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Minireview

Toxicity of aminochromes

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SUMMARY

The first part of the present review deals with the chemical and enzymatic synthesis of adrenochrome and other aminochromes from the corresponding catecholamines. A description of the most significant pathways of formation and the reactivity of the aminochromes is presented.

In the second part the toxicity of aminochromes, mainly at the cardiac and CNS level, is described and some of the molecular mechanisms of the toxic action are outlined.

The toxicity of the aminochromes appears to depend mainly on the production of reduced oxygen species through redox cycling. The interaction of aminochromes with sulfhydryl groups and the induced depletion of oxygen, ascorbate and glutathione are additional mechanisms resulting in noxious effects at a cellular level.

CHEMISTRY OF ADRENOCHROME AND RELATED AMINOCHROMES

Formation of aminochromes

More than a century ago it was observed that aqueous extracts of suprarenal capsules develop a rose-carmine color on standing in the air, in the presence of oxidizing

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Abbreviations and trivial names used: adrenaline, 1-(3,4-dihydroxyphenyl)-2-methylaminoethanol; adrenochrome, 3-hydroxy-5,6-dione-*N*-methylindoline; adrenolutin, 5,6-dihydroxy-*N*-methylindoxyl; aminochromes, indoline-5,6-quinones; DOPA, *β*-(3,4-dihydroxyphenyl)-alanine; dopamine, 1-(3,4-dihydroxyphenyl)-2-aminoethane; epinine, 1-(3,4-dihydroxyphenyl)-2-methylaminoethane; isoproterenol, 1-(3,4-dihydroxyphenyl)-2-isopropylaminoethane; leukoadrenochrome, 3,5,6-trihydroxy-*N*-methylindoline; noradrenaline, 1-(3,4-dihydroxyphenyl)-2-aminoethanol; DTPA, diethylenetriamine pentaacetic acid; ESR, electron spin resonance; GSH, reduced glutathione; HPLC, high-pressure liquid chromatography:

agents or with small amounts of alkalies [1–3]. The specific color of this solution is due to the formaton of a red oxidation product of adrenaline called 'adrenochrome' by Green and Richter [4] who first isolated the product after enzymatically oxidizing adrenaline with tyrosinase. The oxidation of adrenaline to adrenochrome is a complex process where many intermediates, some of which are free radicals, can be formed [2–12] (Fig. 1). Adrenaline is first converted to an *o*-semiquinone which subsequently yields an *o*-quinone, probably through disproportionation of the primary semiquinone [5]. The unstable adrenaline quinone intermediate [2–4,6,8], after de-

