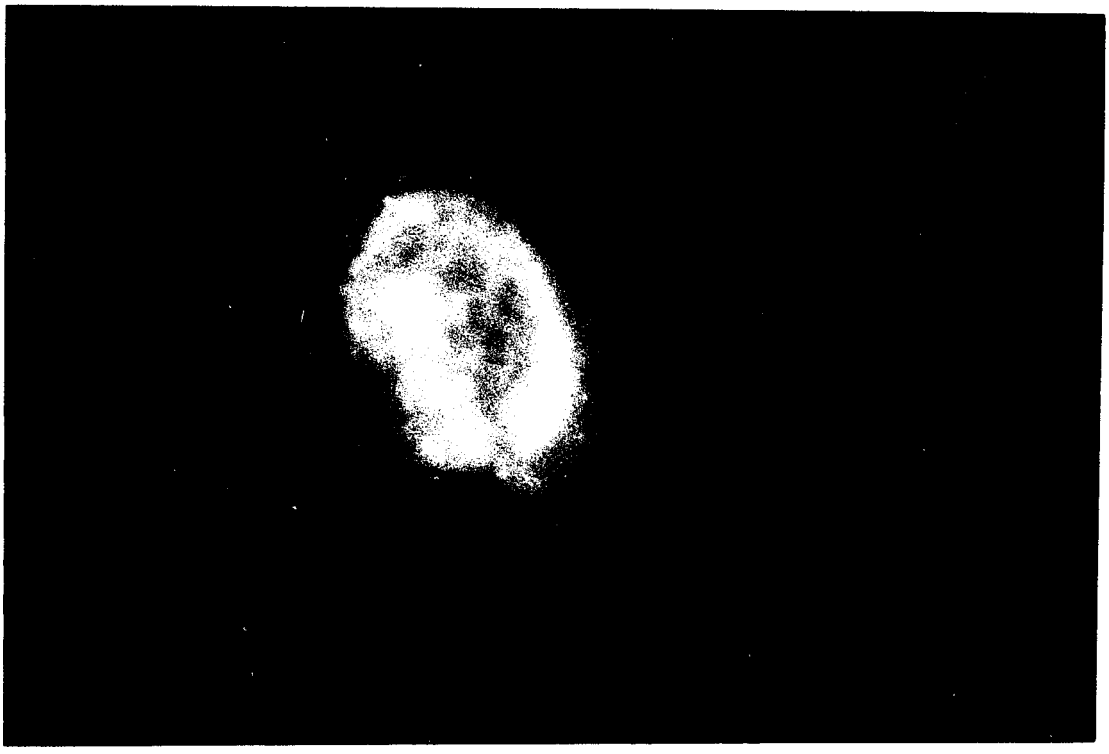
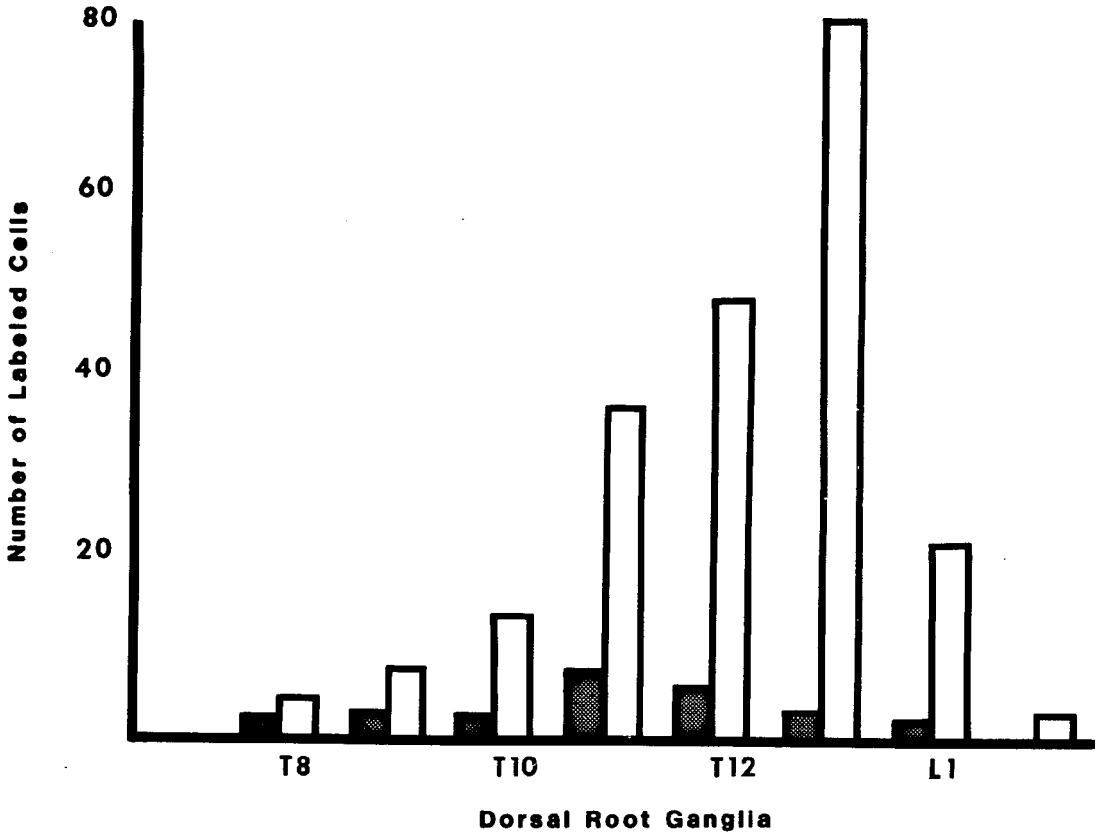


Figure 8. A histogram to demonstrate the number and position of dorsal root ganglion, neuronal cell bodies labeled either by an injection of the kidney alone (open bars) or by both a kidney and a brain stem injection (cross hatched bars) in experiment TB 90.


80
60
40
20
Number of Labeled Cells
T8
T10
T12
L1
Dorsal Root Ganglia

these afferents pass rostrally through as many as 22 segments. Further, although both blue (kidney projecting) and yellow (spinal cord projecting) labeled cells were present in the L2-ganglion, no double labeling was observed in any cell. These two control studies demonstrate that simple diffusion of the dye from the CNS injection site is not responsible for the double labeling observed in this study.

