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CORTISOL AND CORTISONE PRODUCTION IN RAT AND MOUSE ADRENAL INCUBATIONS

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Summary—The presence of 17α-hydroxylase in rodent adrenals is debated. The presence in blood of mice of 11-deoxycortisol together with the absence of cortisol is well known. We demonstrated here the *in vitro* synthesis of 17α-hydroxyprogesterone and cortisol from [^{3}H]progesterone in rat and mouse adrenals. We have shown that these syntheses represented 45 and 28% of those of 11-deoxycorticosterone and corticosterone, respectively, from progesterone. These data clearly suggest the presence of a 17α-hydroxylase activity *in vitro* in these rodents adrenals. In addition, a noticeable synthesis of cortisol (0.87–1.57% per mg tissue, i.e. 52–64% per incubation flask) from 11-deoxycortisol was also observed and was inhibited by 0.1–0.3 μmol of Metyrapone and SKF 12185. These results allow to underline that the adrenals of rat and mouse, two species commonly used in laboratory experiments, may be used for *in vitro* investigations on cortisol metabolism from exogenous radioactive precursors.

INTRODUCTION

It has long been accepted that the adrenal cortex of rat produces mainly corticosterone and only negligible amounts, if any, of cortisol, presumably because of the lack of 17α-hydroxylase [1–3]. The presence (or the absence) of this enzyme and consequently the question of cortisol synthesis by rodent adrenals has been a matter of controversy in the literature with reports either on the absence of 17-hydroxylated steroids [4–6] or on the contrary showing the capacity of rodent adrenals, in normal or pathological conditions, to convert pregnenolone or progesterone to cortisol [7–14].

The study of Badr [15] reporting large circulating concentrations of 11-deoxycortisol in the plasma of different strains of mice prompted us to examine whether the lack of cortisol production in rats and mice as reported by many authors should not be attributed to a lack of conversion of 11-deoxycortisol to cortisol in the adrenal tissue. We therefore tested this hypothesis by incubating rat and mouse adrenals *in vitro* in the presence of tritiated 11-deoxycortisol. Besides, the steroid synthesis from 11-deoxycorticosterone and progesterone has also been documented with the two species.

EXPERIMENTAL

Chemicals

The following [1,2-^{3}H]steroids were used for the experiments: 11-deoxycortisol (sp. act. 58.5 Ci/mmol), 11-deoxycorticosterone (sp. act. 46.8 Ci/mmol) and progesterone (sp. act. 90 Ci/mmol). The following [4-^{14}C]steroids were used: cortisol (sp. act. 55.5 mCi/mol), cortisone (sp. act. 59.8 mCi/mol), corticosterone (sp. act. 52 mCi/mol), aldosterone (sp. act. 55.0 mCi/mol), 17α-hydroxyprogesterone (sp. act. 60 mCi/mol) and 11-deoxycorticosterone (sp. act. 58.5 mCi/mol). These labeled steroids were purchased from New England Nuclear Corporation. Their radiochemical purity was checked by paper chromatography shortly before use.

[4-^{14}C]18-hydroxycorticosterone was obtained from [4-^{14}C]deoxycorticosterone by incubation of rat adrenal homogenates. The radiochemical purity was checked as described previously [16]. [4-^{14}C]11-dehydrocorticosterone was obtained from the chromic acid (20 g/l in water-pure acetic acid, 50:50) oxidation (3 h at laboratory temperature) of [4-^{14}C]corticosterone. The protection of the side-chain of corticosterone by acetylation was not necessary with these conditions [17].

Unlabeled steroids were purchased from Merck (Darmstadt, F.R.G.) and Ikafarm (Israel), malic acid from Sigma (St Louis, Mo., U.S.A.) and sterile Earle's medium from Institut Pasteur (Paris, France). The solvents were of analytical grade as supplied from Merck.

