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OCCIPITAL SOMITES GUIDE MOTOR AXONS OF THE ACCESSORY NERVE IN THE AVIAN EMBRYO

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Abstract—The accessory nerve (nervus accessorius) displays a unique organization in that its axons ascend along the rostrocaudal axis after exiting the cervical spinal cord and medulla oblongata and thereafter project ventrally into the periphery at the first somite level. Little is known about how this organization is achieved. We have investigated the role of somites in the guidance of motor axons of the accessory nerve using heterotopic transplantations of somites in avian embryos. The formation of not only accessory nerve but also the vagal nerve was affected, when a more caudal occipital somite (somites 2–4) was grafted to the position of the first occipital somite. Our study reveals that only the first occipital somite permits the development of ventral projection of accessory axons, a process that is inhibited by more caudal occipital somites.

Key words: motor axon, axonal guidance, nervus accessorius, occipital somites.

INTRODUCTION

The peripheral nervous system (PNS) is composed of spinal nerves in the trunk and cranial nerves in the head. While most of motor axons project ventrally into the periphery immediately after exiting the central nervous system (CNS), branchiomotor (BM) axons in the head–trunk-transitory region display a unique axonal trajectory in that they ascend along the lateral margin of the spinal cord and the medulla oblongata until they bend ventrally into the periphery at the first somite level. These axons form the accessory nerve (reviewed in Chandrasekhar, 2004; Guthrie, 2007).

According to the classical view, the accessory nerve consists of two parts: a cranial portion (Ramus internus, accessory portion) and a spinal section (Ramus externus, spinal portion) (Standring, 2008). The spinal portion innervates the neck and back muscles, the trapezius and sternocleidomastoid muscles (Theis et al., 2010), while the accessory portion innervates laryngeal and pharyngeal muscles. Though not connected (Ryan et al., 2007), fibers of both parts have a common trajectory and exit the hindbrain through the jugular foramen, similar to the vagal nerve. At present, we lack a detailed description of the development of motor axons of the accessory nerve or an understanding of the mechanisms that control their unique trajectory (Pabst et al., 2003; Dillon et al., 2005, 2007; Hirsch et al., 2007). One interesting question is how the trajectory of these axons is controlled. In the trunk, segmental organization of dorsal root ganglia is entirely dependent on the presence of somites (Stern and Keynes, 1987). Since the caudal half of each somite repels neural crest cells and motor axons, their migration takes place through only the cranial somite half (Gammill et al., 2006). The accessory nerve roots are located at the level of somites 1–5, which are called occipital somites because they contribute to the occipital bone (Couly et al., 1993; Christ and Ordahl, 1995; Wilting et al., 1995; Huang et al., 2000). It is conceivable that the first occipital somite (somite 1) allows them to pass through its territory, whereas this process is inhibited by more caudal occipital somites.

In this study, we tested this hypothesis using the avian embryo as it is readily accessible to microsurgical manipulations (Stern, 2005). Indeed our results reveal that the ventral turning of motor axons of both the accessory and vagal nerve is inhibited by all occipital somites, with the exception of the first occipital somite.

EXPERIMENTAL PROCEDURES

Embryos

Fertilized White Leghorn eggs (*Gallus gallus domesticus*) and Japanese quail eggs (*Coturnix coturnix*) were obtained from the Institute for Animal Science at the University of Bonn. Eggs were incubated at 60–70% relative humidity and 37.8 °C. Chick embryos were staged according to Hamburger and Hamilton (1951) and quail embryos according to Ainsworth et al. (2010).

Heterotopic transplantation of somites

Chick embryos at HH stages 9–10 were used for host and stage matched quail embryos for donor. The chick eggs

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were prepared as previously described (Geetha-Loganathan et al., 2010; Wang et al., 2010; Valasek et al., 2011; Pu et al., 2012). Briefly, a window of the egg shell was made over the embryo. A single occipital somite was removed using a combination of tungsten needles and mouth controlled pipettes. To obtain a somite for transplantation, the quail embryo was treated with Dispase. Thereafter, a quail somite was isolated and transplanted into the chick host. Eggs were sealed and reincubated for a further 2 days.

