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Direct and respiratory chain-mediated redox cycling of adrenochrome

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Adrenochrome is reduced by ascorbate in a reaction accompanied by a large and rapid oxygen uptake. The rates of adrenochrome reduction and the concomitant oxygen uptake are decreased in the presence of superoxide dismutase or catalase. The species formed on the one-electron reduction of adrenochrome (i.e., the semiquinone) was shown by pulse radiolysis to rapidly react with oxygen ($9 \cdot 10^8$ $M^{-1} \cdot s^{-1}$), indicating the occurrence of a redox cycling in a system formed by adrenochrome, a reducing agent, and oxygen. Adrenochrome is also reduced to the corresponding semiquinone by complex I of beef heart submitochondrial particles supplemented with NADH, while succinate is unable to support this reduction. The *o*-semiquinone is the intermediate species in the superoxide-generating cycle resulting from both non-enzymatic and enzymatic reduction. The toxic effects of adrenochrome and its pathophysiological role can be explained, at least in part, on the basis of the demonstrated cycle.

Introduction

Several compounds, acting through a redox cycle, can bring about the formation of oxygen reduction products such as the superoxide anion radical and hydrogen peroxide. Among these are anticancer drugs, radiosensitizers, antimicrobial drugs and the herbicide paraquat (for a review see Ref. 1). Since some antitumor antibiotics used in chemotherapy exhibit cardiotoxic effects, it has been proposed that the damage could be ascribed to the reactive oxygen species produced during their redox cycling [2–4], i.e., an autocatalytic process which is self-sustained because of the continuous regeneration of the reactants. For instance, quinonoid compounds can be readily reduced with a direct electron transfer by flavoenzymes, leading to the formation of a semiquinone. The latter can be reoxidized by molecular oxygen with the formation of superoxide anion and secondarily hydrogen peroxide.

Among naturally occurring compounds, catecholamines such as adrenalin, noradrenalin, dopa (3,4-dihydroxyphenylalanine) and dopamine can generate superoxide during oxidation to their respective quinones [5–7]. The rate of autoxidation of the above reported catecholamines is, however, rather low at physiological pH, while compounds such as 6-hydroxydopamine and 6-aminodopamine, widely used as experimental neurotoxins, autoxidize much more rapidly [8–10]. Adrenalin-quinone, in particular, can undergo cyclization to leucoadrenochrome and, subsequently, can be further oxidized to adrenochrome (2,3-dihydro-3-hydroxy-*N*-methylindole-5,6-quinone; Fig. 1). While the chemistry of adrenochrome formation from adrenalin has been extensively investigated [11–19], the evaluation of its interaction with biochemical systems has not received comparable attention. Adrenochrome can be a product of the catabolism of catecholamines and, although direct evidence of its occurrence in vivo is still lacking, its formation might concur to neurotoxic or cardiotoxic conditions [20]. In the past, adrenochrome has been in fact indicated as a causative factor in the development of some forms of mental illness [21], possibly after the formation of complexes with acetylcholine [22]. According to a rather old hypothesis [23], some toxic properties of adrenochrome depend on the formation of adrenolutin, while its reduction to 5,6-dihydroxy-*N*-methylindole was considered to be concomitant with the disappearance of any toxicity. An alteration of the balance between the reductive pathways was consequently related to pathological mental states.

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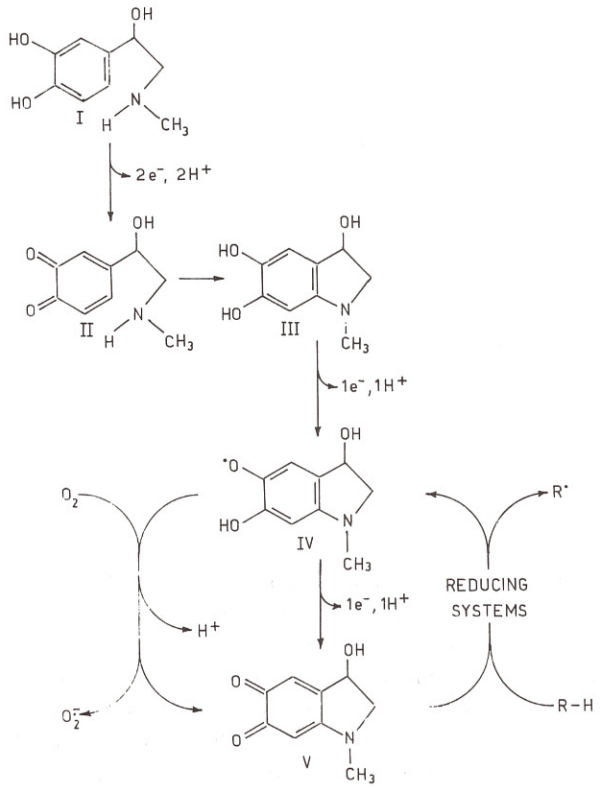

Fig. 1. Pathway of adrenochrome formation from adrenalin and involvement of adrenochrome in a redox cycling process producing superoxide anion. I, adrenalin; II, adrenalin-quinone; III, leuco-adrenochrome; IV, adrenochrome semiquinone; V, adrenochrome. R-H is a generic reducing substrate indicating ascorbate or NAD(P)H, the latter acting in the presence of the microsomal NADPH-cytochrome *P*-450 reductase or of the mitochondrial NADH-ubiquinone reductase.