Adrenals

Male rats of the Wistar WAB 1954 strain (weight 250 g, 12 weeks old) and male mice (weight 35 g, 30 weeks old) from the B6 D2 F1 strain were used for the experiments. They were fed with usual stock condition and raised under laboratory standardized conditions. The animals were killed by cervical dislocation between 0900 and 1000 h and the adrenals quickly removed, cleaned then homogenized in Earle's medium buffer.

Incubation

Incubations of rat adrenals (60–100 mg, wet weight depending on the series of experiments) or mouse adrenals (20–40 mg, wet weight) or without any tissue (blank control) were performed in several series of experiments. Incubation flasks contained exact amounts of the following tritiated precursors: 11-deoxycortisol (7.2 μCi), progesterone (9.9 μCi) and 11-deoxycorticosterone (8.1 μCi) as ethanolic solutions which were evaporated to dryness then dissolved in 2 ml Earle's medium 1 h before starting the incubation. An NADPH-generating system, made up of $NADP^+$ (1 mmol/l) and malic acid (5 mmol/l) neutralized with a 0.1 M potassium hydroxyde solution, was added to each incubation flask [16]. Some incubation flasks received 0.1–0.3 μmol Metyrapone or SKF 12185 to evaluate the inhibitory effect of these drugs on the conversion of 11-deoxycortisol to cortisol by mouse adrenals. The incubations were performed under aerobic conditions in a Dubnoff metabolic shaking incubator at 37°C for 2 h. The proteins were precipitated with 15.0 ml acetone and trace amounts of [4-^{14}C]steroids, depending on the tritiated precursor used, were added to the incubation flasks to be used as internal standards. Extraction of steroids and measurement of radioactivity were performed as previously described [18]. The results were calculated as the percentage conversion per milligram tissue of the total tritiated radioactivity incubated. The data were corrected for procedural losses.

Isolation and characterization of steroids

The steroids were separated by descending paper chromatography. Individual isolated metabolites were further run in suitable chromatographic systems to assess their isopolarity with the carbon-14 internal standards. The isolated steroids were then characterized through derivative formation with the following methods (Table 1). Chromic acid oxidation:

Table 1. Isolation and purification of synthesized steroids

Steroid	Procedure	Derivative	Chromatographic systems
17-Hydroxyprogesterone	Isolation		PC1 (3 h), PC3 (7 h)*
	Chromic acid oxidation	Androstenedione	PC1 (3 h), PC2 (3 h)*
	Sodium borohydride reduction	Testosterone	PC3 (3 h), PC4 (3 h)*
Cortisol	Isolation		PC1 (3 h), PC2 (3 h), PC7 (7 h)*
	Chromic acid oxidation	Adrenosterone	PC2 (3 h), PC4 (3 h), PC6 (3 h)*, PC7 (3 h)*
Cortisone	Isolation		PC1 (3 h), PC2 (3 h), PC5 (48 h)*
	Chromic acid oxidation	Adrenosterone	PC2 (3 h), PC4 (3 h), PC6 (3 h)*, PC7 (3 h)*
11-Deoxycorticosterone (DOC)	Isolation		PC1 (3 h), PC3 (6 h), PC4 (3 h)*
	Acetic anhydride acetylation	DOC acetate	PC1 (3 h), PC3 (3 h)*
	Hydrolysis	DOC	PC1 (3 h), PC3 (3 h), PC4 (3 h)*
Corticosterone (B)	Isolation		PC1 (3 h), PC2 (3 h), PC4 (6 h)*
	Acetic anhydride acetylation	B acetate	PC1 (6 h), PC5 (3 h)*
	Chromic acid oxidation	A acetate	PC1 (6 h), PC5 (3 h)*
11-Dehydrocorticosterone (A)	Isolation		PC4 (6 h), PC5 (6 h)*
	Acetic anhydride acetylation	A acetate	PC1 (6 h), PC5 (3 h)*
	Hydrolysis	A	PC1 (6 h), PC5 (6 h)*
18-Hydroxycorticosterone (18-OHB)	Isolation		PC4 (6 h), PC2 (3 h), PC2 (17 h)*
	Periodic acid oxidation	18 OHB lactone	PC5 (3 h), PC6 (3 h)*
	Chromic acid oxidation	18 OHA lactone	PC1 (3 h), PC5 (3 h), PC6 (3 h)*
Aldosterone (Aldo)	Isolation		PC2 (3 h), PC4 (72 h)*
	Acetic anhydride acetylation	Aldo 18-21 diacetate	PC5 (5 h), PC6 (3 h)*
	Chromic acid oxidation	Aldolactone 21-monoacetate	PC5 (6 h), PC6 (3 h)*

