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SHORT COMMUNICATION

THE INVOLVEMENT OF THE ELECTRON TRANSPORT CHAIN IN UPTAKE OF TESTOSTERONE BY MEMBRANE VESICLES OF *PSEUDOMONAS TESTOSTERONI*

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SUMMARY

Uptake of testosterone by membrane vesicles of *Pseudomonas testosteroni* is inhibited when examined under anaerobic conditions and in the presence of the electron transport chain inhibitors, sodium amytal and antimycin A. These results indicated the involvement of the electron transport chain in steroid uptake by membrane vesicles.

INTRODUCTION

We have shown that the membrane-bound 3β and 17β-hydroxysteroid dehydrogenase of membrane vesicles isolated from *Pseudomonas testosteroni* is involved in the uptake of testosterone*, dihydrotestosterone, and dehydroepiandrosterone by the vesicles [1]. Kaback[2] has demonstrated that D-lactate dehydrogenase and the oxidation of D-lactate and subsequent transfer of electrons along the electron transport chain can provide the energy required for the uptake of several sugars and amino acids in membrane vesicles prepared from *E. coli*. The uptake of testosterone by membrane vesicles of *P. testosteroni* is not affected by D-lactate but requires NAD^+ and the steroid itself may function as the electron donor [3]. The energy for transport may be derived solely from the activity of the enzyme, as in other group translocation systems [4–6]. On the other hand, the electron transport chain may be linked to the enzyme, 3β and 17β-hydroxysteroid dehydrogenase, as a consequence of the reduction of NAD^+. The effect on steroid uptake of several electron transport chain inhibitors was, therefore, investigated.

EXPERIMENTAL PROCEDURES

Materials

P. testosteroni 11996 was obtained from American Type Culture Collection, Rockville, Maryland; unlabelled testosterone from Steraloids, Pawling, New York; [7^3-H]-testosterone and [1,2-^{3}H]-testosterone from New England Nuclear Corporation, Boston, Massachusetts; NAD^+, antimycin A and rotenone from Sigma Chemical Company, Saint Louis, Missouri; sodium amytal from Eli Lilly & Co. (Canada) Ltd, Toronto, Ontario; 2,4-dinitrophenol from Fisher Scientific Company Limited, Montreal, Quebec; oxygen, therapeutic grade, from Canadian Liquid Air, Montreal, Quebec; and nitrogen, pre-purified, from Matheson of Canada Limited, Whitby, Ontario.

* Trivial and systematic nomenclature: Testosterone; 5α-Dihydrotestosterone; Dehydroepiandrosterone; 17β-hydroxy-4-androsten-3-one; 17β-hydroxy-5α-androstan-3-one; 3β-hydroxy-5-androsten-17-one.

Methods

Media used for growth, conditions for induction of transport activity and the steroid transport assay have been previously described [3, 7, 8]. In inhibitor studies, the inhibitor and membrane protein were pre-incubated at 27°C for 10 min in a vol. of 0.1 ml before the addition of testosterone and NAD^+.

Uptake of testosterone under aerobic and anaerobic conditions was carried out in a final vol. of 6.0 ml. The membrane preparation and a mixture of the buffer, testosterone and NAD^+ were pre-flushed separately with either O_2 or N_2 for 15 min in tubes sealed with serum caps through which 2 hypodermic needles were inserted; one to flush the media and one to provide a vent for pressure release. The reaction was initiated by the addition of the

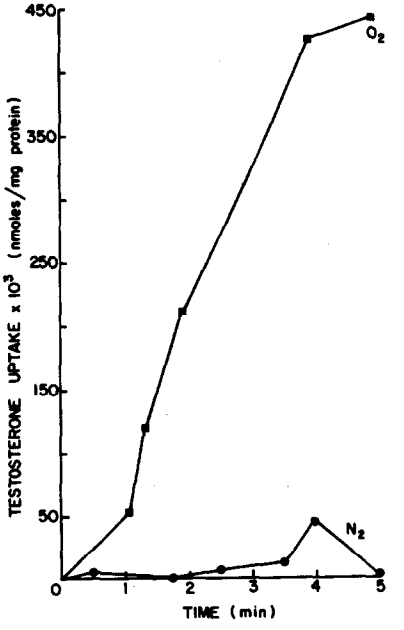

Fig. 1. Effect of anaerobiosis on uptake of testosterone. The experiment was performed as described in Methods.

