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HCG + ACTH stimulation of in vitro dehydroepiandrosterone production in human fetal adrenals from precursor cholesterol and Δ^5-pregnenolone

W. D. Lehmann, C. Lauritzen

Department of Obstetrics and Gynecology University of Ulm

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In earlier investigations we have shown that administration of HCG to newborns stimulates the excretion of DHA in the urine of these children [9, 10]. This increase in urinary DHA concentration by HCG stimulation **in vivo** was even higher in prematures than in newborns at term. Perfusion studies by CHARLES demonstrated that HCG also increases the concentration of DHA in umbilical arterial blood in anencephalics [4]. Intrafetal injections of HCG in utero produced histological evidence of hormone biosynthetic stimulation in the fetal adrenals [7]. HCG crosses to the fetus. It is found in higher concentration in the umbilical vein than in the arteries [8, 9]. A rather high concentration of HCG has been found in fetal adrenal tissues by our group [12]. The concentration of HCG in the fetal organism is highest during the 10th—16th week post menstruationem when the fetal adrenals begin to function. The fetal adrenals are synthesizing DHA-S from the 10th—15th week of pregnancy on during which time a much sharper than normal increase in maternal estrogen excretion occurs [6]. The fetal pituitary production of ACTH begins at about the 16th week and increases up to the end of pregnancy [4, 15]. ACTH also stimulates the production of DHA-S and a great number of other Δ^5-steroids and corticosteroids [10]. From these findings we were led to conclude that HCG might be a natural stimulator of the fetal zone X during the midtrimester of pregnancy, before the evolution of the hypothalamic-anterior pituitary axis occurs and ACTH successively takes over the stimulation of the ripening fetal adrenals.

1 Material and methods

All steroids were kindly given by MERCK (Darmstadt). (4-^{14}C)-DHA with a specific activity of 50 mCi/mMol and (4-^{14}C)-cholesterol with a specific activity of 50 mCi/mMol were supplied by the RADIOCHEMICAL CENTER AMERSHAM (Great Britain). All steroids were tested for their purity with paper and thin layer chromatography.

1.1 Reagents

All reagents were of p. a. purity grade from MERCK (Darmstadt). The solvents were distilled before use. HCG and ACTH were commercial preparations from ORGANON Ltd. The following NADPH generation system was used (BOEHRINGER, Mannheim): 3 mg $NADP^+$, 10 mg glucose-6-phosphate, 2.8 I. E. glucose-6-phosphate-dehydrogenase.

1.2 Tissues

19 pairs of fetal adrenals from fetuses between the 12th and 25th weeks of legally interrupted normal pregnancies were used. The time between recovery of the adrenals and the working up of the tissues was not more than 5 to 10 minutes. The adrenal cortex was separated from the medulla with a micro-manipulator. The cortical

tissue was then homogenized with a glass-teflon homogenator. Microsomes were prepared with a Spinco ultracentrifuge. Alternate tissue slices were prepared according to the method of DEUTSCH.

1.3 Incubations

They were performed under air in a 0.15 M SOERENSEN phosphate buffer (pH 7.35) in an agitated thermal bath heated at a constant 37°. The steroids were dissolved in benzene-methanol (19:1) and after evaporation redissolved in 0.02 ml propyleneglycol. The solution was diluted with 0.15 M SOERENSEN phosphate buffer. 1000 nCi (4-^{14}C)-Δ^5-pregnenolone + 100 μg Δ^5-pregnenolone or 1000 nCi (4-^{14}C-cholesterol + 100 μg cholesterol) were incubated with microsomal fractions or with slices for fetal adrenals, both of 0.5 g fresh weight. The NADPH generating system dissolved in 3.0 ml SOERENSEN phosphate buffer was added. The first incubation was done without tropic hormones, the second with 100 I. U. of HCG, the third with 8 I. U. of ACTH, both dissolved in aqua bidest. At the end of the incubation period the solutions were extracted three times with 10 ml ether-chloroform (3:1) and the extracts were combined and then evaporated **in vacuo**.

