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Prenatal Development of Cutaneous Afferent Connections in the Spinal Cord of Fetal Sheep

A Physiological and Neurochemical Study

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Contents

Abstract

In this study we have examined the shysiological and neurochemical development of the cutaneous afferent pathways from the hindlimb to the spinal cord in fetal sheep. We have shown that somatosensory input from the hindlimb evokes activity in DRG neurons at 87d gestation and in cells in the dorsal horn at 92d (term, 146d). There is evidence of immunoreactivity for substance P, calcitonin gene-related peptide and glutamine several days prior to this at 77–80 days. The implication of these findings are discussed.

Index Entries: Prenatal; development; cutaneous; sensory; spinal cord; synaptic connections.

Introduction

There is now considerable interest in understanding the events that occur prenatally in the development of synaptic connections in sensory systems (Fitzgerald, 1991) and the factors that might influence this development (Shatz and Stryker, 1988). Not only is it important to understand these events as the basis for future organization of sen-sory systems but also to provide information about potential sensory transmission to the brain during fetal life.

In this study, we have examined the physiological and neurochemical development of the cutaneous afferent pathway from the hindlimb to the spinal cord in fetal sheep from 83–110 d of gestation (term = 146 d). Particular interest has been focused on the time of onset of neural activity in the dorsal root ganglia (DRG) and in the dorsal horn cells in the spinal cord, as a result of cutaneous sensory input. This activity has been correlated with the expression of substance P, calcitonin gene-related peptide (CGRP), and glutamate, neurochemicals thought to be concerned with neurotransmission or modulation of sensory information in the spinal cord (Duggan et al., 1988; Morton and Hutchinson, 1989).

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Methods

The ewe and fetus (n = 20) were anesthetised with 1.5–2.0% halothane in 50:50 nitrous oxide in oxygen. A low midline incision was made in the ewe, the dorsal surface of the fetus exposed, and the skin surrounding the vertebral column reflected and sutured to the uterine muscle. The spinal cord was stabilized with vertebral clamps, and a laminectomy was performed to expose the last lumbar (L7) and first sacral (Sl) segments of the spinal cord and associated DRG. The left hindlimb was lifted out of the uterus to allow for application of noxious (pinch, heat > 45°C) and tactile stimuli (touch, brush, and controlled indentation). Glass-insulated tungsten microelectrodes for recording extracellular discharges of single neurons were inserted into the DRG and in later experiments, into the dorsal horn. An earlier histological study enabled us to calculate the extent of the dorsal horn at each of the gestational ages studied. The response properties of these primary sensory neurons and dorsal horn cells to natural cutaneous stimulation or to electrical stimulation of the sciatic nerve were then recorded. At the conclusion of the experiment, fetuses were perfused with 4% paraformaldehyde and 0.3% glutaraldehyde in 0.1*M* phosphate buffer (pH 7.4), and the L7 and S1 segments of the spinal cord were stained for substance P, CGRP, and glutamate immunoreactivity at the light and ultrastructural level.

Results

The first reproducible responses to cutaneous stimulation were recorded from DRG cells at 87 d gestation. Neural activity was produced predominantly by intense squeezing of the skin above the hoof and also by light touch and indentation of the skin of the lower hindlimb. These responses adapted rapidly and consisted of a small burst of 5–10 spikes. It is possible that many of the developing tactile afferents have high thresholds during fetal life and might be indistinguishable at this stage from developing nociceptive afferents. By 97 d, responses were more stable and easier to elicit to light touch and indentation and had clearly defined peripheral receptive fields. At this age, slowly adapting responses to skin indentation were also evident. Responses characteristic of polymodal nociceptors (intense pinching, heat > 45°C) were not evident until 107 d. Rapidly adapting responses to movement of hair shafts were well established by 107 d, approx 5 d after the shafts penetrated the skin surface.

Electrical stimulation of primary afferent fibers in the sciatic nerve first began to evoke activity in the dorsal horn at 92 d of gestation. Bursts of spikes could be reliably recorded from single dorsal horn cells at 96 d. The delay of approx 5 d between the first recording of primary afferent activity in the DRG and the onset of activity in the dorsal horn cells might represent the time required for central synaptic maturation and/or an increase in the extent of afferent terminal arborization.