*Estimation of ^{3}H:^{14}C ratio.

Paper chromatographic systems (PC)—PC1: methylcyclohexane–toluene–formamide (10:10:1 by vol); PC2: dichloroethane–ethylene glycol (10:1 by vol); PC3: hexane–benzene–propylene glycol (10:10:1 by vol); PC4: toluene–propylene glycol (10:1 by vol); PC5: benzene–formamide (10:1 by vol); PC6: benzene–heptane–methanol–water (67:33:80:20 by vol); PC7: benzene–methanol–water (10:5:5 by vol).

Table 2. Purification procedures of steroids isolated from mouse adrenal incubations. All the data represent the $^3H/^{14}C$ ratio of the last radiochromatogram of each purification step. Derivatives are as in Table 1

	Precursor: Progesterone	$^3H/^{14}C$ Ratio	Precursor: 11-Deoxycortisol	$^3H/^{14}C$ Ratio	Precursor: 11-Deoxycorticosterone	$^3H/^{14}C$ Ratio
Steroid synthesized	17-Hydroxyprogesterone	0.40	Cortisol	43.1	Corticosterone	25.2
First derivative		0.35		41.8		24.0
Second derivative		0.34		41.3		23.3
Steroid synthesized	11-Deoxycorticosterone	1.54	Cortisone	24.2	11-Dehydrocorticosterone	14.6
First derivative		1.32		20.3		12.8
Second derivative		1.24		20.1		12.6
Steroid synthesized	Corticosterone	7.60			18-Hydroxycorticosterone	8.51
First derivative		7.02				7.04
Second derivative		6.63				6.65
Steroid synthesized	Cortisol	3.73			Aldosterone	1.82
First derivative		3.01				1.61
Second derivative		2.75				1.60

chromium dioxide (20 g/l) in water-pure acetic acid (50:50), 3 h in darkness at laboratory temperature, extraction with chloroform; periodic acid oxidation: periodic acid (2 g/l in water–pyridine, 98:2), overnight at laboratory temperature, extraction with chloroform; sodium borohydride reduction: sodium borohydride (2 g/l in methanol) 35 min at laboratory temperature, extraction with ethyl acetate; acetic anhydride acetylation: dried extracts dissolved in 3 drops pure pyridine, then addition of 3 drops acetic anhydride, overnight at laboratory temperature, extraction with chloroform; acetate hydrolysis: Na_2CO_3 (5 g/l in water) 1 h at laboratory temperature, extraction with chloroform.

Table 1 shows the paper chromatography systems used for the isolation of synthesized steroids and of conversion derivatives. The radiochemical purity of synthesized steroids and conversion products was assumed from the constancy of ^3H:^{14}C ratios at different stages of purification. Successive ratios for these compounds are given in Table 2.

RESULTS

The synthesis of cortisol from 11-deoxycortisol (Fig. 1) was active in the adrenals of rat (0.87% per mg tissue) and mouse (1.57% per mg tissue). The production of cortisone was also noticeable (rat: 0.23; mouse: 0.41% per mg tissue). Since cortisone was obtained through the oxidation of synthesized cortisol by 11β-hydroxysteroid dehydrogenase, summing the synthesized 11-oxosteroids i.e. cortisol + cortisone provided a reliable index of 11β-hydroxylase activity (rat: 1.10; mouse: 1.98% per mg tissue).

