Morphological adrenarche in rhesus macaques: development of the zona reticularis is concurrent with fetal zone regression in the early neonatal period

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Abstract

Human adrenarche is associated with the establishment of a functional zona reticularis (ZR) and increasing secretion of dehydroepiandrosterone (DHEA) in sulfated form (DS). Like most non-human primates, rhesus macaques are not believed to undergo adrenarche, though they clearly establish a functional ZR after birth. Therefore, we investigated the zonal development, steroidogenic enzyme expression and morphology of rhesus adrenals from 1 day to 14 months of age. Immunohistochemistry was conducted to determine expression profiles of the steroidogenic enzymes 17α-hydroxylase/17,20-lyase cytochrome P450, family 17, subfamily A, polypeptide 1 (CYP17A1), cytochrome P450, family 21, subfamily A, polypeptide 2 (CYP21A2), hydroxy-Δ5-steroid dehydrogenase, 3β- and steroid Δ-isomerase 2 (HSD3B2), the redox partner NADPH-cytochrome P450 oxidoreductase (CPR), as well as the accessory protein cytochrome b5 (b5), a marker of the primate ZR. The rhesus ZR is mature by 3 months of age based on differentiation of the innermost zone that lacks HSD3B2, but exhibits increased b5 expression during this period. Further, the ZR develops in neonates from a previously described dense band of cells which we show expresses b5, CYP17A1, CPR, and CYP21A2 throughout maturation. The fetal zone (FZ) is distinguished from the ZR by its lack of CYP21A2, and ZR development proceeded as the FZ regressed with two important implications: neither FZ regression nor ZR maturation can be monitored independently by circulating adrenal androgens, and these events must be induced by different factors in rhesus, and likely humans. Collectively these data demonstrate that ZR development begins before birth in the rhesus, proceeding concomitantly with FZ regression post-natally, suggesting that rhesus experiences morphological adrenarche during the first three months of life.


Introduction

Among mammals, the human adrenal gland is unusual in that it develops the capacity to secrete prodigious amounts of the C-19 steroid dehydroepiandrosterone (DHEA) and its sulfoconjugate (DS) during childhood, an event commonly referred to as adrenarche. Adrenarche is associated with differentiation of an inner zone of cells in the adrenal cortex, the zona reticularis (ZR; Havelock et al. 2004). Although many non-human primates also develop a prominent ZR, adrenarche is not thought to occur in the most commonly studied species such as the rhesus macaque (Arlt et al. 2002). This perception derives from the lack of data demonstrating a postnatal increase in circulating DHEA or DS in juvenile and adolescent animals (Cutler et al. 1978, Smail et al. 1982). However, immunohistochemical (IHC) studies confirm that the rhesus ZR has the functional capacity for androgen synthesis (Mapes et al. 1999), similar to the human ZR (Conley et al. 2004). As in humans, rhesus adrenal development is also associated with regression of an equally distinct fetal zone (FZ) during the neonatal period (McNulty et al. 1981). Given the parallels in fetal and adult adrenocortical differentiation in both species, it seems that the rhesus macaque must experience an adrenarche. Thus, a more careful definition of neonatal adrenocortical differentiation may shed light on why adrenarche in the rhesus is not marked by a pre-pubertal increase in DS, and is therefore hormonally cryptic.

