The Role of GalNac Terminal Sugar on Adrenal Gland Development

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Abstract: Glycoconjugates, particularly their sugar side chains, play important roles in embryonic development. This study was conducted to examine sugar or terminal glycoconjugate sugar chains of surface developing medullary cells in mouse embryonic suprarenal glands. Mice embryos at days 13 to 20 of gestation developed by mating of 32 Balb/c mice, were serially cut at a thickness of 5 µm. For observation of suprarenal glands and selection of proper samples, specimens were first stained with hematoxyline-eosine (H and E) methods, then by using lectin histochemistry processing microscopic study was done. Among the used lectins such as; SBA, PNA, ConA, WGA and LTA only SBA and PNA on different embryonic days, with intensity and weakness related to the age, decrease and/or increase of chromaffin cells, showed medullary region of adrenal gland. PNA was used as positive control. The timing and distribution of staining with the lectin SBA suggest that, glycoconjugates containing N-acetyl-galactosamin may play a role in development of chromaffin cells at medullary region of suprarenal gland.

Key words: Lectin histochemistry • chromaffin cells • glycoconjugates • embryogenesis

INTRODUCTION

The suprarenal gland develops from two components: (a) a mesodermal portion which forms the cortex and (b) an ectodermal portion which forms the medulla. During the 13th day of mouse development [1], cells originating in the sympathetic system (neural crest cells) invade its medial aspect and give rise to the medulla of the suprarenal gland. They stain yellow-brown with chrome salts and hence are called chromaffin cells [2]. In relation to chromaffin cells development, Rupik by histological and histochemical methods, demonstrated that the chromaffinoblasts differentiate gradually from neuron-like cells to typical chromaffinocytes. All the chromaffinoblasts contain the chromaffin granules. The size and numerical density of the chromaffin granules increase with development [3]. Some of investigators by histochemical methods, have reported that the activity-related plasticity of chromaffin cells is developmentally and physiologically important [4].

Carbohydrate-containing macromolecules of the cell surface are thought to play a significant role in many developmental phenomena, including cell-cell interaction and differentiation [5, 6]. As in other developing systems, the embryonic suprarenal gland undergoes critical transitions during adrenogenesis [7-9]. It has been proposed that glycoconjugates, particularly glycoproteins, may function as tissue-specific stimulants which could initiate morphogenetic events leading to normal development of the adrenal gland [10]. Glycoconjugates containing Galactose-N-acetyl galactoseamin (Gal-GalNAc) have been of particular interest in developmental studies since these sugars invariably occupy a terminal position on oligosaccharide side chains [11]. Previous studies have demonstrated the incorporation of Gal-GalNAc, as a PNA receptor, into glycoproteins during early developmental stages in the rat adrenal gland [12], but the precise histological distribution and origin of the other glycoproteins have not been established.
The purpose of this study was to localize and characterize specific glycoproteins in situ during medullary of adrenogenesis. To this end, lectin histochemistry was undertaken with some lectins from SBA, WGA, PNA, ConA and LTA.

**MATERIALS AND METHODS**

**Tissue collection and processing:** 32 Balb/c mice were mated and day 0 of gestation was assigned at the appearance of a vaginal plug. Since microscopic suprarenal glands are visible at 13th embryonic day [1], at gestational ages of 10 days through 20 days, pregnant mice were anesthetized with ether and sacrificed by cervical dislocation and embryos were freed as rapidly as possible from the uterus and extra embryonic membranes. Embryos or abdominal segments were fixed in a solution of 6% mercuric chloride, 1% sodium acetate and 0.1% glutaraldehyde [13] for 18 hr at room temperature. The tissues were dehydrated through graded alcohols and xylene and embedded in composite paraffin blocks in sagittal, frontal and transverse orientation.

**Histochemical staining:** Paraffin-embedded sections were serially cut at a thickness of 5 µm. The sections were treated with Lugol’s solution to remove mercuric salts prior to histochemical staining. For observation of suprarenal glands and selection of proper sample, some specimens were first stained with hematoxyline-eosine on a part of the cell surface. Tissue collection and processing:

<table>
<thead>
<tr>
<th>Lectin (agglutinin) tested (Common name)</th>
<th>Abbreviation</th>
<th>Carbohydrate binding specify</th>
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<tbody>
<tr>
<td>Arachis hypogaea (Peanut)</td>
<td>PNA</td>
<td>D-Gal-(β1-3)-D-GalNAc</td>
</tr>
<tr>
<td>Lotus tetragonolobus (Asparagus pea)</td>
<td>LTA</td>
<td>α-L-Fuc.</td>
</tr>
<tr>
<td>Triticum vulgaris (Wheat germ)</td>
<td>WGA</td>
<td>Sialic acid</td>
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<tr>
<td>Glycine max (Soybean)</td>
<td>SBA</td>
<td>α, β-D-GalNAc</td>
</tr>
<tr>
<td>Canavalia ensiformia (Jack bean)</td>
<td>ConA</td>
<td>α-D-Glc, α-D-Man</td>
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Controls for lectin staining included: 1) Incubation of PNA-HRP as a positive tissue control [12], 2) Exposure to HRP and substrate medium without lectin.

