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Central regulation of sympathetic neuron development

(tyrosine hydroxylase/spinal cord/sympathetic ganglia)

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ABSTRACT **The sixth lumbar (L-6) ganglion has been used to study the central regulation of peripheral sympathetic neuron development. During post-natal ontogeny, tyrosine hydroxylase [tyrosine 3-monooxygenase, L-tyrosine, tetrahydropteridine: oxygen oxidoreductase (3-hydroxylating), EC 1.14.16.2] activity increased 60-fold, while total protein rose 10-fold in the ganglion. Transection of the spinal cord at the fifth thoracic (T-5) segment in neonatal rats prevented the normal developmental increase in tyrosine hydroxylase activity of the L-6 ganglion. However, spinal transection did not alter the ontogeny of tyrosine hydroxylase in the superior cervical ganglion, which derives its innervation from spinal segments rostral to the surgical lesion. Thus, spinal transection interfered with the maturation of sympathetic neurons distal to, but not proximal to, the lesion. The effect of transection on the L-6 ganglion persisted for at least one month, the longest time tested. Our observations suggest that trans-synaptic regulation of adrenergic maturation in the periphery is governed by suprasegmental mechanisms in the central nervous system.**

Previous work from this laboratory has demonstrated that presynaptic cholinergic nerves regulate the development of postsynaptic adrenergic neurons in the superior cervical ganglion (SCG) (1–4). Surgical transection of the presynaptic cholinergic trunk in neonatal mice and rats prevents the normal developmental increase in ganglionic tyrosine hydroxylase [tyrosine 3-monooxygenase, L-tyrosine, tetrahydropteridine: oxygen oxidoreductase (3-hydroxylating), EC 1.14.16.2] activity and the normal accumulation of Tyr-OH molecules in each postsynaptic neuron (1, 2, 5). This enzyme, which catalyzes the rate-limiting step in catecholamine biosynthesis (6), is highly localized to adrenergic neurons in the SCG, and may be used to monitor maturation of these cells (7). The trans-synaptic regulation of ontogeny in the SCG may be mediated by acetylcholine, since pharmacologic blockade of ganglionic transmission reproduces the effect of decentralization (4, 8).

The role of *central* synapses in the maturation of peripheral adrenergic neurons has yet to be elucidated. The cholinergic fibers innervating sympathetic ganglia arise from cell bodies lying in the intermediolateral column of the lateral horn of the spinal cord (9). In turn, these neurons receive afferents descending from suprasegmental levels (9). The trans-synaptic regulation of peripheral sympathetic development may be dependent on connections with these descending central fibers and/or may simply depend upon segmental spinal mechanisms similar to those mediating muscle stretch reflexes (10). One approach to this problem involves interruption of the descending central pathways that innervate the intermediolateral column cells. However, this cannot be accomplished in the case of the SCG, since its innervation derives mainly from the first three thoracic spinal segments and lesions rostral to this level result in respiratory paralysis and death. Consequently, a more caudal sympathetic ganglion was selected for this study, the sixth lumbar (L-6) ganglion. This ganglion is innervated by cells from the eleventh thoracic to the second lumbar spinal segments (9). In the present report we describe the effects of mid-thoracic spinal transection on the development of adrenergic neurons in the L-6 ganglion.

Abbreviations: Tyr-OH, tyrosine hydroxylase; L-6, sixth lumbar; T-5, fifth thoracic; SCG, superior cervical ganglion.

MATERIALS AND METHODS

Experimental animals

Litters of Charles River Sprague-Dawley rats were housed with mothers that were fed Ralston Purina lab chow and water ad lib., and exposed to 50–75 footcandles (540–810 lumens/m^2) of cool white fluorescent light from 6 a.m. to 6 p.m. Litters were standardized by assigning control and experimental animals to each mother. Animals were killed by exposure to ether vapor prior to assay.

Surgical procedures

Ganglion Dissection. The L-6 ganglia were identified and removed from rats using an atlas as a guide (11). The surgical approach involved a midline abdominal incision extending from the pelvis to approximately 1 cm inferior to the costal margin. All abdominal and pelvic organs were removed, excluding the kidneys, which were used as anatomical landmarks. By means of a dissecting microscope, the aorta and inferior vena cava were dissected free and retracted. Lateral retraction of the psoas muscle exposed the lumbar sympathetic chain lying anterolateral to the vertebral bodies. The sixth lumbar ganglia lie approximately 1 cm inferior to the left renal artery and were removed as a pair. The SCG was removed as previously described (1).