Clearly, adrenocortical development and differentiation is quite complex in human and non-human primates (Winter 1992), involving changes in patterns of steroidogenic enzyme expression that dictate the profile of steroids produced by each zone (Mesiano et al. 1993, Conley & Bird 1997, Jaffe et al. 1998). The mature primate adrenal cortex synthesizes and secretes aldosterone, cortisol, and DS from the zona glomerulosa (ZG), zona fasciculata (ZF) and the ZR respectively. It has been suggested that the adult ZR, ZF, and ZG develop from histologically distinct zones of the fetal
adrenal cortex, namely the FZ, transitional zone (TZ) and definitive zone (DZ) respectively, based on results of IHC studies (Mesiano et al. 1993). The FZ secretes androgens, primarily DS, though little cortisol or aldosterone is synthesized from the TZ and DZ during fetal life (Seron-Ferre et al. 1978, Doody et al. 1990, Nelson et al. 1990). As expected, the ZR and FZ express the steroidogenic enzymes, and accessory proteins, required for androgen synthesis, including 17α-hydroxylase/17,20-lyase cytochrome P450, family 17, subfamily A, polypeptide 1 (CYP17A1), cytochrome b5 (b5), and NADPH-cytochrome P450 oxidoreductase (CPR; Mesiano et al. 1993, Coulter et al. 1996b, Mapes et al. 1999, 2002, Suzuki et al. 2000, Narasaka et al. 2001, Dharia et al. 2004, 2005). The adult ZF and ZG, and to a limited extent the fetal TZ and DZ as noted above, exhibit expression profiles of steroidogenic enzymes that support glucocorticoid and mineralocorticoid syntheses instead (Mesiano et al. 1993, Coulter et al. 1996b, Coulter & Jaffe 1998, Mapes et al. 1999, Suzuki et al. 2000, Narasaka et al. 2001). These enzymes include cytochrome P450, family 21, subfamily A, polypeptide 2 (CYP21A2), hydroxysteroid-Δ5-steroid dehydrogenase, 3β- and steroid Δ-isomerase 1 (HSD3B2) and 11β-hydroxylase cytochrome P450 (P450c11). Thus, the temporal and spatial expression profiles of key steroidogenic enzymes, redox partners and accessory proteins, together with routine histology, allows the zones of the adult and fetal adrenal to be identified, characterized, and distinguished.

The current study was conducted to investigate the temporal and spatial profiles of expression of a suite of steroidogenic enzymes and accessory proteins, especially those necessary for androgen synthesis in neonatal rhesus adrenal glands. The immediate post partum and early neonatal period is a particularly dynamic one where differentiation of the adrenal cortex of rhesus macaques is concerned (McNulty et al. 1981), but it has not been extensively investigated in this species with respect to functional differentiation. Therefore, it was anticipated that better defining the cellular differentiation and zonation of the rhesus adrenal cortex during the early neonatal period would provide insight into ZR development, the event associated with adrenarche, in humans and non-human primates.

Materials and Methods

Animals

Rhesus macaque adrenals were obtained opportunisticly from approved projects at the California National Primate Research Center. Specimens were obtained from animals that were perinatal (1–14 days old; n = 10) neonatal (1–3 months old; n = 12), juvenile (4–14 months old; n = 11) or adult (8 years or older; n = 4) in age. Adrenal glands were fixed in 4% paraformaldehyde and subjected to graded ethanol baths prior to embedding. Tissues were embedded in paraffin wax (Fisher Scientific, Pittsburgh, PA, USA) and sectioned at 5 μm thickness.