The myocardial injury induced by catecholamines has, on the other hand, been attributed, at least in part, to the action of the respective oxidation products [24]. In particular, adrenochrome was shown to depress the contractile activity [25] and to produce arrhythmias [26]. Catecholamines and hence their oxidation products, are locally released during ischemia and, in general, an excess of catecholamines is known to be produced during the stress syndrome [27]. Although the speculation concerning the pathogenic role of adrenochrome in the malfunctioning of the central nervous system and in myocardial injury has hitherto escaped any direct experimental verification, the physiological role of adrenochrome and other aminochromes in the biosynthesis of melanins appears to be well established [28]. Recently, a cellular mechanism for the formation of adrenochrome was provided by the results of Matthews et al. [29] indicating that oxygen radicals produced by polymorphonuclear leucocytes stimulated the oxidation of adrenalin through the adrenochrome pathway. In addition, the same authors found a compound equivalent to adrenochrome in the synovial fluid of patients with rheumatoid arthritis [30].

At present, the mechanism of the redox changes of the aminochrome molecule relevant to its different physiological effects is rather poorly understood. In previous research on microsomes it has been anticipated that adrenochrome can undergo a redox cycle with production of the superoxide [31]. In this paper we report the existence of a non-enzymatic redox cycle for adrenochrome when ascorbate is the source of reducing equivalents. A similar cycle can arise enzymatically in the presence of the mitochondrial electron transport chain.

Materials and Methods

Beef heart submitochondrial particles were prepared according to the procedure '3' of Smith [32]. Protein content was estimated by the biuret method [33] and oxygen uptake was followed with a platinum electrode assembly of the Clark type [34]. Details of the pulse radiolysis equipment have been described elsewhere [35]. All solutions were prepared in triply distilled water and were saturated with either N_2O or N_2 prior to dissolving adrenochrome. The major free radicals, produced in solution on pulse irradiating, are hydroxyl radicals ($^{\cdot}OH$), hydrogen atoms ($H^{\cdot}$) and solvated electrons (e_{aq}^-) produced according to the general scheme:

$$H_2O \rightsquigarrow {}^{\cdot}OH, H^{\cdot}, e_{aq}^-, H_3O^+, H_2O_2, H_2 \quad (1)$$

The solvated electron can directly react with substrates to produce one electron reduced species. However, the other radicals formed along with the solvated electron may also react with the substrate to form unwanted species and must be removed by the addition of appropriate scavengers, e.g., *t*-butanol (2-methylpropan-2-ol). A much cleaner system can be obtained by using nitrous oxide-saturated solutions containing sodium formate. Solvated electrons react with nitrous oxide to generate $^{\cdot}OH$ radicals (reaction 2):

$$e_{aq}^- + N_2O \rightarrow N_2 + {}^{\cdot}OH + OH^- \quad (2)$$

Both $^{\cdot}OH$ and $H^{\cdot}$, react with the formate anion producing the formate radical anion (reactions 3 and 4 [36]).

$$^{\cdot}OH + HCO_2^- \rightarrow CO_2^{-\cdot} + H_2O \quad (3)$$

$$H^{\cdot} + HCO_2^- \rightarrow CO_2^{-\cdot} + H_2 \quad (4)$$

Consequently, the system is uniradical in the formate radical anion which, in contrast to the hydroxyl radical, is a reducing species and can react with a substrate, e.g.,

adrenochrome (Adr), yielding the corresponding radical anion (reaction 5):

$$Adr + CO_2^{-\cdot} \rightarrow Adr^{-\cdot} + CO_2 \quad (5)$$

The same species is formed by direct reduction with e_{aq}^- (reaction 6):

$$Adr + e_{aq}^- \rightarrow Adr^{-\cdot} \quad (6)$$

The formation of the superoxide anion was estimated by following the superoxide dismutase-inhibitable rate of reduction of acetylated ferricytochrome *c* prepared according to Azzi et al. [37]. The extent of acetylation was determined by the ninhydrin method, while the concentration of total cytochrome *c* was measured at 550 nm using the molar extinction coefficient 29 900 $M^{-1} \cdot cm^{-1}$ [38].

Results

Aqueous solutions of adrenochrome strongly absorb at 480 nm [39] and this absorbance is lost upon reduction to leucoadrenochrome [40]. As shown in Fig. 2, ascorbate is able to reduce adrenochrome as measured by the decrease in absorbance at 480 nm. This reduction, under standard conditions, is preceded by a lag-time (Fig. 2b), the length of which increases in the presence of superoxide dismutase (Fig. 2c) or catalase (Fig. 2d); the presence of both enzymes together leads to an even longer lag period (Fig. 2e). The lag-time completely disappears in a nitrogen atmosphere (Fig. 2a), while it is markedly lengthened in 95% O_2 (Fig. 2f),