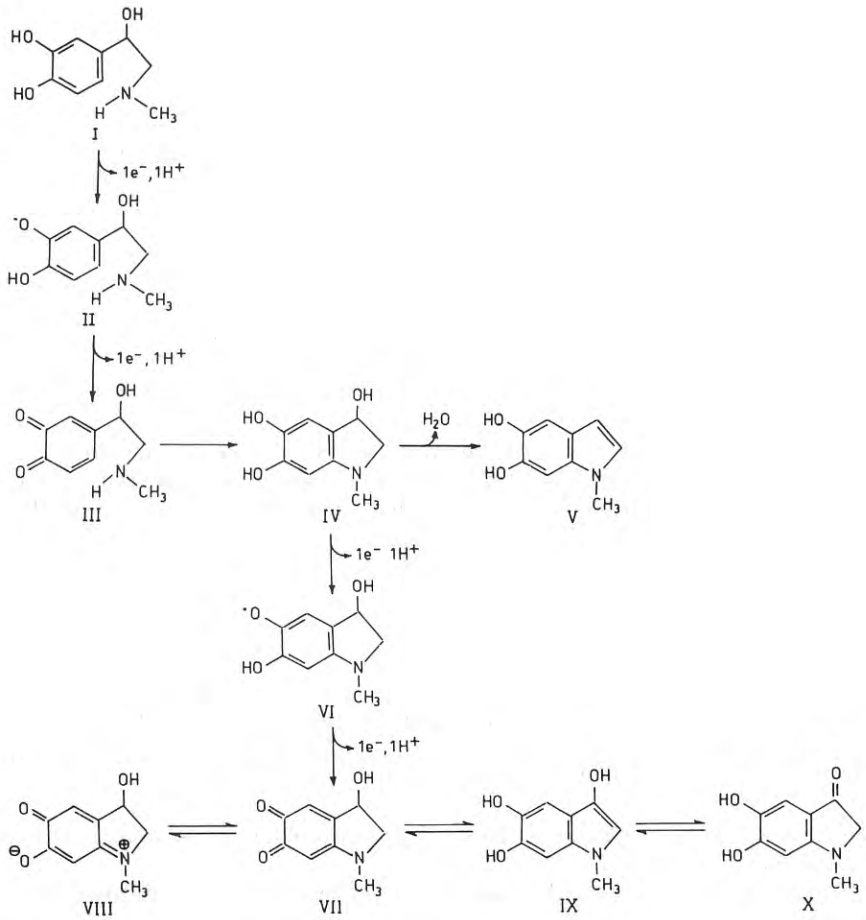

Fig. 1. Pathways of formation of adrenochrome and its derivatives from adrenaline. I, adrenaline; II, adrenaline semiquinone; III, adrenaline quinone; IV, leukoadrenochrome; V, 5,6-dihydroxy-*N*-methylindole; VI, adrenochrome semiquinone; VII, adrenochrome; VIII, adrenochrome (zwitter-ionic structure); IX, adrenolutin; X, adrenolutin (keto-form).

protonation of the side-chain amino group, undergoes a 1,4 intramolecular irreversible cyclization [2–4,8] driven by a nucleophilic attack of the nitrogen atom to the 6-position of the quinone ring [2,7]. The process results in the formation of an unstable leukoadrenochrome rapidly oxidized to adrenochrome by oxidizing species present in the system or by another molecule of *o*-quinone [5,8]. Finally, adrenochrome can be further oxidized and polymerized to melanin-like products. From its physical and chemical properties, adrenochrome appears better expressed in its zwitterionic form [13,14]. By the ESR–spin stabilization approach two types of free radicals were identified during the oxidation of adrenaline and related catecholamines: primary 'open chain' semiquinones, formed by one-electron oxidation of the parent catecholamines, and secondary semiquinones formed after the cyclization reaction [5,15].

Similarly to adrenaline, the various substituted 3,4-dihydroxyphenylethyl amines (catecholamines) can also undergo oxidative cyclization to the corresponding 2,3-dihydroindole-5,6-quinones, called 'aminochromes' with a collective generic name proposed by Sobotka and Austin [16] (Fig. 2). A mechanism for the formation of aminochromes was first postulated by Raper [17,18] to justify the production of melanin from DOPA.

Aminochromes can be obtained from the corresponding catecholamines both by non-enzymatic oxidation in the presence of alkalies, metallic cations, e.g. cupric copper, manganese, nickel and cobalt [2,3,10,19,20], and various oxidizing agents such as silver oxide and manganese dioxide [2,3,10]. Manganic manganese ions complexed by pyrophosphate appear to be particularly effective in carrying out the 4-electron oxidation of dopamine, DOPA, adrenaline and noradrenaline to their corresponding aminochromes [21]. Ferric ions are good catalysts for DOPA oxidation after formation of a 1:1 complex with the catechol moiety of the molecule [22], while ferritin, in the presence of hydrogen peroxide, behaves similarly to the Fenton reagent [24]; apoferritin is inactive [25]. Iron chelates like ferricytochrome *c* are more active than inorganic ferric ions [25]. Hematin and methemoglobin are also effective oxidizing agents of adrenaline [25,26]. Aminochromes can be obtained by enzymatic oxidation performed by the copper-containing catechol oxidases present in both plant and animal tissues [2,4,17,18,27,29–35]. The cytochrome *c*–cytochrome oxidase system [28,36] and ceruloplasmin, a plasma protein [10,37,23], are able to bring about the oxidation of adrenaline to adrenochrome. Interestingly, Fenton's reagent itself is capable of oxidizing adrenaline to adrenochrome [24], indicating that free radicals such as the hydroxyl radical (·OH) can operate the transformation. More recent papers [6,7] have indicated that the conversion of adrenaline to adrenochrome can be stimulated by the superoxide anion as well. The co-oxidation of adrenaline by xanthine oxidase acting on a xanthine substrate was first observed by Valerino and McCormack [38] and explained by McCord and Fridovich [39] who demonstrated that the univalent oxidation of adrenaline by a metal cation or by superoxide anion starts a chain reaction where superoxide anion is the propagating species. This reaction is the basis of a method of assay for superoxide dismutase [7].

