IN VIVO COOPERATION BETWEEN HEPATIC CATALASE AND SUPEROXIDE DISMUTASE DEMONSTRATED BY DIETHYLDITHIOCARBAMATE

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1. Introduction

Despite impressive studies of superoxide dismutases (SOD), the actual extent of O_2^- production in living cells remains unknown [1,2]. Exceptions are granulocytes and alveolar macrophages which release superoxide anions during phagocytosis [3].

Catalase was first described in 1812 [4]; nevertheless, it is still debatable whether this enzyme is essential for life in mammalian tissues. In the liver of the anaesthetized rat that hydrogen peroxide is produced and binds to catalase [5]; however, the source of this H_2O_2 is undetermined.

We have examined the presence of the catalase–peroxide complex (compound I) in the liver of unanaesthetized guinea pigs, by means of the irreversible inhibition of catalase by 3-amino-1,2,4-triazole (AT) administration in vivo. This inhibitor only binds to compound I and not to free catalase [6]. Further to this, the source of hydrogen peroxide was studied by administration of inhibitors of several peroxide producing oxidases. The results indicate that peroxisomal as well as cytoplasmic catalase is active in vivo, and that in unstimulated and physiological conditions superoxide dismutase is providing part of the hydrogen peroxide. Preliminary results have been reported [7].

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2. Material and methods

Male DHPL guinea pigs (300–400 g) had free access to food and water during the experiment. AT 1 g/kg body wt was injected intraperitoneally without anaesthesia, and the animals were sacrificed after 0.5, 1,2,3,4 and 5 h. Catalase activity was assayed in total liver homogenate with the titaniumoxysulfate method [8].

The following enzyme inhibitors were administered by a single injection 1 h prior to AT administration: diethyldithiocarbamate (1.2–1.5 g/kg intraperitoneally), an inhibitor of superoxide dismutase [9–11]; oxonic acid (250 mg/kg i.p.) [12] and trichloropurine (50 mg/kg s.c.) [13], both inhibitors of urate oxidase; allopurinol (150 mg/kg s.c.), an inhibitor of xanthine oxidase [14,15]; iproniazide (50 mg/kg i.p.), an inhibitor of monoamine oxidase [16]; atebrine (100 or 200 mg/kg i.p.), an inhibitor of D-amino acid oxidase [17] and glycolate oxidase [18]; metyrapone (150 mg/kg s.c.), an inhibitor of the cytochrome P450-dependent monooxygenase system [19,20].

3. Results

After AT administration, liver catalase activity declines exponentially, reaching 1.6% after 5 h (fig.1).

When diethyldithiocarbamate (DDC), an inhibitor of superoxide dismutase, is given prior to AT, catalase activity after 4 h is 19%, showing that part of the enzyme is in the free form (table 1).

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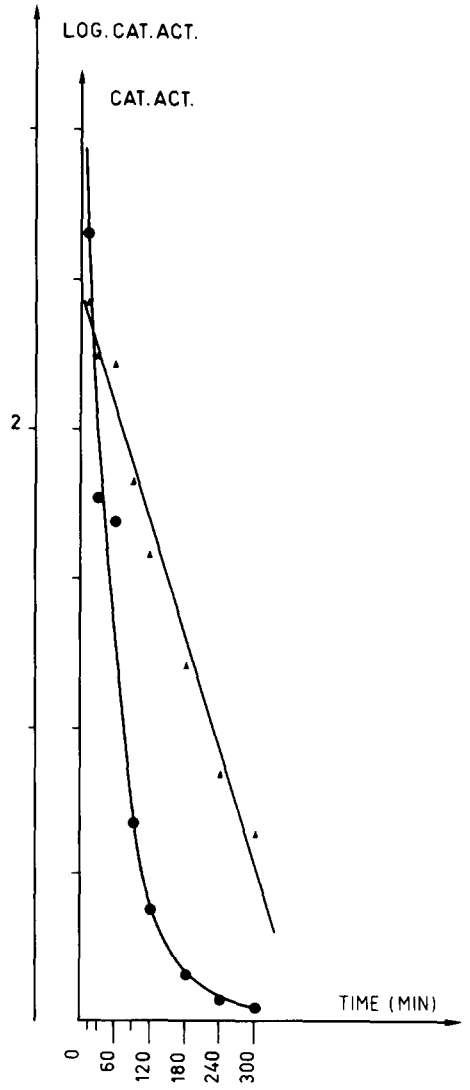

