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Sympathetic preganglionic neurons project to superior cervical ganglion via C7 spinal nerve in pup but not in adult rats

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ABSTRACT

We investigated the distribution of sympathetic preganglionic fibers in each spinal nerve of the brachial plexus, and its correlation with presence of Horner's syndrome in the pup and adult rats. According to surgical intervention to the C7–T1 spinal nerves in the right side, rats of 7 days postnatal (P7), P14 and adulthood (24 for each age group) were subdivided into four subgroups of six each, respectively, i.e., C7 or C8 or T1 spared subgroup — where C7 or C8 or T1 alone was kept intact with avulsion of the other two spinal nerves and division of the sympathetic chain caudal to the stellate ganglion; C7–T1 avulsed subgroup — where C7–T1 were all avulsed but the sympathetic chain kept intact. Fluoro-Gold (FG) was injected bilaterally into the superior cervical ganglion (SCG) for labeling of sympathetic preganglionic neurons (SPNs). Furthermore, Horner's syndrome was examined after avulsion of different spinal nerves for P14 and adult rats. In C7 spared subgroups, FG-labeled neurons accounted averagely for 16.9% in P7, 13.5 in P14 and 1.0 in adult rats, and difference was statistically significant between P7 and adults ($Z=-2.9$, $P=0.004$), P14 and adults ($Z=-2.9$, $P=0.004$). When both C7 and C8 were avulsed, Horner's syndrome was more prone to be produced in pups than in adults ($\chi^2=4.2$, $P=0.04$). These results indicate that some SPNs project to SCG via C7 in the pup, but this pathway disappears during postnatal development. It suggests that in newborns with brachial plexopathy, presence of Horner's syndrome may be correlated with avulsion of C7.

1. Introduction

Sympathetic preganglionic control is critical for regulating the activity of the superior cervical ganglion (SCG), which provides autonomic inputs to the target organs in the head and face such as Müller muscles and pupillary dilator muscle (Chanthaphavong et al., 2003). Clinically, any interruption of this innervation to the SCG, such as avulsion of the C8T1 spinal nerves, may produce Horner's syndrome, appearances of which are ptosis, miosis, and anhydrosis. In rats, sympathetic preganglionic neurons (SPNs) projecting to the SCG originate from segments C8 to T5 of the spinal cord, with main distribution in T1, T2 and T3. The efferent axons from these SPNs leave the spinal cord with the appropriate ventral roots and travel with the spinal nerves briefly, then leave the spinal nerves and rise in the cervico-thoracic sympathetic trunk to synapse upon the SCG (Rando et al., 1981; Strack et al., 1988). Previous studies focused mainly on the distribution and physiological functions of SPNs, and the detailed data regarding the relationship between their preganglionic projection and brachial plexus have not been investigated. Also, there has been little information available concerning developmental changes of projections of SPNs to the SCG. During the normal postnatal development, natural death of SCG neurons usually occurs (Wright et al., 1983). Similarly, there is a significant reduction in the number of corticospinal axons or optic axons from neonates to adults (Reh and Kalil, 1982; Ng and Stone, 1982; Williams et al., 1986). Considering the evidence that target dependence of neurons is maximal during embryonic and postnatal period (Moran and Graeber, 2004), we conjecture that natural death of neurons in the SCG may lead to retrograde death of SPNs, and the relationship between sympathetic preganglionic fibers of the SCG and spinal nerves of the brachial plexus might change during the postnatal development.

The present study was designed to reveal the number and percentage of sympathetic preganglionic fibers projecting to the SCG via each spinal nerve of the brachial plexus in pup and adult rats. As the rat has been generally taken as an ideal animal model for study of brachial plexus injury in human being (Bertelli et al., 1992), elucidation of the relationship between sympathetic preganglionic fibers and brachial plexus, and of its changes with postnatal development may

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help one understand the clinical characteristics of Horner's syndrome in brachial plexopathy of adults and of newborns.