C. Discussion

Recently Kuo et al. (1983) analyzed the central projections of the renal afferent nerves by dipping the cut end of the renal nerve into a horseradish peroxidase (HRP) solution. They demonstrated that the central processes of renal afferents in the cat were localized in various regions of the spinal cord in the segments close to the DRG of origin. However, in their study in the cat and in our own similar study in the rat, no afferents could be followed up to the brain or even into the cervical spinal cord. This failure may be attributable to several factors. First although the most sensitive reaction (tetramethyl benzidine) was used in both studies, the fluorescent dyes are more sensitive for retrograde labeling (Swanson et al., 1980). Second, in the present study only 15-25 DRG cell bodies have been identified as providing direct renal to brainstem projections. This number is quite small in relation to the 2,000 DRG cells projecting other information to the brainstem from the same DRG. Thus improved HRP techniques may be necessary to label such a small number of cells. Third, neither Kuo et al. (1983) nor we have obtained adequate HRP labeling of the central processes of the renal afferents in mature animals. Thus, those studies in which the renal afferents to the spinal cord were identified by orthograde transport of HRP were based on the use of very young animals. It is

possible that the central processes of the renal afferents do not reach the brainstem or do not transport HRP effectively until a later stage (Jacobson, 1978; Kuo et al., 1982).

The present study provides an anatomic basis for the physiologic results recently reported by Simon and Schramm (1983). In that study, renal nerve stimulation resulted in monosynaptic, orthodromic excitation in two areas of the brainstem, i.e., the most medial segment of the nucleus gracilus and the caudal half of the nucleus tractus solitarius. This input was assumed to be carried by large myelinated axons. In the present study, the cell bodies of the axons projecting to the caudal brainstem were found to be medium to large in size (25–45 um). The Simon and Schramm (1983) study also demonstrated that cutting the T10–T11 dorsal roots ipsilateral to the renal nerves that were stimulated resulted in an abolition of the response, whereas sectioning the lower dorsal roots (T12–L1) had little effect on the response. This corroborates our anatomical data, which demonstrate that most of the renal to brainstem projections originate in the T11 DRG.

VII. AN ANALYSIS OF THE SENSORY INNERVATION OF THE URINARY SYSTEM

The motor innervation of the urinary system and adrenal gland have been described previously (Alpert, 1931; Mitchell, 1935; Hollinshead, 1936; Nyo, 1969; Schramm et al., 1975; Schulman, 1975; Weiss et al., 1978) and although it has been assumed that a complementary sensory innervation of each component exists, the only extensive studies of these afferents have been the recent analyses of the kidney and the urinary bladder afferents in the rat (Applebaum, et al. 1980; Donovan et al., 1982, 1983) and cat (Kuo et al., 1982). In this study, the technique of retrograde transport of fluorescent dye has been employed to demonstrate the location of dorsal root ganglion neurons which innervate the urinary system including the bladder. In addition, the study examined the afferent innervation of the adrenal gland, which lies adjacent to the kidney.

A. Experimental Protocol

Twenty-five adult, male, albino rats were each anesthetized and either a midline incision was made for the urinary bladder or ureter injections, or a dorsal incision was made to expose the kidney or adrenal gland. After exposure the bladder, upper, middle or lower ureter, or the adrenal gland was injected with 2 ul of 3% fast blue. Twenty-four hours prior to sacrifice, 3 ul of 5% nuclear yellow were injected into the kidney. All injections were made on the left side. Five to seven days following the initial injection, the animals were

reanesthetized and perfused transcardially. The dye injected organs and the dorsal root ganglia were removed immediately. All tissues were viewed under a fluorescence microscope. In each case, camera lucida drawings (X1100 magnification) of the cell bodies labeled by the blue injections were made. The size of each was then analyzed via a Bioquant (Nashville, TN) planimetric program. Where appropriate, the student's t-test was used to analyze differences between the size of neurons innervating the different organs (Siegal, 1956).

B. Results

In 23 animals the injections resulted in adequately localized labeling of the DRG. The successful cases are documented in Table 1.

1. Neurons Projecting to the Kidney

The nuclear yellow injections of the kidney resulted in a pattern of labeling identical to that previously described (Donovan et al, 1983). Labeled neuronal cell bodies were observed in the T8 to L2 ganglia. The greatest density of neurons was located in the T12 and T13 ganglia (Fig. 9). These injections typically resulted in the labeling of cells from 18–56 um in diameter as demonstrated in Figure 10.

2. Adrenal Afferents

The injections of fast blue into the adrenal gland resulted in the labeling of DRG cell bodies in the T4 through L1 ganglia. The highest density of labeled neurons resided in the T8 ganglion, which contained an average of 35 labeled neurons. These cells (16–42 um in diameter) were significantly smaller than

TABLE I

This table represents the number of rats analyzed in the present study according to the site of fast blue injection. The chief experiment is that described in the text although all other experiments in each class confirmed the results. In all cases the kidney was injected with nuclear yellow.

Injection Site	Number of Injections	Chief Experiment
Adrenal	6	TB 86
Upper ureter	4	TB 116
Middle ureter	5	TB 87
Lower ureter	4	TB 123
Bladder	4	TB 103

Figure 9. Six histograms to demonstrate the position of labeled DRG neuronal cell bodies following injections into the adrenal gland (A/Ad), kidney (B/Ki), upper (C/UI), middle (D/U2) and lower (E/U3) ureter and bladder (F/B1).

