**NOTE** *Anatomy*

# Origin of Efferent Fibers of the Renal Plexus in the Rat Autonomic Nervous System

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ABSTRACT. To clarify the origin of efferent nerves containing renal plexus, the retrograde neuronal tracing was utilized with a new exact closed injection system with microcapsules. The microcapsule was positioned in the rat left renal plexus, and the capsule was filled with fluoro-gold. Retrograde labeled cells were observed in the ipsilateral sympathetic trunk, especially T12 and T13, and the ipsilateral suprarenal ganglia (SrG). There were no labeled cells in the parasympathetic nuclei in medulla oblongata and sacral cords. These results indicated that the origins of efferent nerves in the rat renal plexus are almost all sympathetic ganglia, such as sympathetic trunk and SrG, and cells in other ganglia may be secondary or accessory innervations.

KEY WORDS: autonomic nervous system, fluorogold, kidney.

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The kidney has a significant amount of innervation involving both efferent and afferent fibers. Anatomical analysis of efferent fibers containing sympathetic and parasympathetic nerves has been done utilizing retrograde neuronal tracing methods [2–4, 9, 14]. Innervation of sympathetic nerves in the kidney has been shown to originate from both paravertebral and prevertebral ganglia in the rat [3, 4, 14]; however, the rate of distribution is diverse, especially to the celiac ganglia (CeG). The presence of parasympathetic innervation to the kidney has also been still poorly defined [1, 2]. Norvell and Anderson [9] and Gattone *et al*. [4] have shown that directly injecting tracers to the kidney parenchyma results in labeling the dorsal motor nucleus of vagus (DMV) in the rat; however, these tracer labeled cells persisted after control vagotomies were performed. In addition, the tracing methods for these experiments were carried out under an open state of neuronal treatment with tracers, such as pressure injection to the renal parenchyma or dipping of renal arterial nerve plexus into the tracer. The results from these studies had been examined carefully, but diffusion or ectopic uptake of tracers in peripheral tissues may have occurred as well as pseudo-labeling. In the present study, we examined the neuronal cell body distribution of cells innervated by the renal nerve plexus by injecting neuronal tracers into a microcapsule to minimize tracer diffusion or ectopic uptake.

Twelve male Sprague-Dawley rats, 6 to 8 weeks old and weighing 250–350 g, were used. All surgical procedures were carried out with the animals anesthetized i.p. with medetomidine (0.3 mg/kg), midazolam (4 mg/kg) and butorphanol (5 mg/kg). The Animal Care and Use Committee of Hyogo College of Medicine approved the procedure.

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Fluoro-gold (FG, Fluorochrome, Denver, CO, U.S.A.) was used for retrograde neuronal tracing. The micro Teflon tubes were 1.35 mm outside, 0.75 mm inside and 1.8 mm in length. The left kidney was exposed in anesthetized rats, followed by removal of the anterior fascia of the kidney, exposing renal blood vessels. Renal nerves were carefully separated from the renal artery, tied to a bundle with a silk suture (4–0 U. S. P.), and the distal end was cut. Then, the nerves were passed through a microtube, and the proximal end of the tube was sealed with cyanoacrylate glue (Surgical Aron Alpha®, Toagosei, Tokyo, Japan). The immobilized tube was filled with 1 $\mu l$ of 2% FG, and the distal end of the tube was sealed with glue. For control experiments, 1 to 2 $\mu l$ of 2% FG was poured around the proximal base of a vacant microcapsule.

Three days after applying the neuronal tracer, animals were perfused with saline, followed by phosphate buffer (PB) containing 10% formalin. Prevertebral ganglia, which includes CeG, aorticorenal (ReG) and supurarenal ganglia (SrG), were removed with the aorta and its primary branches. Bilateral sympathetic trunks were dissected from T9 to L2. The medulla oblongata and sacral cords were removed. After washing with PB overnight, they were embedded in 10% gelatin, hardened with 10% formalin and soaked in 20% sucrose. All of these reagents were prepared with PB. The specimens were quickly frozen in −80°C, and 40 $\mu$m thick sections were obtained by cryostat sectioning (CM3050S III, Leica, Wetzlar, Germany). Alternate sections were mounted on glass slides and counted the FG labeled cells in each ganglion under a fluorescent microscope with U excitation filter (BX-51, Olympus, Tokyo, Japan), and photographs were taken with a CCD camera (DP-73, Olympus). All values of each animal were expressed as mean ± SEM, and Student's *t*-test was used for analysis of variance.

