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REDUCTION OF ADRENOCHROME BY RAT LIVER AND BRAIN DT-DIAPHORASE

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Liver and brain exhibit DT-diaphorase activity with adrenochrome as a substrate; the latter is an *o*-quinone derived from the autoxidation of adrenaline exhibiting neurotoxic and cardiotoxic properties. The reaction is strongly inhibited by dicoumarol, a classical inhibitor of DT-diaphorase. DT-diaphorase-reduced adrenochrome undergoes autoxidation as shown by the oxygen uptake occurring during the reaction.

It is proposed that, physiologically, DT-diaphorase might exert a protective role by maintaining adrenochrome in its reduced, non-toxic form.

KEY WORDS: Adrenochrome, DT-diaphorase, quinones.

ABBREVIATIONS: DTPA, diethylenetriamine pentaacetic acid; SOD, superoxide dismutase.

INTRODUCTION

Adrenochrome is an *o*-quinone, derived from the autoxidation of adrenaline, which exhibits neurotoxic and cardiotoxic properties attributable to its interaction with sulfhydryl groups and to the production of oxygen free radicals.[1]

In previous research it was shown that adrenochrome undergoes a one-electron reduction after interaction with the mitochondrial respiratory chain and with the microsomal electron transport chain[2,3] with the concomitant formation of the corresponding semiquinone; consequently, in the presence of oxygen, a redox cycling takes place giving rise to the superoxide anion which subsequently dismutates to hydrogen peroxide. In the present paper the reduction of adrenochrome by NADPH in the presence of the enzyme DT-diaphorase was investigated. DT-diaphorase (NAD(P)H: quinone oxidoreductase, EC 1.6.99.2) is able to reduce several substances, particularly quinones, to the respective reduced form in a two-electron process.[4] DT-diaphorase acts on *p*-quinones, *o*-quinones and also on non-quinonic substrates; benzo- and naphthoquinones, particularly those lacking a side-chain are the most active electron acceptors.[4] 1,4-Naphthoquinone is a better substrate than 1,2-naphthoquinone but the latter is more effective than *p*-benzoquinone.[4] On this ground we have postulated that also adrenochrome might possess structural features for acting as a substrate for this enzyme.

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MATERIALS AND METHODS

DT-diaphorase was prepared from rat liver or brain homogenates essentially as described by Ernster.[5] After differential centrifugations of the 0.25 M sucrose homogenates, the 105,000 × g supernatant ("cytosol") was utilized and fractionated with ammonium sulphate; the 55%–65% fraction was dialysed against 20 mM Tris buffer (pH 7.4) in order to remove the ammonium sulphate. Protein were measured as described by Lowry *et al.*[6] NADPH oxidation was monitored at 340 nm (ε_M: 6,220 $M^{-1} cm^{-1}$), while adrenochrome reduction was followed at 480 nm (ε_M: 4,470 $M^{-1} cm^{-1}$).[7] Oxygen uptake was measured polarographically with a Clark-type oxygen electrode.[8] All experiments were performed at 37°C.

RESULTS

As reported in Table I, adrenochrome acts as a substrate for the enzyme DT-diaphorase obtained from both rat liver and brain. The stimulation of NADPH oxidation is strongly inhibited (ca. 80%) by dicoumarol, which is the best known inhibitor of DT-diaphorase activity[4] indicating the specificity of the reduction process. Nevertheless the reduction rate of adrenochrome, in the experimental conditions used, is less marked (30 nmol · min^{-1} · mg^{-1}) if compared to that obtained when the classical quinones are used as substrates. In fact, the specific activities of duroquinone and naphthoquinones are more than one order of magnitude higher than that measured for adrenochrome (Table I). In the brain the DT-diaphorase activity of adrenochrome is still present, but lower than that found in the liver, in agreement with previous results.[5]

It has been reported by Cadenas *et al.*[9] that several quinones, after DT-diaphorase-mediated reduction, can undergo autoxidation in a process which produces hydrogen peroxide and regenerates the oxidised form of the quinone. Since adrenochrome in its reduced form (leucoadrenochrome) is easily oxidised, we have comparatively measured NADPH oxidation, adrenochrome reduction and oxygen consumption either in the presence or in the absence of DTPA, (Figure 1). It is apparent that, particularly in the absence of DTPA, the amount of NADPH oxidised is larger than the extent of adrenochrome reduction, indicating that autoxidation of reduced adrenochrome (leucoadrenochrome) occurs. In fact, more than 60 nmoles of NADPH are consumed in the fast phase of its autoxidation, while under the same conditions only about

TABLE I
Specific activities of rat liver and brain DT-diaphorase in the presence of different quinones

	LIVER (nmoles · min^{-1} · mg^{-1})	BRAIN (nmoles · min^{-1} · mg^{-1})
Menadione	1,200	600
Duroquinone	1,100	350
1,4-Naphthoquinone	990	447
1,2-Naphthoquinone	650	270
Adrenochrome	28	4

The reaction mixture consisted of 50 mM Na–K phosphate buffer (pH 7.4), 0.2 mM NADPH and 50 μM quinonoid compounds. Liver protein were 0.70 mg/ml for adrenochrome and 60 μg/ml for the other quinones; brain protein were 0.74 mg/ml for adrenochrome and 60 μg/ml for the other quinones.