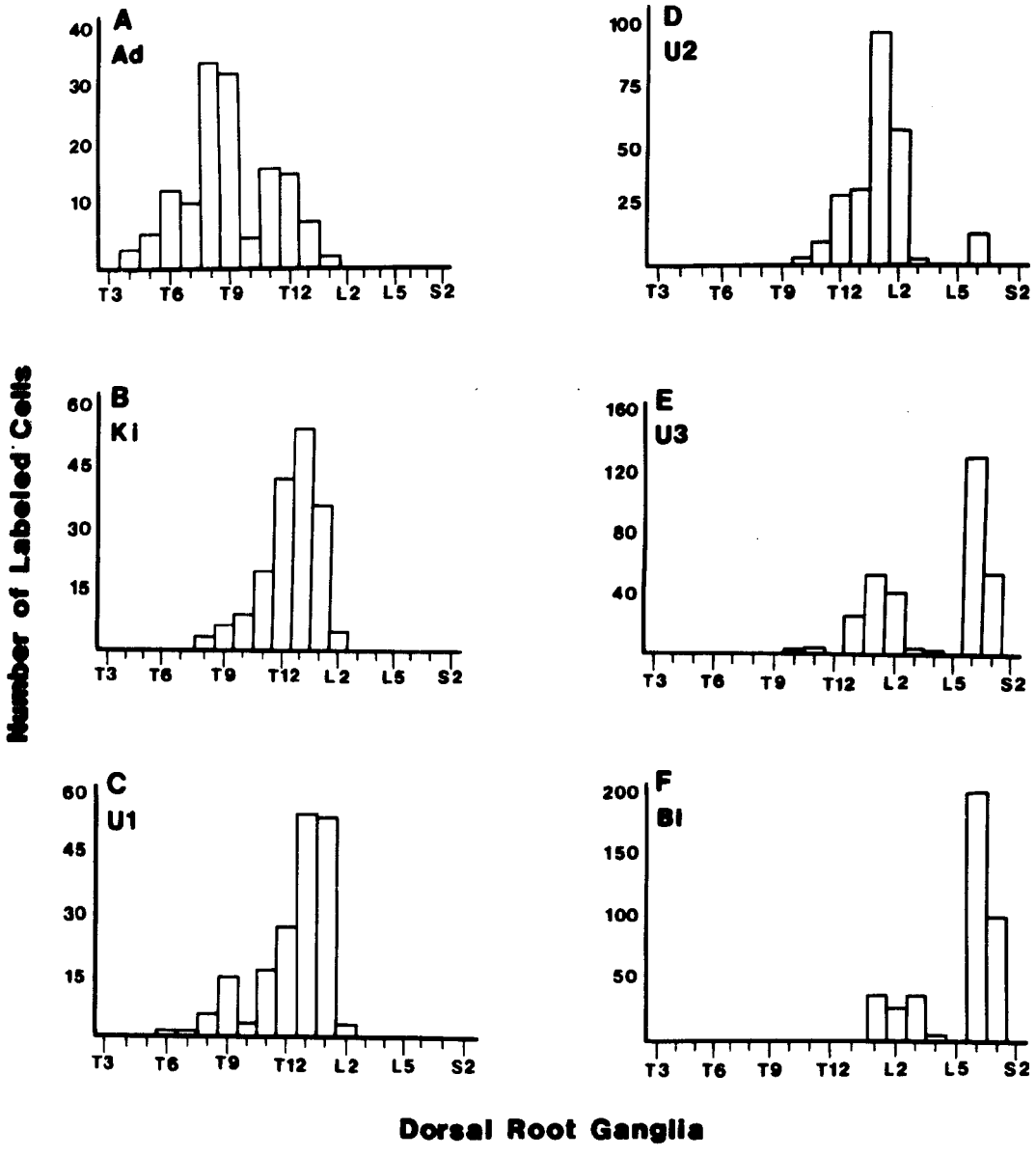

A
Ad
B
Ki
C
U1
D
U2
E
U3
F
Bl
T3
T6
T9
T12
L2
L5
S2
Number of Labeled Cells
Dorsal Root Ganglia

Figure 10. Six histograms to demonstrate the diameter of DRG cell bodies labeled by injections into the adrenal gland (A/Ad), kidney (B/Ki), upper (C/U1), middle (D/U2) and lower (E/U3) ureter and bladder (F/B1). Bin widths are 5um.

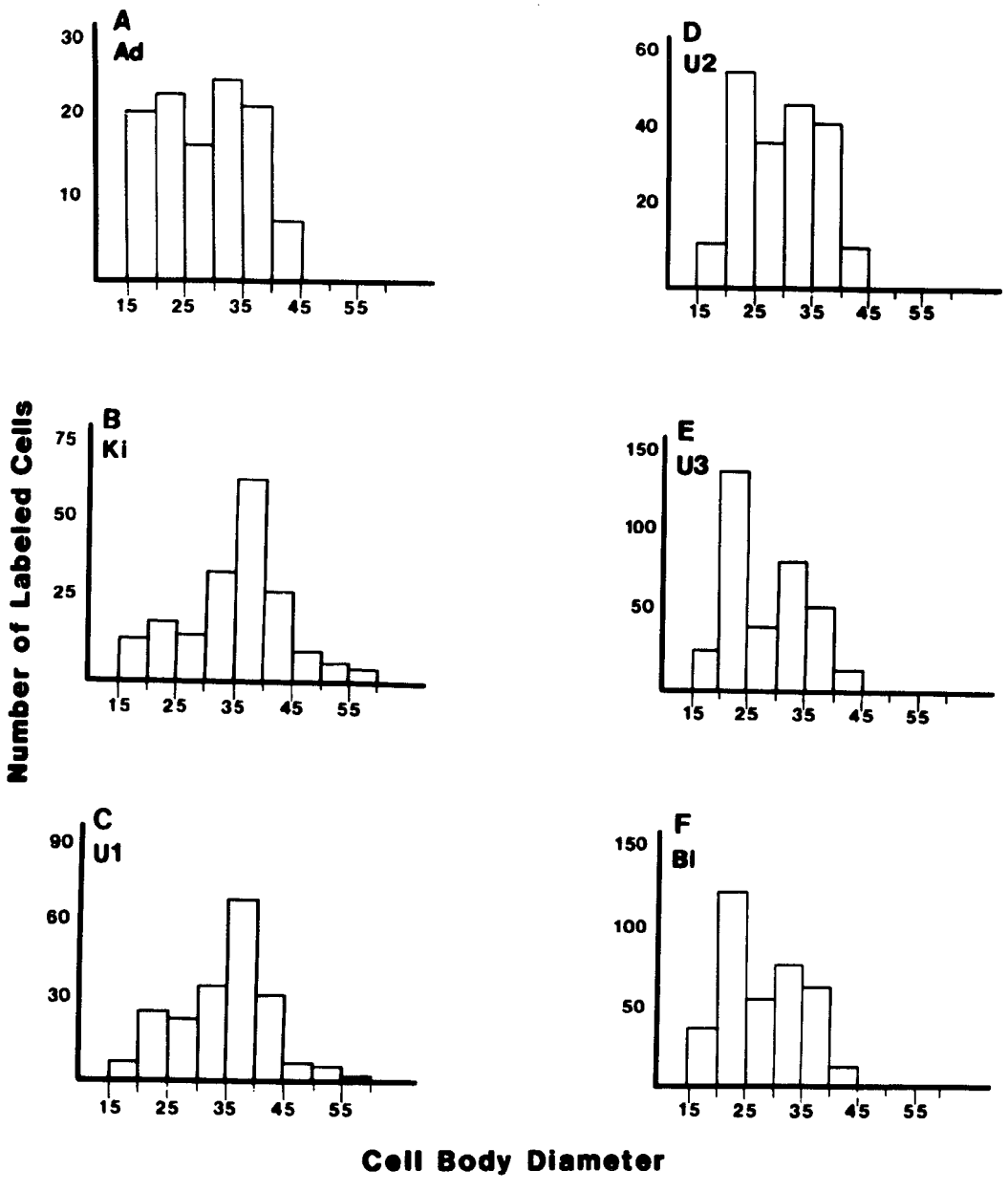
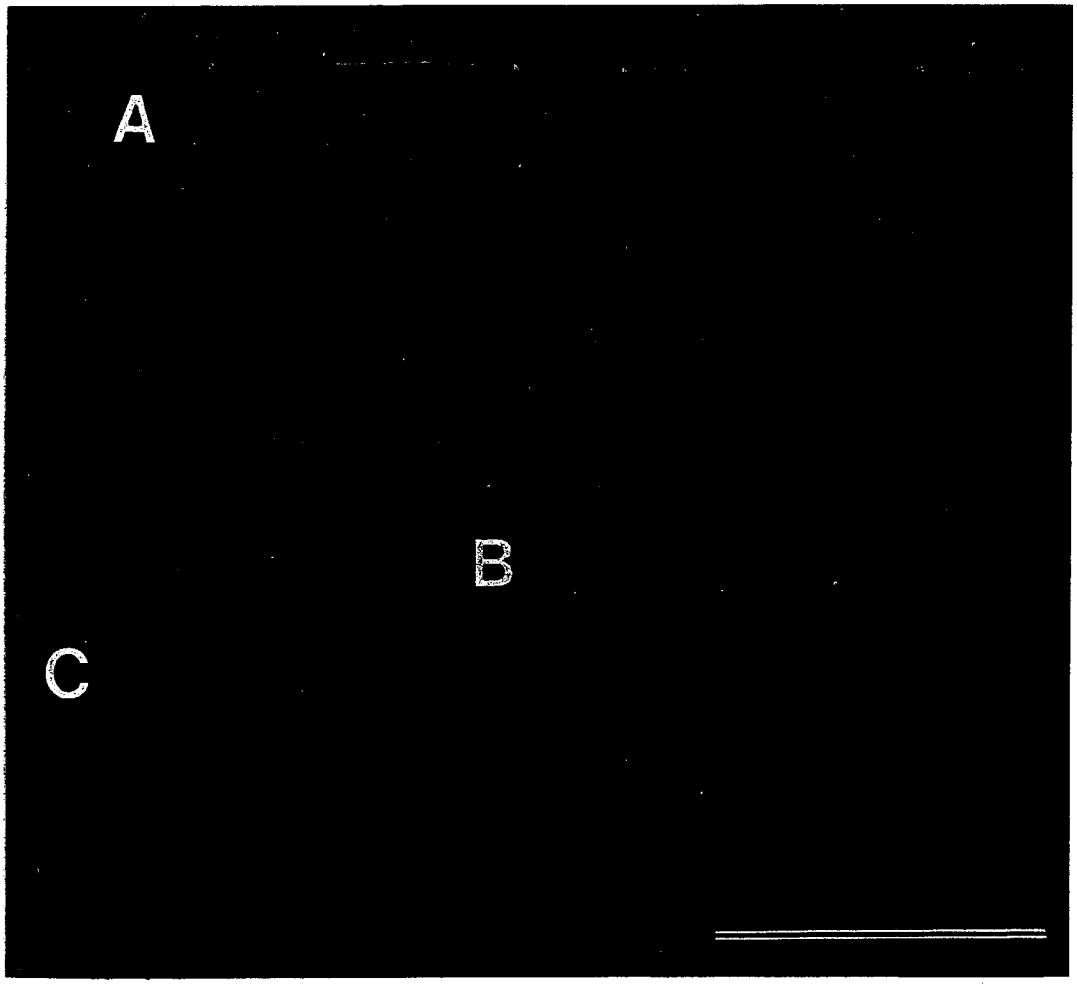