Numerous FG labeled cells were observed in the left paravertebral ganglia T10 to L1, particularly in T12 and T13 (Figs. 1A and 2). A small number of positive cells were observed in the right trunks. Approximately 70.3% of the labeled cells were localized to the ipsilateral (left) ganglia,

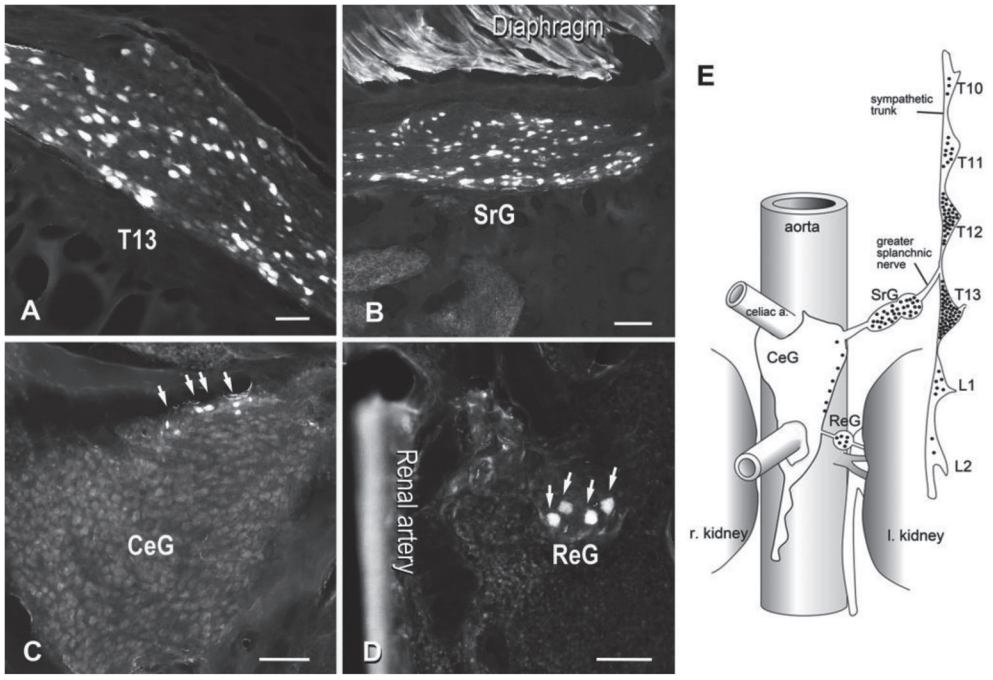


Fig. 1. Fluoro gold-labeled cells in the sympathetic trunk (T13, A), SrG (B), CeG (C) and ReG (D). A large number of labeled cells were observed in T13 and SrG. (A and B) In CeG, labeled cells appeared as a small population in the ganglion (C, arrows). They were unevenly localized to the ipsilateral side (top of photo). A small number of labeled cells were counted in the ReG (arrows) which is a small size ganglion (D). Bar=100 $\mu$m (A and D), 200 $\mu$m (B) and 400 $\mu$m (C). (E) An illustration of the distribution of FG labeled cells in sympathetic ganglia of the rat. The dots reflect the cell populations distributed in each ganglion.

and 2.5% were observed in the contralateral (right) ganglia (Fig. 2). Labeled cells in the prevertebral ganglia were predominantly distributed to the ipsilateral SrG (Figs. 1B and 2), and the total labeled cells were 17.6 ± 3.1% (Fig 2B). There were fewer labeled cells in CeG (Fig. 1C) and ReG (Fig. 1D) (4.9% and 4.6%, respectively). Rate of distribution to the rat sympathetic ganglia in this study showed a value near those in the reports of Chavendra and Weaver [3] or Sripairojthikoon *et al*. [14] rather than that of Gattone *et al*. in which too numerous labeled cells were observed in CeG [4]. Interestingly, labeled cells in CeG were often unevenly distributed in the ipsilateral side of a ganglion, indicating that the cells innervating the kidney may have a tendency to be linearly aligned ipsilaterally (Fig. 1E). In a control experiment in which tracer was placed around the vacant capsule, the number of labeled cells in the CeG significantly increased compared to the experimental group, indicating that there are few CeG cells that uptake the tracer from the renal plexus. The above results suggested that the efferent sympathetic nerves in the kidney primarily originate from the ipsilateral lower sympathetic trunks (T12 and T13) and SrG, and some secondary or accessory cells may be distributed in the CeG, ReG and contralateral sympathetic trunks in the rat.

