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THE EFFECTS OF CORTISONE ON THE INTERCONVERSION OF CORTISOL AND CORTISONE IN THE BABOON

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Summary—In the baboon fetus, the conversion of cortisol (F) to cortisone (E) [80%] exceeds the reverse reaction [15%]. Since the fetus is exposed to high quantities of E throughout most of pregnancy, we determined whether F to E interconversion is altered following acute changes in serum E. Adult female baboons ($N = 3$) were sedated with ketamine, constantly infused for 180 min via an antecubital vein with 15 μCi[^{14}C]E and 15 μCi[^{3}H]F, and saphenous vein blood samples obtained at 70, 80 and 90 min. At 90 min, an infusion of E (166 μg/min) was initiated and blood samples obtained at 160–180 min. This protocol was repeated in the same animals treated 24 and 3 h prior to infusion with 3 mg betamethasone. Metabolic clearance (MCR), transfer constants (%) and serum levels (μg/dl) of F and E were determined. E increased ($P < 0.05$; paired t) MCR–E, and serum F and E levels in control and betamethasone-treated baboons. E also decreased %F to E in betamethasone-treated but not control animals. These findings suggest that acute changes in serum E alter MCR–E, do not influence the conversion of E to F and may decrease the conversion of F to E. Therefore, we suggest that the high conversion of F to E in the baboon fetus is probably not the result of elevated concentrations of E.

INTRODUCTION

Cortisol (F) is important for maturation of the lungs and possibly other organs in the fetus [1]. However, the factors that regulate the formation of F and its catabolism to the less active metabolite cortisone [E] within the fetus are poorly understood. We have previously shown [2] that the conversion of F to E by the baboon fetus and neonate greatly exceeds the reduction of E to F. Similar observations have been noted in the rhesus fetus near term [3]. However, these findings in fetal/neonatal subhuman primates are in contrast to those measured in adult baboons [4] and monkeys [3, 5] in which the conversion of E to F is equal to or greater than the oxidation of F to E. It has been shown, *in vitro*, in baboon [6] and human placenta [7] that E can influence 11β-hydroxysteroid dehydrogenase activity. Since the concentration of E in the circulation of the baboon [8] and human [9] fetus is much greater than that measured in the maternal circulation, it is possible that the differences in F to E interconversion between adult and fetal/neonatal animals is due to the elevated levels of E in the serum of developing fetuses. The present study was designed to examine this possibility.

EXPERIMENTAL

Animals

Three female baboons (*Papio anubis*) weighing 14–18 kg, were housed individually in metabolic cages in air-conditioned quarters under standardized conditions as described previously [10]. Baboons exhibited regular menstrual cycles as judged by menstrual cycle history and turgesence of the external sex skin.

Experiment 1

Three to four days after the onset of menses, animals were briefly restrained and sedated with 100 mg ketamine (Parke–Davis) and catheters were (C.R. Bard, Inc.) inserted into antecubital and saphenous veins. A constant infusion (0.40 ml/min) of 15 μCi [1,2^{3}H]F (sp. act. 55 Ci/mmol; New England Nuclear Corp.) and 15 μCi [4-^{14}C]E (sp. act. 35 mCi/mmol; Research Products International) in 80 ml 0.9% saline–1% ethanol was initiated at time 0 (10:00 h) via the antecubital vein catheter. An infusion of 5% dextrose–water (0.5 ml/min) containing 6 mg/ml ketamine was infused via the saphenous vein to maintain sedation. At 70, 80 and 90 min after onset of the infusion, blood samples (8 ml) were obtained from the saphenous vein. At 90 min, a constant infusion of radioinert E (42 mg/50 ml 4% ethanol–saline; 166 μg/min/0.2 ml) was simultaneously infused with the [^{3}H]F/[^{14}C]E via the antecubital vein and blood samples were obtained from the saphenous vein 70, 80 and 90 min later (i.e. 160, 170 and 180 min from time 0).

Experiment 2

Eight days after completion of experiment 1, animals were injected intramuscularly with 3 mg betamethasone (Celestone; Schering Corp) 24 and 3 h prior to initiation of the infusion protocol described above.

