Cortisol Acetyltransferase from Baboon Brain

By P. J. THOMAS*

Southwest Foundation for Research and Education, San Antonio, Tex., U.S.A.

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Recent studies by various investigators have indicated that acetylation of steroid hormones is an important aspect of their metabolism. Although acetylation of steroid hormones *in vitro* by tissue slices from neonatal rat brain (Grosser & Axelrod, 1967) and mammary gland of rat and mouse (King, Gordon & Smith, 1964) has been reported, there is no information on the enzymes involved in the acetylation and their cofactor requirements. The present paper concerns the isolation of a soluble enzyme from baboon brain capable of acetylating cortisol at C-21 in the presence of acetyl-CoA.

Materials and methods. [4-¹⁴C]Cortisol was purchased from New England Nuclear Corp. (Boston, Mass., U.S.A.) and was purified by chromatography before use. Acetyl-CoA was from PL Biochemicals (Milwaukee, Wis., U.S.A.) and was chromatographically pure. Male baboons, 3–4 months old and weighing about 2 kg. each, were killed by exsanguination under anaesthesia and the brains were perfused *in situ* with 0.9% NaCl injected through a carotid artery. The brains were excised, dissected in a cold-room at 4° and homogenized in 3 vol. of 0.25 M-sucrose containing 0.5 mM-EDTA. The homogenates were centrifuged in a Beckman Spinco model L centrifuge at 100 000g for 1 hr. and the resulting supernatants were used as the source of the enzyme. Protein determinations were carried out by the method of Lowry, Rosebrough, Farr & Randall (1951). Incubations were performed in 50 ml. conical flasks in air at 37°. Each of the incubation flasks contained in addition to the enzyme 10 μ M-cortisol containing 1.4 μ C of [4-¹⁴C]-cortisol suspended in 0.2 ml. of 5% human serum albumin, 0.3 M-NaCl, 0.5 mM-eserine and 10 mM-potassium phosphate buffer, pH 7.4, in a final volume of 6.0 ml. Acetyl-CoA when present was 20 μ M. The incubations were terminated after 1 hr. by the addition of acetone at –20° and the reaction mixtures were stored in a refrigerator overnight. The contents of the flasks were filtered through a sintered-glass funnel and the residues were re-suspended in acetone and washed twice more with acetone. The pooled acetone extracts were evaporated to dryness and the steroids were extracted with chloroform. After the evaporation

of the chloroform the residues were combined with 25 μ g. each of cortisol acetate and cortisone acetate and chromatographed on Whatman no. 1 paper in benzene-formamide (Zaffaroni, 1953) for 8 hr. The radioactivity was detected by scanning the paper chromatogram in a 4 π strip counter and the standard steroids were detected by ultraviolet light and blue tetrazolium reaction. The area corresponding to each of the radioactive zones was eluted with methanol and a sample of the eluate was counted in a Packard Tri-Carb liquid-scintillation counter with automatic external standardization. The ratio of the amount of radioactivity in cortisol acetate to the amount of radioactivity in unchanged cortisol was used to calculate the extent of acetylation. The remainder of the radioactivity obtained from the zone corresponding to cortisol acetate

Table 1. *Acetylation of cortisol by the high-speed supernatant fractions from various parts of baboon brain*

The system contained: 5 ml. of 100 000g supernatant from the source indicated, 10 μ M-[4-¹⁴C]cortisol (1.4 μ C) suspended in 0.2 ml. of 5% human serum albumin, 0.3 M-NaCl, 0.5 mM-eserine and 10 mM-potassium phosphate buffer, pH 7.4, in a final volume of 6.0 ml. After the incubation at 37° for 1 hr. the steroids were extracted and purified by chromatography. The amount of cortisol acetate formed was determined from the ratio of the radioactivity eluted from the area on the chromatogram corresponding to standard cortisol acetate and unchanged cortisol respectively.

System	Acetyl-CoA (20 μ M)	Cortisol acetate formed (m μ mole/mg. of protein/hr.)
Whole brain, dialysed	–	0.00
	+	0.21
Whole brain, boiled	+	0.00
	–	0.05
Brain cortex	+	0.25
	–	0.15
Brain stem	+	0.99
	–	0.07
Cerebellum	+	0.35
	–	0.03
Thalamus-hypothalamus	+	0.62
	–	

* Present address: Central Food Technological Research Institute, Mysore 2, India

was mixed with authentic cortisol 21-acetate and crystallized to constant specific radioactivity.

Results and discussion. Chromatograms of extracts obtained from the incubations of [4-¹⁴C]-cortisol in the presence of acetyl-CoA and the enzyme invariably contained a zone of radioactivity corresponding to standard cortisol acetate in addition to the radioactivity at the origin of the paper corresponding to unmetabolized cortisol. The radioactive material corresponding to cortisol acetate was combined with 10mg. of cortisol 21-acetate and crystallized to constant specific radioactivity. There was no evidence of any radioactive cortisone acetate, indicating the absence of 11 β -hydroxy steroid dehydrogenase from the preparation. As shown in Table 1, the acetylation is considerably enhanced by the presence of acetyl-CoA in the undialysed preparations, and in the dialysed preparations there is virtually no acetylation in the absence of acetyl-CoA. The enzyme is distributed throughout the brain and the activity

seems to be the highest in the brain stem. Most of the acetylating activity could be concentrated in the protein fraction precipitated at 30–40% saturation with (NH₄)₂SO₄. The reaction was found to be linear up to 2hr. and dependent on the concentration of the protein.

Westphal & Ashley (1958) have demonstrated that the acetylation of the C-21 group of certain hormones resulted in profound changes in the interaction of C-3 of that steroid with human serum albumin. However, the physiological significance of the acetylation of cortisol in the brain is not understood.

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