No labeled cells were observed in the parasympathetic nuclei, such as DMV and sacral cord segments (Fig. 2A). Previous tracer studies had occasionally shown labeled cells in DMV [4, 9], and these results supported that vagal innervation is included in the renal plexus. It is likely thought that pseudo-labeling in DMV may occur via peritoneal routes without the vagus [4]. Our tracer method with microcapsules showed the absence of parasympathetic innervation including the cholinergic neurons, leaving the origin of acetylcholine effects to the kidney unknown. Acetylcholine has several effects in the kidney, such as inhibition of ion and water reabsorption [5, 7, 8, 13] and acid/base equilibration [10–12]. In our previous study, it was shown that choline acetylransferase, which catalyzes the synthesis of acetylcholine, was expressed in a part of the cortical collecting ducts in rat kidney [6]. Therefore, it is possible that cholinergic effects in the kidney may be controlled by non-neuronal or other mechanisms.

In conclusion, from the renal plexus, this tracer method exclusively labeled innervated cells in the renal efferent nerves. Sympathetic nerve cells were primarily distributed to the ipsilateral sympathetic trunks T12 to T13 and SrG, and cells in other sympathetic ganglia, such as CeG and ReG, may be secondary or accessory ganglia for the renal plexus. In addition, there was no innervation via the vagus or sacral cord segments to the renal plexus. Thus, the source of acetylcholine may be provided via another unknown route, and further studies are needed to investigate this possibility and to identify the source.

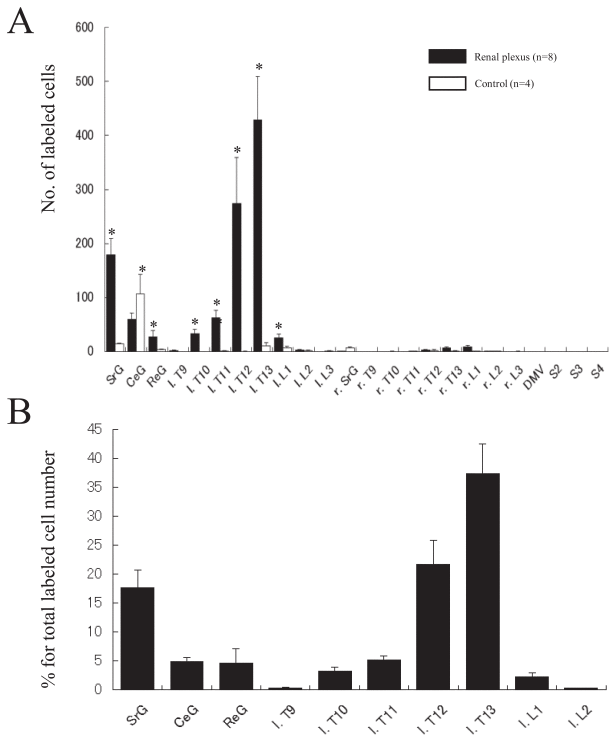


Fig. 2. Mean number of FG positive cells in each ganglion (A) and the mean percentage of labeled cells distributed in each ganglion for the total labeled cells (B) of pre- and paravertebral ganglia in alternate sections. **P*<0.01 represents the significance levels when comparing renal plexus injected experiments with control experiments (mean ± SEM). Typical distribution was observed in the ipsilateral (left) ganglia, such as SrG and sympathetic trunk (T10 to L1). There were no labeled cells in parasympathetic nuclei of medulla oblongata (DMV) or spinal cord segments (S2 to S4). A significant number of labeled cells were counted in CeG in the control experiments compared to those in the experimental groups (A).

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