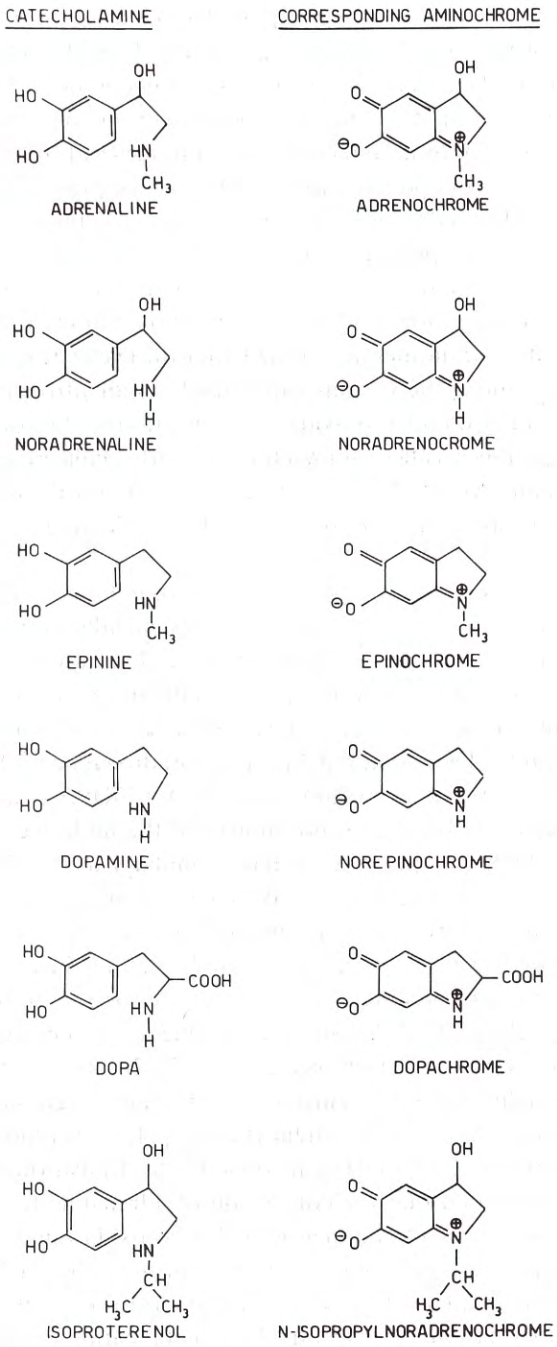

Fig. 2. Formation of some aminochromes from the corresponding catecholamines.

Reactions of aminochromes

Aminochromes undergo 3 main types of reactions, i.e. rearrangement, reduction and oxidation. The rearrangement reaction converts aminochromes to 5,6-dihydroxyindoles or 5,6-dihydroxyindoxyls [2,3,40,41]. The latter are called 'aminolutins' and are tautomers of aminochromes; they are formed by an intramolecular redox reaction bringing about decoloration of the initially red solutions with formation of products exhibiting a yellow-green fluorescence [2,3,40,41]. This rearrangement is a metal-ion or alkali-catalyzed process involving the aminochromes which possess a 3-hydroxy group [2,3,5]. Consequently adrenaline, in the presence of alkali, zinc ions or, to a lesser extent, aluminum ions can rearrange to adrenolutin [40,41] (Fig. 1). Aminolutins occur in the keto-form rather than as trihydroxyindoles [2,3] (Fig. 1) and adrenolutin can be prepared from adrenochrome [12,40] or directly from adrenaline [42,43] by treatment with aqueous sodium hydroxide followed by acidification with acetic acid. Similarly, 5,6-dihydroxy-*N*-isopropylindoxyl, from *N*-isopropyl noradrenochrome, and 5,6-dihydroxy-*N*-methylindole, from epinochrome, were obtained in the solid state [42,44–46] (see also Fig. 2). The rearrangement of aminochromes to 5,6-dihydroxyindoles is greatly favored by the lack of the 3-hydroxyl substituent [2].