A
Ad
30
20
10
15 25 35 45 55
D
U2
60
40
20
15 25 35 45 55
B
Ki
75
50
25
15 25 35 45 55
E
U3
150
100
50
15 25 35 45 55
C
U1
90
60
30
15 25 35 45 55
F
BI
150
100
50
15 25 35 45 55
Number of Labeled Cells
Cell Body Diameter

Figure 11. A photomicrograph of 3 fluorescently labeled DRG neurons. Cells A and B were labeled by transport from an injection into the upper ureter. Cell C was labeled by a nuclear yellow injection of the cortex of the kidney. Note the differential labeling (i.e., cytoplasm versus nucleus) and the close proximity of these neurons. Scale bar = 30um.


A
B
C

those labeled by kidney injections (p. .01) (Fig. 10) and scattered throughout the ganglia. As can be seen in Figure 9, many of the adrenal afferents were located in the same ganglia as renal afferents and were adjacent to the latter neurons (Fig. 11); however, no double labeled neurons were observed.

3. Ureteral Afferents

Injections of the ureter were localized in one of three segments, namely the upper, middle or lower one-third. Following upper ureteral injections, labeled neurons were observed in the ipsilateral T6-L2 ganglia, with the greatest concentration at the T13-L1 levels (Fig. 9). These cell bodies were scattered throughout the respective ganglia, and their size distribution was undifferentiable from the size distribution of the renal afferents (Fig. 10). from T6-L3 and at L6 DRG levels with the highest concentration of labeled neurons at L1 (Fig. 9). These cells (average diameter 29 um) were significantly smaller than the cells projecting to the kidney (cf. Fig. 10). Lower ureter injections resulted in the labeling of ipsilateral neurons from the T10 through S1 ganglia with the highest concentration at the L6 level (Fig. 9). The cell bodies of those latter afferents were significantly smaller (28 um in diameter) than those labeled by kidney injections (Fig. 10). In all three cases, the DRG cell bodies were scattered throughout the ganglia.

4. Bladder Afferents

The injections of the bladder typically resulted in bilateral labeling of DRG neurons. This was true whether the injections were clearly bilateral (n=2) or appeared to be unilateral. These labeled cell bodies were located in the L1 through L4 and L6 through S1 levels of the DRG. The greatest concentration of

cells was observed at the L6 level (Fig. 9). These DRG neurons (28 um in diameter) were significantly smaller than the kidney afferent cell bodies (Fig. 10).

C. Discussion

A major difficulty in interpreting the present fluorescent dye labeling data is the possibility that the dye diffuses to structures in close proximity to the injection, either through the blood supply or through the intermediate tissue, thus resulting in artifactual labeling. The results of this study provide a convincing validation of this technique. Injections of each of the different structures resulted in selective labeling of a unique population of neurons. This was most convincingly demonstrated by a comparison of the bladder and the renal afferents. In that case the afferents were not only differentially localized L1–S1 (bladder) versus T8–L2 (kidney) but also were morphologically dissimilar in that the bladder neurons were markedly smaller than the renal neurons. Injections of the ureter versus the bladder resulted in differential distributions of the labeled DRG neurons but the size differences were not as great. None of the injections resulted in a selective topographical ganglion distribution of labeled neurons.

A critical methodological point which must be addressed in order for our data to be interpreted satisfactorily is the assessment of the ability and sensitivity of the double labeling technique to demonstrate collateralization. In several studies from this laboratory (Donovan and Wyss, 1983; also unpublished analysis of the cingulate afferents) and others (Kuypers et al., 1980; Swanson and Kuypers, 1980), the fluorescent dyes have been shown to double label neurons with collateral projections to different sites within the CNS. The lack

of such labeling in the present study could be a result of failure of the technique to double label neurons of the peripheral nervous system effectively. In a previous study from kidney vs. spinal cord double labeling did result and thus the technique appears to be effective. It should be noted that the blue dye did not appear to decrease the labeling ability of the yellow dye, a problem which has been reported in studies of the olfactory projections (Alheid et al., 1981). Approximately the same number of neurons were labeled whether a second dye was injected or not, suggesting that the failure to observe double labeled neurons in the present study reflects a true separation of the neuronal pools innervating each organ.

