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Short Report

Sympathetic Gangliogenesis and Temporo-Spatial Glycoconjugates's Terminal Sugars Distribution

Mokhtar Jafarpur*, Hasan Mofidpur and Alireza Fazel

Dept. of Anatomy, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

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ABSTRACT

Lectin binding histochemistry was performed on the developing sympathetic ganglionic cells to investigate the distribution and density of defined carbohydrate terminals on the cell surface glycoproteins during autonomic system morphogenesis. Sprague-Dauley rat embryos from 9th gestational day to birth were fixed and paraffinized. Serial sections of these specimens were incubated with different lectins, which were conjugated to horse raddish peroxidase (HRP). Diaminobenzidine was used to localize the HRP on the binding sites of lectins to terminal sugars. Among these lectins, soybean agglutinin reacted with migratory neural crest cells in early stages, from 9th to 13th day of gestation. These cells were moving ventrally in relation to the neural tube. In the late stages from days 14th to 21st, orange peel fungus agglutinin reacted with sympathetic migratory cells. Our results suggest that at each stage of sympathetic ganglia development, a specific glycoconjugate seems to be the key factor for development of the cells. We suggest that only one key specific terminal sugar is active in each stage of development, which might genetically regulated for particular stage of sympathetic gangliogenesis. *Iran. Biomed. J. 7 (4): 183-186, 2003*

Keywords: Neural crest, Sympathetic ganglia, Lectin histochemistry

INTRODUCTION

Extensive studies have been reported on the migration and differentiation of the neural crest cells [1-5]. Researches have defined that a part of neural crest cells, after moving in a specific direction, differentiates into the sympathetic ganglia on each side of the developing vertebral bodies [6-8]. In addition to the process of sympathetic gangliogenesis, the neural crest cells are involved in formation of other parts of the developing embryo including dorsal root and parasympathetic ganglia [3, 4, 9]. Although these cells have essential role in transporting nervous impulses to the smooth muscle, secretory glands and some other organs, the molecular formation of these ganglia has not been studied extensively.

Key role of glycoconjugates in several biological system were studied by several researchers, but, there are few evidence in relation to the gangliogenesis [10-15]. Distribution of terminal sugars and their role on histogenesis of sympathetic ganglia have been poorly investigated because the morphogenesis of these ganglia was unknown partially to elucidate these obscurities.

In this respect, we study the binding distribution of several lectins during sympathetic ganglia morphogenesis using lectin histochemistry.

MATERIALS AND METHODS

Female Sprague-Dauley rats weighing between 350-375 g were obtained from the Razi Institute of Iran (Mashhad). They were mated with similar males. Existance of sperms in vaginal smear was designated as day zero of gestation. Animal house feeding and other circumstances were similar for all animals.

Fetuses from 9th fetal day to birth (and few days after birth were our cases for histochemical studies. Pregnant rats were anesthetized by chloroform in

*Corresponding Author; Tel. (98-511) 854 4081-4; Fax: (98-511) 859 1922; E-mail: jafarpurmokhtar@yahoo.com

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optional gestational days and the fetuses were exited from uterine tubes. Also, newborns (first week) were used for this study. Newborns and fetuses were washed in normal saline and put in the fixatives B4G (6% mercuric chloride, 1% sodium acetate and 0.1% glutaraldehyde), formalin and Buin's fluid. Fetuses or newborns with abnormal appearance (size, shape and weight) were omitted from the study. Using serial section procedure, 15 sections (5-10 micron thickness) were prepared from each specimen. From every case, two slides were stained by Alcian blue and studied by light microscope.

Horse raddish peroxidase-labeled lectins (10 µg/ml PBS) such as orange peel fungus agglutinin (OFA), peanut agglutinin (PNA), soybean agglutinin (SBA), griffonia simplicifolia agglutinin (GSA_1-B_4), wheat germ agglutinin (WGA), maclura pomifera agglutinin (MPA), and dolichos biflorus agglutinin (DBA) were purchased from Sigma and were diluted in PBS (pH 6.8).

