Research paper

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Reduction reactivity of catecholamines and their ability to promote a Fenton reaction

Victoria Melin*[a,b], Adolfo Henríquez [a], Claudia Radojkovic [c], Brigitte Schwederski [d], Wolfgang Kaim [d], Juanita Freer [a,b] and David Contreras*[a,b].

[a] Faculty of Chemical Science, University of Concepción, Concepción, Chile.
[b] Biotechnology Center, University of Concepción, Concepción, Chile.
[c]Faculty of Pharmacy, University of Concepción, Concepción, Chile.
[d] Institute of Inorganic Chemistry, Universität Stuttgart, Stuttgart, Germany.

*** Corresponding authors:**
Biotechnology Center, University of Concepción, Casilla 160-C, Concepción, Chile.
Email address: victoriamelin@udec.cl (Victoria Melin) and dcontrer@udec.cl (David Contreras)

Abstract

Several studies have assigned catecholamines a pro-oxidant role and have therefore correlated catecholamines with the development of different pathophysiological processes. This pro-oxidant effect could be due to the Fenton reaction (i.e., Fe(II) + H_2O_2 → Fe(III) + •OH + OH^-), which is known to be utilized by catecholamines to reduce Fe(III) and O_2. In this work, the ability of a few catecholamines (i.e., dopamine, epinephrine and norepinephrine) to reduce Fe(III) to Fe(II) and O_2 to H_2O_2 and to produce •OH radicals by the Fenton reaction was evaluated at different pH values. The catecholamines were observed to produce Fe(II) and H_2O_2 at different pH values. Therefore, •OH radical production was enhanced at pH values where only Fe(III) reduction was observed. At pH values near 7.0, the catecholamines did not enhance the production of •OH radicals. Instead, the catecholamines acted as antioxidants forming bis-complexes, i.e., $[Fe(LH)_2]^+$. The catecholamines sequestered iron from the reaction system and thereby prevented iron from reacting with other compounds such as H_2O_2.

1. Introduction

Catecholamines, including dopamine, epinephrine and norepinephrine, are hormones and neurotransmitters of the nervous system (Figure 1). The roles of these chemicals in the body is widespread and includes several physiological processes[1]. In addition to their physiological functions, catecholamines can participate in chemical reactions that generate harmful molecules at the cellular level. In autoxidation reactions, catecholamine is oxidized by O_2, resulting in the generation of superoxide radicals ($O_2^{\bullet-}$) and semiquinone, which can be further oxidized to quinone by reducing another O_2 molecule[2, 3]. The radical $O_2^{\bullet-}$ is converted into H_2O_2 and O_2 by a disproportionation reaction. Semiquinone and quinones produced during the oxidation process are also harmful to the cellular environment[4-7]. Because of their ability to produce reactive oxygen species (ROS), catecholamines have been attributed with the development of several diseases associated with oxidative stress, among which include neurodegenerative diseases such as Parkinson's disease[8, 9]. These diseases also affect iron homeostasis[10, 11]. When treated with catecholamines, the iron concentration in *substantia nigra* increased by approximately 35% compared with normal physiological iron levels[12]. In the human body, iron should remain bound to proteins due to the potential for iron to produce free radicals, especially hydroxyl radicals (•OH)[13].

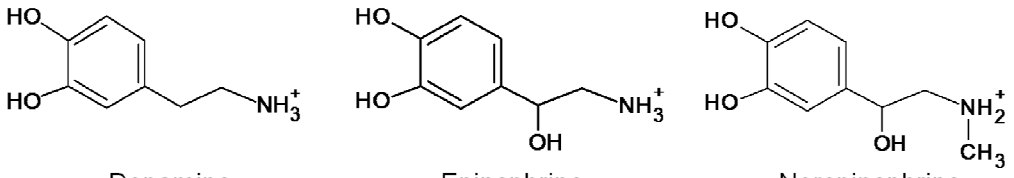

Figure 1: Catecholamines structures.

The main source of •OH radicals in biological systems comes from the Fenton reaction[14]. For this reaction to occur, Fenton reagents, Fe(II) and H_2O_2, are necessary. The reaction mechanism was proposed by Haber and Weiss in 1932. The •OH radicals act as the primary oxidizing species in reaction (1)[15]. During the Fenton reaction, Fe(III) is formed; subsequently, Fe(III) reacts with H_2O_2 to produce Fe(II) by a “Fenton-like” reaction (2)[16]. The Fenton-like reaction is three orders of magnitude slower than the Fenton reaction and thereby represents

the limiting step in the redox cycle of a Fenton system. The reaction mechanism of the Fenton-like reaction involves the production of perhydroxyl radicals (i.e., •OOH)[17].

