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Review Article

BIOCHEMICAL AND TOXICOLOGICAL PROPERTIES OF THE OXIDATION PRODUCTS OF CATECHOLAMINES

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Abstract—The normal catabolism of catecholamines proceeds through enzymatic pathways (monoaminooxidase, catechol-o-methyltransferase, and phenolsulphotransferase). In addition, nonenzymatic oxidative pathways might take place since catechols are readily oxidized. In this review article, the pathways of formation of the oxidation products of catecholamines and their reactions are described. The interactions of these products with different biological systems and their toxicity are examined. Among the reactions known to occur is that with sulfhydryls, which results in either a covalently linked adduct or disulfide production. Another interesting pathway to toxicity involves the oxidation of these catecholamine products by oxygen, with the formation of damaging oxygen-derived species. The action of the oxidation products of catecholamines is outlined, with special attention to the nervous and cardiac systems.

Keywords—Adrenochrome, Aminochromes, Cardiotoxicity, Catecholamine oxidation, Free radicals, Neurotoxicity, *Ortho*-quinones, Redox cycling, Sulfhydryl groups

INTRODUCTION

Natural quinones occur as benzo-, naphtho-, and anthraquinones.[1,2] Ubiquinones are a family of ubiquitous lipid-soluble benzoquinones that can be reversibly oxidized to hydroquinone and consequently act in electron transport in mitochondria. Plastoquinones are benzoquinones involved in the electron transport chain of chloroplasts. Vitamins K are another important group of *para*-quinones characterized by the naphthoquinone ring. They occur naturally as two families: phylloquinones (vitamin K_1) and menaquinones (vitamin K_2). Menadione (vitamin K_3) is a synthetic compound lacking the isoprenoid sidechain but still bearing vitamin activity. Quinones are also present in pollutants,[3,4] essentially as polyaromatic hydrocarbons, and in several antineoplastic drugs.[5,6] Several excellent reviews[1,2,7,8] deal with their functional, toxicological, and antitumor properties, and the reader is referred to them for detailed information.

Quinones can also come from other endogenous sources, such as the oxidative metabolism of the catecholamines. In Figure 1 the structural formulae of some catecholamines and derivatives are reported. Dopa, dopamine, adrenaline, and noradrenaline can be converted to their corresponding quinones. On cyclization, these give rise to the formation of aminochromes and related compounds, such as aminolutins and dihydroxyindoles, which eventually lead to the well-known polymeric products called melanins.

The present review focuses on the chemical properties and the biological effects of the intermediate products formed during the complex and still incompletely defined processes occurring in the oxidative pathway of the catecholamines.

THE OXIDATIVE PATHWAY OF THE CATECHOLAMINES

Redox chemistry of catecholamines

Catecholamines, as is apparent from their name, contain the catechol moiety as an integral part of their structure. Many of the chemical properties of catecholamines are due to the presence of the catechol group; this is certainly the case regarding oxidation reactions, which are a prime concern of this review. A

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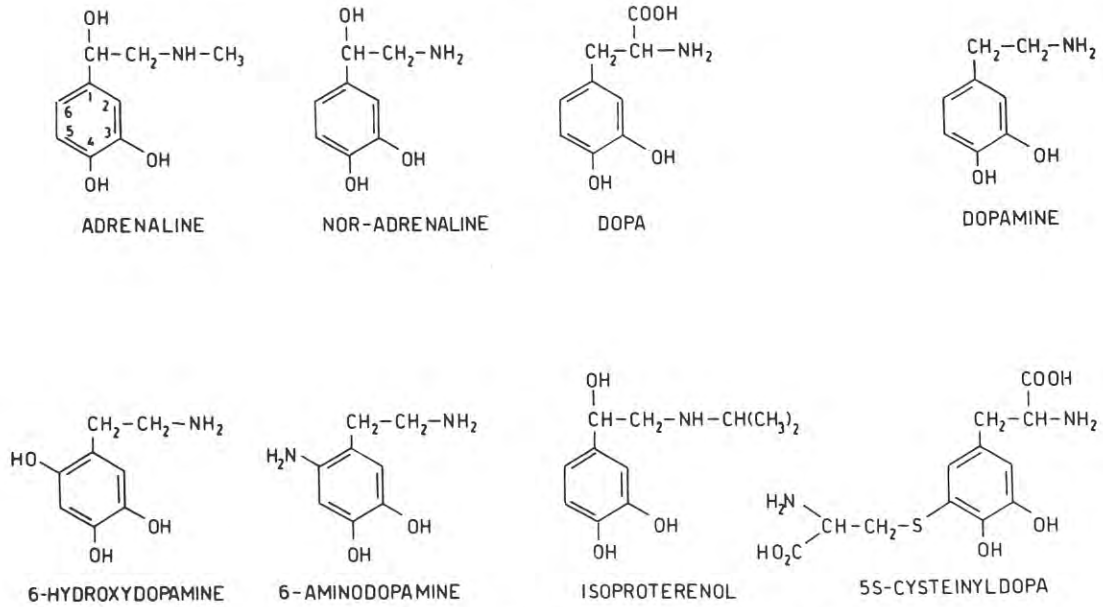


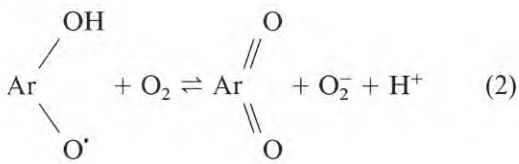
Fig. 1. Structural formulae of various catecholamines and derivatives.

detailed description of the chemistry of catechols is outside the scope of this article; however, a brief outline of some of the relevant reactions is appropriate.

