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Development of adrenal cortical zonation and expression of key elements of adrenal androgen production in the chimpanzee (*Pan troglodytes*) from birth to adulthood

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ABSTRACT

The basis for the pattern of adrenal androgen production in the chimpanzee, which resembles that of humans, is poorly defined. We characterized the developmental zonation and expression of elements of the androgen biosynthetic pathway in the chimpanzee adrenal. The newborn adrenal contained a broad fetal zone (FZ) expressing CYP17, SULT2A1, and Cytochrome B5 (CB5) but not HSD3B; the outer cortex expressed HSD3B but not SULT2A1 or CB5. During infancy, the FZ involuted and the HSD3B-expressing outer cortex broadened. By 3 years of age, a thin layer of cells that expressed CB5, SULT2A1, and CYP17 adjoined the medulla and likely represented the zona reticularis; the outer cortex consisted of distinct zonae fasciculata and glomerulosa. Thereafter, the zona reticularis broadened as also occurs in the human. The adult chimpanzee adrenal displayed other human-like characteristics: intramedullary clusters of reticularis-like cells and also a cortical cuff of zona fasciculata-like cells adjoining the central vein.

1. Introduction

During human fetal development, a specialized zone of the adrenal cortex, known as the fetal zone, comprises about 80% of the mass of the adrenal and produces the large quantities of DHEA and DHEA-sulfate that serve as the principal precursors for placental estrogen formation. The fetal zone of the human adrenal contains large quantities of the enzyme DHEA-sulfotransferase (SULT2A1) and little if any 3β-hydroxysteroid dehydrogenase (HSD3B2) that could direct the adrenal steroid pathway toward delta-4 steroids such as aldosterone and cortisol rather than DHEA/DHEA-sulfate (Barker et al., 1995; Dupont et al., 1990; Mesiano et al., 1993; Parker et al., 1994, 1995). In fact, there appears to be little HSD3B2 in the fetal adrenal until the latter stages of gestation where it is primarily localized to the outer cortex known as the neocortex (Dupont et al., 1990; Mesiano et al., 1993; Parker et al., 1995). The enzyme 17α-hydroxylase/17,20-lyase (CYP17), which can catalyze both the 17α-hydroxylation of pregnenolone and progesterone as well as conversion of such 17-hydroxylated steroids to DHEA and androstenedione, respectively, is characteristic of the fetal and adult human adrenal (Mesiano et al., 1993; Dharia et al., 2005). Cytochrome B5 (CB5), which acts as an accessory protein to CYP17 and promotes its 17,20-lyase activity (Katagiri et al., 1995; Lee-Robichaud et al., 1995), is co-localized with CYP17 in both the fetal zone of the fetal adrenal and the zona reticularis (Dharia et al., 2005), which develops during adrenarche and persists throughout adulthood, in the human adrenal (Dhom, 1973).

Although adrenal androgen production during fetal development and/or during adult life is not common among other mammals, many primate species have been found to bear similarities in the potential for adrenal androgen synthesis to that of the human. Those species that have been found to possess a fetal zone like that of the human also appear to rely on the fetal adrenal to a variable degree to supply precursors for placental estrogen formation during pregnancy (Albrecht et al.,1980; Lanman, 1961; Walsh et al., 1979a, 1979b). Interestingly, however, there is limited information about the morphologic and functional zonation of the fetal and postnatal adrenal of our genetically closest primate relative, the chimpanzee (*Pan troglodytes*).

The available data are suggestive that in the chimpanzee, the fetal adrenal is composed of a morphologically distinct fetal zone and a neocortex (Czekala et al., 1983) and estrogen production during pregnancy follows a similar time course to that of humans

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(Czekala et al., 1983; Reyes et al., 1975; Smith et al., 1999). However, we are not aware of any studies demonstrating the capacity for androgen production by the fetal adrenal or a role for the fetal adrenal in estrogen formation in chimpanzee pregnancy. There are developmental increases in circulating levels of DHEA and DHEA sulfate during adolescence that resemble that seen in human adrenarche (Bernstein et al., 2012; Collins et al., 1981; Copeland et al., 1985; Smail et al., 1982), and there is maintenance in serum levels of DHEA and DHEA sulfate during young adulthood that are within the general range of circulating concentrations noted for humans (Bernstein et al., 2012). Since it is known that DHEA and DHEA sulfate are produced in castrated chimpanzees and are responsive to ACTH (Albertson et al., 1984), these steroids must arise from the adrenal gland. Despite such findings, there are no data concerning the functional phenotype of the adrenal cortical zones of the fetal or postnatal chimpanzee. Based on these limited findings, we sought to better characterize the steroidogenic potential of the adrenal of the fetal, infant, adolescent, and adult chimpanzee by evaluating the zonation and expression of several key elements of the steroidogenic pathway.

2. Methods

Archival adrenal samples from 27 chimpanzees (13 male, 14 female) ranging in age from term newborn to 31 years of age were kindly provided to us by the Yerkes National Primate Research Center and the Southwest National Primate Research Center. At the time of routine autopsy of the chimpanzees, tissues, including the adrenal glands, were removed and were fixed in 10% neutral buffered formalin. Tissues were processed using standard techniques and embedded in paraffin. For our study, five micron sections were mounted on plus slides (corning) and were either stained with hematoxylin and eosin for general morphologic characterization or were utilized for immunohistochemistry using standard techniques in our laboratory.