Immunohistochemistry

Tissue sections were deparaffinized with Citrisolv and rehydrated through a graded ethanol series. Localization of expression of steroidogenic enzymes was visualized by avidin–biotin–peroxidase complex formation (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA, USA), as previously described (Conley et al. 1996, Browne et al. 2006). Additional adrenal tissue sections were also subjected to routine hematoxylin and eosin staining (Fig 1). Steroidogenic enzyme expression was detected in sections from each subject using the following primary antisera: b5 ((1:20 000), polyclonal rabbit anti-human, raised in our laboratory against purified recombinant protein provided by Drs Ron Estabrook and Manju Shet (Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX, USA)), CYP17A1 ((1:10 000), polyclonal chicken anti-human raised in our laboratory against purified recombinant protein), CPR ((1:3000), polyclonal rabbit anti-rat raised in our laboratory against purified recombinant protein provided by Dr Ron Estabrook), CYP21A2 ((1:25 000), polyclonal rabbit anti-human provided by Dr Walter Miller (Department of Pediatrics, University of California San Francisco, San Francisco, CA, USA)), and HSD3B2 ((1:400), polyclonal rabbit anti-human provided by Dr J Ian Mason (Centre for Reproductive Biology, University of Edinburgh Medical School, Edinburgh, UK)). PBS (0·1 M, pH 7·2 with 0·3% Triton-X (Sigma–Aldrich)) was used for tissue washes and antibody dilutions. Endogenous peroxidase activity was quenched by incubation in 0·3% H2O2 (in methanol, room temperature (RT)) for 30 min. Immunolocalization with CYP21A2 and CPR antisera utilized antigen retrieval, performed as previously described (Browne et al. 2006), prior to blocking of non-specific binding. Briefly, sections immersed in Antigen Unmasking Solution (Vector Laboratories) were heated slowly to 95 °C in a steamer, cooled gradually to RT over 45 min, and then subjected to the remainder of the IHC procedure. Non-specific binding was blocked by incubation with 1·5% normal goat serum in PBS (20 min, RT). Tissue sections were incubated with primary antibody incubation overnight at 4 °C for all antisera, followed by 30 min incubations with species-appropriate biotinylated secondary antibody (chicken: Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA; rabbit: Vectastain Elite ABC kit, Vector Laboratories) and avidin–biotin complex solution (Vectastain Elite ABC kit, Vector Laboratories). Primary antibody binding was detected with the peroxidase substrates Vector NovaRed (b5, CYP17A1; 5 min incubation) or 3-amino-9-ethylcarbazole (AEC: CYP21A2, HSD3B2, CPR; 12 min incubation; Vector Laboratories) and counterstained with Gill’s hematoxylin 2 (Fisher Scientific). Tissues stained with Vector NovaRed were dehydrated with graded EtOH series and Citrisolv baths. Vector NovaRed-stained slides were coverslipped using Permount mounting medium (Fisher Scientific); slides stained with AEC were coverslipped using Faramount aqueous mounting medium (Dako, Carpinteria, CA, USA). Specificity


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of all antisera binding was verified by western immunoblotting of rhesus macaque adrenal microsomal samples, all of which demonstrated single bands of the expected molecular size (Figs 2–4). Normal serum was utilized instead of primary antisera in negative controls. Micrographs were taken using brightfield illumination on a DMRB microscope (Leica Corp., Rockleigh, NJ, USA) equipped with a MicroPublisher 3.3 RTV (Qimaging Corporation, Surrey, British Columbia, Canada) and Windows QCapture Suite (Qimaging Corporation). IHC runs for each enzyme included specimens from all ages to minimize variation in staining that might have occurred between different runs.