Spinal Transection. In 10 to 11-day-old rats anesthetized with 2% halothane in 100% oxygen, the spinal cord was transected at the level of the fifth thoracic (T-5) vertebra under an operating microscope. After a posterior midline incision over the mid-dorsal vertebral column, the soft tissues and muscles were reflected, and hemorrhage was controlled with Gelfoam and microcautery. Laminectomies were performed from the fifth thoracic (T-5) to the seventh thoracic (T-7) vertebra, and the spinal cord, with the dura and meninges, was exposed. Spinal transection was accomplished by removing the spinal segment at the T-5 vertebral level, and the resulting space was packed with Gelfoam. This area is far superior to the spinal segments that innervate the L-6 ganglia. Surgical wounds in the neonates were closed with collodion and metal clips and the pups were returned to their mothers. This procedure resulted in a mortality rate of approximately 25–50%, due primarily to failure of mothers to accept pups. The success of spinal transection was assessed by noting postoperative paraparesis. Surgery in rats less than 10 days of age resulted in a prohibitively high mortality rate. Sham-operated littermates served as con-

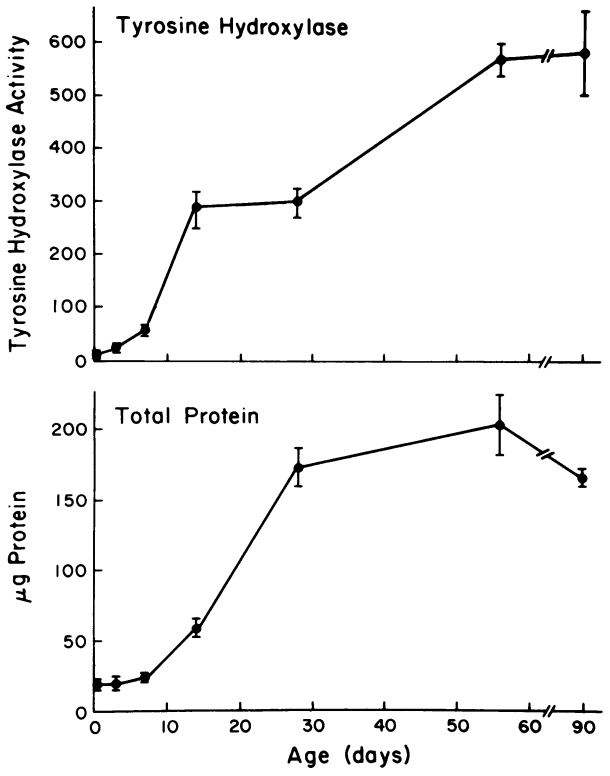

FIG. 1. Developmental increases of tyrosine hydroxylase activity and total protein in L-6 ganglia. Groups of five rats were taken from litters of varying ages, and ganglion pairs from each animal were assayed for enzyme activity and total protein (see *Materials and Methods*). Tyrosine hydroxylase activity is expressed as mean pmol of product per ganglion pair·hr ± SEM (vertical bars). Total protein is expressed as mean μg per ganglion pair ± SEM.

trols in all experiments. The sham procedure consisted of dissecting to the level of the vertebral laminae and spinous processes as described above.

Biochemical procedures

Tyrosine hydroxylase activity was assayed by minor modifications of methods previously described, with tetrahydrobiopterin as cofactor (1, 4, 12). Total ganglion protein was assayed by the method of Lowry *et al.* (13), with bovine serum albumin as standard.

RESULTS

Normal development

To define normal postnatal ganglion development, we randomly selected groups of rats from litters of different ages and L-6 ganglia were assayed for tyrosine hydroxylase activity and total protein. Tyr-OH activity increased 60-fold during postnatal ontogeny. From low levels on day one, enzyme activity rose rapidly during the first 2 weeks and then more gradually, reaching a plateau at approximately 60 days (Fig. 1). Total ganglion protein increased 10- to 11-fold, rendering the rise in specific enzyme activity highly significant (Fig. 1).