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + {\bullet}OH + OH^- \quad k \approx 70\ L{\bullet}mol^{-1}{\bullet}s^{-1}\ [18] \quad (1)$$

$$Fe(III) + H_2O_2 \rightarrow Fe(II) + {\bullet}OOH + H^+ \quad k = 0.01\ L{\bullet}mol^{-1}{\bullet}s^{-1}\ [19] \quad (2)$$

Like other catechols, catecholamines (LH_3^+) can form complexes with iron[20-22]. Figure 2 shows the primary iron complexes that are typically present in aqueous solutions. The formation of the bidentate mono-complex ($[Fe(LH)]^{2+}$) is not dependent on any specific iron species in acidic aqueous solutions (i.e., $[Fe]^{3+}$, $[Fe(OH)]^{2+}$ or $[Fe(OH)_2]^+$)[20]. The formation of a monodentate complex ($[Fe(LH_2)]^{3+}$) was suggested by Xu and Jordan[23]. However, the formation of $[Fe(LH)]^{2+}$ is favorable due to a chelating effect. As the pH value increases, the formation of the bis-complex ($[Fe(LH)_2]^+$) is favored at pH close 7.0. At approximately pH 10 the formation of a tris-complex ($[Fe(LH)_3]$) is predominant. Furthermore, $[Fe(LH)]^{2+}$ has a short half-life due to the reduction of Fe(III)[24].

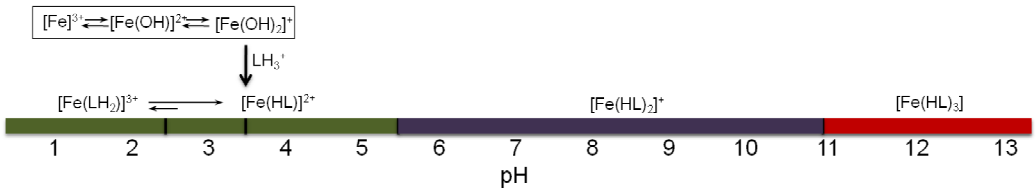

Figure 2: Primary Fe(III)-catecholamine complexes present in aqueous solutions. LH_3^+: triprotic catecholamine.

At physiological pH values, the formation of iron-catechol complexes is sufficiently favorable to extract iron from stored proteins in the body[25-29]. Catecholamines behave as non-innocent ligands, i.e., Fe(III) is reduced within the coordination sphere[25]. Therefore, catecholamines could amplify oxidative pathological states by promoting the bioavailability of iron for the production of free radicals by the Fenton reaction[23].

Paris et al. studied the effects of dopamine iron complexes *in vitro* under physiological conditions[26]. The study highlighted the cellular toxicity of these complexes due to ROS formation (the production of •OH radicals was observed).

Based on studies of pH influence on the oxidation of benzyl alcohol by catecholamine/Fe(II)/H_2O_2 systems, the oxidation of benzyl alcohol was found to be dependent on iron speciation in the catecholamine complexes[27]. The maximum level of oxidation of benzyl alcohol was observed at pH values near 3.4, which was similar to the optimal pH value observed for other catecholate-iron systems[28]. In these reports, the reactivity was determined only by the oxidation of an aromatic substrate. However, the roles of Fe(III) reduction and •OH radical production were not explored. Furthermore, the ability for catecholamines to induce oxidative stress through the Fenton reaction in a biological system was not tested. In the present study, the capacity for catecholamine-driven systems to produce ROS was tested on human umbilical vein endothelial cells (HUVECs), a thin monolayer of cells that acts as the first barrier between blood and tissue.

2. Material and Methods

2.1. Reagents

Catechol, dopamine hydrochloride, (±)-epinephrine hydrochloride, DL-norepinephrine hydrochloride, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-*p,p'*-disulfonic acid (ferrozine), (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Life Technologies) and 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) were all purchased from Sigma. Potassium fluoride, [*bis*(2-hydroxyethyl)imino]-*tris*(hydroxymethyl) methane (BIS-tris, Calbiochem), dichloro-dihydro-fluorescein diacetate (DCFH-DA, Calbiochem), H_2O_2 30%, ferric nitrate, ferrous sulfate, sodium acetate and acetic acid 100% were supplied by Merck.

All reagents were used without additional purification.

2.2. General Procedures

All reagent solutions were prepared in the dark under argon atmospheres. The ionic strengths of all solutions were adjusted to 0.10 mol•L^{-1} with KNO_3. All experiments were performed at 20 ± 0.1°C in triplicate (n= 3).

For experiments where a pH adjustment was required, different buffers were used. A 0.050 mol•L^{-1} BIS-tris buffer was used to maintain pH values from 6.0-7.0; a 0.050 mol•L^{-1} acetate

buffer was used for pH values of 4.0-5.5; and HNO_3 was used to regulate pH values lower than 4.0. The pH of each solution was adjusted prior to experimentation using a 3 Start Thermo Orion pH meter.