2. Materials and methods

2.1. Retrograde tracing of SCG

2.1.1. Animals

72 Sprague–Dawley male rats were divided, on the basis of age, into three groups of 24 each, i.e., two pup groups at the age of 7 days postnatal (P7) and of 14 days (P14), and one adult (3 months old, 250–300 g) group. According to surgical intervention to the C7–T1 spinal nerves at the right side, each age group was subdivided into four subgroups of six each, that is, C7 spared one — where C7 alone was kept intact with avulsion of both C8 and T1 and division of the sympathetic chain caudal to the stellate ganglion, so that the SCG was connected to the spinal cord only by sympathetic chain cranial to the stellate ganglion and C7 spinal nerve; C8 or T1 spared one — where C8 or T1 was kept intact with avulsion of the other two spinal nerves and division of the sympathetic chain caudal to the stellate ganglion; C7–T1 avulsed subgroup — where the C7–T1 spinal nerves were all avulsed but the sympathetic chain kept intact, so that the SCG was connected to the spinal cord merely by sympathetic chain and related spinal nerves caudal to T1 (Fig. 1). In our preliminary experiment for 18 rats at three different ages as mentioned above (six for each age), where only the C5 and C6 spinal nerves were kept intact with avulsion of C7C8T1 and division of the sympathetic chain caudal to the stellate ganglion, no labeled neuron was observed in the sections of the spinal cord for both pup and adult rats after Fluoro-Gold (FG) injection into the ipsilateral SCG. This has indicated that no sympathetic preganglionic fiber projects to SCG via the C5 or the C6 spinal nerve (unpublished data). In this retrograde tracing study, therefore, C5 and C6 were spared to lessen trauma to the rats in all subgroups, so that the experimental animal was liable to be alive. All surgical procedures and protocols used were in accordance with the Guidelines for Ethical Care of Experimental Animals approved by the International Animal Care and Use Committee.

2.1.2. Surgery

All rats were anesthetized with pentobarbital (50 mg/kg, i.p.). By aseptic techniques, the rat was placed in a stereotaxic frame and the spine was exposed through a posteriormiddle incision along the neck, using a 10× (adult rats) or 16× (pup rats) microscope. The C7C8T1 spinal nerves of the brachial plexus at the right side were located by the landmark of the T2 spinal process (Cao and Ling, 2003), and two or three of them were avulsed from the spinal cord according to grouping. For subgroups of C7, C8 or T1 spared, the stellate ganglion, which locates ventral to the rib heads 1 and 2, was approached retropleurally, and the sympathetic chain caudal to the stellate ganglion was divided between rib heads 2 and 3. The rat was then placed in supine position. SCGs on both sides were exposed bilaterally through an anterior longitudinal incision of neck. 4% FG (Biotium, Hayward, CA, USA; cat# 80014) dissolved in distilled water was, in three portions, pressure-injected into the rostral, middle and caudal parts of the bilateral ganglions through 1.0-mm pipettes pulled to tip diameters of 16–18 mm to label the SPNs. The left side was used as control. Care was taken to minimize leakage of the fluorescent dye by swabbing injection sites with cotton sticks and rinsing with saline. After recovery from anesthesia, pup rats were returned to their mothers and adult rats to cages.

2.1.3. Histological procedure

Following survival of 24 to 48 h for the pup and three days for the adult, the rat was deeply re-anesthetized and the left ventricle of the heart was exposed and penetrated with the tip of cannulae ranging in size from insulin syringes (for the pup) to 18 gauge (for the adult). The rat was perfused transcardially with 100 ml of 0.1 M phosphate-buffered 0.9% saline (PBS, pH 7.4) at room temperature (21 ± 1 °C), followed by 300 ml ice-cold 4% paraformaldehyde in PBS. After 20 min of perfusion with fixative, the spinal cord from C7 to T6 was removed with the dorsal root ganglia attached, which were used as the