Catechol (1,2-dihydroxybenzene) is a phenolic compound. Phenols as a group are susceptible to oxidation; of the various oxidizing agents in living systems, oxygen is probably the most important, being virtually ubiquitous and present in relatively high concentrations. Phenolic solutions undergo oxidation by oxygen at physiological pH, and such oxidation is normally termed autooxidation. A quick glance at a list of the reduction potentials[9] of a number of phenols in their protonated state (e.g., phenol, catechol, hydroquinone, pyrogallol) suffices to show that under the conditions likely to be encountered in biological systems (viz., air-saturated solutions, pH ca. 7, and less than 10^{-2} M phenolic material), autooxidation is thermodynamically infeasible. This means that the equilibrium represented by Eq. 1,

$$\mathrm{ArOH} + \mathrm{O_2} \rightleftharpoons \mathrm{ArO^{\bullet}} + \mathrm{H^+} + \mathrm{O_2^-} \qquad (1)$$

which is the first step in autooxidation, lies well to the left. As would be expected, the deprotonated form of the phenols have appreciable lower one-electron reduction potentials; for instance, the one-electron reduction potential of catechol ($\mathrm{ArO^{\bullet}/ArOH}$) is +530 mV at pH 7, while it is far lower (+98 mV at pH 11) for the mono-deprotonated catechol ($\mathrm{ArO^{\bullet}/ArO^-}$).[9] At physiological pH, most phenols exist mainly in their protonated form. Although deprotonation shifts the position of the equilibrium toward the right, it nevertheless remains markedly in favor of the reactants ($E_0(O_2/O_2^-) = -155$ mV).[10,11] In spite of the very small fraction of phenol converted to oxidation products at equilibrium, autooxidation will proceed if these oxidation products are removed from the equilibrium by their participation in other reactions. An excellent example of this is provided by homogentisic acid (2,5-dihydroxyphenyl acetic acid), whose autooxidation in the synovia of patients suffering from alkaptonuria is believed to cause inflammatory arthritis.[12] The autooxidation of homogentisic acid is accelerated by the presence of superoxide dismutase (SOD), which effectively removes O_2^- from equilibrium (see Eq. 1). At the same time, a second equilibrium involving the reaction of oxygen with *para*-semiquinone radical to form the quinone and O_2^- (Eq. 2) is also pulled to the right by the SOD-mediated removal of O_2^-, reducing the concentration of the semiquinone with the concomitant effect of causing even more phenol to react with oxygen to restore the equilibrium.

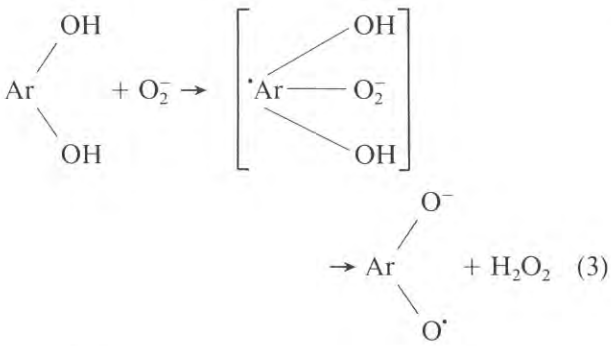


$$\mathrm{Ar(OH)(O^{\bullet})} + \mathrm{O_2} \rightleftharpoons \mathrm{Ar(=O)(=O)} + \mathrm{O_2^-} + \mathrm{H^+} \qquad (2)$$

The reaction of oxygen with *para*-semiquinone radicals seems to be quite general.[13,14] The ability of transition metal ions, such as iron and copper, to react with O_2^- and phenoxyl radicals ($\mathrm{ArO^{\bullet}}$) is one explanation for their widespread catalysis of autooxidations.

The fairly simple picture drawn, so far, is appar-

ently destroyed by the observation that, contrary to its predicted enhancing effect, SOD actually inhibits the autooxidation of several reduced quinones;[15–19] multiple mechanisms appear involved in this inhibitory effect.[20] Adrenaline is a well-known example of the inhibition exerted by SOD;[18] also, 3,4-dihydroxymandelic acid (α-hydroxy-3,4-dihydroxy phenylacetic acid)[19] shows the same behavior. According to Deeble et al.,[21] a reaction with a relatively high rate constant (k ca. 10^4 M^{-1} s^{-1} for catechol) takes place between O_2^- and 1,2-dihydroxybenzenes. The products of this reaction are hydrogen peroxide and a semiquinone radical in a process probably involving the initial formation of a short-lived O_2^- adduct (Eq. 3):



(3)

The occurrence of Eq. 3 removes O_2^- from equilibrium (Eq. 1) as well as causing more ArOH to be oxidized. When SOD is present, Eq. 3 no longer occurs, and this additional route for substrate oxidation is removed.

A striking difference in the chemistry of catechols and 1,4-dihydroxybenzenes is the ability of their respective semiquinones to react with oxygen. The 1,4 semiquinone (*para*-semiquinone), as already mentioned, reacts with oxygen to set up the equilibrium shown in Eq. 2, while catechol semiquinones appear to react with oxygen with very much lower rate constants. Initial studies by Kalyanaraman et al.,[22] measuring the rates of reaction of molecular oxygen with various *ortho*-semiquinone intermediates of simple catecholamines (4-methylcatechol, dopa, dopamine, adrenaline, and 5S-cysteinyldopa), set an upper limit for the rate constant for such a reaction as $< 10^5$ M^{-1} s^{-1}. It has been proposed that the low reactivity of the *ortho*-semiquinones is due to the semiquinone species being stabilized by an intramolecular hydrogen bonding.[23] On the other hand, catecholamines such as 6-hydroxydopamine and 6-aminodopamine are readily oxidized at physiological pH with a large production of superoxide anion and hydrogen peroxide.[24] Their oxidation appears to be enhanced by the 6-hydroxy group and leads to an *ortho*-hydroxy-*para*-quinone, which does not undergo cyclization like simple catecholamines.[25] At variance with the aforementioned *ortho*-semiquinones, the semiquinones derived from hydroxysubstituted catecholamines react at an appreciable rate with molecular oxygen ($k = 10^6 - 10^7$ M^{-1} s^{-1}).

The oxidation of catechols, including catecholamines, by O_2^- could be of enormous importance in living systems where O_2^- is commonly found. The impact of these processes may be enlarged by the occurrence of chain reactions.[18,19] A direct outcome of the low reactivity of the 1,2 semiquinones toward oxygen[19,22,23] is that the lifetime of these radicals in aerated systems, a group to which most living organisms belong, is sufficiently long for them to enter into reactions with each other as well as with other compounds.

