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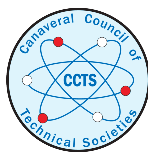

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Spacelab-J Investigation:

Developmental Appearance of Neuron-specific Enolase in the Embryonic Chick's Basilar Papilla

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Abstract

The basilar papilla is the hearing portion of the bird's inner ear and serves as a model for human inner ear development. We used neuron-specific enolase (NSE) as a marker for the chick's auditory nerve (statoacoustic ganglion) development. The chick hatches after an incubation period of 3 weeks. At stage 38 (12th embryonic day), the earliest stage examined in the present experiments, we observed faint NSE staining (immunoreactivity) in neurons of the statoacoustic ganglion. By stage 39- (<13th embryonic day), stronger NSE immunoreactivity appeared in most neuronal cell bodies (perikarya). By stage 46 (21st day [hatching]), neurons stained strongly for NSE. Thus, NSE levels (immunoreactivities) increase during development and correspond to neuronal differentiation.

Introduction

The objective of this preliminary study was to examine the developmental appearance of neuron-specific enolase (NSE) in the embryonic chick's basilar papilla. NSE is a soluble neuronal isoenzyme of the glycolytic enzyme enolase, which converts 2-phosphoglycerate to phosphoenolpyruvate (1,7). It is expressed shortly after the first immature synapses form (8,11,12) and serves as a marker to to correlate synaptogenesis with differentiation of neurons and sensory receptors (hair cells) (3,10,12). Hence, NSE is a sensitive indicator of developmental changes (8), and is closely linked to the final functional differentiation and activity of neurons (7).

Methods

White leghorn eggs (<u>Gallus domesticus</u>) were obtained from Hendry Hatchery, Lake City, Florida. The eggs were incubated at 36.5-37.5°C, sacrificed after different incubation periods, and staged according to Hamburger and Hamilton's scheme (6). For the present study, three embryonic stages and one hatched chick were examined: stages 38, 39-, 41+, and 46 (newly hatched). The auditory lagenae were fixed in cold (3-5°C) 2.5% paraformaldehyde buffered in 0.05 M sodium cacodylate, pH 7.4, and dissected from their bony encasements. After 24 hours of fixation, the lagenae were washed in 0.05 M sodium cacodylate buffer containing 2.5% sucrose, dehydrated in an ethanol series, and then infiltrated and embedded in polyester wax (9). The embedded specimens were sectioned with a microtome. Sections (7-8 μm) were collected on coated slides. After drying, the sections were dewaxed and treated with avidin and biotin blocking reagents (Vector Laboratories, Burlingame, CA). Sections were then processed by immunoperoxidase staining methods (DAKO Quick Staining Kit System 40, Santa Barbara, CA). Experimental sections were exposed to NSE antiserum (rabbit anti-human) and controls sections to non-immune serum for 8 minutes, and to 5% non-fat dry milk for 5 minutes prior to avidin treatment

(4). The secondary antibodies were biotinylated swine anti-rabbit. Coverslips were mounted onto the slides with Permount mounting medium. Slides were examined and photographed under Nomarski interference contrast (NIC) optics.

Results and Discussion

Our results show that NSE staining (immunoreactivity) increases during development. At stage 38 (12th embryonic day), the earliest stage examined in the present study, the neurons of the statoacoustic ganglion exhibited faint NSE staining (Figure 1). By stage 39- (<13th embryonic day), slightly stronger NSE immunoreactivity appeared in perikarya (Figure 2). By stage 41+ (>15th embryonic day), NSE staining intensity increased noticeably and was intermediate between the earlier and older stage 46; neurons enlarged. By stage 46 (21st day [hatching]), neurons stained strongly for NSE (Figure 3), although neurons within a microscopic field stained with varying intensities, giving a mosaic pattern. Diameters of neuronal cell bodies (perikarya) enlarged considerably during development (Compare neuronal sizes in Figures 1 and 3). Because of the much larger sizes of perikarya (ganglion cell bodies) than dendrites and axons, we have presently only detected NSE in the perikarya.

