Differences in utero in activities of catecholamine biosynthetic enzymes in adrenals of spontaneously hypertensive rats

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Summary

1. We sought to determine if catecholamine biosynthetic enzymes of spontaneously hypertensive rats (SHR) differed from those of normotensive Wistar-Kyoto (WKY) and Sprague-Dawley (SD) control rats before birth.

2. By immunocytochemical and biochemical methods we compared strains for the time of appearance and maturation of the enzymes tyrosine hydroxylase (TH), dopamine-β-hydroxylase (DBH) and phenylethanolamine-N-methyltransferase (PNMT) in sympathetic ganglia and adrenals.

3. The time of appearance of enzymes was identical in all three strains: TH and DBH first appeared in sympathetic ganglia on embryonic day 11 (E11) and in adrenal medulla on E16. PNMT, restricted to adrenal medulla, appeared later on E18.

4. The activity of adrenal TH prenatally on E18 and E21 and at day of birth (P1) in SHR was approximately two fold that in WKY or SD rats. In contrast PNMT was lower in SHR but only on E18.

5. Thus, although the timing of the first expression of adrenergic phenotypes is similar in SHR and normotensive controls, the differences in TH activity in adrenals suggest an enhanced biosynthetic capacity for catecholamines in this strain before birth.

6. We conclude that SHR differ from normotensive rats from the first expression of some of the genes controlling catecholamine biosynthesis.

Key words: adrenal, catecholamines, dopamine-β-hydroxylase, foetus, phenylethanolamine-N-methyltransferase, tyrosine hydroxylase.

Abbreviations: DBH, dopamine-β-hydroxylase; PNMT, phenylethanolamine-N-methyltransferase; TH, tyrosine hydroxylase.

Introduction

It is now established that during early postnatal development spontaneously hypertensive rats of the Okamoto strain (SHR) differ with respect to vasomotor regulation from strain-matched Wistar-Kyoto (WKY) control rats. Differences in arterial pressure and vascular reactivity have been detected by at least the third week of postnatal life [1, 2]. The fact that the hypertension of SHR depends upon sympathetic autonomic outflow [3, 4], and that SHR differ from non-hypertensive controls with respect to the activity of several enzymes subserving catecholamine biosynthesis in the adrenal gland [5, 6] and some sympathetic ganglia [7], suggest that processes involving peripheral catecholaminergic systems may in fact play a role in triggering the onset of hypertension in this strain.

It remains to be established whether the differences in the development of the autonomic nervous system in SHR appear postnatally, possibly in response to environmental signals (8–10), or appear prenatally in utero, conceivably under the influence of early gene expression.

We have recently characterized the timing of the embryonal development of catecholaminergic cells in the Sprague-Dawley (SD) rat [11–13]. By the use of immunocytochemical techniques for visualizing in vivo the catecholamine biosynthetic enzymes tyrosine hydroxylase (TH), dopamine-β-hydroxylase (DBH) and phenylethanolamine-N-methyltransferase (PNMT), as well as by measuring the activities of these enzymes biochemically [13], we have been able first, to chart the time at which primitive cells differentiate to express a catecholaminergic phenotype, and, secondly, to...
establish the pattern of migration and maturation of these systems.

In the present study we have sought to determine whether or not rats of the SHR strain differ from WKY and SD control rats with respect to the prenatal development of the sympathetic nervous system.

Methods

The methods have been described in detail elsewhere [11-13]. They are summarized here.

Rats of SHR, WKY and SD strains with timed pregnancies were commercially obtained and housed in light-cycled animal quarters, and fed ad libitum. On various days of gestation, mothers were anaesthetized with pentobarbitol (40 mg/kg) and the embryos removed. The tissues were frozen and sections (16 μm) obtained with a cryostat microtome. The sections were immunostained by the peroxidase-antiperoxidase (PAP) technique of Sternberger et al. [14] with antibodies against TH, DBH and PNMT. The procedure for preparation of the antibodies and the criteria used to judge their specificity have been described elsewhere [11]. In some embryos, the sympathetic ganglia and the adrenal glands were excised and the activities of TH and PNMT were determined by standard methods [e.g. 15].

Results

The time of biochemical development of peripheral sympathetic ganglia and adrenal medulla early in embryogenesis was assessed by the appearance of immunoreactive TH, DBH and PNMT [11-13]. There were no differences in the timing of appearance of the enzymes between embryos of SHR and WKY rat strains, which in turn were similar to that observed in SD rats [11-13].

In summary: (a) no catecholamine enzymes were seen in cells of the neural crest; (b) TH and DBH were first seen on embryonic days (E) 10-5-11 in the most rostral portions of the primary sympathetic ganglion and on the following days in more caudal segments (thus these sympathoblasts express a noradrenergic phenotype); (c) from E11 to E14, cells containing TH and DBH transiently populate the gut; (d) by E13 migrating cells from primary ganglia have colonized the para-aortic plexii; (e) on E16 the first pioneer catecholaminergic cells invade the adrenal anlage (these cells express only TH and DBH and hence are also noradrenergic); (f) PNMT first appears 2 days later on E18 in adrenal medullary cells.