Formation of the oxidation products of catecholamines

The catabolism of catecholamines proceeds through two major pathways involving the mitochondrial enzyme monoamine oxidase and catechol-o-methyltransferase in addition to a minor pathway catalyzed by phenolsulfotransferase.[26] However, a non-enzymatic oxidative pathway should also be taken into account since the catechol moiety can easily undergo oxidation. It was observed some time ago that catecholamine solutions can give rise to the formation of pink oxidation products on standing in air or in the presence of alkali.[27–30]

The oxidation of the catecholamines is a complex process in which quinones, hydroquinones, and semiquinone free radical intermediates are formed.[28–36] The basic oxidation process for the catecholamines was proposed many years ago by Raper[37] to explain the mechanism of melanin formation. Catecholamines are first converted to *ortho*-semiquinones (Fig. 2A) that, after disproportionation, give rise to the corresponding *ortho*-quinones[28,29,31–33] (Fig. 2B). The quinone intermediate, formed after oxidation of the catechol moiety of the various catecholamines, undergoes an irreversible 1,4-intramolecular cyclization; this reaction occurs through a nucleophilic attack of the nitrogen atom at the 6-position of the quinone ring,[28,29,33] leading to the formation of an unstable leucoaminocrome (Fig. 3). The latter is rapidly oxidized to an aminochrome (Fig. 4)* by interaction with another molecule of ortho-quinone or through other oxidizing species present in the system.

The uncatalyzed autooxidation of most of the cate-

* Aminochromes is a collective generic name[73] indicating the 2,3-dihydroindole-5,6-quinones formed on cyclization of the various catecholamines (Fig. 4).

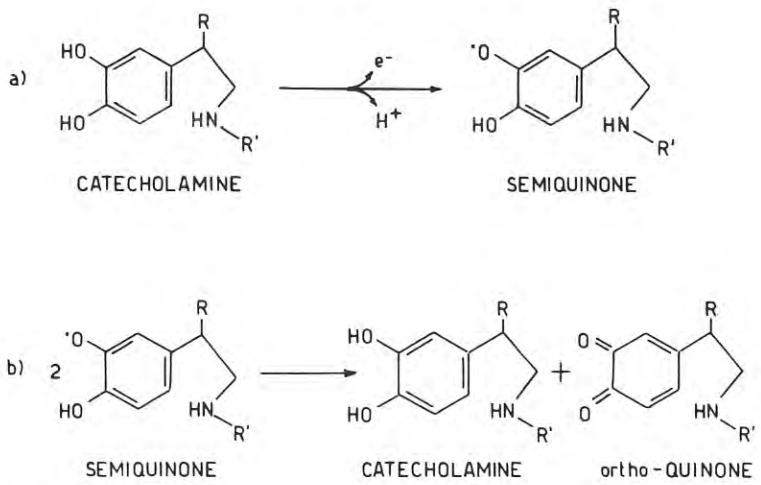


Fig. 2. Oxidation of catecholamines to the corresponding *ortho*-quinones. R: H, OH; R': H, CH_3, isopropyl.

cholamines is extremely slow at physiological pH and therefore is unlikely to be a significant source of oxygen free radicals.[40] However, trace metals as well as alkaline conditions strongly catalyze the oxidation of the catecholamines;[28,29,32,34] in fact, several metal ions, such as copper, manganese, nickel, and cobalt, were shown to promote the oxidation of the catecholamines;[28,29,41,42,43,52–56] The complex of manganic manganese with pyrophosphate is very active at inducing the oxidation of dopamine and other catecholamines to their corresponding aminochromes.[57,100] In the presence of Cu^{2+} the rate of oxidation of adrenaline is linearly dependent on the concentration of copper, which appears to form a Cu^{2+}-adrenaline complex.[41] The formation of adrenochrome from adrenaline was also accelerated by several copper(II) chelates of aminoacids or salicylate, capable of exerting SOD activity.[58,59] The latter enzyme can interact with oxidizing adrenaline forming a ternary complex involving the semiquinone, Cu(II), and superoxide anion.[59] Ceruloplasmin, a plasma protein containing copper[41–43] along with several copper-containing catechol oxidases present in both plant and animal tissues,[28–30,37–39,44–51] are able to initiate the oxidation of the catecholamines. Particularly relevant from the biological point of view is the oxidation of the catecholamines by iron ions and their complexes; many of these reaction mechanisms still have not been conclusively elucidated and need a more thorough investigation. Iron forms a violet-colored complex on interaction with the catechol moiety of the molecule;[54,60–62] this appears to cause the oxidation of catecholamines at a slower rate and to a lower extent compared to other catecholamines. Trautner and Bradley[56] reported a slow catalysis of adrenaline oxidation by Fe^{3+} ions, while other authors[63] found that Fe^{3+} is an effective catalyst of dopamine oxidation. Moreover, iron chelates like ferricyanide, ferricytochrome *c*, hematin, and methemoglobin are effective oxidizing agents of the catecholamines.[28,29,43,60,65] Fenton reagent (a mixture of hydrogen peroxide and ferrous ions) was shown to catalyze the oxidation of adrenaline to adrenochrome,[64] and ferritin, in the presence of hydrogen peroxide, acts in the same way, suggesting that the hydroxyl radical ($^{\cdot}OH$) is involved in the oxidation.[64] The reaction between catecholamines and hydroxyl radicals was confirmed by Bors et al.[32] using pulse radiolysis. The heme enzyme horseradish peroxidase, in the presence of H_2O_2, is also an efficient oxidizing system for the catecholamines;[66] here a high oxidation iron complex, rather than the $^{\cdot}OH$ radical, is likely to be the active agent.

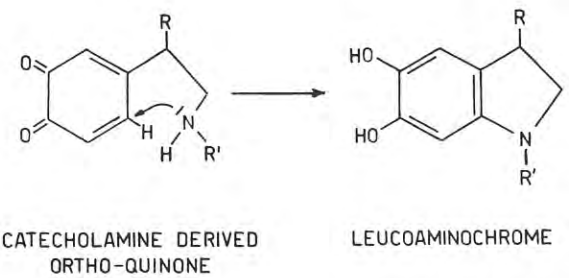


Fig. 3. Cyclization of catecholamine quinones. R: H, OH; R': H, CH_3, isopropyl.