Results

Perinatal zonation

The perinatal (1–14 days old) rhesus adrenal cortex (Fig. 1) was comprised of four distinct regions. Directly underlying the capsule was the DZ, consisting of a band of small cells,
Figure 2  Cytochrome b5 expression in the developing rhesus adrenal cortex (1 day old (DO)–1 month old (MO; A–D) and 2 MO–8 years of age (YO; E–H)). (A and B) Perinatal period (1 and 3 DO respectively); there is patchy expression throughout the fetal zone (FZ) and the transitional zone (TZ) adjacent to it, but expression is more intense in the positive cells of the TZ. The dense band (DB-ZR) is evident between the TZ and FZ in the 3 DO tissue, consisting of compact cells with intense b5-expression, much greater than cells of either the FZ or the TZ. The outermost definitive (DZ) has no detectable b5 expression by comparison. (C) Perinatal period (14 DO); there is increased intensity of b5 expression in the FZ, although expression in the DB-ZR is still greater. The DZ and medulla (M) are negative for b5 expression. A small number of TZ cells near the DB-ZR show b5 expression. (D) Neonatal period (1 MO); nearly all DB-ZR and FZ cells are positive for b5 expression in the region between a distinct b5-negative zona fasciculata (ZF) and M. The FZ, while still b5-positive, is decreasing in size, and there is no detectable b5 expression in the zona glomerulosa (ZG). (E) Neonatal period (2 MO); all DB-ZR and FZ cells are positive for b5 expression in the region between a distinct b5-negative ZF and M. Compact cells of the DB-ZR exhibit increased intensity of expression, similar to perinatal adrenals. The FZ, while still b5-positive, continues to shrink in size. There is no detectable b5 expression in the ZG or M. (F) Neonatal period (3 MO); there is uniform b5 expression throughout the zona reticularis (ZR), as seen in adrenal cortex of mature rhesus. Between the b5-positive compact cells of the ZR and the b5-negative cells of the M is a thin layer of large, vacuolated b5-positive cells of the diminished FZ. (G) Juvenile (1-3 YO); expression of b5 is similar to that observed in the ZR of neonates 3 MO and older. (H) Adult (8 YO); note expression is uniform in intensity in all cells, similar to that seen in the 3 MO ZR. In adults, no cells exhibiting FZ morphology were detected within the b5-positive region between the b5-deficient ZF and M. Bars = 50 μm, all panels same magnification.
Figure 3 Adrenocortical expression of HSD3B2 (A–C) and CYP21A2 (D–F) in the developing rhesus from 5 days (DO)–8 months old (MO). (A) Perinatal (5 DO); expression of HSD3B2 is restricted to the outer definitive zone (DZ) and the outermost region of the transitional zone (TZ). (B) Neonatal (2 MO); HSD3B2 is expressed in all cells of the zona glomerulosa (ZG) and zona fasciculata (ZF), with greatest expression in the ZG. Non-specific staining due to a fold in adrenal tissue is seen at the dense band (DB-ZR)/fetal zone (FZ) border. No HSD3B2 expression was detected in the DB-ZR, FZ, or medulla (M). (C) Juvenile (8 MO); HSD3B2 is expressed in all cells of the ZG and ZF, but is the greatest in the ZG and outermost ZF. No HSD3B2 expression was detected in ZR, FZ, or M cells. (D) Perinatal (5 DO); CYP21A2 is expressed in the DZ, TZ, and faintly in some cells of the DB-ZR. A few FZ cells show faint CYP21A2 staining. (E) Neonatal (2 MO); CYP21A2 expression was intense throughout the ZG, ZF, and outermost DB-ZR, with no detectable expression in the FZ (based on morphological assessment) or M. (F) Juvenile (8 MO); there is strong expression of CYP21A2 in all cells of the ZG and ZF, and strong but patchy expression in cells of the ZR. There was no detectable expression in the vacuolated cells of the FZ or in M. Arrows denote clusters of medullary cells. Bar = 50 μm, all panels same magnification.
Figure 4 Developmental expression of 17α-hydroxylase/17,20-lyase cytochrome P450 (CYP17A1; A–C) and NADPH-cytochrome P450 oxidoreductase (CPR; D–F) in the developing rhesus adrenal (5 day old (DO)–8 months old (MO)). (A) Perinatal (5 DO); there is faint expression of CYP17A1 in some large, vacuolated cells of the fetal zone (FZ) and intense expression in all transitional zone (TZ) cells. Cells of the dense band (DB-ZR) between the TZ and FZ exhibit intermediate expression relative to these zones. No detectable expression in the definitive zone (DZ) was observed. (B) Neonatal (2 MO); all cells of the ZF and the outermost cells of the DB-ZR exhibit uniform expression of CYP17A1. FZ cells exhibit faint, if any, CYP17A1 expression. There is no detectable expression of CYP17A1 in the zona glomerulosa (ZG) or medulla (M). (C) Juvenile (8 MO); CYP17A1 was expressed throughout the ZF and zona reticularis (ZR). The cells at the ZG/ZF border exhibit strong, uniform P45017 expression, patchy expression is seen throughout the innermost ZF and the ZR. There is very faint, if any, expression of CYP17A1 in the diminished FZ and no detectable expression of CPR in the ZG or M. (D and E) Perinatal/Neonatal (1DO/2 MO); CPR was detected throughout the adrenal cortex, with no detectable expression in the M. (F) Juvenile (8 MO); CPR is detected throughout the adrenal cortex with cells of the ZF at the corticomedullary junction and the ZG exhibiting the greatest intensity of CPR expression. There is no detectable expression in the M. Arrows denote clusters of medullary cells. Bar = 50 μm, all panels are the same magnification.
densely packed in columnar arrangements. Immediately adjacent to the DZ were the cells of the TZ, defined by their larger diameter, compact nuclei and lack of columnar organization (Fig. 1A). The innermost region of the perinatal adrenal cortex was comprised of the large, spongy cells of the FZ that were generally disorganized like the TZ cells (Fig. 1A and C). Separating the TZ and the FZ, and completely distinct from the surrounding regions, was a band of tightly packed cells with little cytoplasm, hereafter referred to as the dense band (DB-ZR; Fig. 1A and B). All perinatal specimens examined exhibited a distinct DB-ZR as illustrated in Fig. 1.

