Journal of Steroid Biochemistry, 1970, Vol. 1, pp. 341–347. Pergamon Press. Printed in Great Britain

RECIPROCAL CORTISOL–CORTISONE CONVERSIONS IN THE TOTAL TISSUE AND SUBCELLULAR FRACTIONS OF FOETAL AND ADULT GUINEA PIG LIVER

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(*Received* 9 *July* 1970)

SUMMARY

A mixture of cortisol-^{3}H and cortisone-^{14}C was administered to guinea pig foetuses *in situ* at mid-term and full-term gestation and to adult guinea pigs. After 30 min the radioactivity recovered in the liver accounted for 3–11% of the administered dose. In the foetal liver at mid-term a significant percentage of cortisol was converted to cortisone, but the reverse conversion did not occur. The latter conversion took place to a small extent at the end of gestation. In the liver of the adult animals conversion occurred in both directions. In the foetal liver the greater part of the radioactivity was unconjugated. On the other hand, in adult liver tissues conjugated radioactive material accounted for 27–30%. Five to fourteen per cent of the total radioactivity in the hepatic cells was associated with the nuclear fraction and 60–80% of the tritiated radioactive material in this fraction consisted of unchanged cortisol; unchanged cortisone accounted for 30–40%.

INTRODUCTION

CORTISOL is the principal adrenocortical hormone produced by the guinea pig [1, 2]. This hormone is extensively metabolized and is excreted in the urine of the animal principally as 2α- and 6β-hydroxycortisol, and 20α- and 20β-dihydrocortisol [3–5]. It is widely accepted that the presence of the 11β-hydroxy function is necessary for adrenocortical hormone activity; after *in vivo* administration to humans and various animal species of the corresponding 11-keto form, e.g., cortisone or 11-dehydrocorticosterone, these steroids are largely reduced to the corresponding 11β-hydroxy derivatives. This transformation takes place principally in the liver [6–8].

In this paper the interrelation between cortisone and cortisol in liver tissue of the foetal guinea pig at the middle and end of gestation is studied and compared with that in adult animals.

EXPERIMENTAL

Biological material

Liver tissues were obtained from adult guinea pigs (Hartley strain) and from guinea pig foetuses at the middle and end of gestation.

Radioactive steroids

Cortisol-1,2-^{3}H* (specific activity: 8 μCi/μg) and cortisone-4-^{14}C* (specific activity: 0·06 μCi/μg) were purified in ethyl acetate : toluene : methanol : water (1 : 9 : 5 : 5, v/v) and benzene : methanol : water (2 : 1 : 1, v/v) systems, and radiochemical homogeneity was confirmed by recrystallization.

Injection of the radioactive steroid

A mixture of cortisol-^{3}H and cortisone-^{14}C† was injected subcutaneously into the foetuses *in*

*Purchased from The Radiochemical Centre, Amersham, England.

†The quantities of radioactivity administered are indicated in Table 1.

situ and intravenously into the adult guinea pigs. Thirty minutes later, the animals were killed and the livers removed. The livers of all animals in each experiment were combined, and one tenth of the total liver tissue was ground with sand and the radioactive material extracted 3 times with ethanol 80% (v/v). The other $\frac{9}{10}$ were homogenized and separated into the different subcellular fractions.

Table 1. Cortisol-^{3}H and cortisone-^{14}C injected into foetal and adult guinea pigs and percentage of the radioactivity recovered in the different liver tissues

		Radioactivity injected*		Radioactivity in liver*	
	N	F-^{3}H μCi	E-^{14}C μCi	^{3}H %	^{14}C %
(A) *Foetuses*					
Experiment I (mid-term gestation) (30–35 days)	2	8·5	1	2·3	2·0
Experiment II (mid-term gestation) (30–35 days)	2	20·0	3	7·1	3·9
Experiment III (full-term gestation) (55–58 days)	4	40·0	6	10·0	7·4
(B) *Adults*					
Experiment IV (6–7 months)	3	26	3	11·0	10·0

N: number of animals
F-^{3}H: cortisol-^{3}H
E-^{14}C: cortisone-^{14}C

*The radioactivity injected corresponds to the total radioactivity injected to the *N* animals in each experiment; the recovery radioactivity in the liver represents the average values in each experiment.

Subcellular fractionation

Liver tissues were homogenized in a 0·25 *M* sucrose solution in a Potter–Elvejhem homogenizer and centrifuged at 900 **g** to separate out the crude nuclear fraction. This fraction was washed in a 0·88 *M* sucrose solution after centrifugation at 2500 **g**. The first supernatant was fractionated into the mitochondrial, microsomal and soluble fractions by successive centrifugations at 25,000 **g** and 105,000 **g** in a Spinco Model L-50 ultracentrifuge.

