MALATE DEPENDENT SYNTHESIS OF PROGESTERONE IN THE MITOCHONDRIAL FRACTION OF HUMAN TERM PLACENTA

W. BOGUSLAWSKI, J. KLIMEK and L. ŻELEWSKI
Department of Biochemistry, Medical School, Gdańsk, Poland

Received 14 July 1972

1. Introduction

Ryan and co-workers[1] demonstrated the important role of the placental cholesterol side-chain cleavage enzyme system in progesterone production. Mitochondria of human placenta contain an oxidative chain associated with cytochrome P-450 for the cleavage of the cholesterol side chain in the biosynthesis of pregnenolone. This system uses NADPH as the reducing source [2]. Mason and Boyd [3] recently confirmed that an NADPH generating system is essential for progesterone biosynthesis in mitochondria of human term placenta.

Therefore, it seemed of interest to look for a stimulation of progesterone biosynthesis by malate; this might be due to the malic enzyme activity in the mitochondria, which would result in the generation of NADPH – a cofactor essential for the conversion of cholesterol to pregnenolone.

The experimental results presented here provide evidence for a stimulatory effect of malate on biosynthesis of progesterone from cholesterol by placental mitochondrial fraction.

2. Materials and methods

Nicotinamide adenine dinucleotide phosphate (NADP), and its reduced form (NADPH) were obtained from Boehringer Corporation London. Malic acid was supplied by Koch-Light Laboratory. [4-^{14}C]-cholesterol (specific radioactivity 55.8 mCi/mmole), [^{3}H] pregnenolone and [^{3}H] progesterone were obtained from Radiochemical Centre, Amersham. Human term placentae obtained after normal delivery were cut into small slices, washed three times with 0.9% NaCl and minced. The minced tissue was then homogenized in 1 vol 0.25 M sucrose, in a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 600 *g* for 10 min, the supernatant was centrifuged at 10 000 *g* for 20 min to sediment the mitochondria. The mitochondrial fraction was suspended in 0.154 M potassium chloride and centrifuged at 10 000 *g* for 10 min. This washing procedure was repeated twice. All the described operations were carried out between 0–5°. The enzymic assay used in this study was based on the conversion of added [^{14}C] cholesterol to radioactively labelled C_{21} steroids.

The incubations were carried out in duplicate in a total volume of 5 ml at 37° in air with constant shaking. Incubations were commenced by the addition of [^{14}C] cholesterol and terminated by the addition of 10 ml methanol with 5000 dpm of [^{3}H] pregnenolone and 3800 dpm of [^{3}H] progesterone to each flask. Tritiated compounds were added as a check of recoveries. Recovery of progesterone in each sample was calculated on the basis of tritium found in the final product. The protein precipitate was centrifuged down and re-extracted with 15 ml boiling ethyl acetate. 15 ml chloroform was added to the combined extracts and the mixture was centrifuged to separate the organic and aqueous phases. The organic phase was taken to dryness. The residue was chromatographed in the presence of carriers on thin layer plates of silica gel impregnated with rhodamine 6G in the following TLC system: i) methylene chloride–ether (5:2), ii) benzene–ethanol (9:1), iii) benzene–ethyl acetate (3:2). Final crystalization of progesterone and pregnenolone fractions was carried out in n-hexane–acetone. The crystals and mother liquor were counted

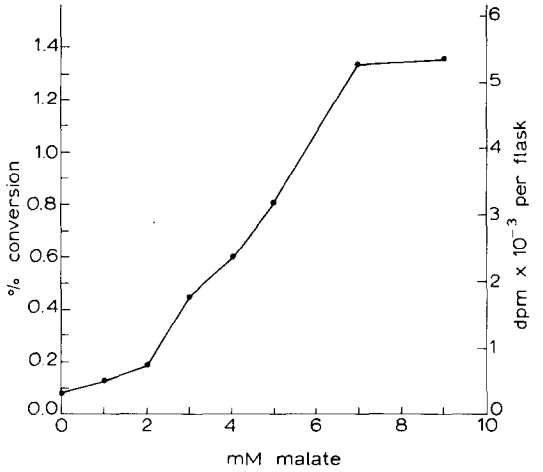

Fig. 1. The effect of malate on [^{14}C]progesterone synthesis in the human placental mitochondrial fraction. The incubation was carried out with 40 mg mitochondrial protein in 5 ml medium of pH 7.4 containing: 0.15 μCi [4-^{14}C]cholesterol, 20 mM phosphate, 10 mM $MgSO_4$, 1 mM $MnSO_4$, 1 mM NADP and appropriate concentrations of malate.

