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Research paper

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

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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., $Fe(II) + H_2O_2 \rightarrow Fe(III) + {}^{\cdot}OH + OH^-$), 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 (Fig. 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 ($O_2^{\cdot-}$) and semiquinone, which can be further oxidized to quinone by reducing another O_2 molecule [2,3]. The radical $O_2^{\cdot-}$ 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].

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].

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

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Fig. 1. Catecholamines structures.

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

Like other catechols, catecholamines (LH_3^+) can form complexes with iron [20–22]. Fig. 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].

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 to 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

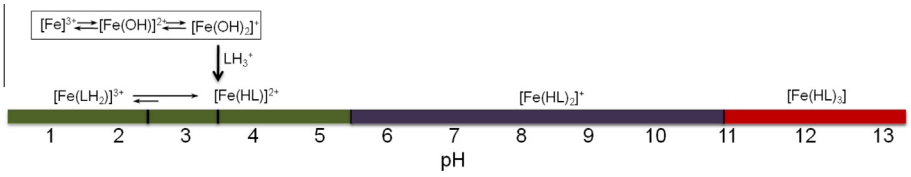

Fig. 2. Primary Fe(III)-catecholamine complexes present in aqueous solutions. LH_3^+: triprotic catecholamine.