2.3. Reduction of Fe(III) by spectrophotometric measurements

The reduction of Fe(III) by each tested catecholamine was determined at pH values between 2.0 and 7.0. The final concentrations in the systems were 0.15 mmol•L^{-1} $Fe(NO_3)_3$ and 1.5 mmol•L^{-1} catechol or catecholamine. The reaction was initiated by adding Fe(III).

Quantification of reduced Fe(III) was determined by spectrometry by measuring the levels of Fe(II) formed at different reaction times (UV-vis diode array spectrophotometer, Agilent 8453). Briefly, the reduction of Fe(III) resulted in the production of colored complexes between the metal and the chelating 3-(pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine (ferrozine). Absorbances were measured at 567 nm[29]. This technique was a variation of that disclosed by Chen and Pignatello[30].

Based on the linear range of the constructed absorbance curve, the initial rate of reduction of Fe(III) was determined. The maximum reduction of Fe(III) was observed 24 h after the start of the reaction.

Furthermore, the relationship between the reduction of Fe(III) at different reaction times with the absorbance of the respectively formed $[Fe(LH)]^{2+}$ was determined at pH 3.4. The absorption band corresponding to the $[Fe(LH)]^{2+}$ complex ($\lambda_{máx}$= 700 nm[21]) was monitored using UV-visible spectrometry. The absorbance was monitored in a "stopped flow" system for fast kinetics (RX2000, Applied Photophysics) using the same reagent concentrations as those used in the reduction of Fe(III).

2.4. Reduction of O_2

The level of O_2 reduction was indirectly determined by monitoring the consumption of O_2 in air-saturated solutions at pH 3.4 and 7.0 ($[O_2]$= 250 µmol•L^{-1} at 20°C[11]).The percentage of O_2 in the solution was monitored every 3 seconds for a period of 24 h in a biological oxygen monitoring system YSI 5300A with a sensitivity of 3 µmol•L^{-1} O_2. The experiments were

performed in the presence or absence of Fe(III). In these systems, the final concentrations were 1.5 mmol•L^{-1} for catecholamine or catechol and 0.15 mmol•L^{-1} for Fe(III).

For all compounds, the initial rate of reduction of O_2 (or O_2 consumption) was determined from the linear range of the O_2 concentration curve early in the reaction. The O_2 consumption after 24 h was also determined.

The concentration of H_2O_2 produced by the reduction of O_2 was determined after 24 h using a reflectometric method (Merck, Reflectoquant) at a detection limit of 5.8 µmol•L^{-1} H_2O_2.

2.5. Hydroxyl radical production

To assess the effect of catecholamines on the •OH radical production by Fenton-like systems, comparative studies were performed in the absence and presence of each catecholamine. Due to the reported ability of catechol to amplify Fenton-like reactions, comparisons between the catecholamine-driven Fenton-like systems and catechol-driven Fenton-like systems were performed for all determinations[28, 31-33]. The studied Fenton-like systems are listed in Table 1.

Table 1. Studied Fenton-like systems.

Systems	**Name**
$Fe(NO_3)_3$ + H_2O_2 + DMPO	Unmodified system
$Fe(NO_3)_3$ + H_2O_2 + DMPO + Catechol	Catechol-driven Fenton-like system
$Fe(NO_3)_3$ + H_2O_2 + DMPO + Catecholamine*	Catecholamine-driven Fenton-like system

Hydroxyl radicals (•OH) were detected using a DMPO spin-trapping method by EPR spectroscopy[34]. The •OH production was determined at pH values between 2.0 and 7.0. The final concentrations in the systems were 0.15 mmol•L^{-1} $Fe(NO_3)_3$, 1.5 mmol•L^{-1} catechol or catecholamines, 1.5 mmol•L^{-1} H_2O_2 and 40 mmol•L^{-1} DMPO. Reactions were initiated by adding Fe(III). Samples were subsequently transferred via syringe to a capillary Aqua X in a Bruker 4108 TMH/9701 instrument. The EPR spectra were recorded on the X band of a Bruker

ESP300 spectrometer equipped with a Bruker ER035M gaussmeter and an HP 5350B microwave counter. The magnetic fields were set at the highest intensity peaks; variations in the peak heights were followed. The amounts of DMPO/•OH adduct produced were considered proportional to the heights of these peaks[35]. The EPR experiments were performed at room temperature (approximately 20°C).

2.6. Determining comparative parameters

The obtained data were normalized according to a method reported by Contreras et al.[31]. The maximum amount of •OH radicals produced in each system and the time required to reach this value were determined and compared.