Adrenochrome can be reduced by various agents to the unstable and colorless form called 'leukoadrenochrome' which spontaneously dehydrates to the more stable 5,6-dihydroxy-*N*-methylindole [2,3,44–47] (Fig. 1); the latter was isolated from the products obtained by reduction of adrenochrome with ascorbic acid and sodium borohydride [48]. On the contrary, leukoadrenochrome was not isolated, but it was nevertheless identified since its solutions can give typical catechol reactions and are optically active [4]. Several agents were employed to reduce adrenochrome to leukoadrenochrome, e.g. sodium dithionite, sodium borohydride, lithium aluminum hydride, leukomethylene blue, thioglycolic acid and 2,3-dimercapto-1-propanol [2,3]. Other reagents of biological interest are also able to decolorize adrenochrome solutions such as ascorbic acid [48–51], glutathione [52,53,94,95] and cysteine [53].

As already reported, the aminochromes without a 3-hydroxyl group such as dopachrome and epinochrome rearrange very easily to the corresponding 5,6-dihydroxyindoles before undergoing reduction to the corresponding leukoaminochromes [2,3,44,45]. Traces of leukoepinochrome (5,6-dihydroxy-*N*-methylindoline) were obtained by Austin et al. [45]. A true leukoderivative (ethyl 2,3-dihydro-5,6-dihydroxy-3-iodoindole-2-carboxylate) was obtained by the corresponding aminochrome; the reaction is reversible and the product undergoes rapid oxidation by air in solution [47].

All aminochromes undergo further oxidation to brown or black insoluble polymeric pigments called 'melanins', of a not yet completely clarified structure [2,3,54]. The rate of oxidation of adrenochrome increases with the temperature and the pH; in alkaline solution it is adrenolutin, the rearrangement product of adrenochrome, that undergoes autoxidation [2]. Noradrenochrome is oxidized to melanitic pigments more readily than adrenochrome [26,55].

The mechanism for the formation of melanins probably proceeds through a rearrangement of the aminochrome to a 5,6-dihydroxyindole which, in turn, oxidizes to 5,6-dihydroxyindolequinone, a compound capable of undergoing a series of self-condensation reactions [35,56,57] (Raper-Mason mechanism). The transformation of adrenochrome to black insoluble melanins can also take place under anaerobic conditions [2,3].

Adrenochrome reacts with reduced glutathione, cysteine and homocysteine, yielding not only reduction products (5,6-dihydroxyindoles) but also substitution products such as 5,6-dihydroxyindol-4-yl sulfides and aminochrome-thiol addition products [2,3,58,60,94,95]. The first two types of reaction are irreversible while the formation of addition products is driven by a reversible reaction [3,59] (Fig. 3).

The occurrence of a reaction between adrenochrome and thiols appears physiologically relevant by the observation that incubation of blood with adrenaline results in a rapid decrease of the non-protein thiols of the erythrocytes, mainly glutathione, referable to the interaction of the latter with an oxidation product of adrenaline [61]. Similarly, the SH-groups of coenzyme A, GSH, or cysteine disappear upon addition of an oxidized catecholamine preparation or in the presence of dihydroxyphenylalanine and tyrosinase [52,53,62]. The irreversible interaction between aminochromes and thiols could be involved in the formation of melanitic pigments while, in the reversible adduct formation, glutathione or other thiols may act as aminochrome carriers able to regenerate the aminochrome under appropriate conditions [94,95].

TOXICITY OF AMINOCHROMES

Cardiotoxicity and neurotoxicity of aminochromes

The major catabolic pathways of adrenaline and noradrenaline depend on the

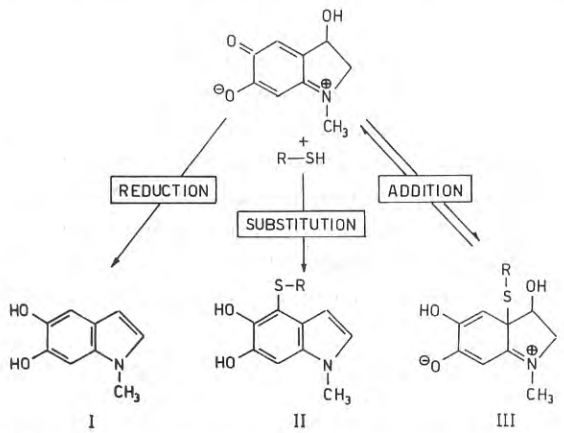

Fig. 3. Products formed after reaction of adrenochrome with cysteine or glutathione (RSH). I, 5,6-dihydroxy-*N*-methylindole; II, 5,6-dihydroxyindol-4-yl sulfide; III, aminochrome–thiol adduct.

monoamine oxidase and catechol-*o*-methyl transferase activities. However, the catechol moiety can also be easily oxidized at physiological pH and, consequently, the oxidative pathways should be taken into account as well. The cellular toxicity of aminochromes and other intermediates of the oxidation of catecholamines has been demonstrated in several studies and appears to involve, in particular, the cardiac and nervous tissue.