The major finding of this study is that although there is considerable overlap in the location of sensory afferents to the adrenal gland, kidney, ureter and bladder, each organ is innervated by a distinct population of neurons, the distribution of which is unique to that organ and/or segment of that organ. The innervation pattern is such that more caudal organs tend to be innervated by more caudal DRG neurons. Following injections into the ureter or kidney, thoracic and lumbar segments of the ipsilateral DRG are labeled; adrenal injections result in mainly thoracic DRG labeling, bladder injections label DRG cell bodies in sacral and lower lumber segments. The size of the afferent cell bodies also differentiate the different populations.

The rostro-caudal position of the DRG neurons innervating the adrenal gland is similar to the position of the sympathetic preganglionic neurons innervating the gland (Holets and Elde, 1982). In this study, efferent adrenal neurons were observed in the intermediolateral cell column of the T1-L2 segments of the spinal cord, with the highest density of labeled cells at the T9

segment. In the present study, the T8 ganglion was found to contain the highest number of adrenal sensory cell bodies.

No detailed comprehensive studies of the location of the cell bodies of the ureteral sensory system have been undertaken previously. Ultrastructural studies have demonstrated the presence of afferent axons and terminals in the musculature and submucosal plexuses of the ureter of the guinea pig (Hoyes et al., 1977; Hoyes and Barber, 1980). In a gross dissection study, Mitchell (1935) observed that each third of the ureter was innervated by a slightly different arrangement of nerve fibers. He reported that the upper third traveled in the renal plexus and/or the intermesenteric nerve, the middle third traveled in the superior hypogastric plexus and the inferior third passed through the inferior hypogastric plexus. This pattern correlates well with the present characterization of the position of afferent cell bodies which innervate each third of the ureter.

The afferent innervation of the bladder has been previously described by Applebaum et al. (1980) using the horeseradish peroxidase technique. Their findings revealed that the bladder afferents reside in the L1-L2 and L6-S1 segments of the DRG. The present findings confirm these results using the fluorescent dye technique.

Finally, no evidence of collateralization was found in this study, despite the close proximity of neurons projecting to each organ. Although this result may be due to methodological limitations, the differences in cell size which were observed suggest that differences in innervation and the lack of collateralization is not the result of such limitations.

VIII. EFFERENT INNERVATION OF THE KIDNEY

An added advantage to the procedures already described is that methods for tracing the renal afferent nerve pathways simultaneously label renal efferent components. It has been demonstrated that the sympathetic outflow in renal nerves plays an important role in the control of renal function (Block et al., 1952; Fink and Brody, 1978; Zanchetti and Stella, 1975). Stimulation of the renal nerves typically causes renal vasoconstriction and a decrease in urine renal nerves typically causes renal vasoconstriction and a decrease in urine output. Renal sympathetic activity is directly involved in renin release (Zanchetti and Stella, 1975) and renal tubular reabsorption of sodium (DiBona, 1978). Despite these interesting findings, little is known about the origin of renal efferent nerve fibers. In the present study the organization of sympathetic efferent pathways to the kidney was investigated using the fluorescent dye technique.

A. Experimental Protocol

Ten animals used in the fluorescent study of the renal afferents were also used to demonstrate renal motor components. This necessitated dissection of the celiac plexus, splanchnic nerves, sympathetic trunk and the spinal cord from the perfused animals. The ganglia and nerves were placed directly on clean slides and the spinal cord was cut transversely through the T4 through the L4 segments in 30 um sections. One of every five sections was placed on clean

slides with adjacent sections stained with thionine solution for topographical reference. All tissue was viewed under fluorescence microscopy.

Also the six animals used in control experiments in the localization study had their celiac plexus, sympathetic trunk, splanchnic nerves and spinal cords removed. These controls included injections of 5 ul 5% true blue in the tail vein of two rats, injections with 2 ul 5% true blue into a previously denervated left kidney of two animals and in order to identify neural elements associated with the retroperitoneal space, two animals were injected with 2 ul of 5% true blue into the connective tissue surrounding the left kidney.