Oxidation of adrenaline to adrenochrome is one of the most important oxidation processes initiated or mediated by superoxide anion. Valerino and McCormack[67] have shown that adrenaline is co-oxidized with xanthine in the presence of the enzyme xanthine oxidase. This observation was explained shortly afterward by McCord and Fridovich,[68] who demonstrated that the superoxide anion (and/or a metal cation) causes the univalent oxidation of adrenaline and initi-

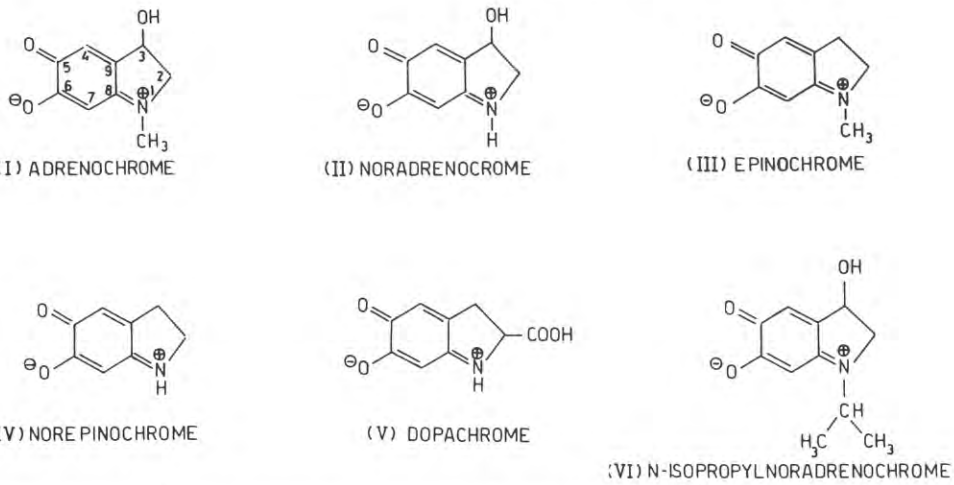


Fig. 4. Structural formulae of some aminochromes. The aminochromes reported are formed respectively from (I) adrenaline; (II) noradrenaline; (III) epinine; (IV) dopamine; (V) dopa; (VI) isoproterenol.

ates a chain reaction in which the superoxide anion is the propagating species. This superoxide-mediated oxidation of adrenaline is inhibited by the enzyme SOD and offers a method for the detection of these oxygen radicals.[18]

Catecholamines can be oxidized by microsomal[69–71] and mitochondrial[72] preparations; in these processes the superoxide anion is involved and is produced by the mitochondrial and microsomal electron transfer chains in the presence of appropriate substrates rather than by the autooxidation of adrenaline.

Aminochromes undergo further oxidation and polymerization to form brown or black insoluble pigments called melanins, the structures of which are still not completely known.[74–76] Their function, with the exception of photoprotection, is also a matter of discussion.[74] The best known pathway describing the formation of melanin is the Raper–Mason mechanism, according to which the formation of melanins proceeds through a rearrangement of the aminochrome to a 5,6-dihydroxyindole which, after oxidation to the corresponding quinone, undergoes a complex series of self-condensation reactions.[37,50,77] Nevertheless, this relatively simple process appears to be more complicated in vivo, where other factors, such as metal ions and sulfhydryl compounds, affect the chemistry of the melanogenesis process.[75] In the sequence of reactions going from aminochrome to melanin, several low-molecular-weight intermediates are formed, and some of these, such as the 5,6-dihydroxyindoles and cysteinyldopas (Fig. 1), can be found in blood and urine.[75,117]

In addition to the skin, hair, choroid and iris of the eye, and the inner ear, melanins are found in the brain, where they are called neuromelanins. The latter originate from dopamine (*substantia nigra*) and noradrenaline (*locus ceruleus*).[74,78,79] Their role has not been clearly defined, and they appear to act as waste accumulation products[80] or free radical scavengers.[74,81] The presence of the melanins of the substantia nigra is largely reduced in patients suffering from Parkinson's disease.[152] Noradrenochrome is oxidized to melanitic pigments more readily than adrenochrome;[55] it should also be noted that the melanization of adrenochrome can also take place under anaerobic conditions.[28]

Aminochromes can rearrange to 5,6-dihydroxyindoles or to 5,6-dihydroxyindoxyls.[28,29,82,83] The lack of the 3-hydroxyl substituent greatly favors the rearrangement of aminochromes to 5,6-dihydroxyindole.[28] In fact, the aminochromes possessing a 3-hydroxy group tautomerize to the corresponding "aminolutins" or 5,6-dihydroxyindoxyls[28,29,31,82,83] (Fig. 5). This rearrangement is an intramolecular redox reaction catalyzed by metal ions, particularly Zn^{2+} and, to a lesser extent, aluminum ions and bringing about a decolorization of the red solution of the aminochromes with formation of products exhibiting a yellow-green fluorescence.[28,29,82,83] Aminochromes, therefore, appear to play a key role in the oxidation of the catecholamines. The decomposition of adrenochrome and the other aminochromes is a complex process in which many factors are involved, such as aerobic/anaerobic conditions, pH, and type and concentration of metal ions.[84] Hence, in addition to the rearrangement reactions, redox exchange, autooxidation, and dimerization reactions occur.[84] At physiological pH and under anaerobic conditions, adrenochrome is converted not only to adrenolutin but also to a dimeric product consisting of an adrenolutin moiety covalently linked to the 9-position of adrenochrome.[84,85] In aerated solution, adrenolutin autooxi-

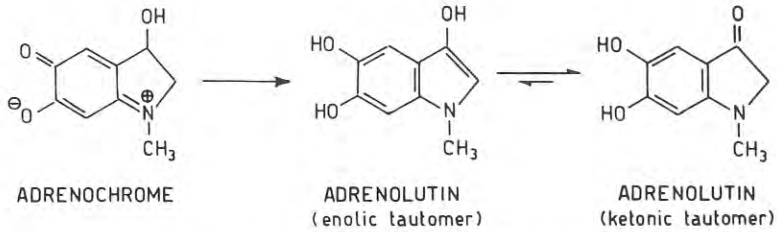


Fig. 5. Rearrangement of adrenochrome to adrenolutin.

dizes to give 5,6-dihydroxy-1-methyl-isatin, which is in turn oxidized to the corresponding 4,4′-dimer.[84]

Finally, a physiological role for the oxidative pathway of adrenaline has been proposed.[86,87] According to this cycling redox mechanism, adrenaline is oxidized to its semiquinone with the concomitant reductive activation of the β-receptor; this oxidation-reduction process appears to be an essential step in the chain of events leading to the activation of adenylate cyclase, where a series of thiol-disulfide interconversions, elicited by the reduced receptor, takes place.[86,87] The reduction potential of all the antagonists tested have values greater than 750 mV, suggesting that they are difficult to oxidize. The β-agonists have reduction potentials in the range 250 mV to 70 mV, somewhat lower than those of the antagonists, and hence they are more amenable to oxidation.[86,87]