Excessive release or administration of catecholamines in doses exceeding the physiological levels may induce cardiotoxic effects such as myocardial necrosis or myocarditis [63,64]. In addition, isoproterenol, a synthetic catecholamine, produces 'infarct-like' myocardial necrosis in experimental animals [63]. Although the pathogenesis of the myocardial damage induced by catecholamines is a multifactorial process [63], a new hypothesis put forward by Dhalla et al. [65–71,73,74] does not attribute to the catecholamines per se but rather to their oxidation products at least part of the observed toxic effects. In anesthetized rats, adrenochrome gives rise to arrhythmias and sudden cardiac death [65]. Perfusion of the rat heart with adrenochrome causes a marked decline in the contractile force, increase in the resting tension and heart failure [66,67] associated with a rapid decrease in the myocardial content of ATP, concomitant with an increase in ADP and AMP [66]. In addition, a decrease in the respiratory control index and state 4 respiration was observed [66]; when rat hearts were perfused with ^{14}C-adrenochrome, a significant amount of the radioactivity was localized in mitochrondria as revealed by autoradiography of the sections prepared from the heart [66]. The toxic effect of adrenochrome in the myocardial cell might be additive to the damaging effect induced by calcium ion overloading [66]. Adrenochrome appears to be taken up by the heart where it causes alterations by irreversibly binding to the various subcellular organelles, as demonstrated by perfusing rat heart with labeled adrenochrome; if the perfusion is continued in the absence of radioactive adrenochrome, about 50% of the radioactivity remains within the tissue [68].

The catecholamine-induced cardiotoxicity is not simply restricted to adrenochrome, but also other oxidation products of adrenaline appear to be effective. In fact, after interaction with ascorbic acid or cysteine, adrenochrome produces damage greater than that observed with adrenochrome alone in isolated and perfused rat heart [69]. Similarly to adrenochrome, oxidized isoproterenol also causes extensive myocardial damage [70,71].

The adrenochrome hypothesis for catecholamine cardiotoxicity was mostly challenged on the basis that relatively high concentrations of adrenochrome (10^{-4} M) are requested to produce cardiac damage, whilst the circulating adrenaline has a concentration of about 10^{-11} to 10^{-12} M [63,72]. Furthermore, according to Rona [63], there is no clear evidence from the electron micrographs that the alterations induced by catecholamines or by their oxidation products are identical; in addition, while the catecholamine-dependent myocardial necrosis brings about a calcium ion overload, adrenochrome inhibits mitochondrial [73] and microsomal [74] calcium ion up-

take and the activity of calcium/magnesium-stimulated ATPase [74]. Bovine heart sarcolemmal membranes are able to form adrenochrome when incubated with adrenaline, particularly when either NADH or NADPH is present [75].

Aminochromes are of marked neurochemical interest, and their involvement in psychiatric disorders has been repeatedly postulated. Adrenochrome, in particular, was considered to be involved in the etiology of some forms of mental illness and an adrenochrome hypothesis of schizophrenia was proposed by Hoffer et al. [76] quite a long time ago. According to these authors, schizophrenia might result from an alteration of the metabolism of adrenaline that results in the formation of a psychotogenic metabolite, e.g. adrenochrome or its rearrangement product, adrenolutin. This rather reductionistic hypothesis was essentially based on the observation that some of the early symptoms of schizophrenia were strikingly close to some effects of hallucinogenic drugs with an indole structure, chemically similar to the indoline-quinone structure of adrenochrome [77]. In addition, adrenochrome can be further metabolized either to adrenolutin, which is considered to be toxic, or to 5,6-dihydroxy-*N*-methylindole, which is reported to be non-toxic [77]. Both adrenochrome and adrenolutin were reported to induce psychic changes in human volunteers, but the results were denied by others (for discussion, see refs. 3,77,78). The adrenochrome hypothesis of schizophrenia was challenged, particularly on the basis that different samples of adrenochrome prepared with different procedures might contain variable amounts of centrally active contaminants responsible for variable psychotic effects [2,3,77]. In addition, ^{14}C-labeled adrenaline and adrenochrome appear to follow different excretion pathways [79]; according to this study, adrenochrome should not be a major metabolite of adrenaline [79].