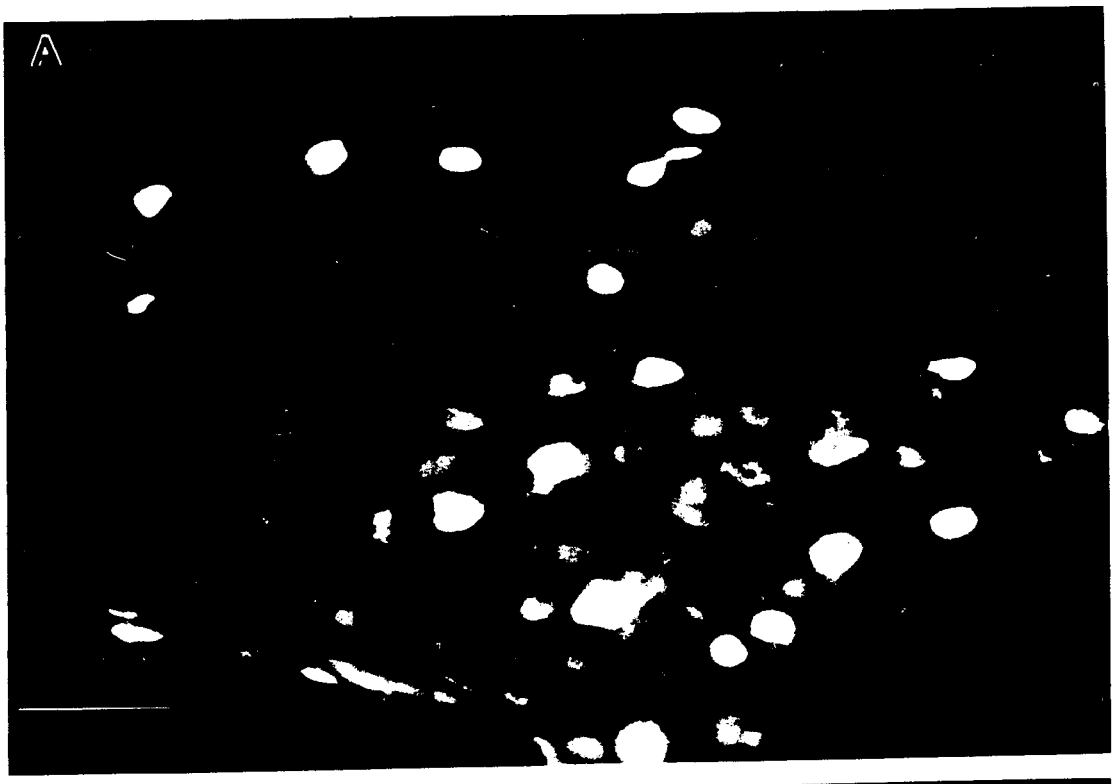
B. Results

After dye injections of either kidney, the majority of labeled cells was observed in the celiac ganglion (Fig. 12A). Injections of fast blue typically labeled an average of 350 cells which were scattered throughout the ganglion in no apparent topographical location. These multipolar cells ranged from 20 um to 49 um in diameter. A smaller number of labeled somata (150 cells) were located in the suprarenal ganglion ipsilateral to the side of injection; the size and shape of these cells corresponded well with those seen in the celiac ganglion. Labeled neuronal cell bodies were observed in the ipsilateral sympathetic trunk from T6 through L2 segments (Fig. 12B). These were smaller in number (total of 80 cells throughout the ganglia) and fusiform in shape with a length of 39 um and a width of 14 um. Thus they appeared as typical postganglionic sympathetic neurons. A small population of labeled cells were located in the intermediolateral cell column of the spinal cord as well.

After tail vein injections, labeled cells were observed bilaterally in the spinal cord at the thoracic and lumbar levels. These labeled neurons were

Figure 12. (A) A photomicrograph of labeled cells present in the celiac ganglion after an injection of true blue into the left kidney of TB 31. (Scale bar = 100 um).

(B) A photomicrograph demonstrating labeled cells in the left sympathetic trunk after a true blue injection of the left kidney (TB 31). (Scale bar = 100 um).


A


B

situated mainly in the intermediolateral cell column. No labeled cells were observed in any of the ganglia (i.e., celiac plexus and sympathetic trunk). These results are identical to those obtained after injections made in the denervated kidneys. Thus, it appears that labeled cells present in the celiac plexus and sympathetic trunk signify a true projection to the kidney, whereas labeling in the spinal cord may be artifactual. The last control experiment (injections of fluorescent dye into the peritoneum surrounding the kidney) labeled many cells in the celiac ganglion as well as bilateral labeling of the sympathetic trunk ganglia.

C. Discussion

The efferent innervation of the kidney is mainly derived from sympathetic fibers (Mitchell, 1950). Electrical stimulation of the renal nerves causes changes in renal function which are similar to those produced by infusion of norepinephrine, the sympathetic neurotransmitter (Kaplan et al., 1953; Zimmerman et al., 1964) to the renal artery. Also histochemical studies indicate the presence of a rich adrenergic innervation of the kidney (Barajas, 1978). The major role of the renal nerves in kidney function has begun to be elucidated (Gill, 1969; Schrier, 1974; Gottschalk, 1979), however except for gross dissections (Green, 1955; Baljet and Drukker, 1979) the origin of these renal efferent fibers had not been described in the rat prior to the initiation of this study. The present study used the fluorescent dye technique to locate postganglionic neurons in the celiac and suprarenal ganglia and the sympathetic trunk and to assess the possibility of the a direct preganglionic innervation of the kidney. The location of the renal postganglionic efferent neurons corresponds well to the route of the splanchnic nerves in the rat. These nerves

arise from preganglionic neurons in the visceromotor centers of the spinal cord and exit the cord at the T6 through L2 levels (Green, 1955). A small proportion of these fibers synapse in the sympathetic trunk (Kuo and Krauthamer, 1981), as demonstrated in our study by labeling of sympathetic cell bodies at these levels after kidney injections. Most of the remaining preganglionic fibers synapse in the celiac plexus and from there postganglionic fibers supply the kidney. The present data demonstrate that the greatest number of labeled renal efferents are in the celiac ganglion. These results correspond with those of Kuo et al. (1982) in which the origin of efferent axons in the renal nerves of the cat was examined using retrograde transport of HRP. In their studies, labeled cells were identified in three locations: (1) centrally along the renal nerve, (2) in the superior mesenteric ganglion and (3) in the ipsilateral sympathetic chain ganglia (T12–L3).

IX. GENERAL DISCUSSION

For several decades the physiological significance of the renal neural supply has been recognized. Although few studies have provided an anatomical basis on which to interpret these physiological findings, the advent of modern tracing techniques (see Robertson, 1978; Heimer and Robards, 1981) has led to a number of recent anatomical investigations of the innervation of the kidney. These include the present study in which the renal afferent and efferent neurons were anatomically characterized in the rat using the fluorescent dye and horseradish peroxidase techniques. The rat renal afferent system is composed of neurons whose cell bodies are located in the T6 through L2 DRG (Donovan et al., 1982, 1983; Ciriello et al., 1982; Lappe et al., 1982), and this innervation is entirely ipsilateral. The neuronal cell bodies innervating the left kidney reside in the T8 through L2 ganglia with the highest concentration of cells at T13. The right kidney receives its afferent supply from neuronal cell bodies located in the T6 through L1 levels with the greatest concentration of cells at T11. The present results demonstrate that this finding did not result from blood borne label or from leakage of dye into the retroperitoneal space. Further, afferent neurons projecting to the urinary system (kidney, ureter and bladder) and the adrenal gland were localized as unique groups of neurons which were often adjacent to renal afferent cell bodies. This indicates that the dyes do not diffuse out of the retrogradely filled cell bodies and label surrounding

cell bodies, a problem which has recently been noted in other studies (see Weidner et al., 1983).

Once the DRG neurons projecting to the kidney were characterized, the second question could be addressed, i.e., what is the CNS course and terminations of the renal afferents. Recently Kuo et al. (1983), using the HRP technique, studied the segmental distribution and central projections of renal afferents in the cat. In their study, the central processes of the labeled DRG cells which were located mainly in the ipsilateral L1 through L3 spinal cord levels (a few neurons in the contralateral DRG were also labeled) were observed to enter the spinal cord near the dorso-lateral sulcus and then pass rostrocaudally in Lissauer's tract sending collaterals to Lamina 1. Other collaterals were reported to course in the lateral or medial part of Lissauer's tract with the medial fibers extending into the contralateral spinal cord and lateral fibers terminating in Lamina 7 and Clark's nucleus. The results of the present study suggest the same general pattern in the rat. Labeled renal afferent projections were observed entering the medial and lateral portions of Lissauer's tract. The medial group was seen to terminate in the vicinity of the intermediomedial cell column and the lateral fibers terminated in the intermediolateral nucleus. It is curious that in both studies labeling of renal central projections could only be produced in very young animals. Further, in neither study could afferents be traced with the HRP technique to levels of the CNS higher than the thoracic spinal cord. Conversely it has been reported that HRP labeling of the main splanchnic nerve in the young kitten results in labeling of axons which reach the caudal brainstem. Even with labeling of such a large nerve, central afferents could not be well differentiated in adult animals. Therefore, in order to assess the adult connections we have employed

the double labeling fluorescent dye method. With this technique, we have identified renal afferent neurons which project directly to the medulla. These results demonstrate that at least 8% of the total number of renal afferent neurons project to the brain.