REDUCTION REACTIONS OF THE PRODUCTS DERIVED FROM THE CATECHOLAMINES

Adrenochrome can be reduced by several agents (e.g., sodium dithionite, sodium borohydride, lithium aluminum hydride, leucomethylene blue, thioglycolic acid, and 2,3-dimercapto-1-propanol) to the unstable and colorless form called "leucoadrenochrome," which spontaneously dehydrates to 5,6-dihydroxy-*N*-methylindole (Fig. 6), a relatively more stable form as compared to the leucoform.[28,29,88–91] While 5,6-dihydroxy-*N*-methylindole can be easily isolated after reduction of adrenochrome with ascorbic acid or sodium borohydride,[92] leucoadrenochrome is not isolatable and there is only indirect evidence for its formation; its solutions give typical catechol reactions

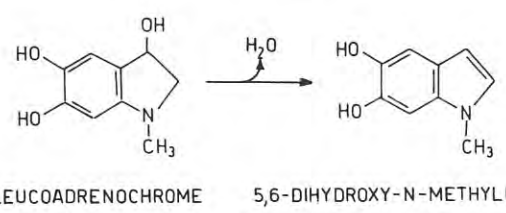


Fig. 6. Formation of 5,6-dihydroxy-*N*-methylindole by dehydration of leucoadrenochrome.

and are optically active.[30] However, Bu'Lock and Harley–Mason[91] do report the isolation of a leucoderivative (ethyl 2,3-dihydro-5,6-dihydroxy-3-iodoindole-2-carboxylate) that, in air, undergoes rapid autooxidation.

Aminochromes without a 3-hydroxyl group (e.g., dopachrome, epinochrome, and norepinochrome) rearrange very easily to the corresponding 5,6-dihydroxyindoles before undergoing reduction to the leuco form.[28,29,88,89] Leucoepinochrome (5,6-dihydroxy-*N*-methylindoline) has been isolated in trace amounts.[89]

In addition to the aforementioned reducing agents, reducing agents of biological interest such as ascorbic acid,[92–95] glutathione,[96–99] cysteine,[97] and the flavoprotein DT-diaphorase[100,107] are very efficient at bringing about the decolorization of aminochrome solutions.

Years ago, Galzigna et al.[101] observed that the reduction of adrenochrome by ascorbate[92–95] is accompanied by a fast oxygen uptake. More recent experiments[102] have demonstrated that this oxidation is not simply due to a trace metal catalysis as it is not inhibited by chelators such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), or desferrioxamine. However, it is inhibited by SOD and catalase. This oxygen consumption appears to be due to a nonenzymatic redox cycling of adrenochrome, where ascorbate is the source of reducing equivalents. A similar cycle can arise enzymatically in the presence of microsomal and mitochondrial preparations. In rat liver microsomes, adrenochrome is able to increase the rate of oxygen consumption elicited by either reduced nicotinamide adenine dinucleotide (NADH) or reduced nicotinamide adenine dinucleotide phosphate (NADPH).[70,103] Similarly, adrenochrome can be reduced to the corresponding semiquinone by complex I of beef heart submitochondrial particles supplemented with NADH; in contrast, succinate is unable to support this reduction,[102] suggesting that the flavin of the mitochondrial complex I is the site of adrenochrome reduction. The formation and characterization of a semiquinone radical anion derived from adrenochrome was directly demonstrated by electron spin

resonance and pulse radiolysis techniques.[35,102,104,105] Similarly to adrenochrome, the oxidation products derived from dopamine also appear to undergo redox cycling.[100,106]

Aminochromes can also act as substrates for the enzyme DT-diaphorase obtained from rat liver and brain.[100,107] The latter enzyme (NAD(P)H:quinone oxidoreductase, EC 1.6.99.2) is able to reduce several subtances, particularly quinones, to their reduced forms in a two-electron process.[108] Consequently, it plays an important role in preventing the formation of semiquinones with the associated production of oxygen reduced forms.[100,109] The stimulation of NADPH oxidation by adrenochrome in the presence of DT-diaphorase is strongly inhibited by dicoumarol, which is the best known inhibitor of this enzyme,[108] indicating the specificity of the process. Adrenochrome can be transformed to its enolic form adrenolutin, considered toxic, or, after two-electron reduction, can dehydrate to the reportedly nontoxic 5,6-dihydroxy-*N*-methylindole.[110] According to this scheme, DT-diaphorase might exert a protective role by keeping adrenochrome in its reduced (nontoxic) form. DT-diaphorase is present in the brain,[111] where the metabolism of the catecholamines is quite active, and its location in various areas of the brain has been mapped.[112]

MOLECULAR MECHANISMS OF TOXICITY OF CATECHOLAMINES AND THEIR OXIDATION PRODUCTS

Any alteration of the metabolism of the catecholamines or disruptions of their transport mechanisms might lead to anomalously high concentrations of these substances, and consequently the enzymes dealing with their catabolism (monoaminooxidase and catechol-o-methyltransferase) are unable to cope efficiently. Therefore, catecholamines may undergo autooxidation in those cell compartments where their concentration has increased, giving rise to potentially toxic products such as free radical species, *ortho*-quinones, and indolic catechols.

During the autooxidation process, a number of organic free radicals are produced and are able to bind irreversibly to the various cell constituents. Ring-substituted *ortho*-semiquinones were detected by electron spin resonance (ESR) spin stabilization techniques when catecholamines were oxidized chemically or enzymatically in the presence of nucleophiles such as aminoacids, peptides, and protein.[113]

5S-derivatives were found after autooxidation of the catecholamines to the respective quinones in the presence of thiol compounds such as cysteine and glutathione.[75,114–117] The glutathionyl adducts are subsequently attacked by peptidases to yield the 5S-cysteinyl derivatives,[75,114–117] and these enzymatic reactions might be fine regulators of the melanogenesis process.[75] In the human brain and in the brain of several mammalian species, 5S-cysteinyldopamine and 5S-cysteinyldopa (Fig. 1) have been found to be present.[114–116] Furthermore, some of the precursors of the biosynthetic process of skin melanins (e.g., cysteinyldopas and dihydroxyindoles) can leak out from the melanosomes and have been detected in the skin, serum, and urine of subjects undergoing active melanogenesis.[117] These compounds are photochemically unstable and, upon photolysis, give rise to the production of a number of free radical species.[117]

