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-7^{3}H and/or II^{14}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 $14{\cdot}2 \times 10^{-6}$ M/L), respectively, however, the V_{max} values were 54·6 and $26{\cdot}0 \times 10^{-7}$ 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 11β-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 11β-hydroxylase activity was found to be extremely low, although 17α- and 21-hydroxylase activities were comparable to eutherians. In echidnas treated for 1 week with frusemide (Lasix), which caused Na^+ depletion and dehydration, there was a marked increase in 11β-hydroxylation of the adrenal homogenates, 60% of the end products from progesterone being 11β-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 11β-hydroxylase for the treated echidna and the rat in these experiments were 0·24 and 0·30 μM and the V_{max} values $8{\cdot}2 \times 10^{-6}$ and $6{\cdot}7 \times 10^{-4}$ μM min^{-1} mg^{-1}, respectively. Thus the efficiencies of 11β-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 11β-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 11β-hydroxylation of ^{3}H-S. Adrenal cells were harvested from 26 control adult male guinea-pigs and from 18 animals treated with ACTH (75 μg $ACTH_{1-24}$ i.v. over 3 h, 24 h previously). The 11β-hydroxylation index [(F/F + S) × 100] determined from F and S adrenal tissue content before cell dispersion, was 82·7 ± 1.1 in controls and 99·5 ± 0·2 24 h after ACTH ($p < 0{\cdot}001$). Incubation of isolated cells with tracer amounts of ^{3}H-S resulted in ^{3}H-F formation, itself undergoing conversion into cortisone (E). The 11β-hydroxylation index, calculated therefore as (F + E/F + E + S) × 100, increased in cells from ACTH-treated animals, averaging after 30 min incubation 88 ± 1 *vs.* 77 ± 2 in controls ($p < 0{\cdot}01$). 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 ($p < 0{\cdot}05$), 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 11β-hydroxylation enzyme system, as judged from ^{3}H-S conversion into ^{3}H-F and ^{3}H-E. In conclusion: (1) No change in 11β-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 11β-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 17α-hydroxypregnenolone, the obligatory cofactor for this reaction NADPH, and by the ratio of $NADP^+$ to NADPH. 17α-hydroxyprogesterone and DHA itself exerted non-competitive inhibition on the conversion. The synthesis of androstenedione from its two immediate precursors, 17α-hydroxyprogesterone and DHA, was also investigated. Both reactions were dependent upon the availability of the substrate and of the obligatory cofactors, NADP and NAD^+, respectively. The conversion of 17α-hydroxyprogesterone to androstenedione was competitively inhibited by pregnenolone, progesterone and 17α-hydroxypregnenolone, while the synthesis of androstenedione from DHA was found to be non-competitively inhibited by oestrone and oestradiol-17β. It is suggested that androgen synthesis is regulated by feedback inhibition at the cellular level.

62. Bovine adrenal cortex 3β-hydroxysteroid dehydrogenase and 3-oxosteroid-Δ5-4-isomerase: phospholipid requirement?

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