Years ago one of us demonstrated [80] that noradrenochrome can give rise to a relatively stable complex with acetylcholine and hypothesized the occurrence of a 'short circuit' [80,81] between adrenergic and cholinergic pathways, that might be a causative factor in the onset of mental illness. This event could take place whenever a catecholamine, once released in a synapse, underwent oxidation to aminochrome instead of being metabolized by its own enzyme system. Such oxidation could be related to a localized drop in the reducing power, but a long cascade of amplifying effects is implicit in the hypothesis that ultimately links an initial biochemical event with a final behavioral change.

Formation of aminochromes in vivo

A major problem is whether or not adrenochrome (and the other aminochromes) can be formed in vivo from the corresponding catecholamines. Even though it was reported that the administration of ^{14}C-labeled adrenaline does not give rise to the endogenous formation of ^{14}C-adrenochrome [79], several soluble and membrane-bound enzymatic systems are able to catalyze the in-vitro conversion of adrenaline to adrenochrome [2,4,10,17,18,23,27–37]. More recently, Matthews et al. [82] provided a demonstration of adrenochrome formation by polymorphonuclear leuko-

cytes in conditions which stimulate their production of oxygen radicals. Polymorphonuclear leukocytes infiltrate tissues during acute inflammation and the massive neutrophil infiltration following acute myocardial infarction is considered to be one of the major causes of tissue damage. By using ^{14}C-adrenaline and HPLC analysis to separate adrenaline from its metabolites Matthews et al. [82] were able to demonstrate that polymorphonuclear leukocytes, in the presence of physiological concentrations of adrenaline, transform the latter mainly to adrenochrome (80%) while the remainder was metabolized through the amine oxidase and catechol-*o*-methyl transferase pathways. The oxidation of adrenaline to adrenochrome can also be observed in the medium isolated after stimulation of the polymorphonuclear leukocytes [82]; in this case, stable substances such as hydrogen peroxide or myeloperoxidase are, at least in part, responsible for the oxidation. Serum taken from patients after acute myocardial infarction show adrenaline oxidase activity [83,84]. Furthermore, a product with a retention time in HPLC similar to that exhibited by adrenochrome was obtained from synovial fluid of patients with rheumatoid arthritis [83].

The possibility of in-vivo formation of catecholamine oxidation products is further strengthened by the recent demonstration of the presence in human brain and in the brain of several mammalian species of 5*S*-cysteinyldopamine and 5*S*-cysteinyldopa [86,87] which are possible intermediates in the melanization process. The 5*S*-cysteinyl adducts are formed after autoxidation of catecholamine to the respective quinones followed by reaction with glutathione. The adducts are subsequently attacked by peptidases to yield the 5*S*-cysteinyl derivatives [86,87].

Although direct clear-cut evidence for the in-vivo occurrence of aminochromes (and other products derived from the oxidative pathway of catecholamines) is still lacking, a physiological role of aminochromes in the biosynthesis of the neuromelanins present in the substantia nigra (melanization of dopamine) and the locus ceruleus (melanization of noradrenaline) appears to be well established [54,88,89].

Molecular mechanisms of aminochrome toxicity

A derangement in the metabolism of the catecholamines or defects in their transport mechanisms can lead to an increased concentration of these substances so that the enzymes acting on their catabolism are unable to cope with their excessive level. Consequently, catecholamines may undergo autoxidation, giving rise to unstable free radical species and more stable aminochromes, both potentially toxic for the cell. According to Fuller and Hemrick-Lueke [85], the toxic action of amphetamines on striatum dopaminergic terminals might be caused by a displacement of dopamine from its vesicular stores into the cytosol, where they undergo a subsequent autoxidation. Very recently it was reported that the ischemic stroke in gerbils gives rise to an increase in dopamine concentration in the extracellular space of the striatum [113].