The incubations with 11-deoxycorticosterone as a precursor allowed to document another pathway utilizing 11β-hydroxylase. The synthesis of corticosterone reached respectively 0.47 and 1.04% per mg tissue in rat and mouse adrenals (Fig. 2). Once again, taking into account the further metabolites of corticosterone (11-dehydrocorticosterone, 18-hydroxycorticosterone and aldosterone) provided a better index

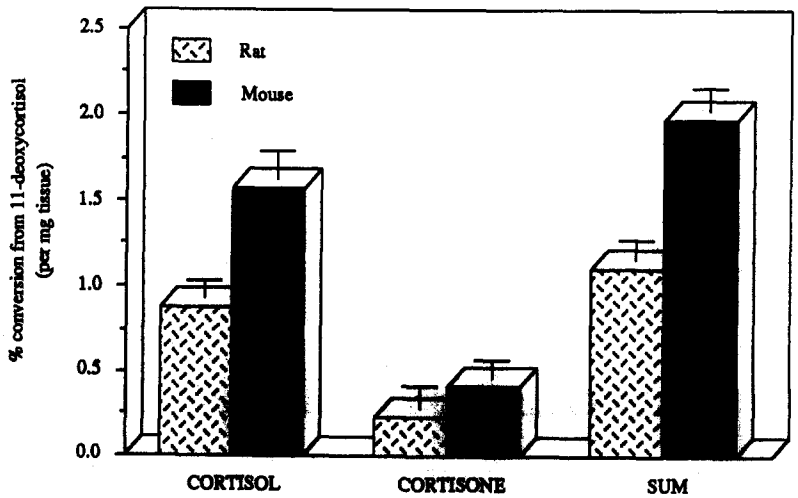

Fig. 1. *In vitro* biosynthesis of cortisol and cortisone from 11-deoxycortisol by mouse and rat adrenals. Incubation lasted for 2 h at 37°C in air. Data are the mean and SD of 10 duplicate incubations. Conversion rates (% conversion per flask) were less than 0.6% in control incubations (without adrenal tissue).

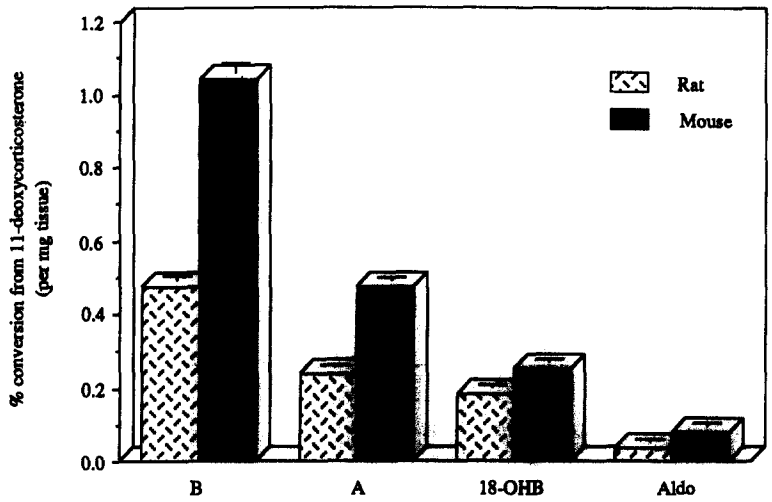

Fig. 2. *In vitro* biosynthesis of mineralocorticoids from 11-deoxycorticosterone by mouse and rat adrenals. Incubation lasted for 2 h at 37°C in air. Data are the mean and range of duplicate incubations. Conversion rates (% conversion per flask) were less than 0.8% in control incubations (without adrenal tissue). B: corticosterone; A: 11-dehydrocorticosterone; 18-OHB: 18-hydroxycorticosterone; Aldo: aldosterone.

of 11β-hydroxylase activity in this pathway, i.e. respectively 0.93 and 1.85% per mg tissue in rat and mouse adrenal incubations.

