Chemically induced antioxidant-sensitive respiration

Relation to glutathione content and lipid peroxidation in the perfused rat liver

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Received 19 September 1984

The interrelations between the hepatic chemically induced antioxidant-sensitive respiration and the contents of malondialdehyde (MDA) and of reduced glutathione (GSH), were studied in the isolated hemoglobin-free perfused rat liver. Antioxidant-sensitive respiration was induced by the infusion of agents such as ethanol, iron, xanthine or *t*-butyl hydroperoxide, or by phenylhydrazine pretreatment in vivo. The development of this respiratory component occurred concomitantly with high levels of MDA in the perfused livers, while those of GSH were diminished.

Antioxidant-sensitive respiration *Glutathione* *Lipid peroxidation* *Malondialdehyde* *Perfused rat liver*

1. INTRODUCTION

The interaction of several toxic substances with the liver tissue has been shown to involve oxidative reactions associated with the process of lipid peroxidation [1,2]. This process seems to be mediated by free radical species which can arise in the metabolism of the agent, as shown for ethanol [3,4]. In fact, NADPH-dependent microsomal oxidation of ethanol has been recently found to involve, in part, hydroxyl radicals generated by NADPH-cytochrome P-450 reductase [4]. The development of an ethanol induced prooxidative condition in the liver has been clearly established by methodologies which are non-invasive for the tissue. These include significant increments in the ethane production by the perfused rat liver [5], the spontaneous chemiluminescence of the in situ rat liver [6] and in the biliary release of malondialdehyde [7] or glutathione disulfide [8] by the anaesthetized rat.

The addition of antioxidants such as (+)-cyanidanol-3, butylated hydroxianisole or ascorbate to the perfused rat liver was found to diminish the rate of oxygen consumption in 110 to 130 nmol · g liver^{-1} · min^{-1} (basal antioxidant-sensitive respiration) [9]. The infusion of increasing concentrations of ethanol (1.8–72.2 mM) was able to further increase this respiratory component, with maximal rates of 200–255 nmol g · liver^{-1} · min^{-1} [9]. Similar results were obtained with iron and high concentrations of 2,4-dinitrophenol. These basal and chemically induced antioxidant-sensitive respiratory components were suggested to be related to oxygen required for one-electron transfer reactions associated with the production of active species of oxygen and lipid peroxidation in the hepatocyte [9]. This suggestion was supported by the powerful free radical scavenging capacity of the antioxidants used and by their lack of effect on the glycolytic rate of the perfused liver [9] and on mitochondrial respiration [10].

The present study examines the possible interrelationship between the hepatic antioxidant-sensitive respiration, as a novel non-invasive method for detecting pro-oxidative conditions in

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00145793/84/$3.00

the perfused liver, and the content of reduced glutathione (GSH), one of the major cellular systems affording antioxidant protection [3,5]. Also, this respiratory component was correlated with the hepatic content of malondialdehyde (MDA), the typical assay for the measurement of lipid peroxidation [2]. Experiments were carried out following the infusion of ethanol, iron, xanthine or *t*-butyl hydroperoxide to the non-recirculated perfused rat liver, or after phenylhydrazine pretreatment in vivo.