2.7. Cell cultures

Cell culture studies were performed using HUVECs (Lonza). Cells were grown in gelatin-coated dishes, using M-199 medium supplemented with 10% newborn calf serum and 10% fetal bovine serum, until approximately 90% confluence. Cell passages were carried out with 0.1% trypsin/EDTA.

All experiments were performed in 96-well plates at 20,000 cells per well cultured at 37°C and 5% CO_2 for 2 days. The Fe(III)-catecholamine complexes were formed by adding 25 nmol•L^{-1} of catecholamine to M-199 medium without phenol red (pH 7.2–7.4) containing 250 nmol•L^{-1} $Fe(NO_3)_3$ and allowing 30 min for complex formation at room temperature. Fe(III)-catecholamine complex formation was verified using a UV-vis diode array spectrometer (Agilent 8453). Prior to testing, the cells were incubated with M-199 1% fetal bovine serum for 4 h[36]. Then,1 nmol•L^{-1} of the Fe(III)-catecholamine complexes was added to the M-199 medium (pH 7.2–7.4) in the presence or absence of 10 nmol•L^{-1} of H_2O_2.

To assess cell viability, the cultures were incubated with MTT reagent following manufacturer recommendations. MTT was added to cultures 4 h after the treatments with the Fe(III)-catecholamine complexes. The MTT reagent is reduced to a purple-derived formazan precipitate in living cells and can be measured at 540 nm after solubilization with DMSO[37]. Before analyzing the results, the correlation between the number of living cells and formazan formation was confirmed (R= 0.985).

The quantification of the produced ROS in HUVECs treated with the Fe(III)-catecholamine complexes was performed using a DCFH-DA probe. In the presence of ROS, fluorescent compounds were formed (λ_{ex}= 485 nm; λ_{em}= 522 nm)[38]. These compounds were detected using a fluorescent spectrometer (Synergy 2 Alpha Biotek Instruments) after 4 h of incubation with the Fe(III)-catecholamine complexes. Fluorescence emission was considered proportional to the amount of intracellularly produced ROS[39].

2.8. Statistical Analyses

To determine the significance of the effect of catecholamines on the parameters analyzed in this study, ANOVA and post-test (Tukey) calculations were carried out to compare differences between means. All statistical analyses were performed using GraphPad InStat 3 (GraphPad Software, Inc.) software.

3. Results and Discussion

3.1. Reduction of Fe(III)

Research studies investigating pollutant removal and wood biodegradation via the Fenton reaction driven by catechols have correlated Fe(III) reduction with the amount of oxidized substrates (mainly by •OH radicals)[33, 40, 41]. To determine the relationship between •OH radical production and the ability for catecholamines to reduce Fe(III), the initial rate of reduction of Fe(III) (I_R) at an optimum pH of 3.4 was measured (Figure 3).

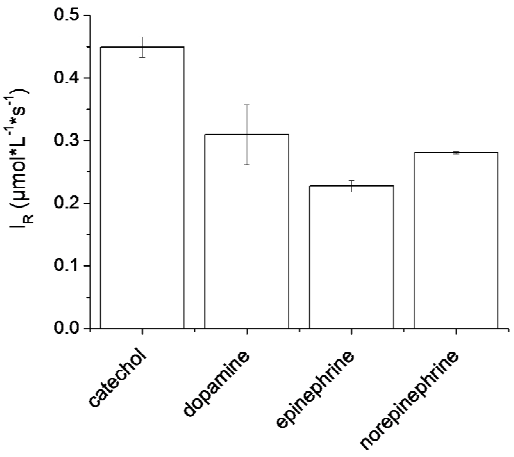

Figure 3. Initial rate of reduction of Fe(III) (I_R) for catechol and catecholamines at pH 3.4 (n=3).

At pH 3.4, catechol reduces Fe(III) more rapidly than catecholamines. Previous studies had reported that the limiting step in the reduction of Fe(III) was an electron transfer between one of the deprotonated oxygen atoms to Fe(III)[20]. Therefore, catecholamines may have a slower electron transfer rate than catechol. This could be due to an electron withdrawing substituent in catecholamines (Hammett constant for dopamine > 0)[42] that may decrease the electron density on the hydroxyl groups in the ring and thereby decrease the electron transfer rate to Fe(III).

The relationship between the kinetics for Fe(III) reduction and changes in $[Fe(LH)]^{2+}$ absorbance was investigated (Figure 4). For all studied compounds, the amounts of reduced Fe(III) increased exponentially until quasi-steady states were reached. For all assayed catechols, the amounts of reduced Fe(III) decreased linearly with $[Fe(LH)]^{2+}$ concentration ($R \geq -0.995$).These results agreed with those of previous works wherein the increases in oxidative capacities of Fenton and Fenton-like systems were related to the presence of $[Fe(LH)]^{2+}$[27].

