

21-OH steroid hydroxylase (21-OH-ase) uses both I and II in corticoid biosynthesis in other species, it was considered of interest to study the comparative interactions which could exist between these two precursors and the rat adrenal 21-OH-ase, determining enzymatic constants for I and II (usual and unusual substrates, respectively). Homogenized adrenals from normal rats were incubated with various combinations of concentrations of I- $^3\text{H}$  and/or II- $^{14}\text{C}$ , acting as substrates and/or inhibitors of 21-OH-ase. The results showed that 21-OH-ase uses II almost as efficiently as I. The  $K_m$  values were about the same for both I and II (13.9 and  $14.2 \times 10^{-6}$  M/L), respectively, however, the  $V_{max}$  values were  $54.6$  and  $26.0 \times 10^{-7}$  M/L/h for I and II, respectively. The amounts of I required to saturate the 21-OH-ase was double than that of II. Further kinetic studies showed that both I and II inhibit the 21-hydroxylation of the other in a reciprocal fashion. While II inhibits the 21-hydroxylation of I by competitive inhibition, I inhibits the 21-hydroxylation of II through a mixed type of inhibition. The results suggest that, rather than the existence of two different specific enzymes (one for I and another for II) as it has been postulated by others, it seems that we are dealing with a 21-hydroxylating system with two active sites. One site uses only I and the other site uses I and/or II indistinctively.

**59. The  $11\beta$ -hydroxylase activity of cell-free adrenal preparations from *Echidnas* (*Tachyglossus aculeatus*) in various physiological states**

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The echidna has a much lower rate of corticosteroid secretion than eutherian mammals and it survives adrenalectomy. Adrenal  $11\beta$ -hydroxylase activity was found to be extremely low, although  $17\alpha$ - and 21-hydroxylase activities were comparable to eutherians. In echidnas treated for 1 week with frusemide (Lasix), which caused  $\text{Na}^+$  depletion and dehydration, there was a marked increase in  $11\beta$ -hydroxylation of the adrenal homogenates, 60% of the end products from progesterone being  $11\beta$ -hydroxylated, compared with less than 1% in controls. This activity was also enhanced by dehydration or treatment of the animal with ACTH. Using purified mitochondrial preparations and deoxycorticosterone substrate the yield of corticosterone was 8.3% in an echidna treated with frusemide, less than 0.1% in a control echidna and 25.5% in a normal rat. The  $K_m$  values from Lineweaver-Burke plots for  $11\beta$ -hydroxylase for the treated echidna and the rat in these experiments were 0.24 and 0.30  $\mu\text{M}$  and the  $V_{max}$  values  $8.2 \times 10^{-6}$  and  $6.7 \times 10^{-4}$   $\mu\text{M min}^{-1} \text{mg}^{-1}$ , respectively. Thus the efficiencies of  $11\beta$ -hydroxylase in the two animals were comparable, but the amount of active enzyme in the echidna was approximately 1% of that in the normal rat. The low corticosteroid secretion rate in the echidna may be a consequence of a relatively meagre mitochondrial enzyme system.

**60. A lasting effect of ACTH on adrenal  $11\beta$ -hydroxylation in guinea-pig**

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The day following i.v. ACTH administration to guinea-pigs, the steroidogenic response to further ACTH stimulation is enhanced, as evidenced by plasma and adrenal tissue levels of cortisol (F), while 11-deoxycortisol (S) is not modified. This suggests a prolonged ACTH effect on late steps of

F biosynthesis. This hypothesis was evaluated on isolated guinea-pig adrenal cells by studying ACTH action on F, S, cyclic-AMP production and on  $11\beta$ -hydroxylation of  $^3\text{H}$ -S. Adrenal cells were harvested from 26 control adult male guinea-pigs and from 18 animals treated with ACTH ( $75 \mu\text{g ACTH}_{1-24}$  i.v. over 3 h, 24 h previously). The  $11\beta$ -hydroxylation index [(F/F+S)  $\times$  100] determined from F and S adrenal tissue content before cell dispersion, was  $82.7 \pm 1.1$  in controls and  $99.5 \pm 0.2$  24 h after ACTH ( $p < 0.001$ ). Incubation of isolated cells with tracer amounts of  $^3\text{H}$ -S resulted in  $^3\text{H}$ -F formation, itself undergoing conversion into cortisone (E). The  $11\beta$ -hydroxylation index, calculated therefore as (F+E/F+E+S)  $\times$  100, increased in cells from ACTH-treated animals, averaging after 30 min incubation  $88 \pm 1$  vs.  $77 \pm 2$  in controls ( $p < 0.01$ ). The cells from both groups were then challenged with ACTH *in vitro* for 2 h, at doses ranging from 1 to 1000 pg/ml cell suspension. The F secretory response of isolated adrenal cells from ACTH-treated animals was enhanced when compared to controls, maximal F production (at 1000 pg ACTH/ml) averaging 1236 and 836 ng F/ $10^5$  cells, respectively ( $p < 0.05$ ), while net S and cyclic-AMP production did not differ in both groups. An acute exposure of cells to ACTH during incubation does not influence any further the activity of the  $11\beta$ -hydroxylation enzyme system, as judged from  $^3\text{H}$ -S conversion into  $^3\text{H}$ -F and  $^3\text{H}$ -E. In conclusion: (1) No change in  $11\beta$ -hydroxylase activity of guinea-pig adrenocortical cells results from acute exposure to ACTH; (2) Activity of this enzyme system increases as a delayed effect of ACTH stimulation, demonstrable the day after infusion with this hormone; (3) The increase in  $11\beta$ -hydroxylase activity could account, at least in part, for the enhanced F secretory response obtained upon repeated ACTH stimulation. In contrast, generation of cyclic-AMP does not seem to be modified under these circumstances.

**61. Regulation of androgen synthesis in the human adrenal gland *in vitro***

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The possibility that local factors at a cellular level might influence androgen synthesis by the human adrenal gland was investigated in an incubation system *in vitro*. The synthesis of DHA was controlled by the availability of its major precursor  $17\alpha$ -hydroxypregnenolone, the obligatory cofactor for this reaction NADPH, and by the ratio of  $\text{NADP}^+$  to NADPH.  $17\alpha$ -hydroxyprogesterone and DHA itself exerted non-competitive inhibition on the conversion. The synthesis of androstenedione from its two immediate precursors,  $17\alpha$ -hydroxyprogesterone and DHA, was also investigated. Both reactions were dependent upon the availability of the substrate and of the obligatory cofactors, NADP and  $\text{NAD}^+$ , respectively. The conversion of  $17\alpha$ -hydroxyprogesterone to androstenedione was competitively inhibited by pregnenolone, progesterone and  $17\alpha$ -hydroxypregnenolone, while the synthesis of androstenedione from DHA was found to be non-competitively inhibited by oestrone and oestradiol- $17\beta$ . It is suggested that androgen synthesis is regulated by feedback inhibition at the cellular level.

**62. Bovine adrenal cortex  $3\beta$ -hydroxysteroid dehydrogenase and 3-oxosteroid- $\Delta^5$ -4-isomerase: phospholipid requirement?**

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