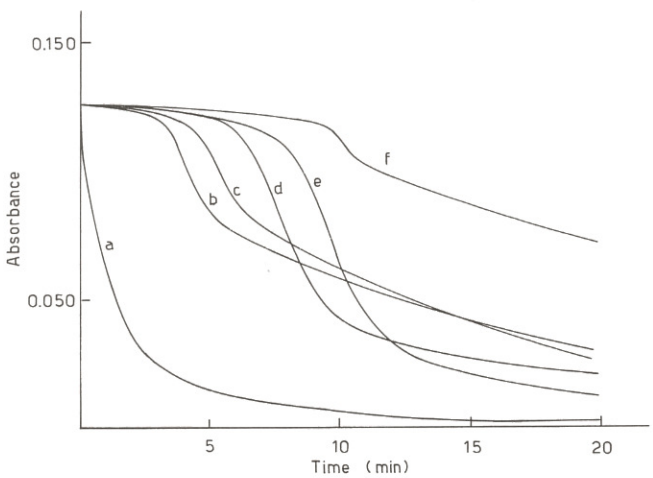

Fig. 2. Reduction of adrenochrome in the presence of ascorbic acid. Ascorbic acid (20 mM) and adrenochrome (40 μM) were added to a 50 mM potassium phosphate medium (pH 7.4). Other additions: (a) nitrogen bubbled for 1 min before adding adrenochrome; (b) none (control); (c) 40 μg/ml superoxide dismutase; (d) 40 μg/ml catalase; (e) 40 μg/ml superoxide dismutase + 40 μg/ml catalase; (f) 95% oxygen bubbled for 1 min before adding adrenochrome. The reactions were performed at 25 °C in closed vessels.

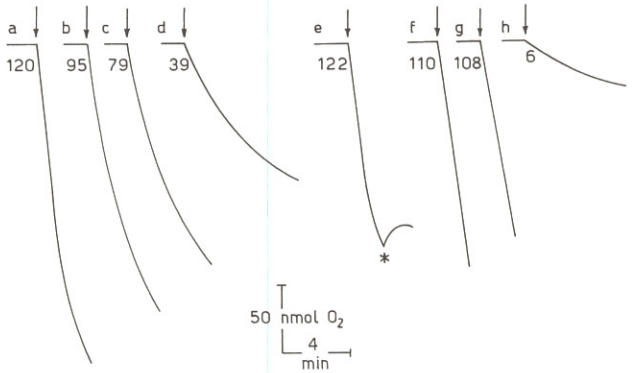

Fig. 3. Ascorbate-induced stimulation of oxygen uptake by adrenochrome. Oxygen uptake was determined at 25 °C in 50 mM potassium phosphate buffer (pH 7.4) containing 2 mM EDTA and 40 μM adrenochrome. Ascorbate 8 mM was added at the arrow. Other additions: (a) control; (b) 130 μg/ml superoxide dismutase; (c) 70 μg/ml catalase; (d) 130 μg/ml superoxide dismutase + 70 μg/ml catalase; (e) 16 μg/ml catalase added at the asterisk; (f) 40 μM butylated hydroxytoluene; (g) 0.8 mM chlorpromazine; (h) adrenochrome monosemicarbazone instead of adrenochrome. The figures under each trace indicate the initial rate of O_2 uptake (nmol · min^{-1}).

as previously demonstrated by Roston [40]. In addition, a rapid oxygen uptake accompanies adrenochrome reduction (Fig. 3) as observed years ago by Galzigna [41]. This oxygen uptake is not due to a trivial autoxidation of ascorbate catalyzed by contaminant metal ions since, under our conditions, the oxygen consumption induced by ascorbate in the absence of adrenochrome is negligible; moreover, the exclusion of EDTA or the addition of other chelating agents such as diethylenetriaminepentaacetic acid (DTPA) or desferrioxamine do not modify the ascorbate/adrenochrome-stimulated oxygen consumption (not shown). The oxygen uptake increases on increasing the pH in the range pH 5–8 (not shown). Either superoxide dismutase or catalase (or the combination of the two) inhibit oxygen consumption (Fig. 3b–d); this effect can be explained on the basis of a continuous restoration of oxygen in the reaction vessel, due to dismutation of the superoxide anion or to the catalase-induced decomposition of H_2O_2. As expected, the addition of catalase, after oxygen depletion, appears to partially restore oxygen in the vessel (Fig. 3e). The addition of antioxidants such as butylated hydroxytoluene (BHT; Fig. 3f) or chlorpromazine (Fig. 3g) induces only a very slight inhibition of oxygen consumption (about 10%). In contrast, when adrenochrome semicarbazone (where an imino group substitutes the quinone moiety at carbon 5) is used instead of adrenochrome, almost no oxygen uptake occurs in the presence of ascorbate (Fig. 3h), indicating that the *o*-quinone structure is essential for the oxygen consumption.

The occurrence of one-electron reduction of adrenochrome to the corresponding o-semiquinone was demonstrated by pulse radiolysis experiments. Fig. 4a shows the transient spectra formed on pulse irradiating