**RESULTS**

In 10th-12th days mouse embryos, the medullary cells were not observed in the embryos. Among the five lectins tested, only SBA and PNA reacted with chromaffin cells at medullary region of suprarenal gland during development. Three other lectins, LTA, WGA and ConA, failed to bind to medullary of the adrenal gland during adrenogenesis.

In 13-day-old mouse embryos, affinity for SBA evidencing terminal GalNAc was observed in a small nest of cells clustered adjacent to suprarenal cortex.

In 14-15 days-old mouse embryos, affinity for SBA evidencing terminal GalNAc was observed in a large than 13th developmental day nest of cells clustered in the medullary of suprarenal gland.

In 16-day-old mouse embryos, affinity for SBA was larger than last developmental days. These cells exhibited strong SBA staining in foci near the nucleus, presumably golgi cisternae.

In 17-19 days old mouse embryos, whole region of medulla was occupied by chromaffin cells and affinity for SBA evidencing terminal GalNAc was observed. They also showed reactivity in cytosol near the cell surface and on a part of the cell surface.

In 20-day-old mouse embryos, the affinity for SBA was the same as 19th Developmental day (Fig. 1 and 2).

None of the lectins with specificity for sugars other than Gal-GalNAc and GalNAc (Table 1) stained the chromaffin cells at any stage of developmental studied (Fig. 3).

At all days of developmental suprarenal medulla, staining of chromaffin cells with PNA were observed. PNA was used as a positive tissue control in the study (Fig. 4). Substitution of exposure to HRP and substrate medium without lectin resulted in no staining.

Table 1: Lectins used in histochemical studies
Fig. 1: Sagittal section through the suprarenal gland in a day 19 mouse embryo. The chromaffin cells within the suprarenal gland medulla reacted with SBA. S = Suprarenal gland, C = Cortex, M = Medulla, Bar = 100 µm

Fig. 2: High magnification from the suprarenal gland of the Fig. 1. More chromaffin cells reacted strongly with SBA. These cells show staining in cytoplasmic foci (arrows). C = Cortex, M = Medulla, Bar = 50µm
Fig. 3: Sagittal section through the kidney and suprarenal gland in a day 19 mouse embryo that was stained with LTA. Only some vessels of kidney (arrows) reacted with the lectin. The other sites, specially suprarenal medulla, in this section were stained by the counterstain. K = Kidney, S = Suprarenal gland, M = Medulla, C = Cortex, Bar = 100 µm

Fig. 4: Sagittal section of kidney and suprarenal gland in a mouse embryo at day 19 of gestation stained lectin from PNA. The chromaffin cells within the suprarenal gland medulla reacted with lectin. K = Kidney, S = Suprarenal gland, C = Cortex, M = Medulla, Bar = 100 µm
Table 2: Reaction of chromaffin cells with lectins during suprarenal embryonic development. The intensity of the staining reaction is based on a subjectively estimated scale ranging from unreactive (-) to intensity reactive (++++)

<table>
<thead>
<tr>
<th>Lectin</th>
<th>13</th>
<th>14</th>
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<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBA</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>WGA</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>LTA</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ConA</td>
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<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
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<tr>
<td>PNA</td>
<td>++</td>
<td>++</td>
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**DISCUSSION**

Cell surface glycoconjugates and in particular their constituent carbohydrate moieties, are known to play an important role in many critical events during embryogenesis. Sugars such as Gal-GalNAc are of particular interest in developmental studies. The terminal position of these sugars in the side chains of many structurally defined glycoconjugates makes them potential candidates as ligands for developmentally regulated recognition molecules such as endogenous lectins, glycosyl transferases, or exoglycosidases on the surface of similar or different embryologic cell types [15, 16].

Katz has been studied that the embryonic sympathoadrenal neural crest cells give rise to a family of related catecholaminergic cell types that include both neuronal and neuroendocrine derivatives. The neuroendocrine and neuronal sublineages can be distinguished by differential expression of carbohydrate epitopes. In the study, PNA, as a control positive, almost exclusively reacted with chromaffin cells (Fig. 4), consistent with Katz observations.

It has been shown here that different terminal sugars-binding lectins vary in their affinity for chromaffin cells at various times during developmental suprarenal medulla in the mouse embryos (Table 2). Of the tested lectins, SBA alone reacted almost exclusively with chromaffin cells. Since only SBA lectin on the days of embryonic study had reaction with the chromaffin cells of suprarenal gland medullary region and specifically binds to the cell surface receptor N-acetyl-galactoseamin (GalNAc), therefore it is proposed that, terminal sugar GalNAc as a recipient of the development inducing factors, be considered an important factor in development of chromaffin cells at medullary region of suprarenal gland.

**REFERENCES**