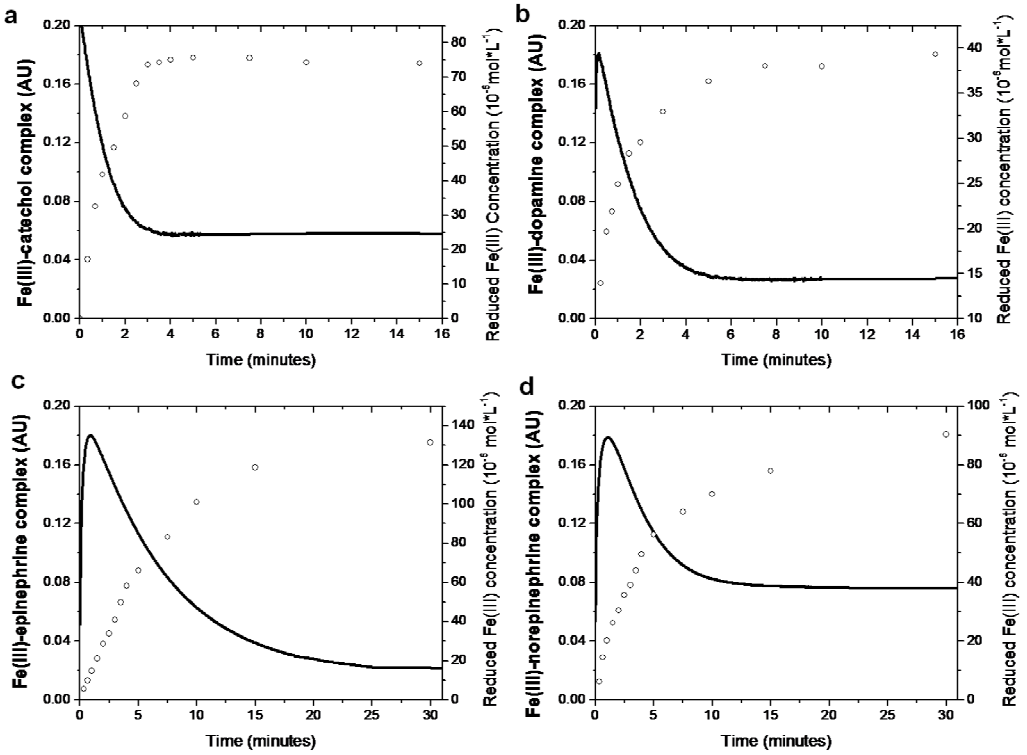

Figure 4. Absorbance of Fe(III)-catecholamine mono-complex ($[Fe(LH)]^{2+}$) (black line) and reduced Fe(III) concentration (white circles) at pH 3.4. a) Catechol; b) dopamine; c) epinephrine; d) norepinephrine (n=3).

To correlate the relationship between catecholamines with oxidative stress under physiological conditions, the ability to reduce Fe(III) at pH 7.0 was determined. At pH 7.0, Fe(III) reduction was not observed for any catecholamine, even in presence of O_2. Thus, it was not possible to determine whether the reduction of Fe(III) occurred. Furthermore, under these conditions, the formation of an intermediate Fe(II)-ferrozine complex was unknown because Fe(II) chelation occurred inside the $[Fe(LH)_2]^+$ complex.

To determine the pH range for the reduction of Fe(III) by catecholamines, I_R as a function of pH was determined (Figure 5).

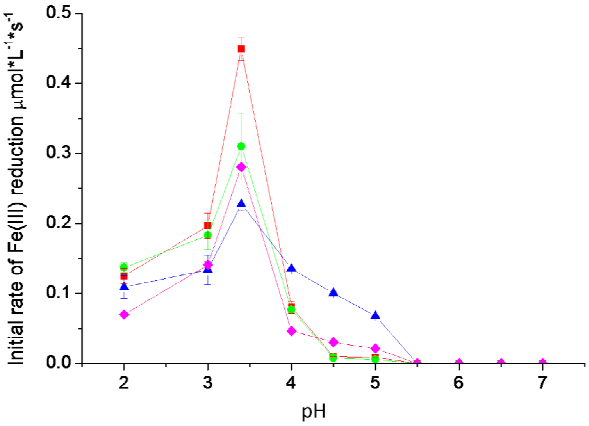

Figure 5. Initial rate of reduction of Fe(III) (I_R) at different pH values. Red square: catechol; green circle: dopamine; blue triangle: epinephrine; magenta rhombus: norepinephrine (n=3).