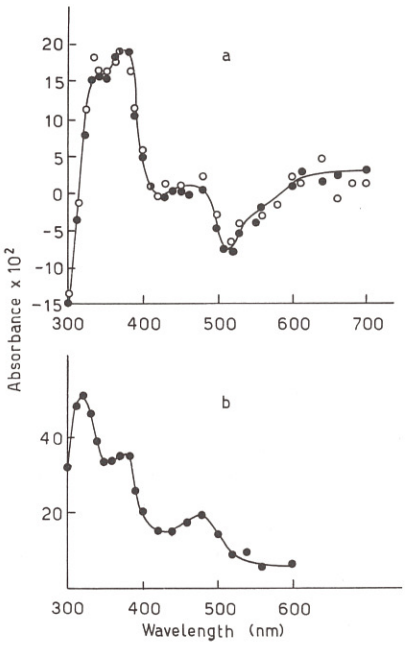

Fig. 4. A comparison of the spectra of adrenochrome semiquinone obtained by reaction of adrenochrome with solvated electrons or formate radicals anions. (a) Transient spectra produced on pulse irradiating 10^{-5} M adrenochrome at pH 7 in nitrogen-saturated solutions with a dose of 1.6 Gy. •, 0.2 M formate (70 μs after the pulse); ○, 0.5 M *t*-butanol (60 μs after the pulse); the spectra are normalized at 370 nm to the value found for formate. Pathlength: 2.5 cm. (b) Spectrum of the adrenochrome transient (0.2 M formate, nitrogen saturated) corrected for adrenochrome depletion, taking G (adrenochrome loss) = $G(CO_2^{-\cdot}) = 0.62\ \mu mol \cdot J^{-1}$ and using the spectrum of a 10^{-5} M adrenochrome in 0.2 M formate solution, measured in 1 cm cuvette at 310 nm using the molar extinction coefficient 21 270 $M^{-1} \cdot cm^{-1}$ [17].

adrenochrome in either N_2O/formate or N_2/*t*-butanol containing solutions. Since the yield of $CO_2^{-\cdot}$ in the formate system is around 0.6 $\mu mol \cdot J^{-1}$ while the yield of e_{aq}^- in the *t*-butanol system is only about 0.28 $\mu mol \cdot J^{-1}$, for comparative purposes the transient spectrum formed by the latter has been normalised at 370 nm to that formed in the case of $CO_2^{-\cdot}$. As can be seen, the transient spectra in the two systems are very similar, indicating that in both cases the one-electron reduced form of adrenochrome (i.e., the semiquinone) is formed. On correcting the transient spectrum formed in the formate system for adrenochrome depletion, the spectrum shown in Fig. 4b is obtained. This is similar to that obtained by Bors et al. [17] who generated the semiquinone using the *t*-butanol system, and further confirms that $CO_2^{-\cdot}$ produces the semiquinone.

The rate constant for the reaction of $CO_2^{-\cdot}$ with adrenochrome was measured by following the absorbance increase at 370 nm in N_2O-saturated solutions containing 10^{-5} M adrenochrome and 0.2 M formate at pH 7 after a pulse of 1.6 Gy. A value of $7 \cdot 10^9\ M^{-1} \cdot s^{-1}$ was obtained. The solvated electron reacts with adrenochrome with an even larger rate constant (approx. $5 \cdot 10^{10}\ M^{-1} \cdot s^{-1}$).

The initial rate of decay of the adrenochrome radical anion is increased in the presence of oxygen. This is illustrated (Fig. 5) by the oscilloscope traces showing the decay of the absorbance signal due to the adrenochrome radical anion decay in the presence (a) and absence (b) of oxygen. In the absence of oxygen the decay follows second-order kinetics with rate constants of approx. $5 \cdot 10^8\ M^{-1} \cdot s^{-1}$ and can be ascribed to dimerisation or dismutation (reaction 7) of the radical anion.

$$2Adr^{-\cdot} \rightarrow Adr + AdrH_2 \quad (7)$$

$$Adr^{-\cdot} + O_2 \rightarrow Adr + O_2^{-\cdot} \quad (8)$$

The enhanced initial decay in the presence of oxygen is first-order and can reasonably be assigned to the reaction of oxygen with radical anion. A first-order rate constant of $2.6 \cdot 10^4\ s^{-1}$ was obtained on curve-fitting the initial oxygen dependent fast decay; after dividing the first-order rate constant by the O_2 concentration a second-order rate constant of $9 \cdot 10^8\ M^{-1} \cdot s^{-1}$ was obtained for reaction 8.

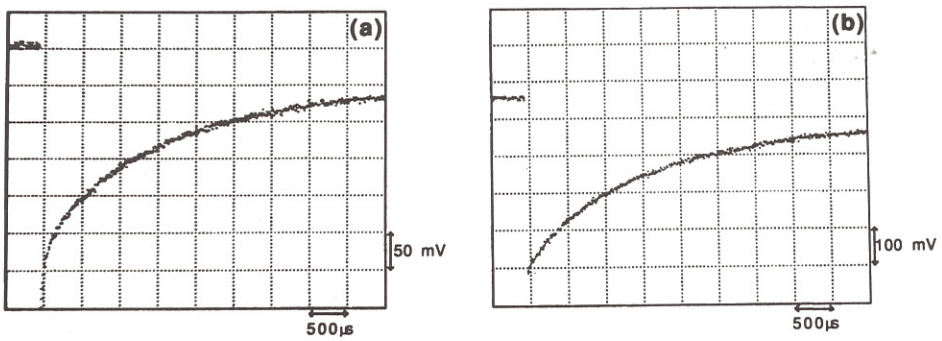

Fig. 5. Oscilloscope traces from pulse radiolysis, showing the effect of oxygen on the decay of the adrenochrome radical anion. The solution of $5 \cdot 10^{-4}$ M adrenochrome was prepared in $4 \cdot 10^{-4}$ M phosphate buffer (pH 7.0) and contained 0.2 M sodium formate. The traces depict the changes in transmitted light intensity, measured as photomultiplier output voltage, as function of time. The pathlength of the flow cell was 2.5 cm and absorption measurements were obtained at 370 nm. The electron pulse (20 ns) was delivered 400 μs after starting the recording. (a) 97.6% N_2 and 2.4% O_2; initial light intensity, 5190 mV; dose, 3.2 Gy. (b) N_2 only; initial light intensity, 5320 mV; dose, 3.4 Gy.

