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FORMATION OF 2-METHOXYOESTRONE FROM OESTRONE IN HUMAN FOETAL ADRENALS *IN VITRO*

L. STÁRKA, J. ŠULCOVÁ, R. KNUPPEN and O. HAUPT
Research Institute of Endocrinology, Prague, Czechoslovakia; and Department of Biochemical Endocrinology, Medical Academy, Lübeck, Germany

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SUMMARY

When [4-^{14}C]-oestrone is incubated with human foetal adrenals from 9 to 16 weeks of pregnancy, a number of metabolites is detected which include 7α-, 15α- and 16α-hydroxyoestrone. Among the products characterized, 2-methoxyoestrone were identified by paper chromatography in various systems, microchemical reaction and crystallization to constant specific activity.

THE FORMATION of 2-methoxyoestrogens in the human was first reported by Kraychy and Gallagher[1] and 2-methoxyoestrone was isolated from human pregnancy urine by Loke and Marrian[2]. The biogenesis and metabolism of 2-substituted phenolic steroids have been extensively studied by numerous investigators. Recently, it was demonstrated that the enzymatic methylation of catechol amines is strongly inhibited by 2-hydroxyoestrogens[3–5].

We would like to report briefly the formation of 2-methoxyoestrone from [4-^{14}C]-oestrone in human foetal adrenals *in vitro*.

At legal interruptions of pregnancy, 7 foetuses of both sexes between the 5th and 12th weeks of gestation were obtained with forceps and 2 foetuses in the 16th week of gestation by sectio minor. The adrenals were excised, quartered and incubated in 3 ml of Krebs–Ringer phosphate buffer, pH 7·4, containing 20 mmol/l of glucose, with 1 μCi [4-^{14}C]-oestrone (spec. activity 32·8 mCi/mmol) at 37°C under oxygen for 60 min. The mixtures were extracted with ether (twice 6 ml) and the extracts taken to dryness. Chromatography was carried out on paper impregnated with formamide according to Knuppen[6]. Besides oestrone and oestradiol-17β, three polar metabolites were detected and characterized by paper chromatography as 7α- ,15α- and 16α-hydroxyoestrones; these metabolites are known to be formed from oestrone in foetal adrenals or liver as demonstrated previously[7–10].

In addition, a radioactive metabolite, less polar than oestrone was localized in the system formamide/monochlorobenzene. On rechromatography on formamide impregnated paper with cyclohexane, the radioactive material was located in the position of authentic 2-methoxyoestrone. After elution of the radioactive material from the paper, 20 μg of non-radioactive 2-methoxyoestrone was added. The mixture was then treated with sodium borohydride in methanol at room temperature. The product obtained had R_F(0·22) as authentic 2-methoxyoestradiol-17β in the system formamide/cyclohexane. For further identification the

radioactive steroid was then eluted and recrystallized with 10 mg of authentic 2-methoxyoestradiol-17β. The criteria of radiochemical homogeneity was met in samples from foetuses from 8 to 16 weeks of gestation, as demonstrated for sample 9 in Table 1.

The yields of 2-methoxyoestrone after the incubation of [4-^{14}C]-oestrone with quartered foetal adrenals are given in Table 2.

Table 1. Radiochemical evidence for the formation of 2-methoxyoestrone in foetal adrenal. The material was reduced with sodium borohydride and after admixture of non-radioactive 2-methoxyoestradiol-17β crystallized to constant specific activity.

Crystallization No.	Solvent	Crystalls dpm/mg
1	benzene	657
2	benzene	662
3	acetone–light petroleum	625
4	acetone–light petroleum	624

Table 2. The yields of [4-^{14}C]-2-methoxyoestrone after the incubation of [4-^{14}C]-oestrone with quartered human foetal adrenals

Sample No.	Foetus age (weeks)	Foetus sex	Adrenals incubated (mg)	Fraction less polar than oestrone (dpm)	Yield of 2-methoxy-oestrone (dpm)	(%)*
1			blank without tissue	25 200	not found	
2	5	?	2·5	49 700	not found	
3	6	?	3·0	45 800	not found	
4	8	?	11·0	53 200	1 946	0·10
5	9	female	9·5	50 500	2 560	0·12
6	9	male	12·0	52 700	9 740	0·44
7	10	male	14·5	88 000	2 470	0·11
8	12	male	32·0	72 500	19 660	0·89
9	16	male	100·5	103 000	29 464	1·34
10	16	male	105·0	46 200	13746	0·62

*Relative to radioactivity incubated.

The present experiment shows conclusively that foetal adrenal tissue metabolizes oestrone to 2-methoxyoestrone. As it is well known that 2-methoxyoestrogens arise by methylation of 2-hydroxylated oestrogens, the intermediate formation of 2-hydroxyoestrone has to be assumed. The fact that no 2-hydroxyoestrone was detected in the present investigation is probably due to the extreme instability of this catechol oestrogen during conventional chromatographic procedures used[11]. Further evidence for the sequence of reaction is the recent observation of the presence of catechol-*O*-methyltransferase activity in the cytoplasmic fraction of the human foetal adrenals, methylating 2-hydroxyoestrone at the phenolic hydroxyl groups[12]. This finding is in good agreement with Acevedo and Beering[13]; they found that oestradiol-17β is transformed to 2-hydroxy- and 2-methoxyoestrogens by human phaeochromocytoma in high

yield. The formation of catechol oestrogens in adrenal tissue and their subsequent methylation to the corresponding methoxy derivatives might be of physiological relevence with respect to the regulation of catechol amine metabolism within the foetal adrenals.

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