At different pH values, catechol and catecholamines showed similar I_R trends. A maximum I_R value was obtained at pH 3.4. Above pH 3.4, I_R values sharply declined and eventually dropped to undetectable levels at pH values greater than 5.5. The I_R trends agreed with the pH range for which $[Fe(LH)]^{2+}$ is the main species[24]. Above pH 5.5, $[Fe(LH)_2]^{+}$ is the main species, and Fe(III) reduction was not observed. Fe(III) reduction was also studied for all systems in the presence of O_2. Under these conditions, no significant changes in I_R were observed.

3.2. Autoxidation of catecholamines and H_2O_2 production

The oxidation of catechols by O_2 or autoxidation, may lead to the formation of H_2O_2 (a Fenton reagent). Thus, the autoxidation of catecholamines was monitored by O_2 consumption in the solution in the presence or absence of Fe(III).

At pH 3.4, no significant O_2 consumption was observed. Fe(III) did not catalyze significant O_2 consumption. The formation and subsequent decomposition of $[Fe(HL)]^{2+}$ was previously verified by spectrometry (Figure 4, black line).

At pH 7.0, catecholamines and catechol were oxidized in air-saturated solutions at different rates (Figure 6a). Epinephrine had the highest initial rate of O_2 consumption and had consumed all O_2 in the solution (250 µmol•L^{-1}). This was consistent with the results reported by Chen et

al.[43], wherein a comparative study by cyclic voltammetry showed that epinephrine had the lowest oxidant potential at pH 7.

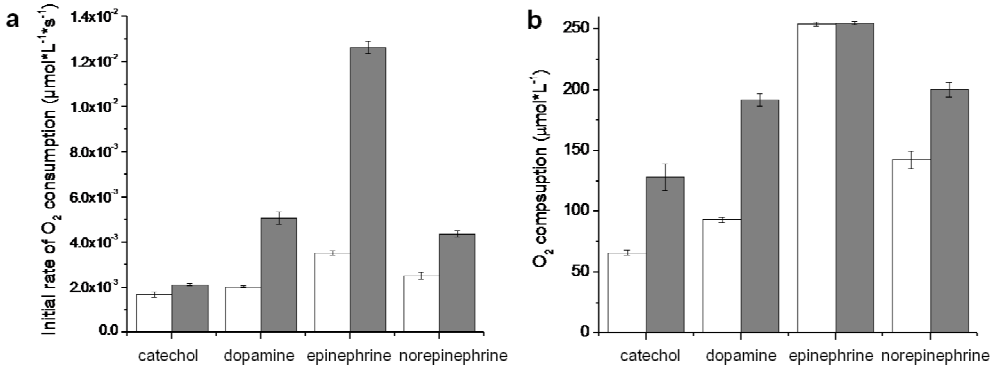

Figure 6. a. Initial rate of consumption of O_2 for catechol and catecholamines at pH 7.0 in absence (white bar) or presence (grey bar) of Fe(III). b. O_2 consumption after 24 h of reaction for catechol and catecholamines at pH 7.0 in absence (white bar) or presence (grey bar) of Fe(III) (n=3).

When the autoxidation of catecholamines was performed in the presence of Fe(III), increases in the initial rate of O_2 consumption were observed (Figure 6a, gray bar). These increases were 26% for catechol and exceeded 250% for dopamine and epinephrine (Figure 6). These results were consistent with Halliwell's generalization[44]. The mechanism by which metals, such as Fe(III), promoted catecholamine oxidation has been related to the formation of Fe(III)-catecholamine complexes and interactions with available d-orbitals to overcome spin restrictions[45].

Other researchers have shown that the autoxidation of catecholamines produces semiquinones and other reaction products with the ability to reduce Fe(III) to Fe(II)[46]. Because of this, we expected our reaction system to produce these products by catecholamine autoxidation. However, Fe(III) reduction at pH 7.0 was not observed (i.e., free Fe(II) was not found in solution after 24 h of autoxidation). This could be due to 4 possibilities: a) Fe(III) was not reduced in the $[Fe(LH)_2]^+$ complex; b) Fe(III) was reduced, but Fe(II) was not released from the $[Fe(LH)_2]^+$ complex; c) Fe(III) was reduced and released into the aqueous solution, but was reoxidized to

Fe(III) by dissolved oxygen or d) a few of the oxidation products precipitated and iron was trapped or remained bound in a heterogeneous phase.

As the main product of O_2 reduction, the concentration of H_2O_2 after 24 h was determined. Only quantifiable concentrations of H_2O_2 were found in solutions where dopamine and norepinephrine were oxidized (14±2 and 19±1 μmol•L^{-1}, respectively). The absence or low concentration of H_2O_2 in solutions where catechol or catecholamine were oxidized could be due to the consumption of this reagent by reacting with the semiquinone through an organic Fenton reagent[47]. This could also cause a decrease in the H_2O_2 concentration after 24 h.

