Metabolism of C^{21} Steroids by Incubated Adrenals of ACTH Treated Rats[1]

The increasing number of investigations dealing with enzymic mechanisms in the adrenal of rats exposed to periodical stimuli makes it necessary to establish a standard of comparison for stimuli-enzyme relationships. The effect of periodical administrations of ACTH on the biosynthesis and catabolism of C^{21} steroids by the rat adrenal partially fulfils the requirements for such a standard.

ACTH[2] (1.5 I.U. in 0.002*N* HCl-saline, twice daily) was administered to female rats of 150–200 g of weight for 1 month. As controls, similar females were injected with saline over the same period. Three groups of animals were submitted to this treatment and then nembutalized (50 mg Nembutal/kg) and decapitated. Three types of incubation experiments, one for each group, were then performed.

(a) *Incorporation of tritium from progesterone-7-H^3 into radioactive fractions.* In this group, individual pairs of adrenals were quartered and incubated in Krebs Ringer-bicarbonate-glucose solution contained in 10 ml beakers, under an atmosphere of 95% O_2 and 5% CO_2. In all assays, progesterone-7-H^3 was used as precursor. At the end of incubations (5 h), corticosterone-4-C^{14} was added to the assays. Total lipids were extracted and chromatographed in the propylene glycol-toluene system according to BIRMINGHAM et al.[3]. Radioactive zones were located, eluted and an aliquot of each counted in a liquid scintillation counter. In the corticosterone[4] fraction, the total steroid content was determined by fluorometry (GIVNER et al.[5]), after which the fraction was acetylated and the acetate chromatographed in the Bush A system and in the propylene glycol-toluene system. Total activity recovered was calculated according to the losses of corticosterone-4-C^{14}.

Table I summarizes the results of this experiment: in spite of significantly increased gland weights, radioactivity corresponding to both the 18 OH DOC and the B zones decreases in incubations corresponding to ACTH treated animals. Non-radioactive corticosterone, on the contrary, remains unchanged or even shows a slight tendency to increase. Therefore the specific activity of corticosterone decreases sharply in treated animals.

(b) *Catabolism of corticosterone.* Experiment (a), although demonstrating a reduced production of corticosterone from radioactive progesterone, does not give any information about the nature of this decrease, which may either be due to a reduced biosynthesis or an increased catabolism of radioactive corticosterone. To test the second hypothesis, corticosterone-1,2-H^3 was incubated in the presence of adrenals of ACTH-treated and control rats, in the same conditions as in the former experiment. Isolation, purification and measurement of cold and radioactive B was also performed as mentioned before.

Table II, summarizing the results of experiment (b), shows that unconverted radioactive corticosterone decreases as does tritiated B in experiment (a). Non-radioactive corticosterone formed during incubation remains unchanged. The decrease in radioactive B observed in both experiments is therefore mainly, if not solely, due to an increased catabolism of B.

(c) *Incubations with 2 differently labeled substrates.* In order to investigate whether at least part of the decrease of radioactivity in the B fraction from precursors could be due to a decreased 11 or 21 hydroxylation, experiments were designed in which the precursors were progesterone-7-H^3 and corticosterone-4-C^{14} or DOC-1,2-H^3 and corticosterone-4-C^{14}. In these experiments, duplicate pools of quartered adrenals proceeding from ACTH-treated and normal animals were incubated in the presence of the above-mentioned precursor pairs.

[1] This investigation was supported by U.S.P.H.S. grant No. 1-RO5-TWO0200-01.

[2] Nordic Biochemicals, Montreal (Canada) 80 I.U./mg.

[3] M. K. BIRMINGHAM and P. J. WARD, J. biol. Chem. *236*, 1661 (1961).

[4] The following trivial names have been used: 11,21-dihydroxy-Δ-4 pregnene-dione-3,20; corticosterone or B. Δ-4-pregnene-dione-3,20; progesterone. 21-hydroxy-Δ-4-pregnene-dione-3,20; DOC. 18,21-dihydroxy-Δ-4-pregnene-dione-3,20; 18 OH DOC.

[5] M. L. GIVNER and J. G. ROCHEFORT, Steroids *6*, 485 (1965).

Table I. Incorporation of radioactivity from progesterone 7-H^3 into steroid fractions

	Gland weight (mg/100 g)	Radioactive B (cpm · 10^3)	Non-radioactive B (μg)	Radioactive 18 OH DOC (cpm · 10^3)
Controls	22.85[a] ± 1.70 (6)[b]	229 ± 35 (6)	5.37 ± 0.75 (6)	58.16 ± 5.7 (4)
ACTH treated	35.42 ± 2.75 (7)	121 ± 21 (7)	6.54 ± 1.49 (7)	35.51 ± 2.3 (4)
P	< 0.01	< 0.05	non-significant	< 0.01

Individual quartered adrenal pairs were incubated in presence of progesterone-7-H^3, 546.000 cpm. [a] Mean ± S.E.M., [b] No. of animals.