Using progesterone as a precursor (Fig. 3), the main products found in the incubation media were corticosterone (rat: 0.17; mouse: 0.23% per mg tissue) and cortisol (rat: 0.04; mouse: 0.05% per mg tissue). Respectively in rat and mouse only 0.01 and 0.02% per mg tissue of incubated progesterone were recovered as 17α-hydroxyprogesterone and 0.02 and 0.03% per mg tissue recovered as 11-deoxycorticosterone. But again, taking into account the further metabolite of 17α-hydroxyprogesterone i.e. cortisol provided a better approximation of the activity of 17α-hydroxylase i.e. 0.05 and 0.06% per mg tissue in rat and mouse adrenals incubations respectively.

The addition of Metyrapone (0.1–0.3 μmol) to mouse adrenal incubations resulted in a 54% to 73% inhibition of 11β-hydroxylase activity (cortisol + cortisone), whereas the addition of 0.2 μmol SKF 12185 resulted in a 98% inhibition (Fig. 4).

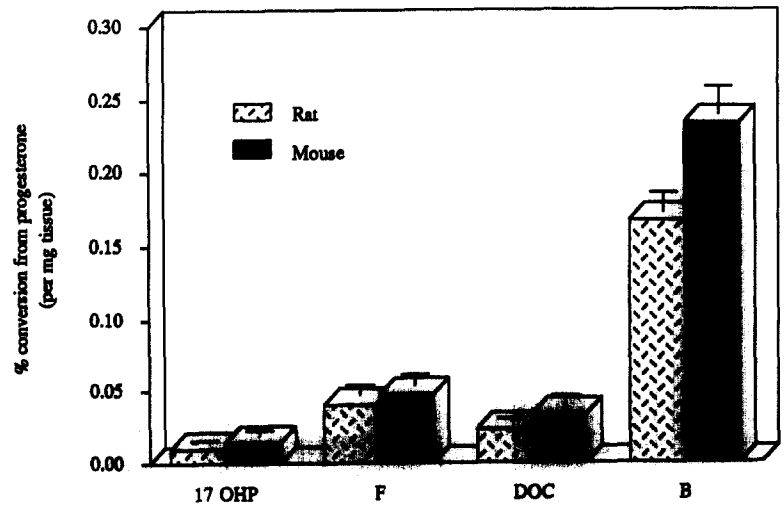

Fig. 3. *In vitro* biosynthesis of 17α-hydroxyprogesterone (17 OHP), cortisol (F), 11-deoxycorticosterone (DOC) and corticosterone (B) from progesterone by mouse and rat adrenals. Incubation lasted for 2 h at 37°C in air. Mouse: mean and range of duplicate incubations; Rat: mean and SD of 3 duplicate incubations. Conversion rates (% conversion per flask) were less than 0.18% in control incubations (without adrenal tissue).