1.4 Paper-chromatography

All experiments were performed at a temperature of 25—27° Celsius. The residues were redissolved in methanol and chromatographed on propyleneglycol impregnated paper (SCHLEICHER a. SCHÜLL, 2043 bMgl). The first chromatography was done in a system containing propyleneglycol-methylcyclohexane, the subsequent rechromatography in a Bush 6 system.

1.5 Quantitative determinations of the steroids

They were done on paper-chromatograms after measuring radio-activity in the PACKARD-Radiochromatogram-Scanner, model 7201. The counting yield after preparing curves with 4-^{14}C-DHA and (4-^{14}C)-Δ^5-pregnenolone and propyleneglycol impregnated paper amounted to 22% of the substance used.

Tab. I. 1000 nCi (4-^{14}C) **Δ_5-pregnenolone** + 100 μg Δ_5-pregnenolone were incubated with **slices from human fetal adrenals**. The first incubation was done without tropic hormones, the second with 100 I. U. of HCG, the third with 8 I. U. of ACTH.

Age of the fetus week	tissue of adrenals	substrate	addition of tropic hormone to incubate	Δ^5pregnenolone substrate recovered nCi	17 α-OH-Pregnenolone generated nCi	DHA generated nCi
12.	slices from 0,5 g fresh tissue	1000 nCi Δ^5-pregnenolone	without addition	565	154	100
			100 I. U. HCG	365	211	158
			8 I. U. ACTH	490	184	123
15.	slices from 0,5 g fresh tissue	1000 nCi Δ^5-pregnenolone	without addition	388	212	124
			100 I. U. HCG	267	303	177
			8 I. U. ACTH	305	267	140
19.	slices from 0,5 g fresh tissue	1000 nCi Δ^5-pregnenolone	without addition	322	222	156
			100 I. U. HCG	240	392	195
			8 I. U. ACTH	300	297	175
22.	slices from 0,5 g fresh tissue	1000 nCi Δ^5-pregnenolone	without addition	303	299	200
			100 I. U. HCG	122	412	281
			8 I. U. ACTH	195	403	225
25.	slices from 0,5 g fresh tissue	1000 nCi Δ^5-pregnenolone	without addition	260	280	167
			100 I. U. HCG	198	372	204
			8 I. U. ACTH	87	421	297

Tab. II. 1000 nCi (4-^{14}C) Δ_5-**pregnenolone** + 100 μg Δ_5-pregnenolone were incubated with the **microsomal fractions from human fetal adrenals.** The first incubation was done without tropic hormones, the second with 100 I. U. of HCG, the third with 8 I. U. of ACTH.

Age of the fetus week	tissue of adrenals	substrate	addition of tropic hormone to incubate	Δ^5-pregnenolone substrate recovered nCi	17 α-OH pregnenolone generated nCi	DHA generated nCi
12.	microsomal fraction	1000 nCi Δ^5-pregnenolone	without addition	401	301	141
			100 I. U. HCG	420	317	162
			8 I. U. ACTH	385	335	150
15.	microsomal fraction	1000 nCi Δ^5-pregnenolone	without addition	367	313	139
			100 I. U. HCG	352	311	121
			8 I. U. ACTH	277	369	170
19.	microsomal fraction	1000 nCi Δ^5-pregnenolone	without addition	100	508	245
			100 I. U. HCG	110	532	211
			8 I. U. ACTH	91	547	296
22.	microsomal fraction	1000 nCi Δ^5-pregnenolone	without addition	178	473	159
			100 I. U. HCG	162	501	171
			8 I. U. ACTH	112	522	203
25.	microsomal fraction	1000 Δ^5-pregnenolone	without addition	110	467	200
			100 I. U. HCG	98	489	221
			8 I. U. ACTH	52	577	298

Tab. III. 1000 nCi (4-^{14}C) **cholesterol** + 100 μg cholesterol were incubated with **slices or the microsomal fractions** from human fetal adrenals. The first incubation was done without tropic hormones, the second with 100 I. U. of HCG, the third with 8 I. U. of ACTH.