2. MATERIALS AND METHODS

Male Wistar rats weighing 200–250 g (liver/body weight ratio of 3.65 ± 0.08 ($n = 23$); mean ± SE), kept on a standard chow diet and water ad libitum, were anesthetized with 50 mg nembutal/kg (i.p.) prior to surgery. Livers were perfused as in [11], using a perfusion fluid containing 118 mM NaCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$; 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$ and 10 mM glucose, equilibrated with a O_2/CO_2 mixture (19/1, v/v) to give pH 7.4, at constant flow rates (3.6 ± 0.1 ml · g liver^{-1} · min^{-1} ($n = 23$)). Perfusions were carried out at 35–37°C without recirculation of the perfusate. The oxygen uptake was measured polarographically [12] in the effluent perfusion fluid collected via a cannula placed in the vena cava and flowed past a Clark-type oxygen electrode. Livers were perfused for 15–20 min prior to the addition of the agents under study. Ethanol (45 mM) (fig.1), iron (200 μM iron-dextran-Imferon, Fisons), xanthine (0.5 mM) or *tert*-butyl hydroperoxide (0.5 mM) were added to perfused livers either in the absence or presence of 2 mM (+)-cyanidanol-3 as in [9]. The effect of phenylhydrazine was assessed in livers from rats treated with daily doses of 25 mg/kg (i.p.) for 3 days, perfused without and with 2 mM (+)-cyanidanol-3. The content of hepatic GSH [13] and of MDA [14] were determined in tissue samples (80–120 mg) obtained 25 min after the addition of the agents under study, at which time changes in oxygen uptake reached a new steady-state condition (fig.1). As previously defined [9], antioxidant-sensitive respiration corresponds to the difference in oxygen uptake in the absence and presence of 2 mM (+)-cyanidanol-3, during either the control period (no addition) or 25 min after the addition of the agents under study (fig.1). Lactate

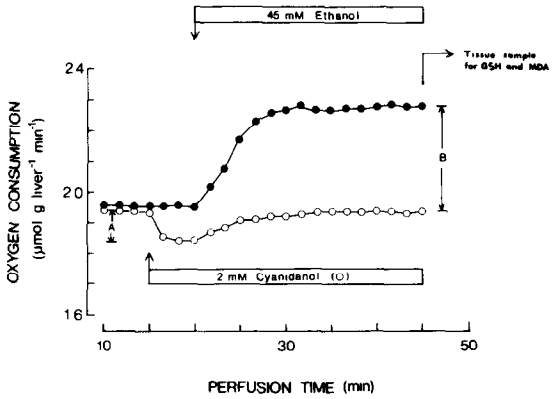

Fig.1. Effect of ethanol on the oxygen consumption by perfused rat liver in the absence (●) and presence (○) of 2 mM (+)-cyanidanol-3. B–A corresponds to the ethanol-induced (+)-cyanidanol-3-sensitive respiration.

dehydrogenase activity [15] in the effluent perfusate (50–200 munits · g liver^{-1} · min^{-1}) was found to be less than 0.2% of activity in the tissue (160–175 units · g liver^{-1}). Proteins were determined as in [16]. Results are expressed as means ± SE.

3. RESULTS AND DISCUSSION

A typical experiment showing the effect of ethanol on the rate of oxygen consumption by livers perfused in the absence and presence of 2 mM (+)-cyanidanol-3 is presented in fig.1. As can be observed, following 15 min of stabilization, the infusion of 45 mM ethanol enhanced hepatic oxygen uptake from 1.95 to 2.28 μmol · g liver^{-1} · min^{-1}, representing an increase of 330 nmol · g liver^{-1} · min^{-1}. In the presence of 2 mM (+)-cyanidanol-3, the enhancement in hepatic respiration induced by 45 mM ethanol amounted to 100 nmol · g liver^{-1} · min^{-1} (from 1.84 to 1.94 μmol · g liver^{-1} · min^{-1}; fig.1). From these results, a net ethanol-induced (+)-cyanidanol-3-sensitive respiration of 230 nmol · g liver^{-1} · min^{-1} can be calculated (B–A in fig.1). Significant increases in the antioxidant-sensitive respiration were also observed when perfused livers were exposed to 200 μM iron, 0.5 mM xanthine or 0.5 mM *t*-butyl hydroperoxide (table 1), agents known to be associated with free radical-related reactions [7,10,17,19]. Furthermore, livers from animals treated with phenylhydrazine in vivo, a powerful

Table 1

Chemically-induced antioxidant-sensitive respiration, malondialdehyde formation and glutathione content in the non-recirculated hemoglobin-free perfused rat liver