Table 1. Effect of energy poisons on the 3β and 17β-hydroxysteroid dehydrogenase and on the uptake of testosterone

Addition	Concentration mM	3β and 17β-Hydroxysteroid dehydrogenase Rate nmol NADH/min/mg protein	% Control	Testosterone uptake Rate nmol/min. mg protein	% Control
None		9.27 ± 0.46	100	1.89 ± 0.20	100
KCN	0.5	5.84	63	0.70	37
	1.0	2.32	25	0.38	20
Sodium amytal	12.5	9.27	100	0.70	37
	25.0	10.38	112	0.13	7
Rotenone	0.075	8.06	87	1.68	89
	0.150	4.72	51	1.06	56
Antimycin A	0.1	9.27	100	1.40	74
	0.2	9.54	103	0.87	46
Dinitrophenol	2.5	9.27	100	0.87	46
	5.0	6.95	75	0.72	38

The assays were performed as described in the text. Values shown for 3β and 17β-hydroxysteroid dehydrogenase are means derived from 4 experiments. Values shown for uptake of testosterone are means derived from 6 experiments. Standard errors are shown for the experiments in which no additions were made.

membrane preparation to the mixture of buffer, testosterone and NAD^+. The reaction mixture was sealed with a serum cap through which three hypodermic needles were inserted; the extra needle was used for sampling.

The assay for 3β and 17β-hydroxysteroid dehydrogenase was performed as described by Talalay[9] except that sodium pyrophosphate buffer was replaced with 0.75 ml of 1 *M* Tris–HCl, pH 9.0. In inhibitor studies, inhibitor and membrane protein were pre-incubated at 27°C for 10 min in a vol. of 1.5 ml before the addition of testosterone and NAD^+.

RESULTS AND DISCUSSION

Uptake of testosterone was inhibited when performed under anaerobic conditions (Fig. 1). Respiration, therefore, appeared to be necessary for the transport of testosterone across the vesicle membrane of *P. testosteroni*.

Results recently presented [1, 3] indicated that the activity of the membrane-bound 3β and 17β-hydroxysteroid dehydrogenase is linked to steroid transport. Therefore, an inhibition of the enzyme activity would be reflected in a decrease of the transport capability of the membrane vesicle. To ensure that electron transport chain inhibitors were exerting their effect on components of the electron transport chain and not on the dehydrogenase, metabolic inhibitor studies were carried out on both the 3β and 17β-hydroxysteroid dehydrogenase and on the uptake process (Table 1).

Rotenone which blocks electron transfer from NAD^+ to cytochrome b, caused similar inhibition of both activities. Potassium cyanide, which blocks the terminal cytochrome a + a_3, caused a greater inhibition of testosterone uptake than of the dehydrogenase activity suggesting that another component of transport was being affected by KCN in addition to the 3β and 17β-hydroxysteroid dehydrogenase.

Only sodium amytal and antimycin A caused a decrease in transport activity but no change in the activity of the 3β and 17β-hydroxysteroid dehydrogenase. Sodium amytal acts between NAD^+ and cytochrome b, and antimycin A acts between cytochrome b and c. These inhibitors which act at different points on the electron transport chain, and inhibition of transport of steroids in the absence of oxygen, indicates that the electron transport is involved in steroid transport.

It is known that oxidative phosphorylation is not required for transport of various substrates by membrane vesicles [2]. It is interesting, therefore, that the inhibition by dinitrophenol of steroid transport in membrane vesicles of *P. testosteroni* can be partially accounted for by inhibition of the 3β and 17β-hydroxysteroid dehydrogenase and may reflect a change in membrane conformation.

In conclusion, both the electron transport chain and the 3β and 17β-hydroxysteroid dehydrogenase are involved in steroid uptake by membrane vesicles of *P. testosteroni*. The two activities may be linked *via* the production of NADH and studies are currently in progress in order to investigate the relationship.

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