**Localization of b5**

In the perinatal adrenals, b5 immunostaining was detected in the TZ and throughout the FZ. Expression of b5 was patchy in both inner zones, with immunostaining more intense in the compact cells of the TZ than the large vacuolated FZ cells (Fig. 2A). The DB-ZR at the boundary between the FZ and TZ also stained strongly for b5 expression during this early perinatal period (Fig. 2B). The proportion of b5-positive cells and the intensity of expression in the TZ, FZ, and DB-ZR increased throughout these first 4 weeks of life (Fig. 2A–C). By 1 month of age (defined as the onset of the neonatal period), the cells of the DB-ZR expressing b5 persisted in between the large vacuolated cells characteristic of a functional ZF and the large, spongy b5-positive cells of a diminishing FZ (Fig. 2D). Expression was greater in the outermost cells of the DB-ZR and decreased toward the FZ, while the DB-ZR persisted and increased in relative width throughout the neonatal period (Fig. 2E and F). By 3 months of age, the DB-ZR exhibited uniform, intense expression of b5, similar to the ZR of the adult rhesus macaque (Fig. 2F and H). A thin layer (1–2 cells thick) of b5-positive FZ cells (characterized based on the morphology previously described) persisted in juveniles (4–14 months; Fig. 2G). No FZ cells were observed in adrenal glands from adult animals (Fig. 2H), and no b5 immunostaining was detected in the DZ, ZG, ZF, or medulla of any adrenal sample. The pattern of expression of b5 expression as described above for each age was evident and consistent in all specimens examined of similar age.

**Localization of HSD3B2**

Expression of HSD3B2 was restricted to the DZ and the outermost region of the TZ during the perinatal period, but did not extend into the DB-ZR or FZ (Fig. 3A). As differentiation proceeded, expression of HSD3B2 was confined to the ZG and ZF of the neonatal and juvenile adrenal cortex, but remained consistently absent from the DB-ZR and FZ (Fig. 3B and C). In all specimens examined, HSD3B2 was expressed intensely in all cells of the DZ, and in sections from older animals, and nearly all ZG and ZF cells were positive. The intensity of expression was similar among cells of the outermost TZ and the ZF cells. No immunostaining was detected in cells of the medulla, DB-ZR, FZ, or ZR of any specimen. The pattern of HSD3B2 expression described above was consistent among all similarly aged specimens.