3.3. •OH radical production by Fenton-like systems

Catecholamines can reduce Fe(III) and O_2 and thereby produce reagents for a Fenton reaction. However, Fe(III) reduction was only observed at pH values below 5.5, and O_2 reduction with H_2O_2 was observed only at pH values near 7.0 or higher. In this way, •OH radical production was evaluated at an optimal pH= 3.4 and at pH 7.0.

The maximum signal of the DMPO/•OH adduct observed, S_{max}, and the time required for each system in reaching that signal, T_{max}, were used to compare the •OH radical production in the Fenton-like systems (Table 2).

Table 2. Comparative parameters of •OH radical production by Fenton-like systems at pH 3.4.

System	t_{max} (min)	S_{max} (AU)
Unmodified system	1.02 ± 0.01	2.15 ± 0.07
Catechol-driven Fenton-like system	1.35 ± 0.03	31.93 ± 3.55
Dopamine-driven Fenton-like system	7.84 ± 0.17	13.16 ± 0.16
Epinephrine-driven Fenton-like system	23.6 ± 0.10	8.08 ± 0.21
Norepinephrine-driven Fenton-like system	13.6 ± 0.65	10.58 ± 0.11

The driven systems reached higher values of S_{max} than that for the unmodified system. According to the literature for similar systems[27, 31], these results indicate that catecholamines increased the reactivity of Fenton-like systems. Catechol-driven systems produced the highest

amounts of •OH radicals. This was reflected in the S_{max} values, which were at least 240% higher than those obtained in other systems. Catecholamine-driven systems required longer time to reach S_{max} with epinephrine-driven systems requiring the most time to reach S_{max}. A logarithmic relationship between S_{max} and I_R was observed (R=0.9984; Figure 7). This confirmed Fe(III) reduction as the primary mechanism that promotes •OH radical production in the Fenton reaction by catechol compounds.

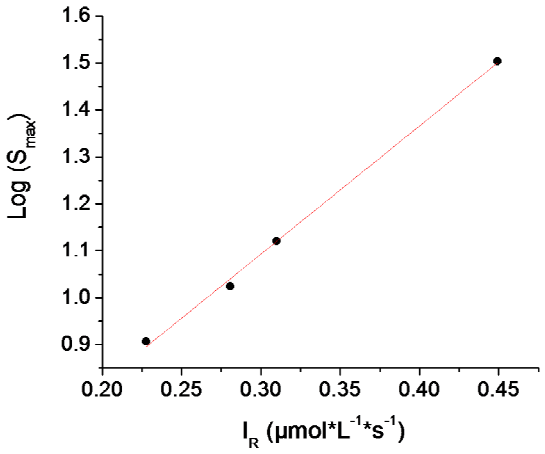

Figure 7. Relationship between maximum signal value of DMPO/•OH adduct (S_{max})and I_R at pH 3.4.

At pH 7.0, all Fenton-like systems showed slower •OH radical production than at pH 3.4. Although the signal of the DMPO/•OH adduct was detectable, it was below the limit of quantification. This was consistent with results obtained in studies on Fe(III) reduction, which showed that the amount of reduced Fe(III) was significant only at acidic pH below 5.5.

Although catecholamine oxidation can produce H_2O_2, the pH at which this happens was not consistent with the pH at which the $[Fe(LH)]^{2+}$ complex, which was responsible for increased reactivity, was formed. Together, these results indicate that at approximately pH 5.5, catecholamines coordinated with iron in aqueous solutions to form $[Fe(LH)_2]^{+}$. The formation of this complex prevented the entry of H_2O_2 into the metal coordination sphere and therefore, prevented the formation of •OH radicals through the Fenton reaction. This antioxidant

mechanism of catecholamines confirmed the reports of other studies that have also shown the ability for catecholamines to scavenge several other radical species[48-50].

3.4. ROS production in cell cultures

To verify the inability to increase ROS production in Fenton-like systems under physiological conditions, an in vitro study was performed. The concentration of reagents used in cultures was the same order of magnitude as the concentration of catecholamines found in blood[51, 52].At this concentration, the Fenton-like reagents did not produce significant decreases in the number of cells in each culture ($p>0.05$, Figure 8a).

ROS production in HUVECs incubated in the presence of Fe(III)-catecholamine complexes showed no significant differences between the controls and the systems with Fenton-like reagents ($p>0.05$, Figure 8b). The exposure to Fenton-like reagents did not result in increases in ROS production beyond normal cell metabolism. These results were consistent with those observed in •OH radical production at pH 7.0, where no quantifiable radical signals were observed.