The indolinequinone ring of aminochromes, once formed in a biological system, can interact with various nucleophiles in the cell, particularly with the protein sulfhydryl groups and with acid soluble thiols such as GSH [91,94,95]. Aminochromes

are therefore able to inactivate the enzymes depending on sulfhydryl groups, either through covalent attachment of the quinone to the enzyme [91,102] or by oxidation to the corresponding disulfides of paired and sterically vicinal thiol groups [100]; in this way intermolecular or intramolecular disulfide bonds can be formed. For instance, the aminochromes formed after oxidation of dopamine, adrenaline and noradrenaline are reversible inhibitors of human brain dihydropteridine reductase, an enzyme dependent on sulfhydryl group integrity for activity, while the corresponding catecholamines are without effect [99,100]. After rearrangement of the various aminochromes to the corresponding aminolutins, the inhibitory properties toward dihydropteridine reductase are lost [99]. The inhibition by aminochromes of such an important enzyme involved in the rate-limiting phase of the biosynthesis of the catecholamines can have serious neurological consequences. Adrenochrome also inhibits guanylate cyclase obtained from human caudate nucleus; it is a more powerful inhibitor than adrenaline but less efficient than dopamine [101]. Furthermore, adrenochrome is able to rapidly and irreversibly inactivate the enzyme catechol-*o*-methyl transferase possibly through an interaction with its sulfhydryl groups [102]. Similarly, an inhibition of actomyosin ATPase was attributed to the reaction between the sulfhydryl groups of the enzyme and the oxidation products of adrenaline, i.e. adrenochrome itself or an isomer of it [96]. Inhibition of polypeptide synthesis by low concentrations of aminochromes and their soluble oxidation products was observed by using an in-vitro amino acid polymerization system from rat brain [97,98]. The authors concluded that the metabolic products of catecholamines might be involved in the regulaton of synthesis of brain enzymes, particularly in specific areas of the brain such as the substantia nigra which contains significant concentrations of catecholamines and their derivatives [97,98].

As far as biological membranes are concerned, adrenochrome was reported to interact with mitochondrial energy processes by decreasing oxygen uptake, ATP formation [66,103] and phosphate uptake [103]. It appears also to act, at least in part, as an uncoupling agent [103]. As already reported, adrenochrome exerts an inhibitory action on the mitochondrial [73] and microsomal [74] systems of calcium ion uptake.

It has recently been demonstrated that during the biosynthesis of skin melanins, some of the precursors (cysteinyldopas and dihydroxyindoles) can leak out from the melanosomes and be detected in skin, serum and urine of individuals undergoing melanogenesis [90]. A similar leakage might also occur during the biosynthesis of neuromelanins. It was also demonstrated that these intermediates are photochemically unstable and their irradiation results in the production of a variety of free radical species [90].

Autoxidation products of DOPA, dopamine, adrenaline and noradrenaline were shown to be cytotoxic for neuroblastoma cells by following the incorporation of tritiated thymidine into DNA [91]. Similar results were found for melanoma cells [92]. These observations indicate a possible use of aminochromes, or their precursors, as potential antitumor agents [93]. In fact, aminochromes, like a number of antitu-

mor quinones, can undergo one-electron reduction to semiquinones, a property which is relevant to their toxic effects (see below).

The free radical forms, produced as intermediates during the autoxidation process, can irreversibly bind to the various constituents of the cell. Kalyanaraman et al. [104], using ESR spin stabilization techniques, were able to detect ring-substituted *o*-semiquinones obtained by chemical or enzymatic oxidation of catecholamines in the presence of nucleophiles such as amino acids, peptides and proteins. Nevertheless, as with the interaction of these free radical intermediates with oxygen, it should be noted that, at variance with *p*-semiquinones, the *o*-semiquinone intermediates of simple catecholamines (e.g. 4-methylcatechol, DOPA, dopamine, adrenaline and 5*S*-cysteinyldopa) do not react with molecular oxygen at an appreciable rate [105] and consequently do not give rise to a significant redox cycling process as described in the following section.

Redox cycling of aminochromes

The production of oxygen free radicals is tremendously increased when redox cycling takes place, as in the case of the quinonoid compounds. The latter can be readily reduced in a one-electron process by flavoenzymes, leading to the formation of a semiquinone which, under aerobic conditions, can be reoxidized with the formation of superoxide anion [106]. Among naturally occurring compounds, catecholamines can generate superoxide through oxidation to their respective quinone form [106,107]. The rate of autoxidation of adrenaline, noradrenaline, DOPA and dopamine, however, is rather low at physiological pH; on the other hand, the related compounds 6-hydroxydopamine and 6-aminodopamine, largely utilized as experimental neurotoxins, autoxidize much more rapidly [107,113]. As shown in Fig. 4, aminochromes are likely candidates for such a process of redox cycling leading to the formation of oxygen free radicals such as the superoxide anion, which, after dismuta-

