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Original Article

Pre and postganglionic innervation of rat adrenal gland by fluorescent tract tracer – Fast blue☆

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ABSTRACT

Aims: To search for pre and postganglionic neurons innervating the adrenal gland by injecting retrograde tract tracer fast blue in the adrenal medulla.

Methods: The motor innervation of rat adrenal gland was studied by a fluorescent tract tracer fast blue. 5 μl of 2% aqueous suspension of fast blue was injected into left adrenal gland. After a survival period of 4–5 days, spinal cord, sympathetic ganglia, suprarenal ganglion, coeliac ganglion and left adrenal gland were dissected out and 15 μm thick plastic sections (JB4 Polysciences) were examined under a fluorescent microscope.

Results: Retrogradely labeled preganglionic neurons were observed in the ipsilateral intermediolateral column of spinal cord from T3 to L2 spinal segments with maximum concentration of labeled neurons from T6 to T11. The labeled neurons were multipolar, spherical or fusiform in shape with transverse diameter 10–20 μm and vertical diameter varying from 12 to 30 μm. Postganglionic labeled neurons were also observed in the left suprarenal ganglion and left sympathetic ganglia (T5 –L2) with maximum concentration from T6 to L1. Labeled neurons varied from 12 to 30 μm in diameter and were randomly distributed throughout the ganglion.

Conclusion: The preganglionic neurons from T3 to L2 spinal segments and postganglionic nerve fibers from ipsilateral sympathetic ganglia (T5 –L2) and suprarenal ganglion supplying the adrenal gland might be responsible for the hormone release by regulating blood flow and also by directly innervating the parenchymal cells.

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1. Introduction

It is generally accepted that chromaffin cells of adrenal medulla are supplied by preganglionic cholinergic fibers, the cell bodies of which are located in the intermediolateral column of the spinal cord. Noxious chemical stimulation of thoracic and lumbar interspinous tissue in anesthetized rats is associated with large increase in adrenal sympathetic efferent nerve activity and catecholamine secretion.[1]

The review of literature shows lot of variation regarding the extent of intermediolateral column supplying the adrenal gland.[2–4]

The presence of encephalin, substance P and vasoactive intestinal polypeptide like immunoreactivity has been seen in the nerve terminals of human adrenal medulla, which suggests that innervation to adrenal medulla may not be purely cholinergic.[5–7] Acetylcholinesterase positive nerve plexus have also been demonstrated in the human adrenal cortex.[8,9]

Adrenergic innervation has been demonstrated in cat adrenal medulla,[10] whereas the postganglionic nerve endings have also been seen in the adrenal gland of dogfish and rat.[11,12]

Therefore the present work was planned to search for postganglionic neurons innervating the adrenal gland and to confirm the extent of intermediolateral column of spinal cord supplying it by injecting retrograde tract tracer fast blue in the adrenal medulla.

2. Materials and methods

The study was conducted on 25 adult albino rats of either sex weighing between 200 and 250 g. The animals were kept under standard laboratory conditions with food and water ad libitum. Under ether anesthesia, laparotomy was performed and left adrenal gland was exposed. 5 μl of 2% aqueous solution of fast blue was slowly injected over a period of 15 min. The needle of the microsyringe was kept in the gland for 5 min and then slowly withdrawn after the completion of injection to allow the tracer to pass from the syringe into the gland. The intestine was replaced in position and the anterior abdominal wall was sutured. After a survival period of 4–5 days, the rat was perfused with 10% formaldehyde. Anterior abdominal wall was opened. Left adrenal gland, celiac ganglion, suprarenal ganglion and C8 to L4 sympathetic ganglia of both sides were dissected out. Laminectomy was performed to expose the spinal cord. The spinal cord was taken out from C7 to L4 spinal segments.

The tissues were immersed in 10% sucrose cacodylate buffer for 24 h and embedded in plastic resin (JB4 kit polysciences) without prior dehydration. The 15 μm thick serial sections of the spinal cord (longitudinal and transverse), sympathetic ganglia, suprarenal ganglion and celiac ganglion were examined under a fluorescent microscope using an excitation filter of 365 nm.

3. Results

Screening of sections of the left adrenal gland showed a needle track with maximum concentration of fluorescent dye in the medulla of the adrenal gland. In three animals, the leakage was seen in the connective tissue outside the adrenal gland and were discarded from the study.

Retrogradely labeled neurons were seen in the intermediolateral column of the spinal cord from third thoracic to second lumbar spinal segments. Labeling was exclusively ipsilateral to the site of injection and no labeled neurons were seen on the opposite side.

Maximum concentration of labeled neurons was observed from T6 to T11 spinal segments. The labeled neurons were multipolar and showed bright blue fluorescence in the cytoplasm with a negative nuclear shadow. Fig. 1 shows transverse section of the spinal cord (T9 segment) with a labeled neuron in the left intermediolateral column of spinal cord. The labeled neurons were spherical or fusiform in shape with transverse diameter varying from 10 μm to 20 μm.

Longitudinal section of the spinal cord showed vertical disposition of the labeled neurons with bright blue fluorescence in the cytoplasm and a negative nuclear shadow (Fig. 2). The neurons vary from 20 μm to 30 μm in length.

Retrogradely labeled neurons were also observed in the suprarenal ganglion and from fifth thoracic to second lumbar sympathetic ganglia with maximum concentration from T6 to T11. Labeling was ipsilateral to the site of injection. Labeled neurons were 12 μm–30 μm in diameter and were arranged singly or in groups of 2–3 neurons throughout the ganglion without any specific localization (Fig. 3). No labeling was seen in the celiac ganglion.

4. Discussion

In the present study, fluorescent tract tracer fast blue was picked up by the nerve terminals in the adrenal medulla and the adjoining part of the adrenal cortex and labeled the neurons retrogradely in the spinal cord, sympathetic ganglia and suprarenal ganglion. These observations clearly demonstrate that the adrenal gland is supplied by both pre and postganglionic sympathetic nerve fibers.

It has been observed that the unilateral destruction of the adrenal medulla or the nerve terminals supplying the adrenal medulla leads to disappearance of preganglionic neurons of the intermediolateral column of T7 to T10 spinal

Fig. 1 – Transverse section of the spinal cord (T9) showing a labeled neuron in the left intermediolateral column of spinal cord. ×300.

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