in 10 ml of scintillation fluid containing 4 g of 2.5-diphenyloxazole and 0.2 g of 1,4 di[2(5-phenyloxazolyl)] benzene per liter of toluene. Radioactivity of steroids was measured in a Nuclear Chicago Mark I spectrometer with an efficiency of 49% for ^{14}C and 52% for ^{3}H.

3. Results and discussion

The results presented in fig. 1 show a characteristic dependence on malate concentration for the cholesterol conversion to progesterone. A marked increase of progesterone synthesis is noted in the presence of malate at concentrations from 2 to 7 mM. As may be seen from the data presented in table 1 increased concentrations of NADPH caused also a stimulation of progesterone biosynthesis. [^{14}C]pregnenolone, usually regarded as the product of the cholesterol side-chain cleavage reaction was not detected at the presented level of counting in the presence of NADPH, malate or NADPH plus malate. The results obtained are similar to those observed by Mason and Boyd [3] in the presence of the NADPH generating system. The additive effect of NADPH and malate on the biosynthesis of progesterone, the decrease of progesterone biosynthesis in the presence of malate in the incubation medium deprived of NADP or Mn^{2+}, and the previously presented evidence for malic enzyme activity in placental mitochondria [4], suggest that malate plays an important role in the system regenerating reductive equivalents, which regulates the steroid biosynthesis in human term placental mitochondrial fraction.

Table 1
The effect of triphosphopyridine nucleotides and malate on progesterone and pregnenolone synthesis in human placental mitochondrial fraction.

Incubations	Progesterone biosynthesis					Pregnenolone biosynthesis		
	iii) TLC	Crystals			^{14}C dpm per flask	iii) TLC	Crystals	
	^{14}C/^{3}H	^{14}C dpm	^{3}H dpm	^{14}C/^{3}H		^{14}C/^{3}H	^{14}C dpm	^{3}H dpm
Control	0.081	*	290	*	0	0.097	*	690
+ 0.5 mM NADPH	0.167	50	310	0.160	610	0.200	*	890
+ 1.0 mM NADPH	0.630	210	340	0.617	2340	0.220	*	630
+ 1.5 mM NADPH	1.250	460	400	1.150	4270	0.180	*	810
+ 10.0 mM malate	0.113	*	360	*	0	0.098	*	760
+ 10.0 mM malate, 1.0 mM NADPH	1.320	390	330	1.180	4480	0.138	*	770
+ 5.0 mM malate, 1.0 mM NADP	0.820	-	-	-	3120	0,095	-	-
+ 5.0 mM malate, 1.0 mM NADP, without Mn^{2+}	0.310	-	-	-	1180	0.155	-	-

Incubation conditions as in fig. 1. Other additions or omissions as indicated in the table.
*The counts of ^{14}C did not exceed twice the background values.

The results of this investigation suggest that malic enzyme may be the source of NADPH for the mitochondrial mixed-oxidations function of conversion cholesterol to pregnenolone in the placenta as in adrenal [5] tissue.

Acknowledgements

This work was supported in part by a grant from the Committee of Biochemistry and Biophysics Polish Academy of Sciences.

References

[1] K.J. Ryan, R.A. Meigs and Z. Petro, Amer. J. Obst. Gynec. 96 (1966) 676.
[2] R.A. Meigs and K.J. Ryan, Biochim Biophys Acta 165 (1968) 476.
[3] J.I. Mason and G.S. Boyd, European J. Biochem. 21 (1971) 308.
[4] Z. Aleksandrowicz, J. Swierczyński, W. Bogusławski, J. Klimek and L. Żelewski, Abst. Commun. 9th Meet. Polish Biochem. Soc. (1971) p. 178.
[5] E.R. Simpson and R.W. Estabrook, Arch. Biochem. Biophys. 129 (1969) 384.