Table 1. Toxicity of the Oxidation Products of Catecholamines

Interaction with cellular sulfhydryl groups (covalent adducts, oxidation to disulfides)
Formation of oxygen free radicals by redox cycling
Inhibition of enzyme activity:
Dihydropteridine reductase
Guanylate cyclase
Catechol-*o*-methyltransferase
Actomyosin ATPase
Polypeptide synthesis
Impairment of mitochondrial energy processes:
Uncoupling
Inhibition of ATP formation
Inhibition of phosphate and calcium uptake
Impairment of calcium uptake by sarcoplasmic reticulum
Cytotoxicity:
Neuroblastoma cells
Melanoma cells
Induction of some forms of mental and nervous illness (schizophrenia, Parkinson's disease, manganese toxicity)
Induction of myocardial injury (depression of contractile activity, arrhythmias)

Interaction with nucleophiles

One of the major mechanisms of quinone toxicity arises from their interaction with cellular sulfhydryls with the formation of stable adducts[8,118] (Table 1). For instance, *ortho*-benzoquinone, formed by a peroxidase-mediated oxidation of catechols, binds covalently to the sulfhydryl groups of proteins.[119] Similarly, 1-naphthol is converted by tyrosinase mainly to 1,2-naphthoquinone, with a small amount of 1,4-naphthoquinone also being formed. The former is mostly responsible for the covalent binding to sulfhydryl groups. 1-Naphthol is considered a potential therapeutic agent in the treatment of cancer cells having a high tyrosinase activity, such as occur in certain melanomas.[120]

Aminochromes, by virtue of their indolinequinone ring, are able to interact with various nucleophiles of the cell; of particular importance are the reactions with acid-soluble thiols such as cysteine, glutathione,

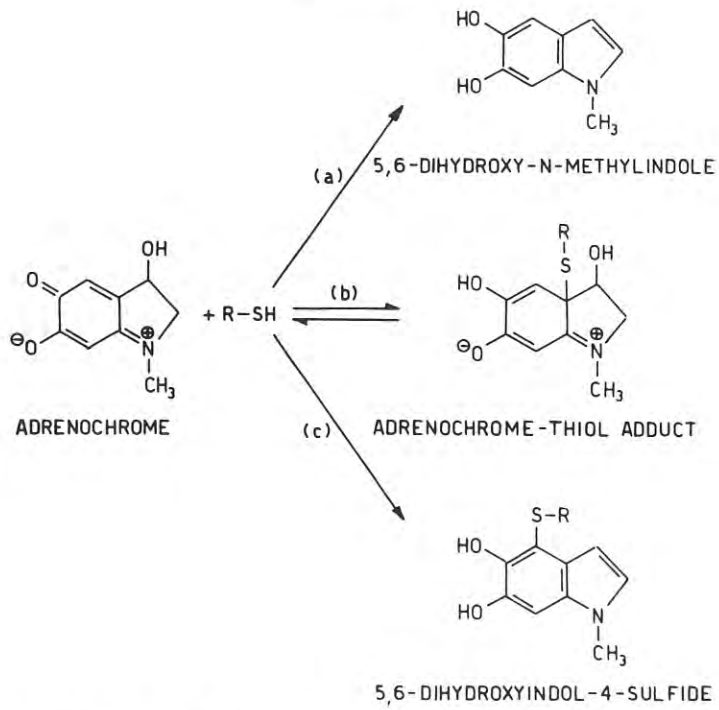


Fig. 7. Reaction of adrenochrome with thiols. Adrenochrome interacts with thiol compounds such as glutathione, cysteine, and homocysteine (R-SH) giving rise respectively to reduction (a), addition (b), and substitution products (c).

and protein sulfhydryl groups.[98,99,121] Adrenochrome, for instance, after interaction with glutathione, cysteine, and homocysteine, gives rise to reduced, substituted, and thiol-addition products[28,29,98,99,122,123] (Fig. 7). The last type of reaction is reported to be reversible,[124] and consequently glutathione and other thiols might act as aminochrome carriers.[98,99] It was observed that the sulfhydryl group of Coenzyme A, reduced glutathione (GSH), and cysteine disappears upon addition of the catecholamine oxidation products obtained from the tyrosinase-catalyzed oxidation of dopa.[96,97,125] Similarly, the incubation of blood with adrenaline induces a rapid decrease in the glutathione content of the erythrocytes, again due to reactions involving adrenaline oxidation products.[126]

Aminochromes were found to inactivate the SH-dependent enzymes either through the formation of a covalent adduct[121,127] or by oxidation, to the corresponding disulfides, of paired and sterically vicinal thiol groups.[128] Both intermolecular and intramolecular disulfide bonds are formed. Several enzymes are reported to be inhibited by the interaction of aminochromes with their SH groups: dihydropteridine reductase,[128,129] guanylate cyclase,[130] catechol-o-methyltransferase,[127] actomyosin ATPase,[131] and polypeptide synthesis[132,133] (Table 1).

Biological membranes can be severely affected by the oxidation products of the catecholamines. Mitochondrial energy processes are altered by adrenochrome, as observed by a decrease in oxygen uptake, adenosine 5′-triphosphate (ATP) formation,[134,135] and phosphate uptake[135] (Table 1). Adrenochrome also inhibits the mitochondrial[136] and microsomal[137] calcium uptake system.