At the light microscope level, there is evidence of immunoreactivity for substance P and CGRP at 77 d and glutamate at 80 d in fibers in the spinal cord, particularly in laminae I, II, and V, the region of termination of primary afferent fibers subserving nociception and touch. Ultrastructural studies showed that at 87 d, axon terminals containing small spherical and large granular vesicles, immunoreactive for substance P, were observed in laminae I and II of the dorsal horn. These terminals predominantly made axodendritic synapses (Fig. 1A), although occasionally axosomatic synapses were observed . The terminals had the characteristic scalloped appearance of primary afferent endings. A similar result was obtained for terminals immunoreactive for CGRP (Fig. 1B). The appearance of neuropeptides in axon terminals well in advance of the onset of neurotransmission at 92 d might suggest a trophic action of these substances on synaptic maturation. It is of interest that, at the developing neuromuscular junction, motor-neuron-derived CGRP appears to have a trophic action on acetylcholine receptor synthesis (New and Mudge, 1986).

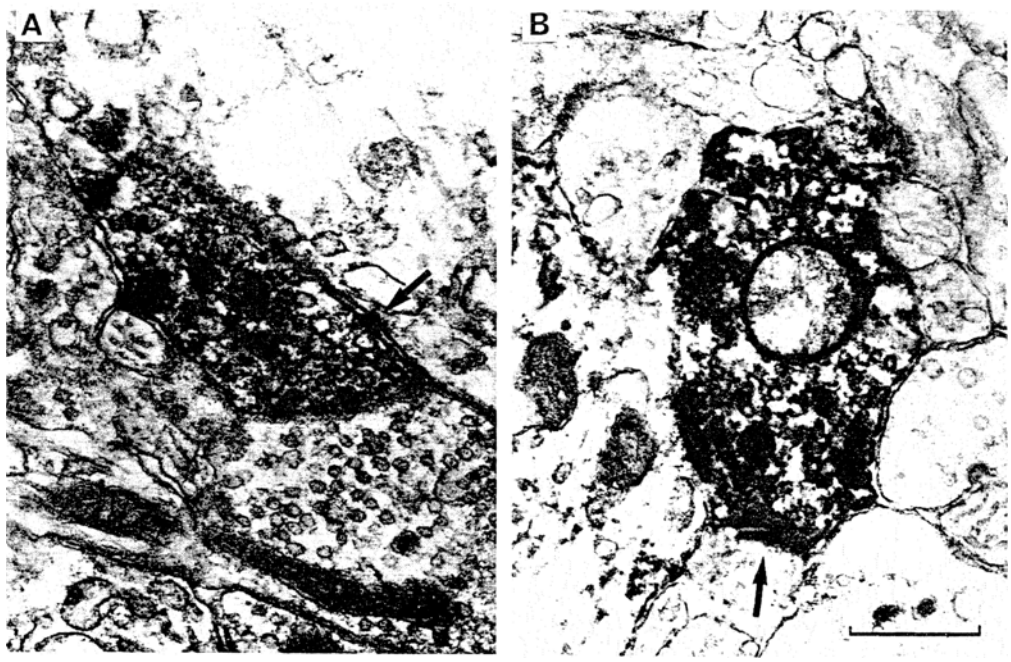

Fig. 1. Electronmicrographs of axon terminals packed with small spherical and large granular vesicles in the substantia gelatinosa (laminae I and II) of the dorsal horn of the spinal cord at 87 d gestation. **(A)** Axodendritic synapse (arrow) immunoreactive for substance P; **(B)** axodendritic synapse (arrow) immunoreactive for CGRP. Note the scalloped appearance characteristic of primary sensory afferent terminals. Bar = 0.5 μm.

Conclusions

Thus, we have shown that, in fetal sheep, cutaneous somatosensory input from the hindlimb evokes activity in the dorsal horn cells at about 60% of the way through gestation. We have yet to determine when this information reaches the brain. Sensory input at this early stage in fetal life might be a necessary requirement for the appropriate development of synaptic connections and subsequently, for the successful postnatal maturation of this system.

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