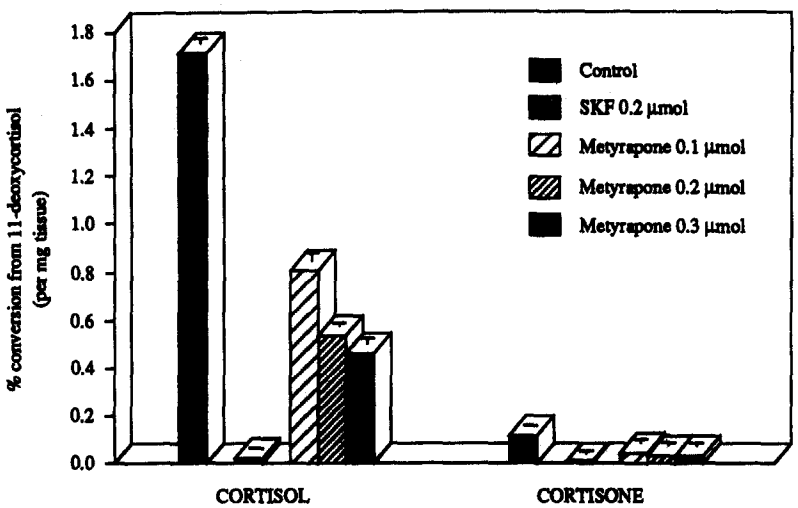

Fig. 4. Effect of SKF and Metyrapone on the *in vitro* biosynthesis of cortisol and cortisone from 11-deoxycortisol by mouse and rat adrenals. Incubation lasted for 2 h at 37°C in air. Data are the mean and range of duplicate incubations. Conversion rates (% conversion per flask) were less than 0.6% in control incubations (without adrenal tissue).

DISCUSSION

Corticosterone is considered as the main glucocorticoid in rat blood whereas cortisol is absent or in negligible amounts in blood of rodents. This has been attributed to the lack of 17α-hydroxylase activity in the adrenals of these species although contradictory results have been published on the matter.

In the present study the synthesis of 17α-hydroxyprogesterone and cortisol from progesterone was 45 and 28% of the synthesis of 11-deoxycorticosterone and corticosterone respectively in rat adrenals and 43 and 20% in mouse adrenals. These results clearly show the presence of an *in vitro* 17α-hydroxylase activity in the studied rodents which agrees with the data from Vinson *et al.* [14], although these authors found lower conversion rates. It has to be noted that neither Nishihara *et al.* [18] nor Perkins *et al.* [19] could find cytochrome *P*-450-17α in rat and mouse adrenals respectively. However the age of the animals must be taken into account. Indeed, these authors have used prepubertal (7–8 weeks) animals whereas our study was performed with glands from adult rats and mice (12 and 30 weeks respectively). Recent studies have shown drastic variations (and even disappearance) of the cytochrome *P*-450-17α and of the synthesis of cortisol in bovine and sheep adrenal cortex during gestation [20, 21]. Lastly, the synthesis of cortisol from progesterone observed in our experiments might be mediated by other enzymes (including cytochrome *P*-450 enzymes) having gratuitous 17α-hydroxylase activity as postulated by Miller [22] about the 21-hydroxylase activity observed by Casey *et al.* [23] in human extraadrenal tissues.

The synthesis of 11-oxosteroids (cortisol + cortisone) from 11-deoxycortisol observed in the present study was similar (rat: 66% and mouse: 81% per incubation flask) to that obtained when incubating, with the same experimental conditions, adrenals of species in which cortisol is the main glucocorticoid, e.g. sheep adrenals [24]. This comes in addition to the data previously published by Baumann and Müller [25]. Thus, the apparent inefficiency of 11β-hydroxylase in mice adrenals as observed *in vivo* from high levels of blood 11-deoxycortisol and very low levels of cortisol was not found here *in vitro* from exogenous tritiated precursors. In addition, the enzyme activity could be impaired by Metyrapone and SKF 12185, two potent inhibitors of 11β-hydroxylase adrenal steroid synthesis. In both species, 11β-hydroxysteroid dehydrogenase was active since cortisone accounted for 21% of the 11-oxosteroids obtained from 11-deoxycortisol and 11-dehydrocorticosterone accounted for 33% of the 11-oxosteroids obtained from 11-deoxycorticosterone.

The *in vitro* conversion of progesterone to 17α-hydroxyprogesterone and cortisol was not negligible as it represented 30% of the conversion to 11-deoxycorticosterone and corticosterone. Since the 11β-hydroxylation of 11-deoxycortisol was apparently even more efficient than that of 11-deoxycorticosterone, the limiting step of cortisol synthesis in these

rodents would be indeed 17α-hydroxylase. The reasons why cortisol is not found in significant amounts in the plasma of rats and mice are not very clear.

Nevertheless this study allows us to point out that both species can apparently be used for studies dealing with cortisol and cortisone metabolism *in vitro* from radioactive exogenous precursors.

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