Effects of spinal transection

To examine the role of central pathways in the development of tyrosine hydroxylase activity in L-6 ganglia, we randomly assigned 10- to 11-day-old rats to an unoperated control group, a sham-operated group, or a group that was subjected to spinal

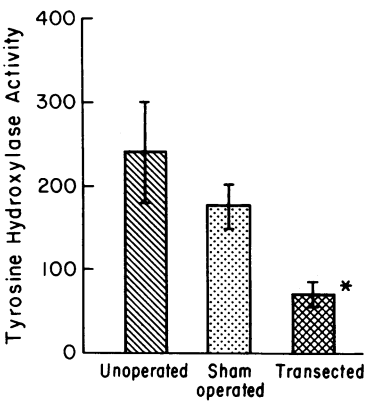

FIG. 2. Effect of spinal cord transection on the development of tyrosine hydroxylase activity. Groups of rats were subjected to spinal cord transection (see *Materials and Methods*), and littermates served as unoperated and sham-operated controls. All animals were killed at 17 days of age. Tyrosine hydroxylase activity was determined in ganglia from groups of six to eight animals. Enzyme activity is expressed as in legend of Fig. 1.

* Differs from both the unoperated and sham-operated groups at $P < 0.001$. There is no significant difference between other groups ($P > 0.05$).

transection. Spinal transection prevented the normal developmental increase in Tyr-OH activity of L-6 ganglion (Fig. 2). However, there was no significant difference in enzyme activity between the unoperated and sham-operated groups (Fig. 2). Additionally, preliminary studies indicate that the intermediolateral column neurons distal to the site of transection are histologically unaltered.

Spinal transection may have prevented normal ganglion Tyr-OH development due to such "nonspecific" factors as altered mobility or nutrition consequent to the procedure. To examine this possibility, the development of tyrosine hydroxylase activity in the SCG, which is innervated by segments rostral to the level of transection, was compared to that of L-6 ganglia after transection at T-5. Spinal transection did not alter the ontogeny of Tyr-OH activity in the SCG, but did prevent the developmental increase in activity in the L-6 ganglia. Tyr-OH activity failed to increase normally in the lumbar ganglia whether expressed per ganglion pair or per microgram of protein (Fig. 3). Thus, spinal transection interfered with the maturation of sympathetic neurons distal to, but not proximal to, the lesion. Consequently, spinal transection itself, and not other "nonspecific" factors, appeared to prevent the normal development of tyrosine hydroxylase activity of L-6 ganglion.

To more fully define the effect of interruption of central pathways on ganglion development, we determined enzyme activity at various times after surgery. Spinal transection resulted in long-lasting inhibition of the ontogeny of tyrosine hydroxylase activity in L-6 ganglia (Fig. 4). Twenty-four hours after surgery there was no alteration in activity. Thereafter, however, tyrosine hydroxylase failed to develop normally, remaining depressed through one month of age, the longest time examined (Fig. 4). Tyr-OH did not rise normally, whether expressed as total activity per L-6 ganglion pair (Fig. 4) or as activity per μg of ganglion protein (not shown).

DISCUSSION

Previous work has demonstrated that presynaptic cholinergic nerves regulate the ontogeny of postsynaptic adrenergic neurons in sympathetic ganglia (1–4). The present studies have investigated the role of central pathways in this regulation.