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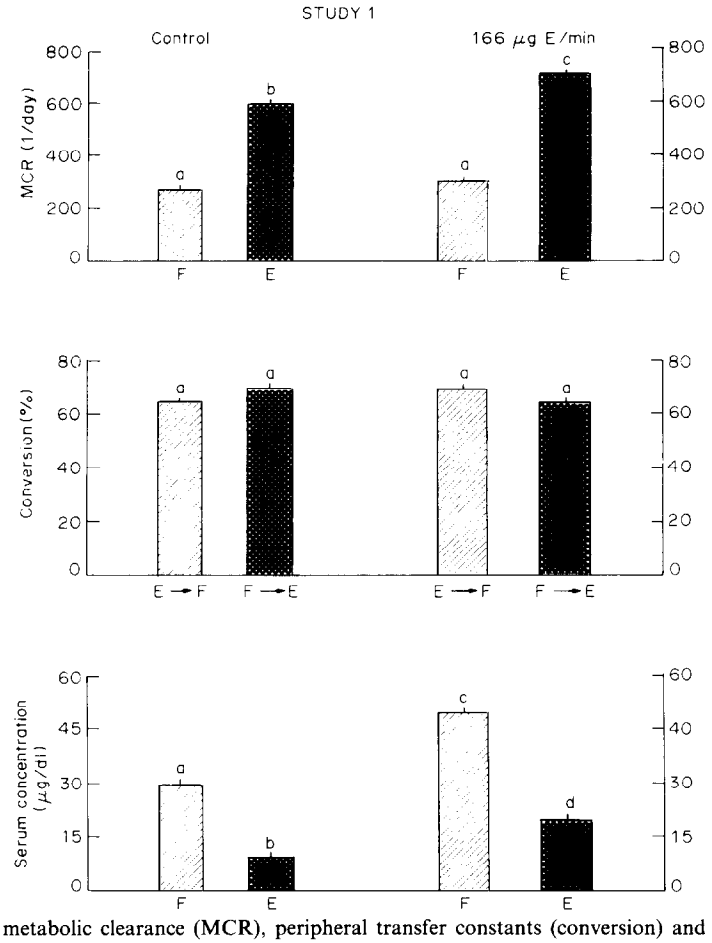

Fig. 1. The metabolic clearance (MCR), peripheral transfer constants (conversion) and serum concentrations of cortisol (F) and cortisone (E) in 3 female baboons before (control) or during infusion of 166 μg/min of exogenous E. Values = mean ± standard error. Different letter superscripts indicate values different from each other at $P < 0.05$ (dependent or independent t tests).

Analyses

All blood samples were kept on ice and serum, separated by centrifugation at 3,000 rpm (4°C), stored at −20°C until analyzed. Radiolabeled F and E were extracted with ethyl acetate from serum following the addition of 200 μg radioinert F and E for estimation of procedural losses. Steroids were purified by paper chromatography essentially as described previously [8]. The ^{3}H and ^{14}C content of F and E were determined by liquid scintillation spectrometry and values corrected for contributions of 14C (9.8%) to the ^{3}H channel; the ^{3}H in the ^{14}C channel was <0.2%. Procedural losses were estimated by the Porter–Silber reaction [4, 11]. The endogenous F and E concentrations were estimated by radioimmunoassay after column chromatography essentially as described previously [8]. Metabolic clearance rates (MCR) and peripheral transfer constants (p) were calculated using the formulas defined by Baird *et al.*[12] and previously described by Pepe *et al.*[4]. Data were analyzed for statistical differences using Student's t tests for dependent or independent observations.

RESULTS

In untreated baboons, MCR–E (562 ± 29 L/D; $x \pm SE$) exceeded ($P < 0.05$) MCR–F (250 ± 16) while the transfer constants for the interconversion of F and E were not statistically different from each other (Fig. 1). However, the serum concentrations of F (31 ± 4 μg/dl) were greater ($P < 0.05$) than those of E (8 ± 1) (Fig. 1). Infusion of E (166 μg/min) into otherwise untreated baboons had no effect on the MCR–F but increased ($P < 0.05$) the MCR–E (704 ± 56) and the serum levels of both F (54 ± 7) and E (21 ± 2). In contrast to the latter, infusion of E had no significant effect on the peripheral interconversion of these corticosteroids. Compared with values in the control period, betamethasone treatment significantly ($P < 0.05$) reduced the serum levels of F (4 ± 2) and E (1 ± 0) and also resulted in a marked decline ($P < 0.05$) in the MCR of F (171 ± 15) and E (271 ± 61; Fig. 2). Although the peripheral transfer constant for the conversion of E to F following betamethasone treatment was greater ($P < 0.05$) than that measured in the untreated control period (Figs 1 and 2), the conversion of F to E