The anti-human SULT2A1 antiserum was developed in the rabbit against purified human liver DHEA sulfotransferase by Dr. Charles Falany (Department of Pharmacology, University of Alabama at Birmingham). The anti-human CB5 antiserum was developed in the rabbit by Dr. Alan J. Conley (School of Veterinary Medicine, Univ. California at Davis), utilizing recombinant human CB5 donated by Ron Estabrook and Manju Shet, (Univ Texas Southwestern Medical School, Dallas TX). The anti-human HSD3B antiserum (which we find to react with both isozymes of the human, HSD3B1 and HSD3B2, which share 95% sequence homology and are both coded for on chromosome 1 in the human) was developed in the rabbit by Dr. Richard Parker against purified human placental 3β-hydroxysteroid dehydrogenase/delta 5–4 isomerase kindly donated by Dr. James Thomas (Mercer University School of Medicine, Macon GA). The mouse monoclonal anti-human CYP17 antibody was developed by Dr. Richard Parker against recombinant human CYP17 that was generated by an expression plasmid kindly donated by Michael Waterman (Vanderbilt University, Nashville TN). Chimpanzee CYP17 differs by only two amino acids from that in the human, and there is 95% homology between these and the enzyme in rhesus and baboon (Arlt et al., 2002). The antisera used in this study of the chimpanzee adrenal have been previously utilized in several published studies on human and rhesus steroidogenic tissues (Dharia et al., 2004; Mapes et al., 2002; Parker et al., 1995; Parker et al., 2000b, for example).

Briefly described, after the tissue sections were subjected to deparaffinization in xylene and progressive hydration in ethanol, 95% ethanol, 75% ethanol, and distilled water, the sections were incubated with 3% hydrogen peroxide for 10 min at 25 °C to block endogenous peroxidase. Then, sections were rinsed in distilled water and Tris buffer, pH 7.6, and incubated for 20 min at 25 °C in 5% goat serum to block non-specific antibody binding. The sections were then drained and incubated for 1hr at 25 °C with antisera diluted in PBE buffer, ph 7.6 (0.1 M phosphate buffer that contained 1% bovine serum albumin, 1 mM EDTA, 1.5 mM sodium azide). The dilutions of antisera used for immunohistochemistry were as follow: a 1:12,000 dilution of rabbit anti-human CB5, a 1:40 dilution of mouse anti-human CYP17, a 1:500 dilution of rabbit anti-human HSD3B1, and a 1:500 dilution of rabbit anti-human SULT2A1. These dilutions were based on our prior studies of human and rhesus steroidogenic tissues. We also immunostained some sections for a neuroendocrine marker of the adrenal medulla by use of rabbit anti-human Chromogranin A antiserum (DAKO, Cat # N1535), diluted 1:500 in PBE buffer and incubated as above. After being rinsed in Tris buffer, all sections were incubated with linking and labeling reagents contained in the Multi-Species Ultra Streptavidin Horseradish peroxidase detection kit with DAB as chromogen (Covance). Sections were then counterstained with hematoxylin and coverslipped after dehydration using graded ehanols to xylene.

Digital photography of the slides was accomplished through the use of a Zeiss Axio Imager M2. Images were captured as Tiff files and composite figures were prepared using Power Point software. Magnifications cited in this paper are the product of the eyepiece lens magnification (10×) and those of the objective lenses (2.5, 5, and 10×).

3. Results

Although adrenal sections from all specimens were usually found to contain regions that were immunopositive for HSD3B, CYP17, SULT2A1, and/or CB5, the distribution and abundance of these elements of the steroidogenic pathway varied with morphologic zonation and developmental stage. There were no apparent differences in the patterns or intensity of immunostaining between the adrenals of male and female chimpanzees at similar ages.

3.1. Newborn

In the newborn chimpanzee ($n = 3$), the cortex was found to contain a narrow outer cortical zone (approximately 25% of the total cortical thickness) resembling that of the human that has been termed the neocortex and a broad inner area previously described as the fetal zone (Fig. 1A). The outer portion of the neocortical area in the chimpanzee resembles the zona glomerulosa whereas the inner part of the neocortex appears to be similar to the cell groupings in the human fetal adrenal characterized as the transitional zone (likely precursor to the zona fasiculata). HSD3B staining was mainly restricted to cells in the outer 2/3rds of the neocortex; minimal, if any, immunostaining for this steroidogenic enzyme was noted in the fetal zone (Fig. 1B). CYP17 staining was noted throughout the fetal zone and the inner neocortex but was absent from the outer neocortex (Fig. 1C). The immunostaining for CB5 (Fig. 1D) was found throughout the fetal zone but not in the neocortex. The immunohistochemical staining pattern for SULT2A1 (not shown) was similar to that of CB5 in the adrenals of newborn chimpanzees.

3.2. Early infancy

In the adrenal of a 1 month old chimpanzee, the outer cortex area was broadened compared to that of the newborns and was immunopositive for HSD3B but not the other steroidogenic markers. Immunostaining for SULT2A1 and CB5 persisted in the broad fetal zone, as did that for CYP17, but not in the neocortex.