To examine whether the strains differed in enzyme activity, TH and PNMT were measured on E18, E21 and first postnatal day in whole adrenals and the superior cervical ganglion of SHR, WKY and SD rats. As indicated in Table 1: (a) foetal adrenals of SHR have 50-80% more TH activity over these 3 days than in those of WKY or SD embryos; (b) in SHR the activity of PNMT is significantly less on E18 than that of WKY or SD rats (thereafter enzyme activity does not differ): (c) there are no strain differences in TH activity in the superior cervical ganglion; (d) at no time do WKY and SD rats differ with respect to the activities of the catecholamine enzymes.

<table>
<thead>
<tr>
<th>Day</th>
<th>SHR (n = 4)</th>
<th>WKY (n = 4)</th>
<th>SD (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adrenal gland PNMT (pmol of product/h per adrenal)</td>
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</tr>
<tr>
<td>E18</td>
<td>13-19 ± 0.95 (P &lt; 0.05)</td>
<td>24-4 ± 1.17 (N.S.)</td>
<td>22-91 ± 9.42</td>
</tr>
<tr>
<td>E21</td>
<td>132-69 ± 10-2 (N.S.)</td>
<td>152-82 ± 9-37 (N.S.)</td>
<td>164-96 ± 11-21</td>
</tr>
<tr>
<td>P1</td>
<td>335-1 ± 17-1 (N.S.)</td>
<td>305-8 ± 19-7 (N.S.)</td>
<td>352-18 ± 17-3</td>
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<tr>
<td></td>
<td>Adrenal gland tyrosine hydroxylase (nmol of product/h per adrenal)</td>
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<td></td>
</tr>
<tr>
<td>E18</td>
<td>1-17 ± 0-072 (P &lt; 0.005)</td>
<td>0-29 ± 0-015 (N.S.)</td>
<td>0-35 ± 0-024</td>
</tr>
<tr>
<td>E21</td>
<td>3-30 ± 0-17 (P &lt; 0.002)</td>
<td>2-17 ± 0-19 (N.S.)</td>
<td>2-39 ± 0-088</td>
</tr>
<tr>
<td>P1</td>
<td>6-11 ± 0-12 (P &lt; 0.002)</td>
<td>3-42 ± 0-24 (N.S.)</td>
<td>3-61 ± 0-094</td>
</tr>
<tr>
<td></td>
<td>Superior cervical ganglia tyrosine hydroxylase (nmol of product/h per ganglion)</td>
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<td></td>
</tr>
<tr>
<td>E18</td>
<td>0-40 ± 0-04 (N.S.)</td>
<td>0-56 ± 0-07</td>
<td>—</td>
</tr>
<tr>
<td>E21</td>
<td>1-24 ± 0-05 (N.S.)</td>
<td>1-29 ± 0-27</td>
<td>—</td>
</tr>
<tr>
<td>P3</td>
<td>2-32 ± 0-09 (N.S.)</td>
<td>2-56 ± 0-10</td>
<td>—</td>
</tr>
<tr>
<td>P5</td>
<td>4-17 ± 0-20 (N.S.)</td>
<td>4-26 ± 0-19</td>
<td>—</td>
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</tbody>
</table>
enzymes in adrenals and superior cervical ganglion.

Discussion

This present study demonstrates that differences can be detected in the maturation of the autonomic nervous system in rats of the SHR strain during late embryogenesis. The difference is characterized by an augmented activity of TH in the adrenal medulla of SHR, which is present at birth. The elevation in TH activity is anatomically restricted to the adrenals in SHR since it is not seen in the sympathetic superior cervical ganglion.

The mechanisms for accounting for the augmentation in TH activity in the adrenal of SHR is unknown. Conceivably it could be a consequence of the presence of a greater number of adrenal medullary cells expressing the enzyme, enhanced survivability of these cells, or simply greater amount and/or activity of enzyme per cell than in their non-hypertensive counterparts. Thus, although there are no substantial differences in the timing of differentiation of the developing adrenal chromaffin cells, they do differ in the regulation of TH once the enzyme is expressed.

One possible mechanism to account for the difference could relate to the pituitary adrenal control systems. It has been proposed on the basis of histochemical observations that in early postnatal development SHR have an over-activity of the pituitary—adrenal cortical axis [16]. However, while it is well known that glucocorticoids regulate the activity and amount of PNMT in the adrenal medulla [17, 18] and such control is exerted during late embryogenesis [13], the facts, first, that the strains do not differ in activity of PNMT, and, secondly, that glucocorticoids regulate TH activity in adult [19] but not foetal adrenal glands [20], make it unlikely that glucocorticoids are involved. On the other hand, recent evidence that ACTH may exert a relatively selective action on TH activity [20] raises the possibility than an overabundance of this hormone in SHR might raise TH activity over that of the other strains.

The relationship between the differences in the development of the autonomic system of SHR and the ultimate expression of hypertension in that strain is not known. There is evidence from studies of catecholamine biosynthesis in the peripheral sympathetic system and in brain that although SHR may differ from their controls in the amounts and activities of these enzymes, such differences may be regional and transient [21]. However, the increasing evidence that the magnitude of hypertension in SHR may be altered by maternal and non-maternal environment [8–10] raises the prospect that some preconditioning of autonomic development may in fact amplify environmental signals and be a predisposing, rather than causative, factor in the evolution of the hypertension.

The observation that differences of autonomic function between SHR and their controls appear in utero is of interest for it sets the earliest appearance of differences between SHR and non-hypertensive controls back to embryonic life. The finding indicates that factors operating in utero, either controlled by gene expression or even conceivably by maternal factors [17], may influence the ultimate development of autonomic function in the strain.

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References