Age of the fetus week	tissue of adrenals	substrate	addition of tropic hormone to incubate	cholesterol substrate recovered nCi	Δ^5-pregnenolone generated nCi	17 α-OH pregnenolone generated nCi	DHA generated nCi
15.	slices	1000 nCi cholesterol	without addition	502	58	67	80
			100 I. U. HCG	400	97	102	120
			8 I. U. ACTH	470	72	87	91
	microsomal fraction	1000 nCi cholesterol	without addition	520	67	92	101
			100 I. U. HCG	541	56	100	89
			8 I. U. ACTH	384	102	165	147
25.	slices	1000 nCi cholesterol	without addition	420	103	99	107
			100 I. U. HCG	312	157	142	160
			8 I. U. ACTH	298	173	156	171
	microsomal fraction	1000 nCi cholesterol	without addition	401	112	123	127
			100 I. U. HCG	419	101	112	104
			8 I. U. ACTH	321	156	176	184

1.6 Characterization of steroids

The chromatographic peaks of the main metabolites Δ^5-pregnenolone, 17α-hydroxypregnenolone and DHA were cut out from paper, washed out with warm methanol and evaporated. The isolated steroids were recrystallized from a benzene-light petroleum mixture to constant specific activity.

2 Results

The addition of HCG resulted in highly significant increases in 17 α-hydroxypregnenolone and dehydroepiandrosterone production from cholesterol and Δ^5-pregnenolone substrate in slices from the 12th week of pregnancy on (Tabs. I, III). The results were statistically significant on the 5% level as compared to controls without added tropins. The effect of adding ACTH to tissues from the 12th—25th weeks was much less than that of adding HCG. In incubations from the 25th week the effect of HCG seemed to decrease while that of ACTH increased. When microsomal fractions were used, no effect of HCG on the generation of 17 α-hydroxypregnenolone and dehydroepiandrosterone from pregnenolone or cholesterol could be seen (Tabs. II, III).

3 Discussion

Our investigations have shown that not only **in vivo** but also **in vitro** HCG has a stimulatory effect on the biogenesis of dehydroepiandrosterone from precursors such as Δ^5-pregnenolone and cholesterol in fetal adrenals. **The stimulatory effect of HCG on the DHA production of fetal adrenals is first noted in the 12th week of pregnancy and increases up to the 22nd week.** It seems that at the 25th week the effect of HCG begins to decrease, while that of **ACTH, which is very low between the 12th—22nd week, increases after the 25th week.** HCG seems to act only when intact adrenal cells are present.

Our results are additional **in vitro** evidence that HCG stimulates the fetal adrenals to produce the estrogen precursor DHA. The circumstantial evidence for this adrenocorticotropic effect of HCG on zone X of the fetal adrenals in midpregnancy has been given in the introduction. There are some other arguments for this probable physiological action of HCG. In anencephalics for example, zone X of the fetal adrenal cortex usually will develop normally up to the fifth month of pregnancy in spite of the absence of an ACTH secretion, while the ACTH dependent outer zones will regress [1, 13, 14]. In tissue incubations of fetal adrenals DHA is only produced for three days without ACTH. Cortisol and DHA are produced when ACTH is added but only from the definite cortex. The fetal zone, however, will regress [2].

Additional and more extensive investigations have been planned in an attempt to answer two important questions: at what point does HCG have an influence in the biogenesis of DHA, and is there a reverse feedback system between fetal adrenal DHA-production and placental HCG?

Summary

Fetal adrenal glands were obtained from legal abortions in the 14—22 gestational weeks. The adrenal cortex was separated from the medulla using a micromanipulator. The cortex was homogenized in a glass-teflon homogenizer and the microsomal fraction was isolated by centrifugation. Tissue slices were also prepared by the method of Deutsch. The microsomal fraction or the slices were incubated with (4-^{14}C) pregnenolone or (4-^{14}C) cholesterol in the presence of an NADPH regenerating system and oxygen. ACTH or HCG was added. After extraction and paperchromatography radioactivity was determined in a scanner.