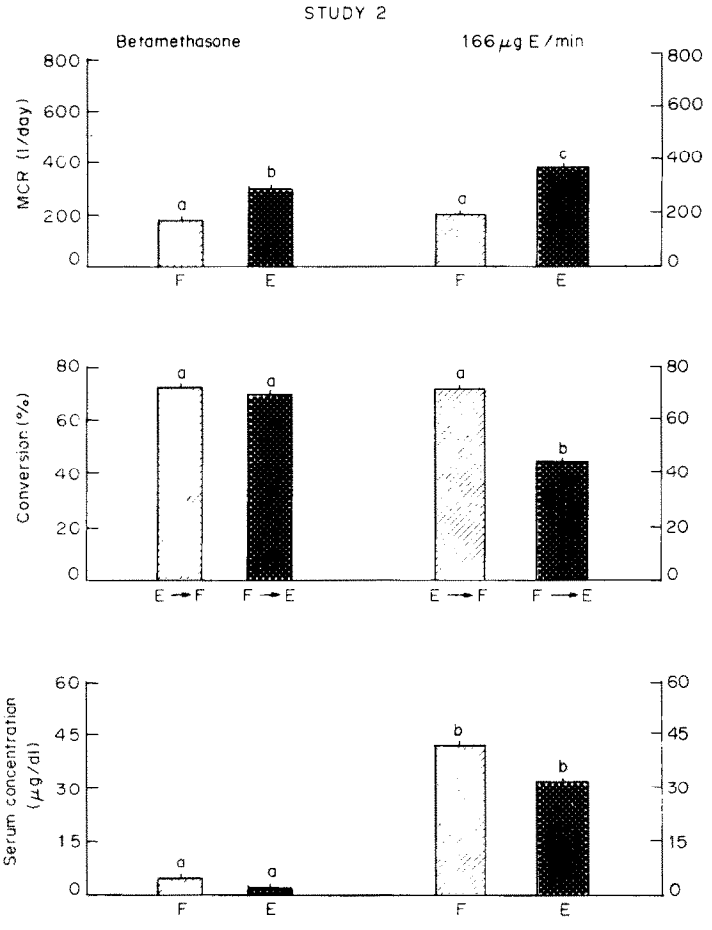

Fig. 2. The metabolic clearance (MCR), peripheral transfer constants (conversion), and serum concentrations of cortisol (F) and cortisone (E) in 3 female baboons before or during infusion of 166 μg/min of exogenous E. Baboons are the same animals examined in Study 1 (Fig. 1) but injected i.m. with 3 mg betamethasone (Celestone) 24 and 3 h prior to experimentation (Study 2). Values = mean ± standard error. Different letter superscripts indicate values different from each other at $P < 0.05$ (dependent or independent t tests).

was not significantly affected. Infusion of E into betamethasone-treated baboons resulted in increased ($P < 0.05$) clearance of E (369 ± 80) but not F (199 ± 7) while the serum levels of both F (41 ± 6) and E (34 ± 8) were dramatically ($P < 0.05$) elevated (Fig. 2). Compared to values prior to infusion, E had no effect of the conversion of E to F whereas the peripheral transfer constant for the oxidation of F to E was significantly ($P < 0.05$) reduced (Fig. 2).

DISCUSSION

This study clearly demonstrated in adult baboons that altering the serum levels of E either by acute infusion of E or by short-term treatment with exogenous betamethasone had no effect on the peripheral transfer constants for F to E interconversion. Moreover, it appears that when the serum concentrations of E are increased and approximate those of F, as occurred in the betamethasone-treated animals infused with exogenous E, the conversion of F to E is actually decreased. These findings suggest that in adult baboons the enzyme system catalyzing the oxidation of F to E is sensitive to product inhibition. This suggestion is consistent with our recent findings [6] which demonstrated *in vitro* that addition of E to homogenates of term baboon placenta significantly inhibited the conversion of F to E catalyzed by 11β-hydroxysteroid dehydrogenase. Similar findings have been observed in human term placenta [7] and in human liver [13]. Although studies remain to be performed in the perinate, collectively, our findings permit the suggestion that the extensive conversion of F to E measured in the baboon fetus and neonate probably does not result solely from the elevated concentrations of E characteristic of the intrauterine and perinatal period. Since it has been demonstrated that there are probably two enzymes regulating F to E interconversion in the lung [14] as well as in the human [7] and baboon [6] placenta, it is possible that the extensive and preferential conversion of F to E in the baboon fetal/neonate [2] is due to a difference in the tissue concentration of two enzymes regulating F to E interconversion.

