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Production of Reduced Forms of Oxygen by Adrenochrome in the presence of Ascorbate, Microsomes and Submitochondrial Particles.

A. Bindoli, M.P. Rigobello and L. Galzigna
Centro Studio Fisiologia Mitocondriale, CNR and Istituto di Chimica Biologica, Università di Padova (Italy).

Abstract

Adrenochrome, an oxidation product of adrenaline with psychotomimetic properties, can be reduced by ascorbate in a reaction that shows a rapid oxygen consumption. Superoxide dismutase and catalase strongly inhibit this oxygen uptake. Similarly, the addition of adrenochrome to rat liver microsomes or beef heart submitochondrial particles supplemented with reducing substrates determines a large consumption of oxygen. The reported results are compatible with a redox cycle where reducing equivalents supplied by ascorbate or the electron transport chains of microsomes or mitochondria are able to reduce adrenochrome which is subsequently reoxidized by oxygen with the production of superoxide anion and hydrogen peroxide.

Introduction

Catecholamines can easily undergo an oxidative cyclization to indole-5,6-quinones, generally referred to as aminochromes. In particular adrenochrome is the oxidation product of adrenaline and the adrenaline-adrenochrome transformation can also be induced by the superoxide anion (1).

Adrenochrome has been reported to be involved in the development of some forms of mental illness (2) possibly after formation of a complex with acetylcholine (3). Some toxic properties of adre-

nochrome appear to depend on the formation of adrenolutin, while its reduction to 5,6-dihydroxy N-methyl indole is thought to eliminate its toxicity. According to an old hypothesis (4), schizophrenia might in fact result from an alteration of the balance between the two pathways. Although the speculation concerning the pathogenetic role of adrenochrome in the malfunctioning of the central nervous system hitherto escaped any attempt of an experimental verification, the physiological role of adrenochrome and other aminochromes in the biosynthesis of melanins is well established (5). Despite this, the mechanism of redox changes of the aminochrome molecule is still rather poorly understood.

In the present communication we wish to describe the interaction of adrenochrome with ascorbate and the involvement of the microsomal and mitochondrial electron transport chains in its reduction. This occurs in the presence of reducing equivalents and is accompanied by a marked oxygen uptake.

Materials and Methods

Rat liver microsomes were prepared essentially as described by Ernster and Nordenbrand (6) and beef heart submitochondrial particles according to the procedure "3" of Smith (7). Protein content was estimated by the biuret method (8) and oxygen uptake was followed with a platinum electrode assembly of the Clark type (9).

Results and Discussion

Several years ago it has been reported that ascorbate is able to reduce adrenochrome (10, 11). In addition, concomitantly with this reduction, a rapid oxygen uptake occurs as observed by Galzigna (12). The oxygen uptake (Table 1) increases by increasing pH and does not depend on an autoxidation of ascorbate catalyzed by contaminant metals since, in our conditions, the oxidation of ascorbate in the absence of adrenochrome is negligible (not shown).

Table 1. Effect of superoxide dismutase, catalase and antioxidants on ascorbate-induced stimulation of oxygen uptake by adrenochrome.

Additions	Oxygen uptake (nmol O_2/min•mg prot)	% Inhibition
None	120	-
Superoxide dismutase	95	21
Catalase	79	34
Superoxide dismutase + Catalase	39	67
Butylated hydroxytoluene	110	8
Chlorpromazine	108	10
Adrenochrome semicarbazone	6	-

Reactions were carried out in 50 mM K-phosphate buffer (pH 7.4) at 25°C, in the presence of 8 mM ascorbate and 40 μM adrenochrome. When indicated were also added: 130 μg/ml superoxide dismutase, 70 μg/ml catalase, 0.04 mM butylated hydroxytoluene and 0.8 mM chlorpromazine. When adrenochrome semicarbazone (40 μM) was added, adrenochrome was omitted.

Consequently, the observed oxygen uptake appears to arise from an autoxidation of a partially reduced adrenochrome free-radical intermediate. The latter form may autoxidize with the production of superoxide anion which, secondarily, after dismutation, originates hydrogen peroxide. In fact either superoxide dismutase or catalase (or the combination of the two) strongly inhibit oxygen consumption (Table 1) and this can be explained on the basis of a continuous restoration of the oxygen in the reaction vessel, due to the dismutation of superoxide or to the catalase activity. On the other hand, typical antioxidants such as butylated hydroxytoluene (BHT) or chlorpromazine appear to be scarcely effective since their inhibitory effect does not exceed 10% (Table 1). When adrenochrome semicarbazone (where an imino group substitutes a quinone moiety) is used instead of adrenochrome, almost no oxygen uptake occurs in the presence of ascorbate, indicating that the o-quinone structure is essential for the phenomenon.

The redox cycling, which we postulate as an explanation of such phenomenon, is not restricted to the chemical system ascorbate-adrenochrome but also occurs when adrenochrome is added to rat liver microsomes in the presence of NADH and even more in the presence of NADPH (Table 2). In the latter case, the microsomal NADPH-cytochrome P-450 reductase evidently delivers electrons that are able to reduce adrenochrome to its semiquinonic form. The consumption of oxygen, in excess if compared to the amount of the adrenochrome present, is once again suggestive of a redox cycling.