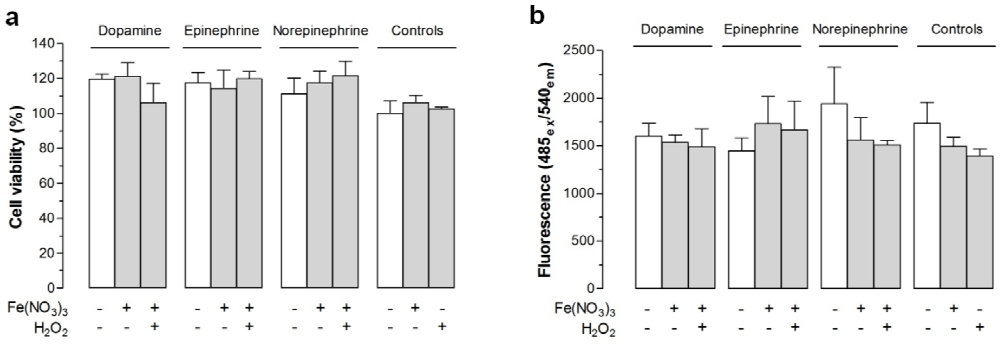

Figure 8. a. Cell viability of HUVECs and b. production of reactive oxygen species (ROS) in HUVECs incubated for 4 h in the presence of Fe(III)-catecholamine complex in M-199 medium (pH7.2-7.4; n = 3).

4. Conclusion

Catecholamines have been associated with the development of oxidative stress by Fenton reactions. These compounds reduce Fe(III) to Fe(II) and O_2 to H_2O_2 and thereby produce Fenton reagents. However, the presence of catecholamines in the studied systems did not increase the production of ROS at physiological conditions. Accordingly, catecholamines could not promote the Fenton reaction at pH values near 7.0, even in presence of excess of H_2O_2. At this pH, the $[Fe(LH)_2]^+$ complex did not have the ability to reduce Fe(III). However at acidic pH of 3.4 the ability to reduce Fe (III) was closely related to the change in the amount of •OH radicals that can be produced by a driven Fenton reaction. The $[Fe(LH)_2]^+$ complex also did not allow the participation of iron in Fenton-like reactions. According to these results, oxidative stress mediated by catecholamine-driven Fenton reactions requires microenvironments with pH values below 5.5 and external sources of H_2O_2.

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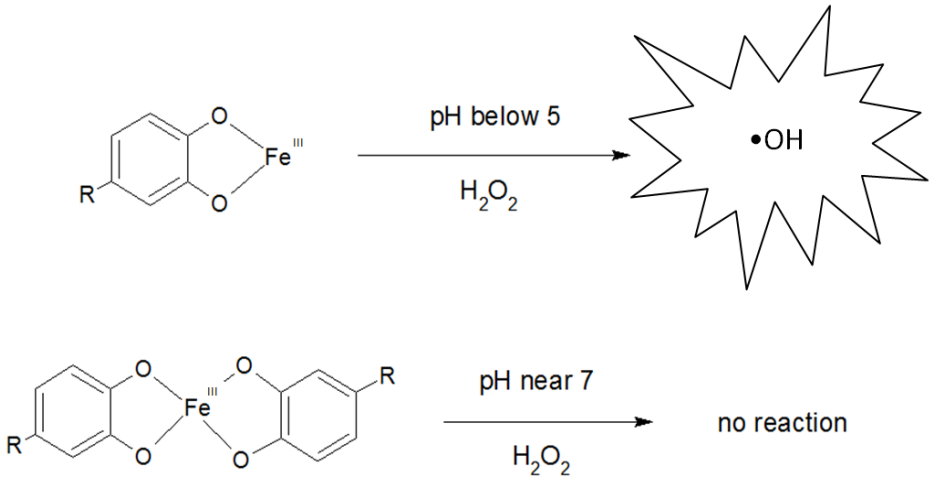
Graphical abstract



R=CH_2-CH_2-NH_2 (dopamine); CHOH-CH_2-$NHCH_3$ (epinephrine); or CHOH-CH_2-NH_2 (norepinephrine).

Graphical abstract

The reduction of Fe(III) and O_2 by catecholamines occurrs at different pH range. Although catecholamines can produces H_2O_2 by their autoxidation, these compounds only promote •OH radical production by Fenton reaction at pH value wherein Fe(III) is reduced. This is because •OH radical production is related to initial rate of Fe(III) reduction.

Highlights

- Catecholamines can reduce Fe(III) at acidic pH values up to pH 5.5
- Fe(III) reduction by catecholamines is correlated with •OH production by Fenton reaction
- Catecholamines promote O_2 reduction to H_2O_2 at pH near 7.0
- Catecholamines cannot promote Fenton reactions at pH near 7.0