Immunohistochemistry

All operated embryos were fixed in Dent's fixative (1 h to overnight) and then bleached in Dent's Bleach overnight before immunohistochemical staining. BEN- and 3A10-antibodies were used to identify axons. Quail cells were detected with a monoclonal QCPN-antibody. The antibodies were purchased from the Developmental Studies Hybridoma Bank, Iowa City, IA, USA. Goat-anti-mouse-Cy2 and -Cy3 secondary antibodies were used to detect the primary antibody. The stained embryos were photographed using a Nikon digital camera DXM1200C, mounted on a Nikon SM 21500 microscope.

RESULTS

Accessory nerve exits the hindbrain through the territory of the first somite

During morphogenesis, axons of the accessory nerve join the vagal nerve and collectively ascend along the rostrocaudal axis and turning ventrally before reaching the otic vesicle. At this stage axons of these nerves are hardly distinguishable from each other. Using histological sections of embryos in which the first somite was marked, the vagal nerve was seen to pass through the territory of the developing somite 1 (Hamilton and Hinsch, 1956; Huang et al., 1997). In this study, we reinvestigated the topography of the accessory and vagal nerve using whole mount immunohistochemistry with 3A10 and desmin antibodies (data not shown). The desmin antibody marks the myotome. Compared to the myotome of the second somite, the myotome of the first one is considerably smaller. The nervus glossopharyngeus (IX), vagus (X) and accessorius (XI) pass through the space between the second somite and the otic vesicle.

Heterotopic transplantation of the first somite does not alter the axonal pathfinding of the accessory nerve

The observation that the accessory nerve passes through the first somite (the first occipital somite) led us to ask whether the first somite is sufficient to induce the ventral projection of accessorius axons. To address this question we heterotopically grafted the first somite from quail to chick embryos. The first somite is not completely segmented. No segment boundary is found between the first somite and the head mesoderm. The first intersomitic cleft is located between first and second somites (Hamburger and Hamilton, 1951; Hamilton and Hinsch, 1956). To isolate the first somite, a cut was made between the head mesoderm and the first somite. Thereafter, the somite was released from the adjacent tissue with help of Dispase and transferred to the position of other occipital somites of the chick host. The transplantation was performed at stages 9–10 prior to the neural crest migration (Tosney, 1982).

After a 2-day reincubation period, the host embryos were fixed and stained in whole mount with the BEN antibody. BEN is a surface glycoprotein which is expressed in various developing tissues (Pourquie et al., 1992) and the BEN-antibody was used to mark motor axons of the accessory nerve (Schubert and Kaprielian, 2001; Dillon et al., 2005). We observed that the BEN-antibody labeled also both motor and sensory axons in cranial nerves and spinal nerves (Fig. 1). The position of the grafted quail somite was detected using QCPN antibody (quail nuclei marker). While the grafted somite tissue was seen to be located at the middle level of the running course of the nervus accessorius, no ectopic axonal projection could be detected in operated embryos ($n = 4$, Fig. 1). These results show that the first somite is not sufficient to induce the ventral projection of motor axons of the nervus accessorius.

Transplantation of a more caudal occipital somite (somites 2–4) to the somite 1 position affects the formation of the nervus vagus and accessorius

Motor axons of the nervus accesorius exit the neural epithelium at about stage 18. Then they ascend along the lateral surface of the neural epithelium rather than passing through the somites 2–5. These observations led to the question whether the somites 2–5 constrain motor axons of the accessory nerve to make an anterior course using repel mechanisms. To address this question, the first occipital somite (somite 1) of a chick host embryo was replaced by a more caudal occipital somite (somites 2–4) of a stage matched quail embryo. The somite transplantation was performed at stages 9–10 prior to the neural crest cell migration ($n = 6$). Two days after reincubation, grafted quail cells were detected in the position of the first somite in all chick host embryos. The accessory and vagal nerve failed to form normally in all operated embryos ($n = 6/6$, Fig. 2). No axons of the anterior–posterior fascicle projected ventrally at the level of the graft. Axons from the placode-derived ganglion (nodose ganglion) did not project medially and truncated within the quail graft. These observations indicate that ventral projections of motor axons were inhibited by all occipital somites with the exception of the first somite (Fig. 3).

DISCUSSION

In this study, we have investigated the influence of somites on pathfinding of accessory motor axons. We found that the second to fourth occipital somites inhibit the ventral projection of motor axons of the accessory nerve. It has been shown that these somites induce the degeneration of the developing sensory ganglion (Lim et al., 1987; Avivi et al., 2002; Kant and Goldstein,