Concerning the efferent innervation of the kidney, both the parasympathetic and the sympathetic renal supply were studied. There have been reports of a direct projection to the kidney from brainstem parasympathetic nuclei (nucleus of the vagus or nucleus of the solitary tract) (Ciriello et al., 1982; Lappe et al., 1982). Our data do not give evidence for the presence of direct parasympathetic innervation. Control experiments performed in our laboratory indicate that the labeling in the brainstem following intrarenal injections of fluorescent dye results in part if not totally from nonspecific sources (Donovan et al., 1982, 1983). The control experiments included dye injections into previously denervated kidneys and injections into the tail vein of the rat, both of which labeled approximately the same number of cells in the same region of the medulla as the experimental kidney injections. This pattern of labeling is also seen after intravenous injections of HRP (Broadwell and Brightman, 1976). Other techniques must be employed to determine whether a renal parasympathetic supply exists and, if so, whether it originates in the medulla.

The sympathetic efferent supply to the kidney is more easily assessed. Labeled neuronal cell bodies were identified in the celiac plexus and the ipsilateral sympathetic trunk after kidney injections of the tracer. These results are similar to those observed by Kuo et al. (1982) after HRP renal nerve tracing studies in the cat. In this study labeled neuronal perikarya were seen in the

superior mesenteric ganglion, the ipsilateral sympathetic trunk and centrally along the renal nerve.

A large number of physiological studies have investigated the functional significance of the neural input to the kidney. The results have demonstrated that the renal efferent nerves are important in the control of fluid balance, cardiovascular homeostasis and the pathogenesis of hypertension (Winternitz and Oparil, 1982). As regards the afferent innervation of the kidney, both mechano- and chemoreceptors have been demonstrated in several species (Astrom and Crafoord, 1968; Nijima, 1971; Recordati et al., 1980) and a variety of stimuli have been shown to result in alterations in the afferent renal nerve activity. The results of the present anatomical study demonstrate that the renal afferent neuronal cell bodies are of variable sizes, a finding that is consistent with the physiological observation that there are several types of receptors involved in transmission of renal sensory impulses.

The specific functions attributed to the afferent renal nerve supply include the following. First, several investigators have reported that the renal afferent nerves may influence cardiovascular homeostasis. Decreases in systemic pressure have been described during electrical stimulation of these nerves (Ueda et al., 1967; Aars and Akre, 1970), while one study reported no change in arteriopressure during stimulation (Astrom and Crafoord, 1968) and still another study indicated that stimulating renal afferent nerves increases arterial pressure and heart rate (Calaresu et al., 1976). These apparent discrepencies have been attributed to differences in anesthesia and in the species of animals used (Calaresu et al., 1976). Second, stimulation of the renal afferents in one kidney has been shown to produce a reflex increase in efferent nerve activity and arterial vasoconstriction in both the ipsilateral (Recordati et al., 1980) and

contralateral kidney (Calaresu et al., 1978; Colindres et al., 1980; Brody and Johnson, 1980). In a preliminary experiment, Recordati et al. (1980) reported that activation of renal chemoreceptors elicited excitatory reflexes to the ipsilateral kidney and adrenal gland both in rats with intact nervous systems and rats with the spinal cord transected at the first cervical segment. In contrast, the contralateral reflex appears to be subserved by supraspinal pathways, since this response to electrical stimulation of afferent renal nerves was abolished by spinal cord sectioning (Brody and Johnson, 1980; Calaresu et al., 1978; Colindres et al., 1980). These findings suggest that the reno–renal reflex is mediated by spinal (ipsilateral) and supraspinal (contralateral) neurons.

In the spinal cord many of the preganglionic sympathetic neurons reside in the intermediolateral cell column. Our data and those of Kuo et al. (1983) suggest a close relationship between the central processes of some renal afferent fibers and neurons within the intermediolateral cell column. The medial group of renal afferent fibers appears to terminate in the area of the intermediomedial nucleus. This nucleus has been shown in previous studies to receive a small number of fibers from the dorsal roots at all levels (Shriver et al., 1968; Carpenter et al., 1968) and may serve as an intermediary relay in transmission of impulses to visceral motor neurons (Petras and Cummings, 1972). Other studies have further suggested that many preganglionic sympathetic neurons are located in this region (Cummings, 1969; Petras and Cummings, 1972; Chung et al., 1975).

Both physiological and anatomical studies support the existence of supraspinal connections of renal afferent neurons. Fernandez et al. (1974) observed that renal denervation in the rat resulted in an increase in norepinephrine content of the whole hypothalamus without a significant change