Autooxidation products of dopa, dopamine, adrenaline, and noradrenaline exert a cytotoxic action on neuroblastoma cells as revealed by a decrease in the incorporation of tritiated thymidine into DNA.[121] A similar behavior was found for melanoma cells,[138–140] indicating a possible role for aminochromes or their precursors as potential antitumor agents.[105]

Production of free radicals

In addition to the interactions with nucleophiles present in the cell, quinonoid compounds can give rise to the production of oxygen free radicals in biological systems. This occurs, as already described, when a reducing system, usually a flavoprotein, is able to reduce the quinone to a semiquinone or hydroquinone, which, under aerobic conditions, can be reoxidized with the formation of the superoxide anion. The catecholamine-related compounds 6-aminodopamine and 6-hydroxydopamine,[24,161–163] reduced adrenochrome,[102] and norepinochrome,[100,106] unlike many other catecholamines,[22] autooxidize very rapidly and therefore are likely candidates for such a redox cycling process. The resulting superoxide anion dismutates to

hydrogen peroxide. In the presence of iron (II), hydrogen peroxide forms the hydroxyl radical ($^{\bullet}OH$), which is highly reactive and can lead to DNA and protein damage.[141]

Lipid peroxidation is another of the molecular alterations caused by oxidative stress. Quinonoid compounds, depending on their specific properties and on the experimental conditions, can either stimulate or inhibit lipid peroxidation. For instance, anthracycline antibiotics stimulate lipid peroxidation,[142] while menadione[143] and coenzyme Q[144,145] act as powerful antioxidants. Adrenochrome inhibits lipid peroxidation induced with different systems that may be either dependent or independent of iron ions.[146] A reduced form of adrenochrome (leucoadrenochrome or the corresponding semiquinone) can act as a particularly efficient antioxidant; a partial antioxidant effect is also exhibited by the oxidized form.[146] Consequently, the toxicity of adrenochrome is not readily ascribable to a direct peroxidative process since, in fact, it possesses antioxidant properties. However, toxic action through an oxidative stress-linked mechanism cannot be ruled out.

In conclusion, the toxicity of catecholamine oxidation products can be attributed either to the formation of a covalent linkage with the cellular sulfhydryl groups or to the production of oxygen free radicals which then induce oxidative stress (Table 1). In addition, the redox cycling process can lead to a large oxygen consumption, which might create hypoxic conditions, as well as depletion of glutathione and thiol levels.

In vivo formation of aminochromes

A major reservation regarding the potential toxicity of the oxidation products of the catecholamines concerns the likelihood of the in vivo formation of these intermediates, particularly the aminochromes. However, the presence of cysteinyl-dopas derivatives in brain, blood, and urine[75,117] indicates the occurrence of the oxidative pathway for the catecholamines in vivo.

As already discussed, in addition to the metal-catalyzed formation of aminochromes, several enzymatic systems, either soluble or membrane bound, are able to catalyze the conversion of adrenaline to adrenochrome.[28–30,38,39,43–51] Nevertheless, according to Schayer and Smiley,[147] ^{14}C-adrenaline does not result in the endogenous formation of ^{14}C-adrenochrome. On the other hand, more recently, Matthews et al.,[148] using ^{14}C-adrenaline and high-pressure liquid chromatography (HPLC) analysis, have shown that polymorphonuclear leucocytes, stimulated to produce oxygen radicals, are able to convert physiological concentrations of adrenaline into adrenochrome as the major product (80%). The remaining adrenaline was metabolized through the usual pathways (monoamino oxidase and catechol-o-methyl transferase). This oxidation is probably catalyzed by the enzyme myeloperoxidase in the presence of hydrogen peroxide.

An "adrenaline oxidase" activity was observed in the serum of patients with acute myocardial infarction,[149,150] while adrenochrome was found in the synovial fluid of patients with rheumatoid arthritis.[149] Recently, an HPLC assay technique for the measurement of plasma adrenolutin was developed.[151] A relatively high level of adrenolutin was found in the plasma of rat, dog, rabbit, and pig. Furthermore, this level was increased by the administration of different catecholamines, suggesting the presence of an efficient mechanism for the oxidation of catecholamines under in vivo conditions.[151] Adrenochrome, unlike adrenolutin, was not detected in plasma by these methods.[151]

NEUROTOXICITY

The neurons of *substantia nigra* form dopamine as their neurotransmitter, and their pigmentation is due to neuromelanin formed after polymerization of dopamine.[152] These neurons project their bodies to the striatum. Nigrostriatal neurons are progressively lost with the natural ageing process; in Parkinson's disease there is an accelerated loss of the pigmented neurons in the *pars compacta* of the *substantia nigra.*[153] The typical symptoms of Parkinson's disease such as rigidity and tremor do not appear until about 80% of the neurons have been destroyed by aging, disease, or environmental chemical insult,[154] indicating that the neuromotor system is highly compensated. It has been suggested[36,80,152,153,155–157] that in Parkinson's disease an intermediate metabolite involved in the synthesis of neuromelanin might exert a toxic action on the neuron, leading to its demise. Cohen,[158] meanwhile, proposed that an increased dopamine turnover can elicit an oxidative stress that could contribute to the pathobiology of Parkinson's disease. Increased turnover of dopamine might be mediated by monoamine oxidase, an enzyme present in the mitochondrial outer membrane and responsible for hydrogen peroxide formation, according to Eq. 4:

$$\text{Dopamine} + O_2 + H_2O \rightarrow \text{3,4-dihydroxyphenylacetaldehyde} + NH_3 + H_2O_2 \quad (4)$$

An alteration on the uptake mechanism of the catecholamines could produce high extracellular concen-

trations that may then undergo oxidation.[158] Indeed, it was reported[159] that amphetamines exert their toxic action on *striatum* dopaminergic terminals by a displacement of dopamine from its vesicular stores into the cytosol, whereupon autooxidation could occur. More evident for this type of mechanism is that the ischemic stroke in gerbils induces a rise in dopamine concentration in the *striatum.*[106] The in vivo occurrence of catecholamine oxidation products is also demonstrated by the recovery of 5S-cysteinyldopamine and 5S-cysteinyldopa in human brain and in the brain of several mammalian species.[114–116] The 5S-cysteinyl adduct is formed by the reaction of the catecholamine quinone with glutathione, followed by peptidase-induced hydrolysis of the initial adduct to yield the 5S-cysteinyl derivative.[114–116]

The experimental neurotoxin 6-hydroxydopamine induces Parkinsonism in experimental animals by destroying the catecholaminergic neurons after selective accumulation of catecholamines as a result of changes in the catecholamine uptake mechanism.[160] Unlike most naturally occurring catecholamines, 6-hydroxydopamine undergoes rapid autooxidation at or near neutral pH, giving rise to quinoidal compounds.[22,24,25] In the presence of reducing agents such as ascorbate, redox cycling takes place,[121,161] with a large consumption of oxygen and the production of superoxide radicals[162] and other reactive species such as the hydroxyl radical.[163]