The conversion of pregnenolone to 17 α-hydroxypregnenolone and dehydroepiandrosterone was increased by ACTH. In the presence of HCG dehydroepiandrosterone production only was increased, — and this occurred in slices only. In the microsomal fraction HCG was without effect on steroid biogenesis from dehydroepiandrosterone.

Keywords: Adrenal cortex (fetal), dehydroepiandrosterone production (fetal).

Zusammenfassung

HCG und ACTH Stimulation der Dehydroepiandrosteron-Produktion der menschlichen fetalen Nebennierenrinde in vitro aus den Präkursoren Cholesterol u. Δ^5-Pregnenolon

Fetale Nebennieren der 14.—22. Gestationswoche wurden durch legale Interruptiones gewonnen. Mit Hilfe eines Mikromanipulators wurde die Rinde vom Mark der Nebennieren getrennt. Das Rindengewebe wurde in einem Glas-Teflon-Homogenisator homogenisiert und durch Ultrazentrifugation die Mikrosomenfraktion gewonnen. Außerdem wurden Gewebeschnitte nach der Methode von Deutsch angefertigt. Das Gewebe (Mikrosomenfraktion oder Schnitte) wurde mit (4-^{14}C) Pregnenolon oder (4-^{14}C) Cholesterol in Gegenwart des NADPH generierenden Systems und Sauerstoff inkubiert. ACTH oder HCG wurden den Inkubationen hinzugefügt. Es erfolgte die Extraktion und die papierchromatographische Auftrennung. Die Messung der Radioaktivität erfolgte im Radiochromatogramm-Scanner.

In den Inkubationen sowohl mit der Mikrosomenfraktion wie auch mit den Gewebeschnitten wurde **die Verstoffwechselung von Pregnolon zu 17 α-Hydroxypregnenolon und Dehydroepiandrosteron durch den Einfluß von ACTH gesteigert.** Wurde dagegen **HCG den Inkubationen hinzugefügt, ließ sich nur bei der Inkubation mit Gewebeschnitten eine höhere Ausbeute von Dehydroepiandrosteron feststellen, wenn Pregnenolon oder Cholesterol als Substrat eingesetzt wurde.** Bei Inkubation mit der **Mikrosomenfraktion hatte HCG keinen Einfluß auf die Steroidbiogenese von Dehydroepiandrosteron.**

Schlüsselwörter: Nebennierenrinde (fetale), Dehydroepiandrosteron-Produktion (fetale).

Résumé

Stimulation par HCG et ACTH de production de déhydroépiandrostérone in vitro dans les capsules surrénales en présence de cholestérol et de Δ^5-prégnénolone

Des capsules surrénales foetales ont été prélevées par interruptions légales entre la 14 ème et la 22 ème semaine de gestation. L'écorce en a été détachée de la moelle à l'aide d'un micromanipulateur. On a alors homogénisé les tissus corticaux dans un homogénisateur de verre téflon et extrait la fraction microsomique par ultracentrifugation. De plus, on a préparé des coupes de tissus en appliquant la méthode de Deutsch. Les tissus (fraction microsomique ou coupes) ont été incubés avec (4-^{14}C) de prégnénolone ou (4-^{14}C) de cholestérol en présence du système générateur de NADPH et d'oxygène. ACTH ou HCG ont été ajoutés aux incubations. On obtint ainsi l'extraction et la séparation chromatographique en papier. La mesure de radioactivité fut effectuée dans le radiochromatogramm-scanner.

Dans les incubations aussi bien avec la fraction microsomique qu'avec les coupes de tissus, **on a observé une transformation de substance accrue du prégnénolone en 17 α-hydroxyprégnénolone et déhydroépiandrostérone sous l'effet de l'ACTH.** Par contre, **en cas d'addition de HCG aux incubations, seules celles des coupes tissulaires ont révélé un taux plus élevé de déhydroépiandrostérone en présence de prégnénolone ou de cholestérol.** Dans les incubations avec **fraction microsomique, le HCG n'a eu aucune influence sur la stéroidbiogénèse du déhydroépiandrostérone.**

Mots-clés: Écorce surrénale foetale, production de déhydroépiandrostérone foetal.

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