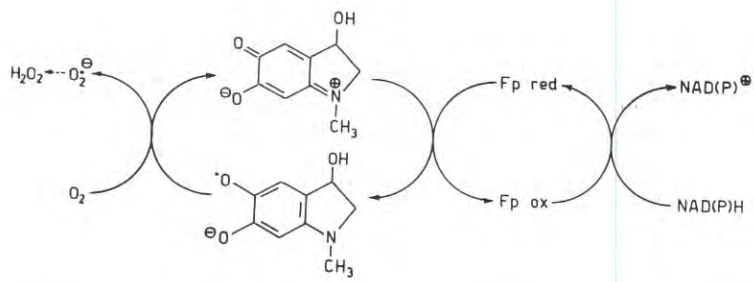

Fig. 4. Redox cycling of adrenochrome. Fp: flavoprotein of microsomal NADPH-cytochrome P-450 reductase or of mitochondrial NADH-ubiquinone reductase (Complex I). Fp ox: oxidized flavoprotein; Fp red: reduced flavoprotein. Cyclic consumption of NAD(P)H and oxygen is apparent and concomitant with the production of oxygen-reduced species. Similar redox cycling can be stimulated by ascorbate acting instead of the reduced flavoprotein.

tion, gives rise to hydrogen peroxide; the latter, through an iron-catalyzed Haber-Weiss mechanism, can produce the hydroxyl radical (·OH) which is particularly damaging to DNA and proteins [108].

Some time ago, it was observed by one of us [109] that, concomitantly with the reduction of adrenochrome by ascorbate [48–51], a large and rapid oxygen uptake occurs. The latter reaction is not modified by the presence of chelators such as EDTA, DTPA and desferrioxamine, while it is strongly inhibited by superoxide dismutase and catalase [110,111]. Consequently, this oxygen consumption appears to arise from autoxidation of a partially reduced adrenochrome-free radical intermediate [110,111] with the production of the superoxide anion, and, secondarily, of hydrogen peroxide. In rat liver microsomal fractions, Powis [112] demonstrated that adrenochrome is able to increase the rate of NADPH-dependent oxygen utilization at a level largely exceeding that reported for the typical substrates of the microsomal mixed-function oxidase. The result was explained on the basis of reoxidation of the adrenochrome semiquinone back to adrenochrome with the formation of superoxide [112]. Similarly, rotenone-insensitive oxygen uptake, largely in excess if compared to the concentration of adrenochrome present, was measured when adrenochrome was added to beef heart submitochondrial particles supplemented with NADH [110]. This oxygen uptake, mediated by the complex I of the mitochondrial respiratory chain, was paralleled by a superoxide-dismutase-inhibitable reduction of cytochrome *c* [111].

The *o*-semiquinone radical anion resulting from one-electron reduced adrenochrome has been characterized by EPR and pulse radiolysis experiments [11,15,93,111] and its reaction rate with oxygen is comparable with that observed for other semiquinones of well-established biological interest [111].

Redox cycling similar to that reported for adrenochrome was also demonstrated for the pre-melanin oxidation products derived from dopamine [113]; the latter, on the contrary, does not initiate redox cycling since its semiquinone form is scarcely reactive with oxygen [105].

In conclusion, it is possible to suggest that the reported cytotoxicity of aminochromes could be, at least in part, related to their ability to undergo redox cycling which, in addition to extensive production of toxic oxygen free radicals, can also lead to a depletion of oxygen, resulting in hypoxic conditions. A concomitant factor could be depletion of ascorbic acid which is particularly concentrated in the gray and white matter of the normal central nervous system [114] and in the adrenal tissue [115] together with a decrease in the level of cellular glutathione and protein sulfhydryl groups. As reported above, the oxidation of catecholamines is a multistep oxidation process in which many redox couples can be formed [5]. Consequently the occurrence of 'extended redox cycling' can be inferred. This extended cycling may involve the reaction of the leukoaminochrome/aminochrome couple, and, in addition, those of catecholamine/catecholamine quinone, aminolutin/aminolutin quinone and 5,6-dihydroxyindole/5,6-dihydroxyindole quinone. This could explain the autocatalytic be-

havior of the system which implies progressive amplification of an initial event involving an oxidative process.

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