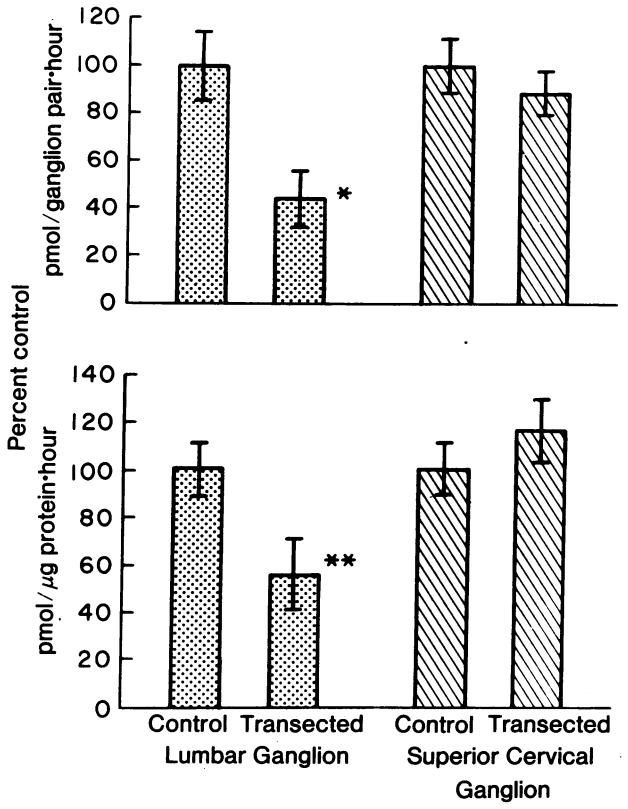

FIG. 3. Comparison of the effects of spinal cord transection on development of tyrosine hydroxylase activity in the superior cervical ganglia and the sixth lumbar ganglia. Spinal transection or sham operation was performed in 11-day-old rats, and the animals were killed at 28 days of age. Enzyme activity is expressed as per cent of sham-operated control of pmol per ganglion pair·hr (top) and pmol/μg of protein·hr (bottom). Control values for the lumbar (L-6) ganglia were 182 ± 27.1 pmol per ganglion pair·hr and 4.1 ± 0.46 pmol/μg of protein·hr. Those for the superior cervical ganglia were 1868 ± 217.5 pmol per ganglion pair·hr and 6.8 ± 0.73 pmol/μg of protein·hr.
* Differs from respective control group at $P < 0.02$.
** Differs from respective control group at $P < 0.05$.

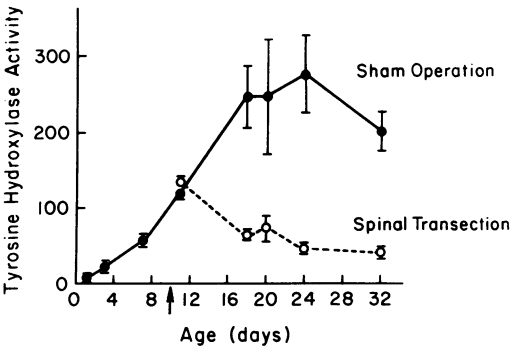

FIG. 4. Time course of the effect of spinal cord transection on the development of lumbar ganglion tyrosine hydroxylase activity. Rats were subjected to spinal transection at 10–11 days of age (arrow) as described in *Materials and Methods*, and groups of 6 to 8 were killed at varying times thereafter. In all instances sham-operated littermates served as controls. Enzyme activity is expressed as pmol per ganglion pair·hr. Subsequent to the 1-day postoperative time, all spinal transection groups differ from sham-operated groups at $P < 0.01$.

Our initial experiments defined the normal maturation of tyrosine hydroxylase activity in sympathetic neurons of L-6 ganglia. The postnatal increases in Tyr-OH activity and total protein were qualitatively similar to those previously described for another sympathetic ganglion, the SCG (1, 5). In both cases there were highly significant increases in specific Tyr-OH activity. These observations suggest that the pattern of development of adrenergic neurons in different sympathetic ganglia is similar. However, the actual time courses of ontogeny may differ. In the SCG, tyrosine hydroxylase activity increases 6- to 8-fold postnatally, reaching adult plateau values by day 14 of life (1, 5). In L-6 ganglia, on the other hand, Tyr-OH activity increased 60-fold, and adult levels were not reached until approximately 2 months of age (Fig. 1). Consequently, there may be a rostro-caudal gradient in the development of ganglion adrenergic neurons in the body. Such temporo-spatial organization corresponds to the previously reported gradient in appearance of neural crest cells, the progenitors of these sympathetic neurons (14).

Interruption of pathways within the central nervous system prevented the normal development of peripheral sympathetic neurons. Spinal transection at T-5 in neonatal rats blocked the ontogenetic increase of tyrosine hydroxylase activity in the L-6 ganglion. Since this ganglion is innervated by segments caudal to the lesion, central pathways appear necessary for normal ganglion maturation. Furthermore, T-5 transection did not affect Tyr-OH activity in the SCG, which is innervated by segments rostral to the lesion. Consequently, abnormal maturation of L-6 ganglia was not simply secondary to "nonspecific" effects of surgery. Moreover, preliminary studies indicate that lumbar intermediolateral column neurons are histologically normal after spinal transection. Thus, surgery does not destroy intermediolateral column neurons and thereby prevent peripheral adrenergic ontogeny. Rather, these results indicate that descending central pathways may influence cholinergic neurons within the spinal intermediolateral column, which, in turn, regulate peripheral adrenergic development (1–4). It may be inferred that trans-synaptic regulation of adrenergic maturation in the periphery is governed by higher centers in the central nervous system. Apparently, the preganglionic, intermediolateral column neurons do not function autonomously in this context, but require information of suprasegmental origin.

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