The sections were washed to omit the chloride pigments. To neutralize the endogenous peroxidase, sections were placed in 1% H_2O_2 solution in methanol for 5-10 minutes and then were washed in PBS for one hour. Then, slides were placed in lectins solution in a moistened chamber for two hours. All sections were placed in a solution containing 0.03g DAB in 100 ml PBS and H_2O_2 for 10 minutes and after working for 5-10 minutes, they were placed in Alcian blue (pH 2.5) for 5 minutes for counterstaining [2]. The brown color shows the existence of specific terminal sugar.

RESULTS

Developing neural crest cells migrate ventrally toward the notochord at early days of their migration. These cells reacted with SBA in 11^{th} gestational day (Fig. 1A) and were tapered gradually. As seen in Figure 1A, these cells were concentrated beside the notochord and mainly stained with counterstain (Alcian blue).

Observation of several serial sections showed similarity in the migratory pathway and localization of masses of ganglionic cells. In the stage of vertebral body formation, these colonies were observed beside the developing vertebral body. At 13^{th} gestational day, masses of differentiating sympathetic ganglia cells were visible (Fig. 1B). These colonies which are precartilage in the developmental stage reacted with SBA and range of reactivity at 12^{th}-14^{th} gestational days was less than 11^{th} day.

SBA was the only lectin reacted with sympathetic cells before the 14^{th} day of development and OFA was the only one that reacted after 14^{th} day. Masses of ganglionic cells were detected by OFA beside and in front of developing vertebral body on 14^{th} gestational day (Fig. 1C). We observed three masses of cells on each side of midline in several sections. Reactivity to OFA was diminished gradually from 14^{th} gestational day. Ganglia were stained mainly by counterstain in these stages.

Observation of serial sections from 15^{th} day, confirmed existence of only one sympathetic ganglion on each side of a developing vertebral body (Fig. 1D). Few sympathetic cells reacted to OFA in this embryonic day and the rest stained only by counterstain Alcian blue.

DISCUSSION

In the present study, we demonstrated the role of glycoconjugates terminal sugars on histogenesis of sympathetic ganglia. Our study elucidated that neural crest cells committed to sympathetic gangliogenesis reacted only with SBA in early days of their migration (Fig. 1A).

There are several reports concerning the key role of glycoconjugate terminal sugars during development [13,16]. Researchers have shown that SBA is specific lectin for detection of GalNac (N-acetyl galactosamine) terminal sugar of glyco-conjugate molecules. They have suggested that decreasing the reaction of lectin is probably due to the masking by sialic acid, or spatial position of molecular structure or changes in the connection of terminal sugars to penultimate molecule of the complex glycoconjugate [17-19]. It seems that GalNac has a critical role in early developmental stages of the sympathetic ganglia. This molecule might act as inducer of migration and cell differentiation during early migratory stages of embryogenesis.

GalNac induces the formation of masses of the ganglionic cells on each side of a vertebral body in early stages (Fig. 1B). One, two and finally three masses were formed on each side (Fig. 1C). Posterior masses were possibly differentiated to sympathetic ganglial chain and two others become preaortic ganglia.

We also detected that from 14^{th} embryonic day until birth, only OFA was reacted with active terminal sugar. Range of reactivity as observed on