Fig.1. Catalase activity in total liver homogenate after administration of AT: (•) activity expressed in units/g fresh liver; (▲) log of these values.

4. Discussion

The inhibition by AT shows that most of the hepatic catalase is present as compound I in physiological conditions; in the rat similar conclusions were reached [21] and in the anaesthetized animal [5]. The presence of sufficient amounts of H_2O_2 is not a mere result of the enzyme inhibition, because the AT effect is roughly proportional to the amount of active catalase (fig.1).

Table 1
Total catalase activity

	Controls	AT (4 h)	AT + DDC (4 h)
Units/g[a]	267.5 ± 7.8	6.9 ± 1.8	50.4 ± 11.9
%	100	2.6	18.8

[a] Means ± SEM; the difference between AT and DDC is significant $0.01 < p < 0.02$

Unlike the situation in the rat, between 56% and 87% of the hepatic catalase in the male guinea pig is localized in the cytosol, the remainder being peroxisomal enzyme. This was calculated from microspectrophotometric data after diaminobenzidine staining for catalase [22]. As a consequence these results prove for the first time that cytoplasmic catalase is provided with substrate under physiological conditions.

Inhibition of SOD is accompanied by a substantial decrease of hydrogen peroxide production, as shown by an increase in free active catalase. Although DDC also inhibits 2 other enzymes, glutathione (GSH) peroxidase [23] and aldehyde dehydrogenase [24], this cannot be the cause of the increased catalase activity. Catalase itself is unaffected by DDC [10,23,25].

The importance of H_2O_2 production by hepatic SOD is probably underestimated because inhibition by DDC is only 71% [9], and also because part of the peroxide possibly reacts with GSH peroxidase and thus escapes detection. The latter enzyme apparently is not implicated in destruction of endogenous H_2O_2 in isolated rat hepatocytes [26].

The efficient cooperation between catalase and SOD must be facilitated by the localisation of both enzymes in the cytosolic compartment of the parenchymal cell. A small amount of SOD is also found in the mitochondria, but this belongs partly to the Mn-type SOD which is not affected by DDC [27–31].

The activities of catalase and SOD have been compared in vitro; the results were not in favour of the existence of cooperation [32–37].

The advantage for the cell of such cooperation can be assumed on the basis of in vitro experiments: H_2O_2 inactivates SOD [38,39]; co-immobilization of catalase and glucose oxidase protects the latter from deactivation by its own reaction product, H_2O_2 [40]; co-immobilization of catalase, SOD and xanthine oxidase similarly prolongs the activity of the latter

enzyme [41]. However, the activity of catalase leads to conservation of O_2, a function that should be particularly valuable under conditions of ischemia.

The lack of any demonstrable effect on catalase after administration of inhibitors of other oxidases, does not necessarily mean that those are inactive in the living cell. The degree of inhibition by some of these substances has been tested only in vitro (atebrine [17,18]; trichloropurine [13]; metyrapone [19,20]). Inhibition of cytochrome P450 by metyrapone is <50% if animals were not pretreated with phenobarbital [19,20]. The effect of oxonic acid on urate oxidase attains its maximum (50% inhibition) after 30 min and then levels off [42]. Finally, H_2O_2 can also be metabolized by GSH peroxidase. This might well explain the negative result during inhibition of monoamine oxidase, an enzyme that is localized in the mitochondria, as is GSH peroxidase. In perfused rat liver an increase in GSH oxidation was demonstrated during perfusion of an exogenous amine; there was no effect on catalase [43].

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