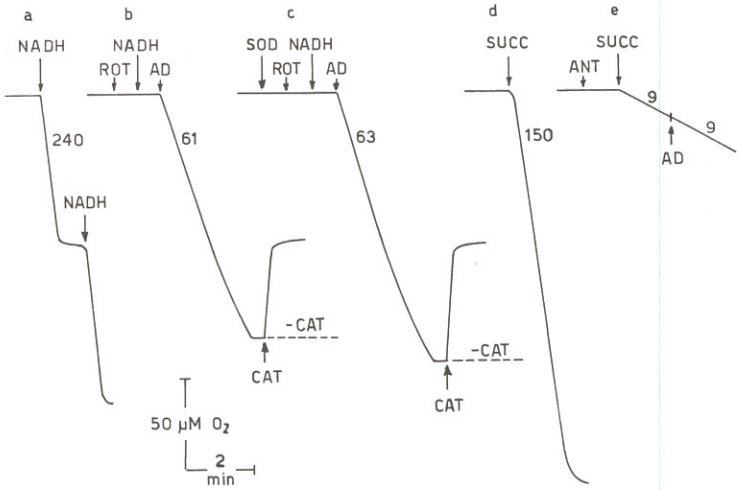

Fig. 6. Effect of adrenochrome on NADH- or succinate-induced oxygen uptake in beef heart submitochondrial particles. Beef heart submitochondrial particles (1.2 mg/ml) were incubated in 50 mM potassium phosphate buffer (pH 7.4) at 25 °C. When indicated were also added. 0.20 mM NADH, 3 μg/ml rotenone (ROT), 0.26 mM adrenochrome (AD), 6 mM succinate (SUCC), 3 μg/ml antimycin (ANT), 15 μg/ml superoxide dismutase (SOD) and 20 μg/ml catalase (CAT). The figures by each trace indicate the initial rate of O_2 uptake (nmol/min per mg protein).

The redox cycling, which is the most likely explanation of the non-enzymatic oxygen consumption of the ascorbate-adrenochrome couple, may also occur when adrenochrome is added to beef heart submitochondrial particles supplemented with reducing substrates. The addition of NADH to submitochondrial particles induces a relatively fast oxygen uptake with a rate of 240 nmol/min per mg protein (Fig. 6a). The extent of O_2 uptake follows a stoichiometry of 2 mol NADH per mol O_2, and another addition of NADH gives the same stoichiometry. The consumed amounts of O_2 can be justified by considering that the flux of electrons along the mitochondrial respiratory chain brings to the formation of water in a 4 e^- process, according to the following basic stoichiometry:

$$2NADH + 2H^+ + O_2 \rightarrow 2NAD^+ + 2H_2O$$

In the presence of rotenone the oxygen uptake induced by NADH is completely inhibited, because of the inhibition of electron flux; moreover, the addition of adrenochrome gives rise to a remarkable O_2 uptake (Fig. 6b) to an extent larger than that observed in Fig. 6a, although with a lower rate (61 nmol/min per mg protein). In this case (Fig. 6b), the adrenochrome semiquinone is formed and, after reacting with oxygen, yields the superoxide anion which dismutates to hydrogen peroxide. The overall process is 2 e^- reduction and seems to obey to the following stoichiometry:

$$NADH + H^+ + O_2 \rightarrow NAD^+ + H_2O_2$$

Catalase, added to the medium at the end of the oxygen uptake, causes a return of oxygen into the incubation medium (Fig. 6b) confirming that hydrogen peroxide was formed. This behaviour is similar to that observed with the ascorbate-adrenochrome system (Fig. 3e). The extent of O_2 uptake, due to adrenochrome in rotenone-inhibited submitochondrial particles, is increased when superoxide dismutase is included in the system (Fig. 6c). This stimulatory effect indicates that superoxide dismutase prevents reactions of superoxide different from dismutation. It is known, in fact, that superoxide might interact with ferricytochrome *c* which, in turn, feeds electrons to cytochrome oxidase and then to oxygen

TABLE I

Adrenochrome-induced oxygen uptake and superoxide production by beef heart submitochondrial particles

Submitochondrial particles were incubated in 50 mM potassium phosphate buffer (pH 7.4) at 25 °C in the presence of 0.200 mM NADH and 3 μg/ml rotenone. Submitochondrial particles were 1.2 mg/ml for oxygen uptake and 0.38 mg/ml for superoxide anion estimations; in the latter case 50 μM acetylated ferricytochrome *c* was present and, in the control experiments, also 10 μg/ml of superoxide dismutase. The reactions were started by the addition of the indicated concentrations of adrenochrome. The initial rates of oxygen consumption or of acetylated ferricytochrome *c* reduction were recorded and measured as indicated under Materials and Methods. The figures shown are typical results of at least three sets of experiments.

Adrenochrome (μM)	Oxygen uptake (nmol/min per mg protein)	Superoxide production (nmol/min per mg protein)
25	13	10
50	23	19
80	28	27
100	37	33
130	45	48
160	53	49
200	54	52
260	60	61
320	66	65
400	78	76

and, in this way, electrons can bypass the rotenone-inhibited site, partly reverting the stoichiometry to 2 mol NADH per mol O_2. The amount of oxygen produced in the vessel after the addition of catalase (Fig. 6b and c) is quantitatively consistent with the hypothesized formation of the superoxide anion, followed by its dismutation to hydrogen peroxide, and subsequent decomposition by catalase. Succinate, used as a substrate instead of NADH, gives rise to a large O_2 uptake (Fig. 6d), which is almost completely inhibited by antimycin (Fig. 6e). In the latter case, no stimulation of oxygen uptake was observed after addition of adrenochrome (Fig. 6e), suggesting that only the flavin of the mitochondrial complex I is a site of adrenochrome reduction.