Table II. Unconverted radioactive corticosterone

	Gland weight (mg/100 g)	Radioactive B (cpm · 10^3)	Non-radioactive B (μg)
Controls	33.20[a] ± 1.87 (5)[b]	158 ± 7.8 (5)	3.43 ± 0.87 (5)
ACTH treated	40.20 ± 1.24 (5)	103 ± 9.1 (5)	3.62 ± 0.43 (5)
P	< 0.05	< 0.01	non-significant

Individual quartered adrenal pairs were incubated in the presence of corticosterone 1,2 H^3, 380.000 cpm. [a] ± S.E.M. [b] No. of animals

Table III. Incubations with double labelled precursor pairs

Precursors	Conditions of rats	B H^3/C^{14}	18 OH DOC H^3/C^{14}
Progesterone 7 H^3 B 4 C^{14}	Controls	11.6[a] ± 0.4	212 ± 38
Progesterone 7 H^3 B 4 C^{14}	ACTH-treated	15.0 ± 0.3[b]	88 ± 11
DOC 1,2 H^3 B 4 C^{14}	Controls	11.7 ± 0.4	195 ± 24
DOC 1,2 H^3 B 4 C^{14}	ACTH-treated	11.6 ± 0.5	30 ± 10[b]

4 Pools of quartered adrenals from 10 ACTH-treated rats and 4 pools of quartered adrenals from 10 control rats were incubated with progesterone 7 H^3 (546.000 cpm)-corticosterone 4 C^{14} (22.000 cpm) and with DOC 1,2 H^3 (574.000 cpm)-corticosterone 4 C^{14} (22.000 cpm). [a] Mean ± S.E.M. [b] $P < 0.05$. Each value represents duplicate assays.

rats in the presence of radioactive progesterone or DOC, find an increase in the yield of radioactive B and a decrease of its metabolites, 18 OH B and aldosterone. His results with treated animals thus resemble our in vitro studies (LANTOS et al.[6]) but differ from the findings of the present investigation. The difference might well be due to different doses employed, VECSEI's being a long-lasting ACTH preparation, while in the present research intermittent doses, resembling periodic stimuli, were administered.

One possible explanation for the 'symmetric' effect in the present work, as compared to our in vitro studies, might be the following: repeated administration of ACTH protects the corticosterone catabolizing systems of the adrenals. Consequently, these adrenals, when incubated post mortem, might be capable of catabolizing corticosterone to a greater extent than those of untreated animals. On the other hand, the increase of 21 hydroxylation, elicited by ACTH treatment – and probably increased at other sites – counterbalances the increased catabolism of B; the total yield of endogenous corticosterone remaining unchanged.

Table IV. Main results obtained by chronic treatment with ACTH and ACTH added to incubation media

	Catabolism of radioactive B	Yield of endogenous B	Yield of radioactive 18 OH DOC	21 Hydroxylation	11 Hydroxylation
Adrenals of ACTH-treated rats	Experiment (a, b) increased	Experiment (a, b) unchanged	Experiment (a, c) decreased	Experiment (c) increased	Experiment (c) unchanged
ACTH added to medium	decreased[6]	increased[6]	increased[6]	unchanged[6]	unchanged[6]

The results (Table III) show that incorporation of tritium from progesterone into corticosterone, as represented by H^3/C^{14} ratios, was increased in ACTH-treated animals, demonstrating an enhanced, instead of a decreased 21 hydroxylating capacity for adrenals of treated animals. Tritium incorporations into B from the precursor DOC remains unchanged. Tritium incorporation into the 18 OH DOC fraction is diminished.

The results of this investigation differ from those obtained in an earlier work (LANTOS et al.[6]) in which ACTH was *added* in vitro to incubations of adrenals of normal rats. Comparative data are shown in Table IV: while the addition of ACTH to the medium decreases the catabolism of B by incubated adrenals, previous treatment of the animals with ACTH increases this catabolism. Similarly, while ACTH in vitro augments the yield of 18 OH DOC from its precursors, ACTH administered chronically diminishes this yield. Moreover, ACTH added to the medium increases the incorporation from progesterone and DOC into the 18 OH DOC fraction, while in the adrenal of the ACTH-treated rat, the contrary is observed[7].

Opposite or 'symmetrical' effects of this kind, between additions of trophins to incubations or normal glands and in vivo stimulation prior to incubation, have been reported earlier. Thus ARMSTRONG et al.[8] find an increased synthesis of endogenous progesterone incubations of ovaries to which LH has been added, but a decreased synthesis in animals chronically treated with LH. MOROZOVA[9] reports a decreased 3-β-ol dehydrogenase activity in adrenal incubations of rabbits chronically treated with ACTH, an effect which decreases instead of increases the corticoid production. In contrast to this, VECSEI et al.[10], incubating adrenals of ACTH-treated

The results obtained by measuring radioactive metabolite production can be misleading if the fate of the non-radioactive moiety is ignored. In the present investigation, non-radioactive corticosterone remains unchanged, or even increases. Therefore, the observed decrease in the yield of radioactive B is not due to a higher concentration (and consequently greater conversion) of this radioactive steroid within a supposedly reduced corticosterone pool.

Resumen. En animales tratados crónicamente con ACTH se aumentan o activan los sistemas de la adrenal que catabolizan la corticosterona y los que hidroxilan en C^{21}, a la vez que disminuye la cantidad de radioactividad incorporada a la 18 OH DOC. Esto indica que el ACTH in vivo tiene sobre la adrenal incubada una acción distinta, a veces opuesta, a la del ACTH exógeno agregado in vitro al principio de la incubación.

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[6] C. P. LANTOS, M. K. BIRMINGHAM and H. TRAIKOV, Acta physiol. latinoam. in press.

[7] H^3/C^{14} values of 18 OH DOC from progesterone-B (Table III) lack significance ($0.1 > P > 0.05$) because of high dispersion of the duplicates.

[8] D. ARMSTRONG, J. O'BRIEN and R. GREEP, Endocrinology *75*, 489 (1964).

[9] M. S. MOROZOVA, Fedn Proc. Fedn Am. Socs exp. Biol. *25*, 163 (1966).

[10] P. VECSEI, D. LOMMER, H. G. STEINACKER, A. VECSEI-GÖRGENYI and H. P. WOLFF, Acta endocr., Copenh. *53*, 24 (1966).