**Localization of CYP21A2**

Expression of CYP21A2 was localized throughout the DZ, TZ, and DB-ZR of the adrenal cortex of perinatal adrenal glands (Fig. 3D), and immunostaining was intense and uniform throughout the DZ and outermost TZ. All cells of the TZ expressed CYP21A2. CYP21A2 expression in the DB-ZR was patchy, with the cells at the TZ/DB-ZR border exhibiting the greatest intensity and the number of positive cells. By contrast, CYP21A2 expression was essentially absent from the FZ, even faintly immunopositive cells were rare (Fig. 3D). This pattern of CYP21A2 expression persisted through the perinatal and neonatal stages with marked expression throughout the ZG, ZF, and DB-ZR, but a relative lack of CYP21A2 expression in the FZ (Fig. 3E and F). By contrast, the proportion of DB-ZR cells that were immunopositive for CYP21A2 greatly increased with age in neonates (Fig. 3E) to include all cells in this band, rather than those primarily at the TZ border, as in the perinatal adrenals. CYP21A2 expression was undetected in the remaining FZ cells (Fig. 3E). In the adrenal cortex of juvenile rhesus macaques (Fig. 3F), CYP21A2 was expressed in nearly all cells of the ZG and ZF, with continued patchy expression throughout the established ZR. The FZ cells of the juvenile adrenal similarly lacked CYP21A2 expression and continued to decrease in cell numbers and general size compared with the neonatal adrenal. No cellular expression was observed in the medulla of any adrenal samples. This was a consistent pattern evident in all specimens examined of similar age.

**Localization of CYP17A1**

CYP17A1 was detected throughout the TZ and FZ of the perinatal adrenal cortex (Fig. 4A). Expression was sparse and faint in the large, vacuolated cells of the FZ, while nearly all cells of the outer TZ exhibited intense staining. Expression of CYP17A1 that was observed in the cells of the DB-ZR was intermediate in intensity compared with FZ and TZ staining (Fig. 4A). A similar pattern of CYP17A1 expression was observed in neonatal adrenal specimens (1–3 months old), with CYP17A1 localized throughout the large cells of the ZF and the compact cells of the DB-ZR (Fig. 4B). Expression of CYP17A1 was barely detectable in the vacuolated cells of the FZ during this period. After the establishment of a morphologically mature ZR (more than 3 months old), CYP17A1 expression was observed throughout the ZF and ZR, with expression in nearly all cells of the ZF and patchy expression throughout the ZR (Fig. 4C). ZF cells exhibited generally greater intensity of CYP17A1 expression than ZR cells. There was no detectable expression of CYP17A1 in the
medulla, DZ, or ZG of samples studied. As for other enzymes examined, the pattern of expression was consistent in all specimens of similar age.

**Localization of CPR**

Expression of CPR was detected in all cells of all zones and throughout the adrenal cortex of all specimens regardless of age. Adrenocortical cells at the corticomedullary junction exhibited the greatest CPR expression in the majority of samples (Fig. 4D–F); otherwise, expression was uniform throughout the cortex. Cellular expression of CPR was undetectable in the medulla of any adrenal specimen.

**Discussion**

As recently described (Havelock et al. 2004), adrenarche occurs in humans as a result of maturation of the ZR, and although rhesus macaques experience similar cortical differentiation (Mapes et al. 1999), they are generally not thought to experience adrenarche (Arlt et al. 2002). Previously, we and others have demonstrated that b5 is an effective marker of ZR differentiation and, along with CYP17A1 expression, androgen synthetic capacity of rhesus and human adrenal tissues including the FZ (Sakai et al. 1993, Yanase et al. 1998, Narasaka et al. 2001, Mapes et al. 2002, Dharia et al. 2004). Neither the origins of the cells of the ZR, nor their relationship to the FZ, is well understood in non-human primate species. The characterization of b5 expression in the neonatal rhesus adrenal cortex presented here helps clarify both these issues. Based on morphology and a general lack of CYP21A2 expression in the FZ compared with the ZR, the cells of these two adrenal zones exhibit different steroidogenic phenotypes. The expression of CYP21A2 in the DB-ZR and its resemblance to the ZR therefore supports the view expressed by McNulty and colleagues based on morphology alone (McNulty et al. 1981) that the ZR develops from their so-called ‘dense band’. ZR development and FZ regression are also clearly coincident events in the rhesus (Fig. 5) as evidenced by the delineation of both of these zones in the perinatal and neonatal adrenal gland. This supports the view that they have different origins and fates. We conclude that the rhesus macaque experiences the same developmental event defined as adrenarche in humans based on histological differentiation of the ZR (Havelock et al. 2004), but that it is concurrent with FZ regression and compressed into the first 2–3 months of life, complicating recognition by analysis of peripheral androgen concentrations.

