Evidence for Change of 11 β -Hydroxysteroid Dehydrogenase Activity during Infancy and Childhood

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ABSTRACT

The conversion of cortisol (F) to cortisone (E) is catalyzed by 11 β -hydroxysteroid dehydrogenase type 2. The present study was designed to investigate the changes of F and E plasma concentration as an indirect measurement of 11 β -hydroxysteroid dehydrogenase activity in infancy and childhood. Plasma samples were obtained from 262 healthy children and adolescents aged 1 d to 18 y. Plasma F and E were measured, using specific radioimmunoassays after extraction and automated Sephadex LH 20 chromatography. The F/E ratio was calculated to assess 11 β -hydroxysteroid dehydrogenase activity. During the first year of life, plasma F levels rose significantly ($r^2 = 0.24$; $p = 0.01$), and thereafter no further increase was seen until adulthood ($r^2 = 0.01$; $p = 0.86$). In contrast, plasma E significantly decreased during the first year of life ($r^2 = -0.35$; $p < 0.001$) and stayed unchanged thereafter ($r^2 = 0.02$; $p = 0.81$). As a consequence, the F/E ratio rose significantly during the first year ($r^2 = 0.67$;

$p < 0.001$) but did not change afterward ($r^2 = 0.001$; $p = 0.99$). During the first year of life, there is a change from the predominance of E, with low mineralocorticoid receptor affinity, to F, with high mineralocorticoid receptor affinity. This shift corresponds to the declining plasma concentrations of aldosterone during infancy. The changes may indicate a not yet recognized, significant change of 11 β -hydroxysteroid dehydrogenase isoenzyme activity or alterations in the secretion of F and E, which may be of relevance for the development of arterial blood pressure in infancy. (*Pediatr Res* 48: 697–700, 2000)

Abbreviations

11 β -HSD, 11 β -hydroxysteroid dehydrogenase

F, cortisol

E, cortisone

MR, mineralocorticoid receptor

F binds as avidly to the MR as aldosterone (1). In contrast, E has no affinity to the MR (2). The conversion of F to E is catalyzed by 11 β -HSD type 2 (11 β -HSD 2) (3). In man, this enzyme is predominantly active in kidney and in placenta (4, 5). Recently, 11 β -HSD 2 expression has also been demonstrated in human lung (6). The enzyme catalyzing the reverse reaction from E to F, called 11 β -HSD type 1, is found predominantly in hepatic tissue (7). 11 β -HSD 1 appears to be of little importance in the origin of hypertensive disorders such as the apparent mineralocorticoid excess syndrome (8, 9). The importance of glucocorticoid metabolism in hypertension associated with Cushing's syndrome (10–12) demonstrates the importance of 11 β -HSD dysregulation in nongenetic disease as well. Childhood is a phase characterized by physiologically rising blood pressure (13). This makes 11 β -HSD a possible candidate enzyme involved in increasing blood pressure during childhood.

Declining plasma concentrations of the most potent physiologic mineralocorticoid, aldosterone, have been known for the last 20 y (14–16). Developmental aspects of glucocorticoid metabolism have, however, been less well examined. In particular, no data are available on the changes of F/E conversion that is catalyzed by the 11 β -HSD isoenzymes during childhood.

The objective of the present study was to examine the changes of F and E plasma concentrations from infancy to adulthood using a highly specific simultaneous microdetermination method and to calculate F/E ratios to assess 11 β -HSD activity indirectly *in vivo*. The second objective of the study was to provide normal ranges for F, E, and F/E ratio during different phases of childhood and adolescence.

METHODS

The study was approved by the local ethics committees. Informed consent was obtained from parents and, as far as possible, volunteers. None of the children had a history or clinical signs of adrenal disease. All children had a normal

Received September 14, 1999; accepted May 16, 2000.

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Figure 1. Plasma F concentrations of 140 healthy children during the first year of life as a function of age.

age-related blood pressure. No medication was taken by any of the children and adolescents that interfered with glucocorticoid or mineralocorticoid metabolism. All patients and their parents had denied the intake of licorice and grapefruit juice at least 3 d before examination. Of the 262 individuals enrolled, 121 were boys and 141 were girls. All infants examined were term newborns. Plasma samples were obtained between 0800 h and 1000 h in all children and adolescents. The plasma samples were kept frozen at -20°C and analyzed when all specimens had been obtained. Plasma E and F were measured by RIA after automated Sephadex LH-20 chromatography [for details see Sippell *et al.* (17)]. The intraassay variation coefficients for E and F were 6.9% and 14.5%, the interassay variation coefficients were 11.9% and 16.3%, respectively.

The activity of 11β -HSD was calculated indirectly using the ratio of F/E. Values are presented as mean and SEM for plasma F and E, which showed Gaussian distribution. When two groups were compared, a *t* test was performed. Multiple groups were compared by one-way ANOVA and *post hoc t* tests according to Bonferroni (18). F/E ratios did not always show Gaussian distribution and are thus presented as median and range. Nonparametric multiple groups were assessed by Kruskal-Wallis test and *post hoc* Dunn's multiple comparison test. Statistical analysis comparing two groups was performed using the Mann-Whitney test. The relationship between two variables was calculated using Spearman's nonparametric correlation for F/E ratios and linear correlation for plasma F and E concentrations.