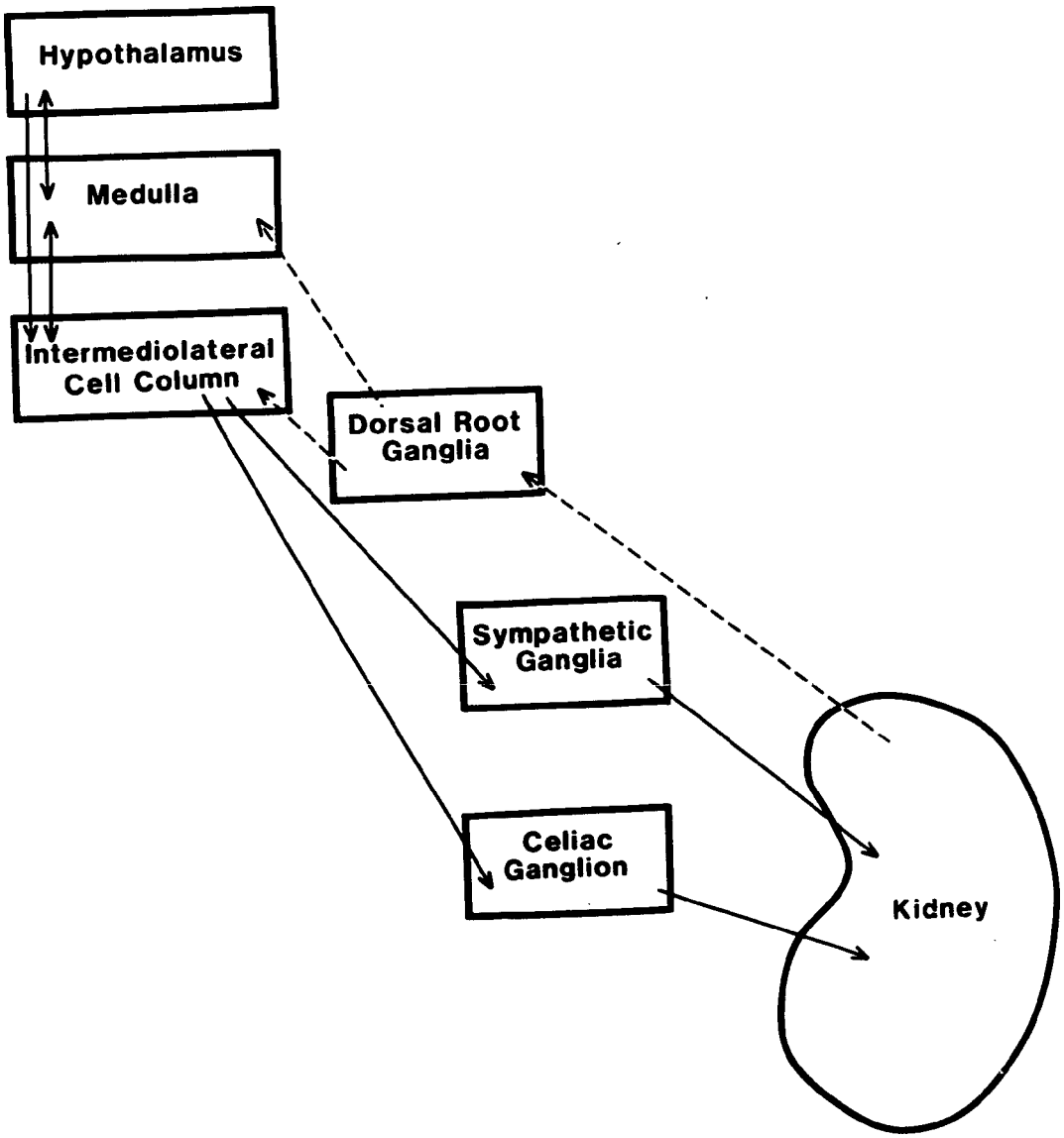
in blood pressure, suggesting that the renal nerves may modulate hypothalamic noradrenergic activity. In addition, Calaresu and Ciriello (1981) measured the electrical activity of spontaneously firing single units in the medulla and hypothalamus during stimulation of the afferent renal nerves of anesthetized cats. These investigators found responsive units in a number of areas, including the lateral tegmental field, the area of the paramedian reticular nucleus, the dorsal vagal complex, the lateral hypothalamic area, the lateral preoptic area and the paraventricular nucleus of the hypothalamus. Many of these regions are thought to be involved in body fluid regulation and cardiovascular function (Ciriello and Calaresu, 1980; Miura and Reis, 1971; Zanchetti and Stella, 1975; Haywood, 1977). Recently investigations have been undertaken to determine whether these renal afferent projections to the brainstem are direct or indirect. Simon and Schramm (1983) found that electrical stimulation of the renal nerve resulted in an apparent direct, orthodromic excitation of the nucleus gracilus and the caudal half of the nucleus tractus solitarius. The present anatomical study has demonstrated that approximately 8% of the total renal afferent neurons project directly to neurons in the caudal brainstem. The effectiveness of this pathway in mediating afferent influences on the hypothalamus (Fernandez et al., 1974; Calaresu and Ciriello, 1981) remains to be tested.

Recent anatomical studies support the existence of both afferent and efferent connections between the medulla and the hypothalamus (McKellar and Loewy, 1981; Swanson et al., 1980). The brainstem afferent connections to the paraventricular hypothalamic nucleus (PVH) were studied using autoradiography (McKellar and Loewy, 1981). This nucleus was demonstrated to receive projections from the parabrachial nuclei, locus coeruleus, ventral medulla and A1 region. In a further study, these authors showed that the A1 catecholamine

cell group was efferently connected not only to the PVH but also to the dorsomedial nucleus, the dorsal hypothalamic area, the supraoptic nucleus and the median eminence (McKellar and Loewy, 1981). Direct hypothalamic projections terminate in the medulla and the spinal cord. These hypothalamic projections originally demonstrated by Kuypers and Maisky (1975) are now known to originate in several regions, and in many cases are directed to the intermediolateral cell column. The caudal brainstem also has efferent projections to the spinal cord and more precisely to the intermediolateral cell column (Amendt et al., 1978). Using the method of retrograde transport of HRP it has been demonstrated that the dorsomedial part of the tractus solitarius, the ventral part of the raphe nuclei, and the ventrolateral reticular formation project to the intermediolateral cell column. In light of these observations and with the data presented in our study, a feedback loop involving the renal nerves, caudal brainstem and hypothalamus can be theorized (see Fig. 13).

There is increasing evidence that the renal nerves are important in the pathogenesis of hypertension in a number of experimental models. In both the deoxycorticosterone acetate (DOCA)-salt and the spontaneously hypertensive rat (SHR) models, renal denervation has been observed to delay the development and blunt the severity of hypertension (Katholi et al., 1980; Kline et al., 1978; Dietz et al., 1978; Winternitz et al., 1980). This delay was associated with increased urinary sodium excretion, suggesting a renal efferent mechanism. In these models renal denervation failed to lower blood pressures of animals with established hypertension. Renal denervation has also been demonstrated to delay the onset of one-kidney Grollman hypertension (Brody and Johnson, 1980) and in preliminary reports these authors have indicated that interruption of renal afferent connections to the anterior hypothalamus may result in the prevention

Figure 13. A schematic diagram demonstrating the neuroanatomical connections involved in renal function.


Hypothalamus
Medulla
Intermediolateral Cell Column
Dorsal Root Ganglia
Sympathetic Ganglia
Celiac Ganglion
Kidney

of hypertension in this model (Brody and Johnson, 1980; Webb et al., 1981). Recent studies of the effects of renal denervation in the one-kidney, one clip Goldblatt model of experimental hypertension (1K1C) suggest that renal afferent nerves are involved in this model of hypertension (Katholi et al., 1981; Winternitz and Oparil, 1982; Katholi et al., 1982). Studies by Katholi et al. (1981) have demonstrated that the renal nerves are necessary for maintenance of 1K1C in the rat and that this effect is not mediated by renin nor is it associated with alterations in sodium intake or excretion, water intake, or renal function. Their data suggest that the effects of denervation in 1K1C are not due to interruption of renal efferent nerves. Further studies have led to the speculation that the major effect of renal denervation in this model may be an interruption of renal afferent nerve activity which by a direct feedback mechanism attenuates systemic sympathetic tone, thereby lowering blood pressure. Support for this hypothesis also comes from the present anatomical studies in that renal afferent fibers were demonstrated to project centrally to the intermediolateral cell column in the spinal cord and to the medulla which is a region involved in cardiovascular regulation. Ongoing studies in our laboratory are making use of the present data in order to determine the effective site of renal nerve action in each hypertension model.

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