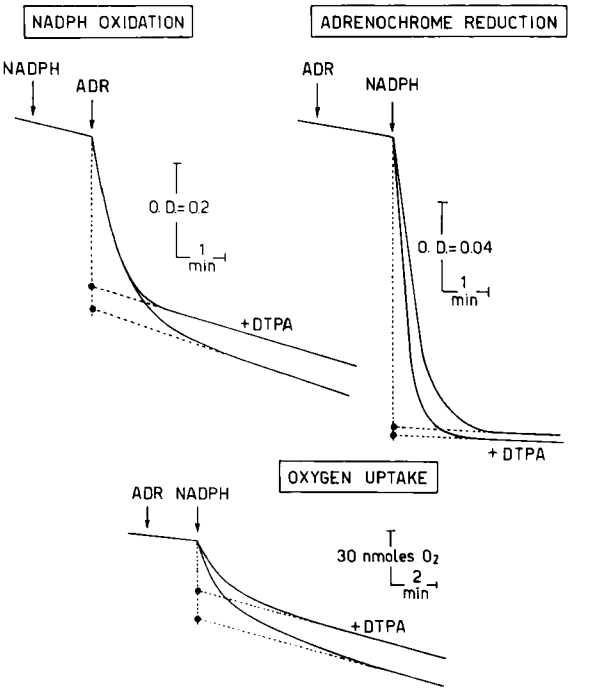

FIGURE 1 Simultaneous measurement of NADPH oxidation, adrenochrome reduction and oxygen uptake during the action of DT-diaphorase on adrenochrome. The reaction mixture was formed by 50 mM Na–K phosphate buffer (pH 7.4), 3 mg/ml protein, 0.2 mM NADPH and 50 μM adrenochrome; when indicated, also 1 mM DTPA was added.

28 nmoles of adrenochrome are reduced; correspondingly an O_2 uptake of approximately 40 nmoles is recorded. Moreover, in the presence of millimolar DTPA, oxygen uptake still occurs but to an extent lower than that observed in the absence of DTPA, indicating that other processes might take place in addition to autoxidation. For instance, a reverse dismutation between leucoadrenochrome and adrenochrome could occur, giving rise to the semiquinone form that accounts for the consumptions of oxygen.

The exclusion of oxygen from the reaction mixture decreases the rate of NADPH oxidation and particularly the slow phase of the reaction (not shown).

DISCUSSION

The main catabolic pathways of adrenaline depend on monoaminoxidase and catechol-*o*-methyltransferase activities. Nevertheless, adrenaline can also undergo autoxidation followed by cyclization giving rise to an unstable leucoadrenochrome that rapidly oxidises to adrenochrome. The latter can be further metabolised to adrenolutin, which is considered to be toxic or, alternatively, can undergo dehydration to a reportedly non-toxic 5,6-dihydroxyoxy-N-methylindole.[10] DT-diaphorase might then exert a protective role by maintaining adrenochrome in its reduced, *i.e.*

non-toxic, form. The presence of this enzyme might be particularly important in the brain where the metabolism of catecholamines is quite active. The presence of DT-diaphorase activity in the brain was described by Giuditta *et al.*[11] and its presence in various regions of rat brain was also detected.[12] A possible role of the enzyme in catecholaminergic cell connected to the production of stable and easily conjugatable catechol hydroquinones, thus preventing free radical formation from semiquinones, was suggested.[12]

DT-diaphorase acting as a two-electron reductase plays an important role in preventing the formation of semiquinones and the associated production of oxygen reduced forms.[13] Recently Cadenas *et al.*[9] found a new activity displayed by superoxide dismutase involving the reduction of a semiquinone by the superoxide anion. *p*-Quinones appear to be detoxified by the combined action of DT-diaphorase and superoxide dismutase. On the contrary, *o*-quinones such as 1,2-naphthoquinone behave in the opposite way, and SOD appears to stimulate the autoxidation instead of inhibiting it.[9] Adrenochrome autoxidation appears only slightly inhibited by SOD under our experimental conditions (not shown); on the other hand, we used a partially purified preparation of DT-diaphorase, probably contaminated by SOD.

Similarly to adrenochrome, also the various catecholamines can undergo autoxidation and cyclization to the corresponding aminochromes; the role of DT-diaphorase on the reduction of these endogenously formed quinones, particularly at the level of the central nervous system, appears to be worthy of further investigation.

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