RESULTS

During the first year of life, there was a significant increase in the morning plasma F levels of healthy children ($r^2 = 0.24$; $p = 0.01$; Fig. 1). Plasma F in the first year of life was 184 ± 16 nM ($n = 98$) versus 277 ± 32 nM ($n = 18$) in the second year of life ($p = 0.03$). There was a significant difference between the plasma F concentration in the first month of life in every age group > 1 y (Table 1). After the first year, no further increase in F plasma concentration was seen until adulthood ($r^2 = 0.01$; $p = 0.86$). Plasma F concentration was 268 ± 11 nM for children and adolescents between 1 and 18 y. There was no difference in F concentrations of male and female individuals for the complete group (230 ± 13 versus 257 ± 13 nM; $p = 0.19$). Likewise, plasma F concentrations did not differ between boys and girls of the different age groups shown in Table 1. Detailed ranges and percentiles of plasma F concentrations at different ages are shown in Table 1.

Plasma E significantly decreased during the first year of life ($r^2 = -0.35$; $p < 0.001$, Fig. 2). Mean E in the first year was 66 ± 4 nM ($n = 98$) and dropped to 43 ± 6 nM ($n = 18$) in the second year of life ($p = 0.05$). Like plasma F, E concentrations did not change after the first year ($r^2 = 0.02$; $p = 0.81$). There was a mean plasma E concentration of 43 ± 2 nM in the age group > 1 y. Plasma E concentrations in the first month of life were significantly higher than in all ages > 6 mo (Table 2). As for F, there was no difference between plasma E concentrations of male and female children and adolescents (47 ± 2 versus 52 ± 3 nM, respectively; $p = 0.20$). Plasma E concentrations did not differ between boys and girls of the different age groups shown in Table 2. Detailed ranges and percentiles of plasma E concentrations at different ages are shown in Table 2.

The F/E ratios rose from 2.3 (range, 0.1–17.0) in the first year of life to 7.2 (range, 3.3–15.7) in the second year ($p < 0.001$). The increase was significant during the first year of life ($r^2 = 0.67$; $p < 0.001$; Fig. 3). As for plasma F and E, there was no further change in the F/E ratios after the first year of life ($r^2 = 0.001$; $p = 0.99$). The median F/E ratio was 5.7 (range, 0.4–36.8) for the latter age group. Infants < 1 mo of life had significantly lower F/E ratios than any age group > 6 mo of age. There was no significant difference between boys and girls with regard to F/E ratio [1.4 (range, 0.04–10.3) versus 1.5 (range, 0.07–10.3), respectively; $p = 0.43$]. Likewise, plasma F/E ratios did not differ between boys and girls of the different

Table 1. Age-related medians, ranges, quartiles, means, and SEM for plasma F concentrations in 262 healthy children and adolescents

Variable	Age							
	< 1 mo	1–3 mo	3–6 mo	6–12 mo	1–2 y	2–4y	4–10 y	10–18 y
Number	34	26	19	20	18	23	69	53
Mean	137	173	231	233	277	253	288	246
SEM	25	30	41	36	32	22	18	18
Minimum	4	9	11	32	48	127	79	61
25% percentile	34	68	97	106	166	171	179	147
Median	88	129	171	177	274	233	264	219
75% percentile	226	224	410	322	340	298	372	316
Maximum	720	597	584	585	529	545	763	548
<i>p</i> value (vs < 1 mo)		NS	NS	NS	< 0.05	< 0.05	< 0.001	< 0.01

Concentrations are shown in nM. There is a significant increase of the plasma F concentration during infancy as assessed by one-way ANOVA ($p < 0.001$). *Post hoc t* tests according to Bonferroni show a significant difference between the first month of life and the older age groups > 1 y.

Figure 2. Plasma E concentrations of 140 healthy children during the first year of life as a function of age.

Figure 3. F/E ratios of 140 healthy children during the first year of life as a function of age. The F/E ratio assesses the activity of the 11 β -HSD isoenzymes.

age groups shown in Table 3. Detailed ranges and percentiles of F/E ratios at different ages are shown in Table 3.

DISCUSSION

The present study shows a reverse development in plasma F and E concentrations during the first year of life. Whereas F concentrations increase, plasma E decreases during the same period of time. As a consequence, the endogenous glucocorticoids gradually shift to more mineralocorticoid activity during the first year of childhood. It is of interest that this change parallels two typical developments in early childhood, *i.e.* the rising blood pressure (13) and the diminution of the ratio between extracellular and intracellular fluid. As to the blood pressure increase, the efficiency of MR action to elevate blood pressure is well known from diseases with an excess of mineralocorticoids, such as Conn's syndrome, as well as F excess caused by genetic disease (4), adrenal overproduction (10–12), and alimentary inhibition of 11 β -HSD 2 by licorice (19). Thus, the rising concentrations of F may contribute to the physiologic increase of blood pressure in early childhood.