In Table I the oxygen uptake induced by adrenochrome in rotenone-inhibited beef heart submitochondrial particles in the presence of saturating amounts of NADH and adrenochrome is reported, along with the rate of superoxide formation under the same experimental conditions. The rate of oxygen consumption is in keeping with the rate of formation of superoxide. Both oxygen uptake and superoxide production show saturation kinetics with similar K_m (178 and 188 μM, respectively). Only native superoxide dismutase is an effective inhibitor of the acetylated cytochrome *c* reduction, since denatured superoxide dismutase is almost completely ineffective; in addition, when heat-denatured submitochondrial particles are used, the production of superoxide is extremely low, strongly supporting the enzymatic nature of this reaction (not shown).

Adrenochrome semicarbazone, as expected from the oxygen uptake measurements (see Fig. 3h), does not induce any production of superoxide anion (not shown).

Discussion

Adrenochrome can be either reduced in a non-enzymatic process due to ascorbate or, alternatively, in a process catalysed by the mitochondrial respiratory chain in the presence of NADH.

In previous research [31] the *o*-semiquinone form has been postulated as an intermediate in the reduction of adrenochrome catalysed by liver microsomes and NADPH, and the same species appears to be the intermediate in a superoxide-generating cycle [31]. Adrenochrome reduction is a two-step process in which adrenochrome semiquinone appears to be the intermediate; the existence of the latter and its interaction with dioxygen is suggested by the adrenochrome/ascorbate reaction and clearly demonstrated by the pulse radiolysis experiments, while its ability to form the superoxide anion is supported by the rapid reduction of the acetylated cytochrome *c*. It is interesting to note that the semiquinone of catechol only slowly reacts with O_2 ($k < 5 \cdot 10^5$ $M^{-1} \cdot s^{-1}$) [42]; the considerably enhanced rate of reaction in the case of adrenochrome ($9 \cdot 10^8$ $M^{-1} \cdot s^{-1}$) is perhaps due to the electron-donating effect of the nitrogen atom. It is, however, apparent from Fig. 5a that the situation is more complex than would be the case if only reactions 7 and 8 were taking place; in fact, based on the first-order rate constant for the initial rapid decay, the half-life for the overall reaction should be about 25 μs, in which case the reaction should essentially be completed in less than 150 μs and, at this time after the pulse, the light intensity should have returned to its pre-pulse value, which is not the case. One possibility is that the reverse reaction of reaction 8 occurs (i.e., $Adr + O_2^{-\cdot}$), hence leading to an equilibrium. Further work is needed to establish the precise mechanism. In the meantime it is clear from the pulse radiolysis data that the adrenochrome radical anion rapidly reacts with oxygen, although, in view of the probable occurrence of several simultaneous reactions involving the adrenochrome radical anion, the value of the rate constant given above for reaction 8 must be taken as an upper limit. The reaction rate of adrenochrome with O_2 is comparable to that observed for the semiquinones of mitomycin C ($2.2 \cdot 10^8$ $M^{-1} \cdot s^{-1}$), adriamycin ($3 \cdot 10^8$ $M^{-1} \cdot s^{-1}$) [35], daunorubicin ($2 \cdot 10^9$ $M^{-1} \cdot s^{-1}$) [43] and paraquat ($7.7 \cdot 10^8$ $M^{-1} \cdot s^{-1}$) [44]. Radical anions resulting from one-electron-reduced adrenochrome have been characterized by electron spin resonance (ESR) spectroscopy [45,46], and we have also observed their formation by this techniques in the presence of Zn^{2+}; with a mixture of ascorbate-adrenochrome, the semidehydroascorbate radical, which gives a very strong signal, prevents a clear-cut observation of the free radical form of adrenochrome.

The superoxide dismutase- and catalase-dependent increase of the lag-time observed during the ascorbate-induced reduction of adrenochrome (Fig. 2), and the slowing down by the same enzymes of oxygen uptake induced by the ascorbate-adrenochrome system can be explained by considering the specific reactions catalysed by the two enzymes. Such reactions, through the dismutation of the superoxide anion and the decomposition of hydrogen peroxide, are able to supply oxygen to the system, slowing down both the reduction of adrenochrome (Fig. 2) and the apparent rate of consumption of oxygen (Fig. 3). A reaction scheme explaining both the adrenochrome (Adr) reduction by ascorbate (AH^-) and the oxygen uptake might be the following:

$$Adr + AH^- \rightarrow Adr^{-\cdot} + AH^{\cdot} \quad (9)$$

$$Adr^{-\cdot} + Adr^{-\cdot} \rightarrow Adr + AdrH_2 \quad (10)$$

$$AH^{\cdot} + AH^{\cdot} \rightarrow A + AH_2 \quad (11)$$

$$Adr^{-\cdot} + O_2 \rightarrow Adr + O_2^{-\cdot} \quad (12)$$

$$AH^- + O_2^{-\cdot} \rightarrow AH^{\cdot} + H_2O_2 \quad (13)$$

$$2O_2^{-\cdot} \xrightarrow{SOD} H_2O_2 + O_2 \quad (14)$$

$$H_2O_2 \xrightarrow{CAT} H_2O + 1/2\ O_2 \quad (15)$$

In the absence of oxygen (reactions 9–11), adrenochrome is completely reduced by ascorbate, initially to give $Adr^{-\cdot}$, and no lag phase is apparent (Fig. 2a). The semiquinone form of adrenochrome ($Adr^{-\cdot}$) dismutates to leucoadrenochrome ($AdrH_2$) and adrenochrome, while the semidehydroascorbate radical ($AH^{\cdot}$) dismutates to ascorbate and dehydroascorbate (A). In the presence of oxygen, adrenochrome can be reformed in reaction 12, thus leading to a lag-phase in the kinetics until all oxygen is consumed (Fig. 2b). The superoxide anion produced in reaction 12 reacts with ascorbate forming hydrogen peroxide [47] (reaction 13). In the presence of superoxide dismutase (SOD), reaction 14 (which produces O_2) competes with reaction 13 and consequently an increased lag-phase is observed (Fig. 2c). When catalase (CAT) is added, reaction 15 takes place, again leading to oxygen formation and an increased lag-phase (Fig. 2d). The lower increase in lag-phase on SOD addition as compared to catalase addition can be explained by reaction 13 competing with 14.

The negligible effect of antioxidants on the oxygen uptake of the ascorbate-adrenochrome system (Fig. 3f and g) can be explained by proposing that any reaction between antioxidant and the semiquinone is too slow to compete with the reaction of the semiquinone with oxygen.

The strong stimulation by adrenochrome of the superoxide production by rotenone-treated beef heart submitochondrial particles in the presence of NADH, and the lack of effect of succinate, support the assumption that the reduction of adrenochrome might be mediated by the flavin of the NADH dehydrogenase. In fact, the one-electron reduction potential of the $Adr/Adr^{-\cdot}$ couple at pH 7 is -253 mV [48], while that of $NAD^+/NADH$ couple is -320 mV and consequently the formation of $Adr^{-\cdot}$ is thermodynamically favoured. The redox potential of the $Adr/Adr^{-\cdot}$ couple is also compatible with the formation of the superoxide anion, since that of the couple $O_2/O_2^{-\cdot}$ is of -160 mV [49]. The inability of succinate to induce any oxygen uptake could be due to the unfavourable redox potential; in fact the redox potential of the couple succinate/fumarate is of -31 mV, higher than that of the couple $Adr/Adr^{-\cdot}$. Similarly to adrenochrome, also the well known anticancer drugs doxorubicin, daunorubicin and rubidazone can generate oxygen free radicals after reduction by the mitochondrial NADH dehydrogenase [4].

In conclusion, our results support the occurrence of a redox cycle of adrenochrome (Fig. 1), an *o*-quinone that, at variance with most of the above reported quinonoid xenobiotics (1) could be formed endogenously from physiological molecules. The reported neurotoxicity and cardiotoxicity of adrenochrome could be the result, at least in part, of this feature, which, in addition to the production of harmful oxygen free radicals, also causes oxygen depletion, resulting in hypoxic conditions and depletion of ascorbic acid, which is a major constituent of the central nervous system [50] and adrenal tissues [51]. Finally, a decrease in the level of sulphydryl groups and glutathione could also occur because of their interaction with oxygen reduced species [52] and adrenochrome free radical.

The oxidation of adrenalin to adrenochrome and then further to adrenolutin is a multistep oxidation process where many redox couples can be formed [53]. Consequently an extended redox cycling may be envisaged where not only the leucoadrenochrome/adrenochrome couple is active but, also the adrenalin/adrenalin quinone, adrenolutin/adrenolutin quinone and the 5,6-dihydroxyindole/5,6-dihydroxyindole quinone couples can have a role.

Recently, a redox cycling similar to that reported for adrenochrome was also observed in the presence of ascorbate for the pre-melanin oxidation products derived from dopamine [54].

References

1 Kappus, H. (1986) Biochem. Pharmacol. 35, 1–6.
2 Goodman, J. and Hochstein, P. (1977) Biochem. Biophys. Res. Commun. 77, 797–803.
3 Donehower, R.C., Myers, C.E. and Chabner, B.A. (1979) Life Sci. 25, 1–13.
4 Doroshow, J.H. and Davies, K.J.A. (1986) J. Biol. Chem. 261, 3068–3074.
5 Misra, H.P. and Fridovich, I. (1972) J. Biol. Chem. 247, 3170–3175.
6 Uemura, T., Chiesara, E. and Cova, D. (1977) Mol. Pharmacol. 13, 196–215.
7 Schenkman, J.B., Jansson, I., Powis, G. and Kappus, H. (1979) Mol. Pharmacol. 15, 428–438.
8 Heikkila, R. and Cohen, G. (1971) Science 172, 1257–1258.
9 Cohen, G. and Heikkila, R.E. (1974) J. Biol. Chem. 249, 2447–2452.
10 Rotman, A. and Creveling, C.R. (1976) FEBS Lett. 72, 227–230.
11 Harley-Mason, J. (1950) J. Chem. Soc. 1276–1282.
12 Bu'Lock, J.D. and Harley-Mason, J. (1951) J. Chem. Soc. 712–716.
13 Heacock, R.A. (1959) Chem. Rev. 59, 181–237.
14 Hawley, M.D., Tatawawadi, S.V., Piekarsky, S. and Adams, R. (1967) J. Am. Chem. Soc. 89, 447–450.
15 Valerino, D.M. and McCormack, J.J. (1971) Biochem. Pharmacol. 20, 47–55.
16 Heacock, R.A. and Powell, W.S. (1973) in Progress in Medicinal Chemistry (Ellis, G.P. and West, G.B., eds.), Vol. 9, pp. 275–339, North Holland, Amsterdam.
17 Bors, W., Saran, M., Michel, C., Lengfelder, E., Fuchs, C. and Spöttl, R. (1975) Int. J. Rad. Biol. 28, 353–371.
18 Bors, W., Saran, M., Lengfelder, E., Michel, C., Fuchs, C. and Frenzel, C. (1978) Photochem. Photobiol. 28, 629–638.
19 Graham, D.G. (1978) Mol. Pharmacol. 14, 633–643.
20 Bindoli, A., Rigobello, M.P. and Galzigna, L. (1989) Toxicol. Lett. 48, 3–20.
21 Heacock, R.A. (1971) Bull. Chimie Ther. 6, 300–304.