While the FZ and ZR of human and non-human primate (rhesus) adrenal glands have similar potential for androgen secretion, their temporal patterns of development and differentiation differ greatly. In humans, FZ expression of the steroidogenic enzymes involved in androgen synthesis persists from mid-gestation to term, coinciding with increases in secretion of DHEA and DS (Parker et al. 1982, Mesiano et al. 1993, Narasaka et al. 2001). During the first year of life, there is a marked decline in circulating DHEA and DS levels associated with the involution of the human FZ (Benner 1940, de Peretti & Forest 1976, 1978), and with decreased expression of CYP17A1, DHEA sulfotransferase, b5, and CPR throughout the adrenal cortex (Suzuki et al. 2000). By the fifth or sixth year of life, levels of DHEA and DS rise detectably (Rosenfield & Eberlein 1969, Rosenfield et al. 1969), marking the onset of adrenarche (de Peretti & Forest 1976, 1978, Parker et al. 1978). Thus, human ZR differentiation begins years after complete regression of the FZ (Suzuki et al. 2000), and is marked by increased CYP17A1 and b5 expression in the fifth year of life, as well as by a fully differentiated zone with reduced HSD3B2 expression by 8 years of age (Endoh et al. 1996, Gell et al. 1998, Suzuki et al. 2000). Consequently, the long temporal separation between FZ regression and differentiation of the ZR in humans makes the two events easily distinguished by changes in circulating DS after birth.

**Figure 5** Representation of dehydroepiandrosterone (DS) profiles in humans and rhesus macaques with major adrenocortical events demarcated. Green, upward diagonal areas indicate the chronological span of the active FZ. Blue, downward diagonal areas indicate the chronological span of the active ZR. (A) Changing DS levels with age in humans, based on data from published reports (Abraham et al. 1973, Parker et al. 1978, 1982, de Peretti & Forest 1978, Orentreich et al. 1992, Belanger et al. 1994, Lasley et al. 2002). (B) Changing DS levels with age in rhesus macaques, based on data from published reports (Culler et al. 1978, Smail et al. 1982, Koritnik et al. 1983, Seron-Ferre et al. 1983, Walsh et al. 1984, Pepe et al. 1988, Conley et al. 2004). Note the overlap between the active FZ and the active ZR (cross-hatched) during early life in the rhesus.
In comparison to humans, DS concentrations in the rhesus fetus are generally lower during gestation, but increase immediately pre-partum (Seron-Ferre et al. 1983, Walsh et al. 1984). This pre-partum rise in fetal serum DS coincides with increasing adrenal expression of CYP17A1 and b5 during late fetal development (Mapes et al. 2002). Results of the current IHC studies show that the establishment of a morphologically mature ZR in the neonatal rhesus macaque resembles human adrenarche (Havelock et al. 2004), although its appearance coincides with regression of the FZ. The earliest studies investigating this phenomenon in the rhesus reported only that adrenal androgens declined in animals older than 2–4 months of age (Cutler et al. 1978, Smail et al. 1982), and not observing any preceding increase, concluded therefore that this species does not experience an adrenarche. However, our data demonstrate that rhesus ZR development begins in late gestation (Mapes et al. 2002) and as shown here is essentially completed in the third month of life. Whereas regression of the FZ and maturation of the ZR are temporally separated by years in humans, both events occur concurrently, compressed within the first two months of life in the rhesus macaque. In contrast to the onset of ZR differentiation, which is pre-pubertal in both rhesus and human, the peak in DS concentrations differs markedly, preceding puberty in rhesus, but post-dating puberty in humans by more than a decade. This does not indicate any fundamental difference exists between human and rhesus adrenal with respect to ZR development. In fact, it is entirely consistent with the physiological independence of ZR development from gonadal function, as evidenced in normal children and those with precocious puberty (Sklar et al. 1980, Palmert et al. 2002). The accessory protein b5 (Onoda & Hall 1982, 1983, Walsh et al. 1983, Walsh et al. 1987), proopiomelanocortin (POMC) likely stimulates CYP17A1 expression in cultured fetal adrenal cells (Di Blasio et al. 1987), producing androgens (Sakai et al. 1996, 1999). The enzymatic activities of CYP17A1 are differentially regulated, as evidenced in children undergoing adrenarche and patients with isolated 17,20-lyase deficiency (Kaufman et al. 1999, 2002, Suzuki et al. 2007) and in human endocrinological disorders (Sakai et al. 1993), in addition to a potentially important role in the regulation of 17α-hydroxylase activity, increasing the capacity for DHEA production (Pandey & Miller 2005). While b5 facilitates electron transfer with some microsomal P450s (Kurian et al. 2004), evidence suggests this is not the mechanism of action for stimulation of 17,20-lyase activity (Auchus et al. 1998). An allosteric action of b5, thought to promote complex formation between CYP17A1 and CPR, has been suggested (Auchus et al. 1998, Pandey & Miller 2005). Co-localization of CYP17A1 and b5 has been demonstrated previously in androgenic tissues of human and non-human primates (Mapes et al. 1999, 2002, Suzuki et al. 2000, Dharia et al. 2004, Pattison et al. 2007) and in human adrenal disease (Yanase et al. 1998). Increased expression of b5 and CYP17A1 may account for increased adrenal androgen production with endocrinological disorders (Sakai et al. 1993), in addition to the role of b5 in adrenarche. However, the role of b5 in adrenarche has not been as carefully evaluated in human tissues, and further studies are necessary.
necessary to determine its contribution to changes in adrenal androgen secretion during development.