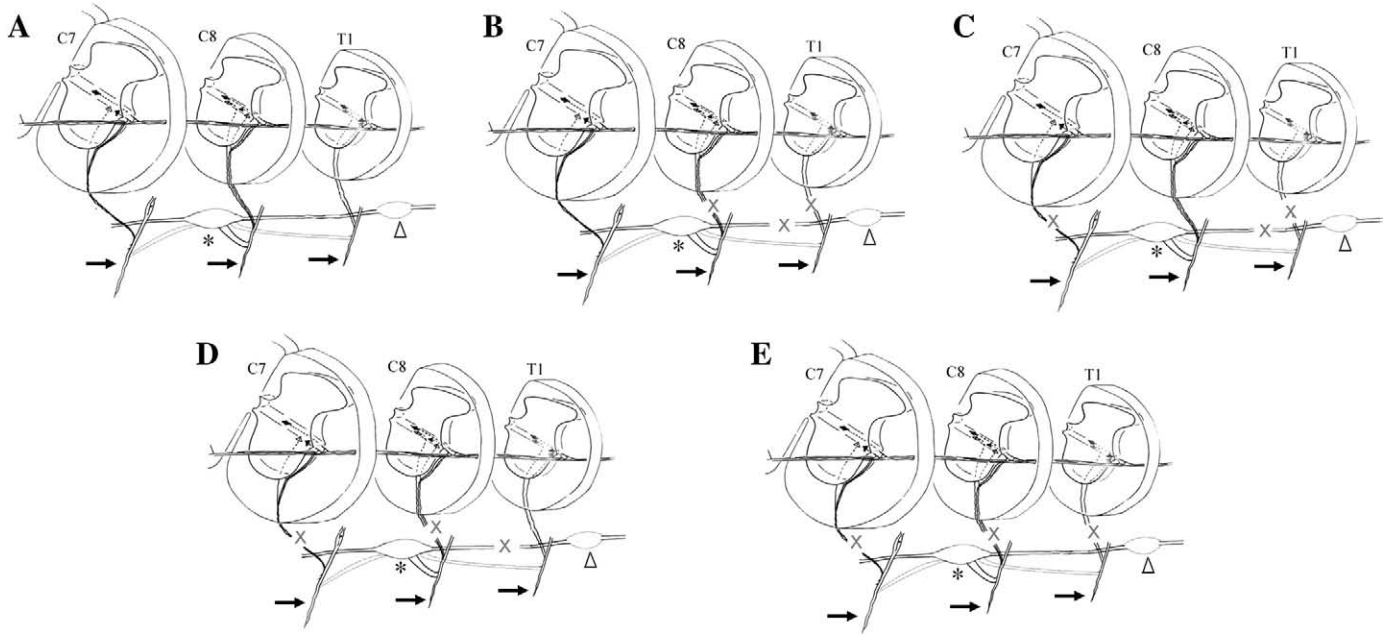

Fig. 1. The diagrams show different surgical intervention of spinal nerves of the brachial plexus for retrograde tracing study in 4 subgroups of each age group. A. The normal efferent pathway of SPNs projecting to the SCG. B. C7 spared subgroup: C7 keeps intact with avulsion of both C8 and T1 and division of the sympathetic chain caudal to the stellate ganglion. C. C8 spared subgroup: C8 keeps intact with avulsion of both C7 and T1 and division of the sympathetic chain caudal to the stellate ganglion. D. T1 spared subgroup: T1 keeps intact with avulsion of both C7 and C8 and division of the sympathetic chain caudal to the stellate ganglion. E. C7C8T1 avulsed subgroup: C7, C8 and T1 are avulsed so that the SCG is only connected to the spinal cord by sympathetic chain and related spinal nerves caudal to T1. Crosses in A to E express avulsion of the spinal nerve or division of the sympathetic chain. Asterisks in A to E express the stellate ganglion. Triangles in A to E express the T2 ganglion. Arrows in A to E express the C7 to T1 spinal nerves.