INHIBITION OF HUMAN LACTATE DEHYDROGENASE ISOENZYMES BY OESTRADIOL-17-β *IN VITRO*

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1. Introduction

Lactate dehydrogenase (LDH) is composed of 2 types of subunits i.e. muscle type (M) and heart type (H) which combine, in most mammalian tissues, into 5 tetrameric isoenzymes (LDH 1–5) [1]. H-type isoenzymes, unlike M-type, are significantly inhibited by excess pyruvate and this has been suggested as a physiological basis for the existence of two types of LDH subunits [2]. There have been relatively few reports on the influence of oestrogens on LDH isoenzymes; Goodfriend and Kaplan [3] found that oestradiol induced the synthesis of M-type LDH in rat uterus. In this communication we show that oestradiol-17-β, (10^{-6} M) selectively inhibits H-type LDH isoenzymes from human placenta and that the inhibition can be reversed by various compounds containing thiol groups.

2. Materials and methods

Normal human placentae were obtained immediately after delivery; cord, chorion and amnion were removed and the remainder was thoroughly washed with cold 0.9% NaCl and either used immediately or stored at $-20°$. Placenta was then homogenized in 1 vol of cold 0.02 M Tris-HCl buffer pH 7.4 in a Sorval omnimix for 2 min at speed 7 and the homogenate was centrifuged at 2° for 25 min at 12,000 *g*. The clear supernatant was freeze-dried and approx. 1,000 I.U. of LDH in 2 ml 0.02 M Tris-HCl buffer was applied to a column (2.8 × 30 cm) of DEAE-Sephadex A-50 and LDH isoenzymes were eluted with NaCl (fig. 1) as described by Fritz et al. [4]. Each isoenzyme fraction was free of other LDH isoenzymes when tested by starch gel electrophoresis for 18 hr [5]. To study the effects of steroids on LDH isoenzymes the following mixture was incubated at 37° for 30 min; 0.5 ml of each isoenzyme (3 mg protein), 1.3 ml 0.02 M Tris-HCl buffer pH 7.4 and 0.15 ml steroid in 1% ethanol. LDH was then assayed spectrophotometrically at 340 nm by adding 0.02 ml NADH (final concentration 3.3×10^{-4} M), to the latter mixture. Control tubes contained 0.15 ml of ethanol without steroid. Enzyme activity is expressed in International Units [6]. Protein was estimated by a modified Biuret method [7].

Oestradiol-17-β and diethystilboestrol (DES) were the purest grades available. The purity of oestradiol and DES was checked by gas chromatography (4 ft glass column; stationary phase, 3% OV210 on diatomite CQ 100–120 mesh, JJS – Chromatography, Kings Lynn, England; N_2 flow rate 50 ml/min; column temp. 260°) and also by thin layer chromatography in 3 different solvent systems [8]. Oestradiol was pure but DES contained a second unidentified component.

3. Results and discussion

Oestradiol (10^{-6} M) inhibited LDH-1, LDH-2 and LDH-3 but not LDH-4 or LDH-5; the inhibition progressively decreased from LDH-1 to LDH-3 (table 1).

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Table 1
Inhibition of human placental LDH isoenzymes by oestradiol-17-β (10^{-6} M)*.

Isoenzyme	Inhibition relative to control** (%)
LDH-1	94
LDH-2	71
LDH-3	25
LDH-4	0
LDH-5	0

* Each isoenzyme was incubated with oestradiol as described in Methods. No oestradiol was added to controls. The above figures represent mean values for LDH isoenzymes purified on separate occasions from 6 normal placentae.
** Activity of control was expressed in International Units/ml (Bergmeyer, 1965).

Table 2
Ability of various substances to protect LDH-1 from inhibition by oestradiol-17-β*.

Substance added with oestradiol	Final concentration in assay mixture (M)	LDH activity as percentage of control**.
Control (buffered enzyme incubated for 30 min with nothing added)		100
Ostradiol alone	10^{-5}	0
NADH	10^{-3}	94
Cysteine	5×10^{-3}	133
Na pyruvate	10^{-3}	0
NAD	10^{-3}	0
ATP	5×10^{-3}	0
Adenine	10^{-2}	0
AMP	10^{-2}	0
EDTA	5×10^{-3}	97

* The above substances were added together with oestradiol (final concentration 10^{-5} M) to the assay mixture described under Methods; the mixtures were then incubated at 37° for 30 min after which time the tubes were assayed for LDH activity. Each figure is the mean of 5 separate experiments.
** Activity of control was expressed in International Units/ml (Bergmeyer, 1965).