Also beef heart submitochondrial particles supplemented with NADH and rotenone display an oxygen consumption due to the presence of adrenochrome (Table 3); catalase, added to the medium after most of the oxygen uptake has taken place, determines a return of oxygen into the incubation medium, indicating that hydrogen peroxide was formed (not shown). Nevertheless, when succinate was used as a substrate in the presence of antimycin, no stimulation of the

Table 2. Effect of adrenochrome on oxygen uptake in rat liver microsomes.

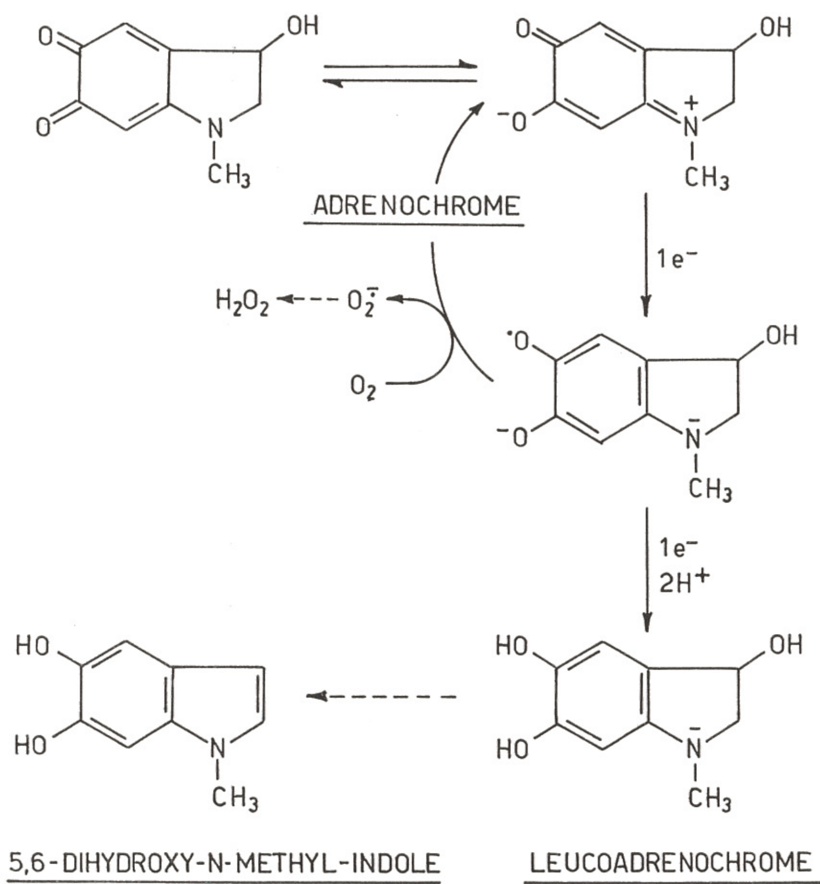
Additions	Oxygen uptake	
	Initial rate	Total O_2
	(nmol O_2/min•mg prot)	(nmol O_2/10min•mg prot)
NADH	5.3	22.8
NADH + Adrenochrome	20.4	72.2
NADPH	7.6	29.7
NADPH + Adrenochrome	47.1	83.6
NADH + NADPH	7.6	36.5
NADH + NADPH + Adrenochrome	52.2	147.7

Microsomes (1 mg/ml) were incubated in 50 mM K-phosphate buffer (pH 7.4) at 25°C. When indicated were also added: 1.6 mM NADH, 1.6 mM NADPH and 80 μM adrenochrome.

Table 3. Effect of adrenochrome on oxygen uptake in beef heart submitochondrial particles.

Additions	Initial rate of O_2 uptake (nmol O_2/min·mg prot)
NADH	151.4
NADH + Rotenone	3.1
NADH + Rotenone + Adrenochrome	41.3
Succinate	23.7
Succinate + Antimycin	4.5
Succinate + Antimycin + Adrenochrome	4.5

Beef heart submitochondrial particles (2.5 mg/ml) were incubated in 50 mM K-phosphate buffer (pH 7.4) at 25°C. When indicated were also added: 0.5 mM NADH, 2μg/mg prot rotenone, 0.2 mM adrenochrome, 5 mM succinate and 2μg/mg prot antimycin.


OH
OH
N
CH3
ADRENOCHROME
1e⁻
H2O2
O2
O2
OH
CH3
1e⁻
2H⁺
HO
HO
N
CH3
HO
OH
HO
CH3
5,6-DIHYDROXY-N-METHYL-INDOLE
LEUCOADRENOCHROME

oxygen uptake was observed (Table 3) suggesting that the NADH dehydrogenase flavin of complex I is the mitochondrial site of adrenochrome reduction.

The reported results are all compatible with the hypothesis of a redox cycling (Figure 1) in which the reducing equivalents supplied by ascorbate, or the electron transport chains of microsomes and mitochondria, are able to reduce adrenochrome which is subsequently reoxidized by oxygen with the production of superoxide anion and hydrogen peroxide.

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