As already reported, manganese is a good catalyst for the oxidation of dopamine and other catecholamines.[28,29,43,52,53,55,57,100] When relatively large amounts of manganese compounds are given to experimental animals, various symptoms, including muscular tremors associated with a decrease of dopamine in the brain, are observed.[164] A neurological disorder called *locura manganica* (manganese madness) has been observed in Chile among the miners of manganese ores. This disease has a rough resemblance to Parkinson's, but the damage is mostly to the *striatum* and the *pallidum* and not to *substantia nigra.*[141,165]

The involvement of aminochromes, and in particular of adrenochrome, in psychiatric disorders has been the object of several studies and, in particular, adrenochrome was considered to be responsible for some forms of mental illness.[166] According to Hoffer,[166] schizophrenia might result from an altered metabolism of adrenaline giving rise to psychotogenic metabolites, proposed to be adrenochrome or adrenolutin. This hypothesis was essentially based on the observation of a close similarity between the indoline quinone structure of the aminochromes and the indole structure of hallucinogenic drugs able to induce some of the early symptoms of schizophrenia.[167] The same hypothesis allows adrenochrome to be further metabolized to adrenolutin, which is considered to be toxic, or to 5,6-dihydroxy-*N*-methylindole reported to be nontoxic.[167] There is, in fact, no general agreement on the ability of adrenochrome and/or adrenolutin to induce psychic changes.[29,167,168] One particular criticism is that samples of adrenochrome prepared using different procedures might contain different contaminants which may be responsible for the observed psychotic effects.[28,29,167]

According to Galzigna, noradrenochrome is able to form a relatively stable complex with acetylcholine, thus establishing a kind of "short circuit" between adrenergic and cholinergic pathways causing the onset of mental illness.[169,170] It is tempting to speculate that this interaction might take place when a catecholamine, released in a synapse, undergoes autooxidation to adrenochrome instead of being metabolized by its own enzymes.

CARDIOTOXICITY

High levels of plasma catecholamines, either the result of excessive release or their administration in doses exceeding physiological concentrations, have been found to induce morphological and functional alterations in the heart, ultimately leading to myocardial necrosis.[171–173] Catecholamines are released during ischemia/reperfusion conditions.[174] The catecholamine-induced myocardial damage is a multifactorial process, and several mechanisms were proposed to explain it.[172] As reported earlier, catecholamines are only slowly oxidized in the absence of a catalyst;[40] however, catecholamines can give rise to free radicals through catalyzed oxidations involving enzymatic systems or metal ions.[40] Dhalla et al.[175–177] suggested that the cardiotoxic effects of catecholamines are not referable to the catecholamine per se but rather to their oxidation products, such as adrenochrome. Perfusion of rat heart in the presence of the latter, in addition to ultrastructural damages,[176] gives rise to arrhythmias and sudden cardiac death[175,177] associated with a marked decline in the contactile force and increase in the resting tension.[134,175] A rapid depletion of ATP and a parallel increase in adenosine 5′-diphosphate (ADP) and adenosine 5′-monophosphate (AMP) was also observed.[134]

At the subcellular level, a decrease in the mitochondrial respiratory control index and an increase in state 4 respiration was reported.[134] In addition, adrenochrome depresses mitochondrial calcium uptake[136] with a mixed-type inhibition not readily reversible, indicating a strong binding of adrenochrome to the mitochondrial membrane. This binding appears to be of a covalent type occurring through interaction with sulfhydryl groups.[136] Adrenochrome decreases micro-

somal calcium binding, calcium uptake, and inhibits calcium/magnesium-stimulated ATPase activity.[137] In like manner, it significantly decreases calcium ATPase, sodium/potassium ATPase, and adenylate cyclase of rat heart sarcolemma.[178] The mitochondrial, microsomal, and sarcolemmal fractions taken after perfusion with adrenochrome showed similar depressions of these enzyme activities.[134,137,178] After perfusion with ^{14}C-adrenochrome, the sarcolemmal fraction showed the highest uptake of adrenochrome, followed by the microsomal and mitochondrial fractions.[179] On reperfusion, approximately 50% of the radioactivity remains in the heart, indicating an irreversible binding of adrenochrome to the tissues.[179] It is interesting to note that, after reaction of adrenochrome with reducing agents, such as ascorbic acid or cysteine, the resulting products induce, in isolated and perfused rat heart, damage greater than that observed with adrenochrome alone.[180] This suggests that the catecholamine-induced cardiotoxicity is also due to oxidation products other than adrenochrome.

It was reported that the synthetic catecholamine isoproterenol can induce, in experimental animals, an "infarct-like" myocardial necrosis[172,181] and, similarly to adrenochrome, oxidized isoproterenol causes extensive myocardial damage.[181–183]

The role of adrenochrome in eliciting catecholamine cardiotoxicity has been questioned on the basis that the alterations induced by catecholamines are not identical to those induced by adrenochrome.[172] Furthermore, high concentrations of adrenochrome (10^{-4} M) are needed to induce the toxic effects, while the circulating adrenaline is about 10^{-11} – 10^{-12} M (Refs. 172, 173). Recently, Dhalla et al.[151] have shown that adrenochrome rapidly disappears upon its exposure to blood, possibly because of its transformation to other metabolites, such as adrenolutin. The latter, at least in rat blood, was found present in a relatively large concentration (10^{-4} M) and did not undergo degradation by blood cells. Consequently, the adrenolutin concentration perhaps accurately reflects the extent of oxidation of the circulating catecholamines.[151] Nevertheless, according to Hegedus and Altschule,[184] adrenolutin, incubated with plasma, changes to a plasma-soluble compound of unknown structure.[184]

CONCLUSIONS

The metabolic and toxicological effects of the oxidation products of the catecholamines appear to be of relevance to biological systems. The mechanism of action and the involvement of these endogenous toxins especially at the nervous and cardiac level are still a matter of discussion. In particular, the in vivo formation of aminochromes and other oxidation products of the catecholamines awaits definite confirmation, although the finding of sulfhydryl adducts of the catecholamines in the brain and biological fluids along with the presence of fluorescent material (believed to be adrenolutin) in blood are a strong indication of the in vivo formation of these compounds.

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DEFINITIONS

Adrenochrome: 2,3-dihydro-3-hydroxy-N-methyl-indole-5,6-quinone

Adrenolutin: 5,6-dihydroxy-N-methyl indoxyl

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-methylaminoethane

Leucoaminochrome: 2,3-dihydro-5,6-dihydroxy-N-methyl-indole

Leucoadrenochrome: 2,3-dihydro-3,5,6-trihydroxy-N-methylindole