Recently, we [15] measured F to E interconversion in baboon neonates delivered to mothers treated during the final third of gestation with the anti-estrogen ethamoxytriphetol (MER-25) and compared values with those from untreated controls [2]. Although values for the MCR of F and E as well as the reduction of E to F were similar in the 2 groups, the transfer constant for the conversion of F to E decreased from 69% in untreated animals to 29% following treatment with MER-25 [15]. Assuming that MER-25 crossed the placenta and interfered with the action of estrogen in the fetus, we suggested that estrogen may regulate the enzyme catalyzing the oxidation of F to E. In light of our present findings showing a lack of effect of exogenous E to increase the oxidation of F to E, it appears more likely that estrogen, and not E, in the fetus modifies and possibly regulates the activity of the enzyme catalyzing the oxidation of F to E.

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REFERENCES

1. Liggins G. C. and Howie R. N.: A controlled trial of antepartum glucocorticoid treatment for the prevention of the respiratory distress syndrome in premature infants. *Pediatrics* **50** (1972) 515–525.
2. Pepe G. J.: The production and secretion of cortisol by baboon neonates. *Steroids* **33** (1979) 251–260.
3. Mitchell B. F., Seron-Ferre M. and Jaffe R. B.: Cortisol–cortisone interrelationship in the late gestation rhesus monkey fetus *in utero*. *Endocrinology* **111** (1982) 1837–1842.
4. Pepe G. J., Ehrenkranz R. A. and Townsley J. D.: The metabolic clearance rates and interconversion of cortisol and cortisone in pregnant and nonpregnant baboons. *Endocrinology* **99** (1976) 597–601.
5. Kittinger G. W.: Feto–maternal production and transfer of cortisol in the rhesus (*Macaca mulatta*). *Steroids* **23** (1974) 229–243.
6. Pepe G. J. and Albrecht E. D.: Comparison of cortisol–cortisone interconversion *in vitro* by the human and baboon placenta. *Steroids* (1985). In press.
7. Bernal A. L., Flint A. P. F., Anderson A. B. M. and Turnbull A.: 11β-Hydroxysteroid dehydrogenase activity (E.C. 1.1.1.146) in human placenta and decidua. *J. steroid Biochem.* **13** (1980) 1081–1087.
8. Pepe G. J. and Albrecht E. D.: Transuteroplacental metabolism of cortisol and cortisone during mid and late gestation in the baboon. *Endocrinology* **115** (1984) 1946–1951.
9. Murphy B. E. P.: Cortisol and cortisone in human fetal development. *J. steroid Biochem.* **11** (1979) 509–513.
10. Albrecht E. D.: A role for estrogen in progesterone production during baboon pregnancy. *Am. J. Obstet. Gynec.* **135** (1980) 569–574.
11. Porter C. C. and Silber R. H.: Quantitative color reaction for cortisone and related 17,21-dihydroxy-20-ketosteroids. *J. biol. Chem.* **185** (1950) 201–207.
12. Baird D. T., Horton R., Longcope C. and Tait J. F.: Steroid dynamics under steady-state conditions. *Recent Prog. Horm. Res.* **25** (1969) 611–664.
13. Meigs R. A. and Engle L. I.: The metabolism of adrenocortical steroids by human tissues. *Endocrinology* **69** (1961) 152–162.
14. Abramovitz M., Branchaud C. L. and Murphy B. E. P.: Cortisol–cortisone interconversion in human fetal lung: contrasting results using explant and monolayer cultures suggest that 11 beta-hydroxysteroid dehydrogenase (E.C. 1.1.1.146) comprises two enzymes. *J. clin. Endocr. Metab.* **54** (1982) 563–568.
15. Albrecht E. D., Johnson D. K. and Pepe G. J.: Effect of maternal administration of the antiestrogen ethamoxytriphetol throughout the last third of baboon gestation upon neonatal corticoid production. *Steroids* **37** (1981) 45–50.