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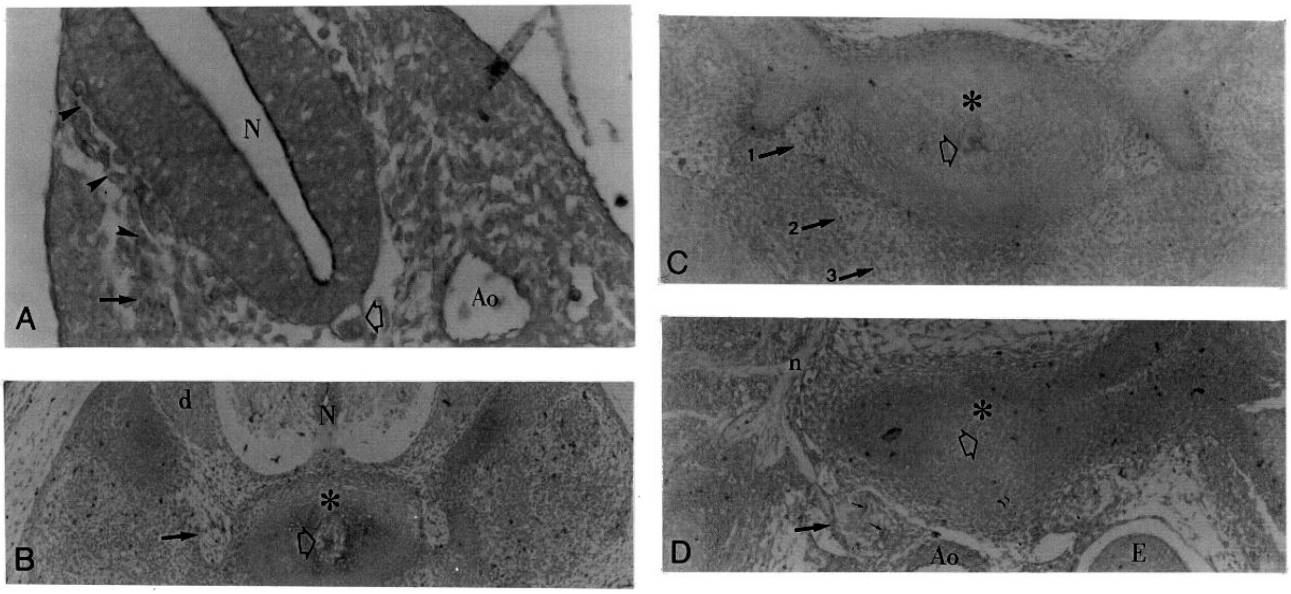

Fig. 1. (A), Transverse section from 11th gestational day of mouse. Arrow indicates differentiating neurons of sympathetic ganglion. Arrow heads, migratory neural crest cells; hollow arrow, notochord; Ao, aorta; N, neural tube. Used lectin is SBA; **(B),** Transverse section from 13th of mouse embryonic day. Arrow indicates differentiating neurons of sympathetic ganglion. Hollow arrow, notochord. N, neural tube ; d, dorsal root ganglia; *, developing vertebral body. Used lectin is SBA; **(C)**, Transverse section from 14th mouse embryonic day. Arrow 1 indicates differentiating sympathetic ganglion. Arrows 2 & 3, differentiating neurons of preaortic ganglia; Hollow arrow, notochord. *, developing vertebral body. Used lectin is OFA; **(D),** Transverse section from 16th mouse fetal day. Big arrow indicates differentiating neurons of sympathetic ganglion. Small arrows, ganglionic neurons; Ao, aorta; E, esophageal wall; Hollow arrow, degenerating notochord; N, spinal nerve; *, developing vertebral body. Used lectin is OFA.

Figure 3 was diminished gradually. Several researches have confirmed that OFA is the only lectin for detection of fucose as terminal sugar [20-21]. Other findings related to OFA and fucose were similar to SBA and GalNac, respectively.

Based on these observations, it seems that fucose has the key role in sympathetic ganglionic development at this stage. As Figure 1D shows, in the last stages of sympathetic ganglionic development, sympathetic chain's ganglions beside the vertebral body were well-developed and their reaction with OFA decreased and only few intraganglionic cells reacted to OFA.

During fetal stage of development spinal nerves appeared. These nerves had connection to the ganglia of the sympathetic chain. In last days of development, there were no detectable active terminal sugar which indicates final stage of ganglionic cell differentiation and therefore, they reacted only with Alcian blue.

We suggest that omission of key terminal sugar in each ganglionic developmental stage for instance exposure to putative teratogens might cause developmental defect of this part of peripheral nervous system.

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