Classically, adrenarche has been defined based on circulating DS concentrations, one of the few easily measured markers of this event. Recent longitudinal studies conducted from early childhood (Remer & Manz 1999, Palmert et al. 2001, Remer et al. 2005), adding to earlier reports (Sizonenko et al. 1976, Parker et al. 1978, Ilondo et al. 1982, Kelnar & Brook 1983), indicate that adrenarche is associated with a gradual, rather than abrupt, rise in adrenal androgen production, but this is not the pattern seen in the rhesus macaque. As noted earlier, DS levels are high in the rhesus soon after birth, but decline rapidly between 2 and 3 months of age then more gradually throughout the rest of life (Koritnik et al. 1983, Seron-Ferre et al. 1983, 1986, Kemnitz et al. 2000). Although our data suggest that the concurrence of FZ regression with ZR differentiation likely make it difficult to distinguish these events from each other based on circulating DS, there may be other equally important confounding factors. For instance, it has been suggested that efficient metabolism of DHEA, specifically 16α-hydroxylation, in early childhood and adolescence may mask a significant portion of the adrenal androgens secreted during early human development, most often estimated from DHEA and DS levels only (Remer et al. 2005). In addition, changes in glucuronidation (Guillemette et al. 1996) and even sulfotransferase itself, which we have shown is expressed in the rhesus adrenal (Parker et al. 2000), along with other routes of androgen metabolism (Remer et al. 2005), may alter apparent adrenal androgen output. All of the above make peripheral hormone levels potentially poor indices of adrenal development in non-human primates. Adrenarche can be defined in histochemical and even biochemical terms if tissues are available for such analyses and, though not clinically useful, are relevant nonetheless because circulating hormone concentrations may not accurately reflect the underlying biology, especially in non-human primate models.

We believe that the late gestational, perinatal, and neonatal rhesus macaque is a suitable model for study of adrenarche, and potentially also for hyperandrogenic syndromes such as polycystic ovarian disease (Abbott et al. 2008). The processes and factors involved in primate adrenal maturation are complex, as evidenced by the gender and gonadal differences in ZR function in marmosets (Pattison et al. 2007). Studies examining the capacity for androgen production during this dynamic period, as well as investigation into the regulation of ZR development, are necessary to properly characterize the morphological adrenarche exhibited by the rhesus.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.


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References