Procedure for the isolation of cortisol and cortisone

The total tissues and the different subcellular fractions were precipitated with ethanol, left at − 10°C for three days, and centrifuged. The supernatant was then evaporated to dryness, and the residue dissolved in water. The radioactive material was extracted successively with dichloromethane, ethyl acetate, and n-butanol. The dichloromethane and ethyl acetate extracts were considered to contain the unconjugated steroids, and the n-butanol extract, the conjugated steroids. The dichloromethane extracts were chromatographed on paper in the chloroform/formamide system and the radioactive material corresponding respectively to the mobilities of cortisone and cortisol was eluted and chromatographed successively in Systems 2, 3 and 4.

Chromatographic systems

The following solvent systems were used:

(1) Chloroform/formamide.
(2) Benzene : methanol : water (2 : 1 : 1, v/v).
(3) Isooctane : t-butanol : water (9 : 5 : 10, v/v).
(4) Ethyl acetate : toluene : methanol : water (1 : 9 : 5 : 5, v/v).
(5) Toluene/propanediol.
(6) Benzene/propanediol.

(7) Ligroin : toluene (1 : 1, v/v).
(8) Isooctane : toluene : methanol : water (1 : 1 : 1 : 1, v/v).
(9) Isooctane : toluene : methanol : water (3 : 1 : 2 : 2, v/v).

Nomenclature and abbreviations

20α-Dihydrocortisol. 11β, 17α, 20α, 21-tetrahydroxy-4-pregnen-3-one.
20β-Dihydrocortisol. 11β, 17α, 20β, 21-tetrahydroxy-4-pregnen-3-one.
2α-Hydroxycortisol. 2α, 11β, 17α, 21-tetrahydroxy-4-pregnene-3,20-dione.
6β-Hydroxycortisol. 6β, 11β, 17α, 21-tetrahydroxy-4-pregnene-3,20-dione.
11β-Hydroxy androstenedione. .11β-hydroxy-4-androstene-3,17-dione.
Adrenosterone. 4-androstene-3,11,17-trione.
dpm. disintegrations per minute.

Measurement of the radioactivity

Radioactivity was measured by liquid scintillation in a Packard Model 3002 spectrometer. The unconjugated portion was measured using toluene scintillator solution, and the total and conjugated radioactive materials were each measured after the addition of 0·5 ml methanol. Quenching tests were carried out by the addition of toluene-^{3}H and/or -^{14}C standard.

RESULTS

Table 1 lists the percentages of the administered radioactivity recovered in the total liver tissues. 2–10% of ^{3}H and 2–7% of ^{14}C were recovered in the foetal livers, while in the adult livers the values were 11% and 10%, respectively.

Table 2 lists the percentages of free and conjugated radioactivity in the different livers. As is indicated, during the foetal life of the guinea pig, the greater part of the radioactivity of the steroids used is unconjugated. On the other hand, conjugated radioactive material accounts for 27–30% in the adult liver.

Table 2. Free and conjugated radioactive material in foetal and adult guinea pig liver tissues after simultaneous administration of cortisol-^{3}H and cortisone-^{14}C. (Values are expressed as percentage of the total radioactive material in each tissue and represent the average values for all the animals in each experiment)

	Fractions			
	Free		Conjugated	
	^{3}H	^{14}C	^{3}H	^{14}C
(A) *Foetus*				
Experiment I	99	99	1	1
Experiment II	95	96	5	4
Experiment III	92	98	8	2
(B) *Adults*				
Experiment IV	70	73	30	27

Table 3 indicates the distribution of the radioactivity among the different subcellular fractions. As this table shows, most of the radioactivity is localized in the supernatant fraction for both foetal and adult liver tissues, but a significant percentage is found in the nuclear fraction.

Table 3. Distribution of the radioactive material in the different subcellular fractions of the liver tissues of foetal and adult guinea pigs after administration of cortisol-^{3}H and cortisone-^{14}C. (Values are expressed as percentage of the radioactivity of the total liver tissue and represent the average values for all the animals in each experiment)

	Foetal liver							
	Mid-term gestation				Full-term gestation		Adult liver	
Subcellular fraction	Exp. I		Exp. II		Exp. III		Exp. IV	
	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C
Nuclear	10	15	6	5	13	14	8	11
Mitochondrial	8	9	4	3	6	6	14	13
Microsomal	10	10	8	7	2	5	11	16
Supernatant	72	66	82	85	79	75	67	60

Identification of the radioactive steroids

Cortisol. Part of the radioactive material corresponding to the mobility of cortisol (System 4) was eluted and acetylated. The acetate had the same mobility as cortisol acetate when chromatographed in Systems 5, 6 and 7.