It has to be borne in mind, however, that, although the F/E ratio increases approximately 4-fold in our study, aldosterone serum concentrations drop to approximately one-fifth in the first year of life (14–16). Therefore, it appears possible that the shift to increased mineralocorticoid activity of the two endogenous glucocorticoids compensates for the physiologic decline of aldosterone activity. The second temporary compensatory

mechanism for the declining aldosterone concentrations is plasma deoxycorticosterone showing a peak around the age of 4 y (16). Alternatively, the decrease in plasma aldosterone and deoxycorticosterone concentration might be a consequence of the rising F-induced mineralocorticoid activity.

In fetal cord blood, there appears to be a rise in E and constant or rising levels of plasma F (20, 21). The relatively high plasma F concentrations right after birth drop within the first week of life before they start rising again (16, 22). Comparative data on F and E plasma concentrations later in childhood have barely been reported before (16). However, the larger number of patients in this study allows for the calculation of more precise data. Also, F/E ratios during childhood and adolescence are here reported for the first time. Moreover, the significantly lower morning F levels in young children have not been recognized in standard textbooks of pediatrics and even pediatric endocrinology.

The increasing F/E ratio in the first year of childhood could imply an altered activity of the 11 β -HSD isoenzymes. Two alternative explanations are possible: either the activity of 11 β -HSD 2, converting F to E, decreases or 11 β -HSD 1 activity, catalyzing the opposite reaction, rises with age. In favor of changes in 11 β -HSD 2 activity is the observation that an increasing activity of 11 β -HSD 1 is of little importance in the genesis of hypertensive disorders (8, 9). However, the F/E ratio does reflect the changes in total activity of the 11 β -HSD

Table 2. Age-related medians, ranges, quartiles, means, and SEM for plasma E concentrations in 262 healthy children and adolescents

Variable	Age							
	< 1 mo	1–3 mo	3–6 mo	6–12 mo	1–2 y	2–4 y	4–10 y	10–18 y
Number	34	26	19	20	18	23	69	53
Mean	71	68	75	44	43	35	45	43
SEM	7	7	13	8	6	3	3	3
Minimum	9	19	7	4	8	14	12	6
25% percentile	32	38	47	31	27	24	31	33
Median	71	61	67	36	44	30	40	43
75% percentile	99	90	93	55	69	44	60	56
Maximum	190	152	272	173	75	74	116	96
p value (vs < 1 mo)		NS	NS	< 0.05	< 0.05	< 0.001	< 0.001	< 0.001

Concentrations are shown in nM. There is a significant decrease of plasma E concentration during infancy as assessed by one-way ANOVA ($p < 0.001$). *Post hoc t* tests according to Bonferroni show a significant difference between the first month of life and the older age groups > 6 mo.

Table 3. Age-related medians, ranges, and quartiles for the F/E ratio in 262 healthy children and adolescents

Variable	Age							
	< 1 mo	1–3 mo	3–6 mo	6–12 mo	1–2 y	2–4 y	4–10 y	10–18 y
Number	34	26	19	20	18	23	69	53
Minimum	0.12	0.14	0.9	1.0	3.3	3.3	0.4	1.990
25% percentile	0.4	1.0	1.7	4.4	4.8	5.0	3.9	3.850
Median	1.7	1.9	2.2	6.6	7.2	7.3	5.3	5.140
75% percentile	2.5	4.6	5.2	8.0	11.3	10.0	7.2	7.675
Maximum	16.3	17.0	8.2	13.4	15.7	15.7	36.8	26.50
p value (vs < 1 mo)		NS	NS	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

The F/E ratio assesses the activity of the 11 β -HSD isoenzymes. There is a significant increase of the F/E ratio during infancy as assessed by Kruskal-Wallis test for nonparametric data ($p < 0.001$). *Post hoc* Dunn's multiple comparison test shows a significant difference between the first month of life and the older age groups > 6 mo.

enzyme but not its activity in the different tissues. In fact, F/E ratios are only indirect markers of 11 β -HSD activity *in vivo*. Therefore, examinations of tissue activity of the two isoenzymes in children would be needed to elucidate where the actual changes take place.

The present study demonstrates that in the first year of life percentiles for plasma F, E, and F/E ratios differ from later in life, although the normal ranges are wide in every age group. However, to exactly classify values for the variables examined in our study, the percentiles shown on the basis of 262 children and adolescents may be helpful. There still is a variation in the values shown, which is because of the fact that samples in our study were drawn between 0800 h and 1000 h and also because of a certain interindividual phase shift of the pituitary-adrenal circadian rhythm.

In summary, there is a developmental change in the plasma concentrations of F and E in the first year of life. This possibly compensates for the loss of mineralocorticoid action caused by the decrease of aldosterone over the same period. The increasing F/E ratio suggests a maturation of the activities of the 11 β -HSD isoenzymes or the secretory process for F and E.

The authors thank Jutta Sigwart for excellent technical assistance.

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