22 Galzigna, L. (1970) Nature 225, 1058–1059.
23 Hoffer, A., Osmond, H. and Smythies, J. (1954) J. Mental. Sci. 100, 29–45.
24 Rona, G. (1985) J. Mol. Cell. Cardiol. 17, 291–306.
25 Yates, J.C., Beamish, R.E. and Dhalla, N.S. (1981) Am. Heart J. 102, 210–221.
26 Beamish, R.E., Dhillon, K.S., Singal, P.K. and Dhalla, N.S. (1981) Am. Heart J. 102, 149–152.
27 Meerson, F.Z., Kagan, V.E., Kozlov, Yu.P., Belkina, L.M. and Arkhipenko, Y.V. (1982) Basic Res. Cardiol. 77, 465–485.
28 Rodgers, A.D. and Curzon, G. (1975) J. Neurochem. 24, 1123–1129.
29 Matthews, S.B., Henderson, A.H. and Campbell, A.K. (1985) J. Mol. Cell. Cardiol. 17, 339–348.
30 Matthews, S.B., Hallett, M.B., Henderson, A.H. and Campbell, A.K. (1984) Adv. Myocardiol. 6, 367–381.
31 Powis, G. (1979) Biochem. Pharmacol. 28, 83–89.
32 Smith, A.L. (1967) Methods Enzymol. 10, 81–89.
33 Gornall, A.G., Bardawill, C.J. and David, M.M. (1949) J. Biol. Chem. 177, 751–766.
34 Estabrook, R.W. (1967) Methods Enzymol. 10, 41–47.
35 Butler, J., Hoey, B.M. and Swallow, A.J. (1985) FEBS Lett. 182, 95–98.
36 Von Sonntag, C. (1987) The Chemical Basis of Radiation Biology, Taylor & Francis, London.
37 Azzi, A., Montecucco, C. and Richter, C. (1975) Biochem. Biophys. Res. Commun. 65, 597–603.
38 Massey, V. (1959) Biochim. Biophys. Acta 34, 255–256.
39 Green, S., Mazur, A. and Shorr, E. (1956) J. Biol. Chem. 220, 237–255.
40 Roston, S. (1962) Nature 194, 1079–1080.
41 Galzigna, L. (1972) Biochem. Pharmacol. 21, 203–207.
42 Deeble, D.J., Parsons, B.J., Phillips, G.O., Schuchmann, H.P. and Von Sonntag, C. (1988) Int. J. Radiat. Biol. 54, 179–193.
43 Houée-Levin, C., Gardes-Albert, M., Ferradini, C., Faraggi, M. and Klapper, M. (1985) FEBS Lett. 179, 46–50.
44 Farrington, J.A., Ebert, M., Land, E.J. and Fletcher, K. (1973) Biochim. Biophys. Acta 314, 372–381.
45 Borg, D.C. (1965) Proc. Natl. Acad. Sci. USA 53, 633–639.
46 Prabhananda, B.S., Kalyanaraman, B. and Sealy, R.C. (1985) Biochim. Biophys. Acta 840, 21–28.
47 Cabelli, D.E. and Bielski, B.H.J. (1983) J. Phys. Chem. 87, 1809–1812.
48 Svingen, B.A. and Powis, G. (1981) Arch. Biochem. Biophys. 209, 119–126.
49 Koppenol, W.H. and Butler, J. (1985) Adv. Free Rad. Biol. Med. 1, 91–131.
50 Halliwell, B. and Gutteridge, J.M.C. (1985) Trends Neurosci. 8, 22–26.
51 Hornig, D. and Hartmann, D. (1982) in Ascorbic Acid: Chemistry, Metabolism, and Uses (Seib, P.A. and Tolbert, B.M., eds.), pp. 293–316, Am. Chem. Soc., Washington.
52 Armstrong, D.A. and Buchanam, J.D. (1978) Photochem. Photobiol. 28, 743–755.
53 Kalyanaraman, B., Felix, C.C. and Sealy, R.C. (1984) J. Biol. Chem. 259, 354–358.
54 Pileblad, E., Slivka, A., Bratvold, D. and Cohen, G. (1988) Arch. Biochem. Biophys. 263, 447–452.