During development, the statoacoustic ganglion's bipolar neurons grow bidirectionally towards their synaptic target populations in the lagenae (hair cells of the basilar papilla) and brain (neurons of the nucleus magnocellularis). The developmental appearance of NSE may be related to and even triggered by early synaptogenesis, when the first immature synaptic endings form. Whitehead et al. (10) reported that synapse formation is related to the onset of NSE immunoreactivity in the chick's auditory and vestibular systems. For example, NSE first appears in the auditory nerve, albeit in low levels (Figure 1), slightly before the developmental period (stage 38) in which the earliest behavioral responses can first be elicited by auditory stimulation (11). By the period of late synaptogenesis, auditory function is well established and NSE immunoreactivity is stronger, resembling the adult pattern (10).

We have previously described histological and cytological changes in the developing basilar papilla (2,5). Our present preliminary results show the ontogenetic appearance of NSE in statoacoustic neurons in the embryonic chick's basilar papilla by stage 38. NSE appearance seems to correspond with morphological events such as synapse formation and neuronal differentiation. Although we cannot presently account for the mosaic pattern of NSE immunoreactivity at stage 46 (hatching) (Figure 3), we offer two possibilities. First, the different neuronal types within the statoacoustic ganglion, based upon size, location and staining patterns, contain different NSE levels. For example, neuronal types in the central nervous system contain different NSE levels; i.e., NSE levels that are characteristic for that neuronal cell type (7). Second, different neuronal types might develop at different rates, so that the darkly staining neurons represent the earlier developing neurons. The fact that Whitehead et al. (10) did not report the mosaic pattern may reflect different preparative procedures. For example, they used 40 μm vibrotome sections, whereas we used 7-8 μm sections. Although their thicker sections may have concealed some of the differences in staining intensities among perikarya, their thicker sections showed the earlier appearance of neuronal staining and the more delicate dendritic and axonal staining;

our thinner sections did not show dendritic and axonal staining.

As part of a larger study, we will be describing the cytochemical profiles of other cellular markers in the hearing and balance portions of the inner ear under conditions of normal gravity. We want to establish baseline data for comparison to embryonic chicks exposed to microgravity (an epigenetic influence) during the SpaceLab-J experiments that are scheduled for July, 1991.

Acknowledgements

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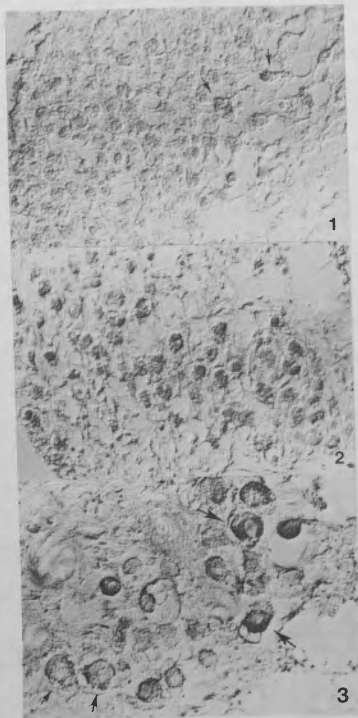

Figure Captions

Figure 1. Developing statoacoustic ganglion of stage 38 chick embryo showing appearance of NSE immunoreactivity. A small group of neurons exhibit stronger NSE staining. NIC optics. (500X).

Figure 2. Distribution of NSE staining in the perikarya (neuronal cell bodies) of stage 39- embryonic chick statoacoustic ganglion. NIC optics (500X).

Figure 3. NSE immunoreactivity in the statoacoustic ganglion of stage 46 (hatched) chick. The perikarya exhibit a mosaic pattern of staining, with some perikarya staining more strongly (large arrows) than others (small arrows). NIC optics. (500X).