Condition	Antioxidant-sensitive respiration (nmol · g liver^{-1} · min^{-1})	MDA formation (nmol · mg protein^{-1})	GSH content (μmol · g liver^{-1})
Control (4)	113 ± 8	32.0 ± 3.8	4.71 ± 0.16
Ethanol (45 mM) (4)	226 ± 11[a] (100)	47.2 ± 3.4 (48)	2.40 ± 0.66[b] (−49)
Iron (200 μM) (4)	313 ± 15[a] (177)	91.0 ± 4.1[a] (184)	1.76 ± 0.16[a] (−63)
Xanthine (0.5 mM) (4)	256 ± 17[a] (126)	43.9 ± 1.9[c] (37)	2.45 ± 0.11[b] (−48)
t-Butyl hydroperoxide (0.5 mM) (4)	259 ± 14[a] (129)	89.5 ± 3.7[a] (180)	1.08 ± 0.07[a] (−77)
Phenylhydrazine (3)	290 ± 12[a] (157)	64.1 ± 2.3[b] (100)	1.46 ± 0.30[a] (−69)

Livers were perfused as described in section 2. The basal rate of oxygen consumption was 1.98 ± 0.05 (n = 20) μmol · g liver^{-1} · min^{-1}. Basal (control) antioxidant-sensitive respiration was determined in livers perfused without and with 2 mM (+)-cyanidanol-3 [9]. Due to the biphasic effect of *t*-butyl hydroperoxide on hepatic respiration [10], the antioxidant-sensitive respiratory component elicited by this agent was assessed after the infusion was ended. Significance studies for the differences between the experimental and the control situation were carried out by Student's *t*-test for unpaired: [a]$P < 0.001$; [b]$P < 0.01$; [c]$P < 0.05$. Values in parentheses indicate the percentage change with respect to control values

hemolytic agent also associated with free radical reactions [20], showed a marked enhancement in the hepatic antioxidant-sensitive respiration (table 1). The chemically-induced antioxidant-sensitive oxygen consumption found in these experimental conditions was observed concomitantly with a significant enhancement in the hepatic content of MDA (table 1), assayed in tissue samples obtained after 45 min of perfusion as indicated in fig.1. These parameters were shown to be significantly correlated (fig.2B), suggesting that the chemically-induced antioxidant-sensitive respiratory component observed must be related to the oxygen required in reactions which initiate lipid peroxidation and/or in those which occur in the process itself.

The development of a pro-oxidative condition in the liver by the agents under study was paralled by a diminished hepatic GSH content (table 1). Moreover, a significant inversed correlation between the antioxidant-sensitive respiratory rates and GSH content was found (fig.2A), indicating an increased GSH demand of the liver tissue to cope with the pro-oxidative conditions imposed. These data further support the contention that the lipid peroxidative process and the GSH protective system of the liver cell are closely interrelated, as found for aliphatic alcohols [21,22] acetaldehyde [22] and iron [7] pretreatments in vivo.

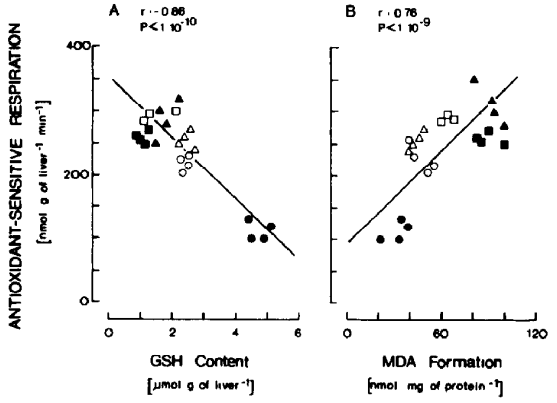

Fig.2. Correlation between chemically-induced antioxidant-sensitive respiration and hepatic GSH content (A) or MDA formation (B). Individual values from average data shown in table 1 were used: control (●), 45 mM ethanol (○), 200 μM iron (▲), 0.5 mM xanthine (△), 0.5 mM *t*-butyl hydroperoxide (■) and phenylhydrazine (□).

ACKNOWLEDGEMENTS

This research was supported by the Departamento de Investigación y Bibliotecas, Universidad de Chile, grant B-1860-8414. The excellent technical assistance of Carmen Almeyda and Felix González is acknowledged. The secretarial assistance of Rosa Garrido is also acknowledged.

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