Table 3
Ability of various substances to reverse the inhibition of LDH-1 oestradiol-17-β*.

Substance added to inhibited enzyme	Concentration (M)	Enzyme activity as percentage of control**
Control (buffered enzyme incubated for 1 hr with nothing added)		100
Oestradiol alone	10^{-5}	0
Cysteine	5×10^{-3}	96
Glutathione	5×10^{-3}	76
Mercaptoethanol	5×10^{-3}	80
EDTA	10^{-2}	38
NADH	5×10^{-3}	0

* The experimental set up was identical with that in table 2 except the activity of LDH-1 was completely inhibited by incubation with oestradiol (10^{-5}M) at 37° for 30 min before the above substances were added. Each figure is the mean of 5 separate experiments.
** Activity of control was expressed in International Units/ml (Bergmeyer, 1965).

In contrast, none of the following steroids (10^{-6} M) inhibited any of the 5 isoenzymes: oestradiol-17-α, oestradiol-3-sulphate, oestradiol-17-sulphate, oestradiol-3-17-disulphate, oestrone, testosterone, androsterone and dehydro-iso-androsterone. DES (10^{-6} M) however behaved very similar to oestradiol-17-β in that it preferentially inhibited H-type LDH isoenzymes.

The ability of various substances, including NADH and compounds containing thiol groups, to protect and reverse LDH-1 inhibition by oestradiol was determined (tables 2 and 3). The results showed that thiols and EDTA protected and reversed inhibition of LDH-1 by oestradiol. NADH was not as effective as compounds such as cysteine and glutathione and neither did NADH reverse the inhibition by oestradiol or DES. The same thiol compounds also protected and reversed inhibition of LDH-1 by DES. In contrast to the results obtained with oestradiol, EDTA had no effect on the inhibition of LDH-1 by DES.

These results showed that human H-type LDH was specifically inhibited by relatively low concentrations of oestradiol *in vitro*. No inhibition of placen-

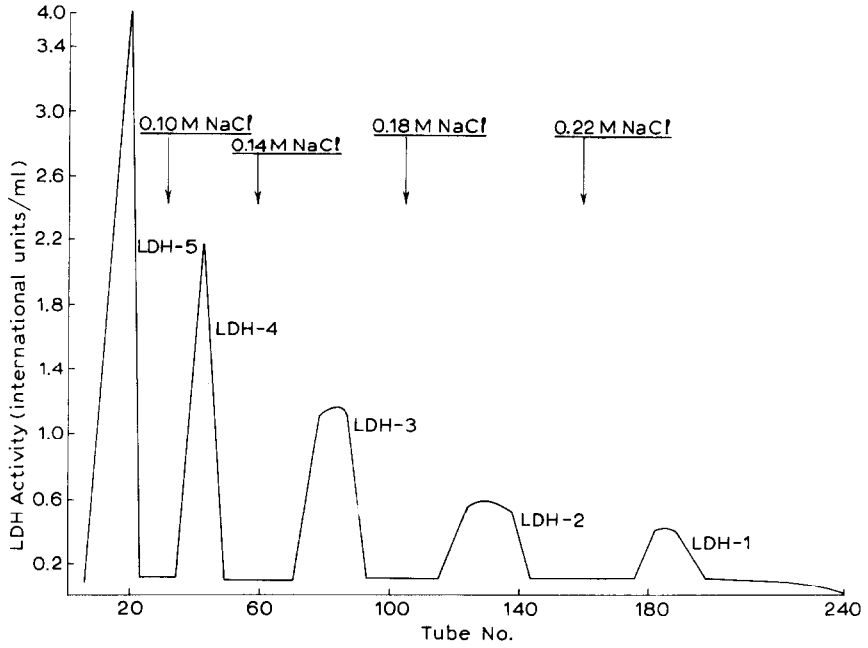

Fig. 1. Chromatography of human placental LDH isoenzymes on DEAE-Sephadex A-50. For experimental details, see text.

tal malate dehydrogenase or glucose-6-phosphate dehydrogenase by oestradiol was detected in these present experiments. Although 10^{-6} M or 10^{-5} M oestradiol was used in all the aforementioned experiments, 20% inhibition of LDH-1 by 10^{-8} M oestradiol was obtained in these studies. The protection and reversal of inhibition by compounds containing thiol groups suggests that inhibition of LDH by oestradiol involves an essential thiol group on the LDH molecule which when modified results in loss of catalytic activity [9].

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