Another portion was oxidized with sodium bismuthate, and the oxidized products had the same R_f as 11β-hydroxy-androstenedione when chromatographed in Systems 8 and 9. Another portion was mixed with authentic carrier cortisol, and

Table 4. Recrystallization to constant specific activity of the radioactive cortisol and cortisone isolated from the liver tissues (dpm/mg)

		Crystals		Mother liquors	
		^{3}H	^{14}C	^{3}H	^{14}C
Cortisol					
(Foetus, mid-term	(a)	310	0	300	0
gestation)	(b)	310	0	295	0
Cortisol					
(Foetus, full-term	(a)	1080	60	1380	170
gestation)	(b)	1050	37	1100	70
	(c)	1100	35	1080	35
Cortisol					
(Adult)	(a)	840	130	2050	250
	(b)	850	110	900	130
	(c)	850	110	870	115
Cortisone					
(Foetus, mid-term	(a)	680	540	750	710
gestation)	(b)	670	520	650	530
Cortisone					
(Foetus, full-term	(a)	1320	1450	1850	2050
gestation)	(b)	1280	1470	1290	1500
Cortisone					
(Adult)	(a)	290	75	650	140
	(b)	305	70	300	75

(a): methanol (b): ethanol (c): methanol : benzene

Table 5. Quantitative distribution of cortisol and cortisone isolated from the total and subcellular fractions of the foetal and adult guinea pig liver tissues after simultaneous administration of cortisol-^{3}H and cortisone-^{14}C. (Values are expressed as percentage of the total radioactivity in each fraction*)

	Foetal liver																											
	Mid-term gestation (Exp. I – Exp. II)										Full-term gestation (Exp. III)										Adult liver (Exp. IV)									
	Total tissue		N		Mit		Mic		S		Total tissue		N		Mit		Mic		S		Total tissue		N		Mit		Mic		S		Control†	
Steroid	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C	^{3}H	^{14}C
Cortisol	27–30	0–0	60–80	0–0	25–30	0–0	30–35	0–0	25–32	0–0	26	3	63	1	30	2	20	2	28	3	8	6	57	12	6	3	5	5	7	5	100	0
Cortisone	3–4	17–10	1–3	28–35	2–3	16–9	4–6	18–15	3–4	15–12	5	30	3	42	6	30	5	32	4	28	10	17	8	36	9	20	9	18	10	15	0	100

*Corrections for losses were made using a parallel standard of cortisol-^{3}H or cortisone-^{14}C.

†A mixture of 1 μCi of cortisol-^{3}H and 0·2 μCi of cortisone-^{14}C was used for control.

N: Nuclear fraction.

Mit: Mitochondrial fraction.

Mic: Microsomal fraction.

S: Supernatant.

the specific activities of ^{3}H and ^{14}C were determined after recrystallization in different solvent systems (Table 4).

Cortisone. A portion of the material corresponding in mobility to cortisone was acetylated, and the corresponding acetate had the same R_f as authentic cortisone acetate in Systems 5 and 6. Another portion was oxidized with sodium bismuthate, and the product obtained had the same polarity as adrenosterone after chromatography in Systems 8 and 9. The results of crystallization are shown in Table 4.

Table 5 gives values for the conversions of cortisol to cortisone and vice versa in the total tissue and in the subcellular fractions of foetal and adult guinea pig liver. As is indicated in this table, in the foetal liver at midterm, a significant part of the cortisol was converted to cortisone, but no cortisone was reduced to cortisol. Slight 11β-reductase activity was noted at the end of gestation; this activity was intense in the adults. It is also interesting to note that, in both foetal and adult animals, the greater part (60–80%) of the radioactivity in the nucleus consisted of unchanged cortisol.

DISCUSSION

The data presented in Table 2 show that the liver of the guinea pig foetus has a very small capacity for the conjugation of cortisol and cortisone; this is in contrast to the data for the adult liver of the same animal, as well as to results obtained with the human foetal liver, where a significant proportion of the radioactive material was found in the conjugated fraction[9]. Furthermore, since at full term most of the radioactivity is still unconjugated, it is concluded that the enzymatic systems for conjugation are developed after birth.

The observation that in the guinea pig foetal liver a significant part of the cortisol was converted to cortisone but no cortisone was converted to cortisol, is similar to results obtained with human foetal liver at mid-term[9]. In agreement with these results, it is interesting to note that a large quantity of cortisone (many times that of cortisol) is found in the human placenta[10] and in the human umbilical cord blood[11].

At present these findings are difficult to explain from the biological point of view, but various authors have found different degrees of transformation of cortisol (particularly to cortisone) by normal and pathological tissues. Berliner and Dougherty[12] found that in leukaemic lymphocytes cortisol is more extensively metabolized than in normal lymphocytes. It has also been established that the growth of fibroblasts is markedly inhibited by cortisol, while cortisone has no effect or a slightly stimulating one[13].

As Table 5 indicates, the formation of cortisone is greater at full-term than at mid-term; similar findings were obtained for this hormone in the brain tissue of foetal baboons, where the transformation of cortisol to cortisone was many times greater in the third trimester of gestation than in the second[14].

It is relevant to note that a significant percentage of the radioactivity is localized in the nuclear fraction and the greater part of this radioactive material consists of unchanged cortisol.

ACKNOWLEDGEMENTS

The expenses of this investigation were defrayed by a research grant from the Centre National de Recherche Scientifique of France.

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