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Prevention of iron- and copper-mediated DNA damage by catecholamine and amino acid neurotransmitters, L-DOPA, and curcumin: metal binding as a general antioxidant mechanism†

Carla R. García, Carlos Angelé-Martínez, Jenna A. Wilkes, Hsiao C. Wang, Erin E. Battin and Julia L. Brumaghim*

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Concentrations of labile iron and copper are elevated in patients with neurological disorders, causing interest in metal-neurotransmitter interactions. Catecholamine (dopamine, epinephrine, and norepinephrine) and amino acid (glycine, glutamate, and 4-aminobutyrate) neurotransmitters are antioxidants also known to bind metal ions. To investigate the role of metal binding as an antioxidant mechanism for these neurotransmitters, L-dihydroxyphenylalanine (L-DOPA), and curcumin, their abilities to prevent iron- and copper-mediated DNA damage were quantified, cyclic voltammetry was used to determine the relationship between their redox potentials and DNA damage prevention, and UV-vis studies were conducted to determine iron and copper binding as well as iron oxidation rates. In contrast to amino acid neurotransmitters, catecholamine neurotransmitters, L-DOPA, and curcumin prevent significant iron-mediated DNA damage (IC50 values of 3.2 to 18 \( \mu \)M) and are electrochemically active. However, glycine and glutamate are more effective at preventing copper-mediated DNA damage (IC50 values of 35 and 12.9 \( \mu \)M, respectively) than L-DOPA, the only catecholamine to prevent this damage (IC50 = 73 \( \mu \)M). This metal-mediated DNA damage prevention is directly related to the metal-binding behaviour of these compounds. When bound to iron or copper, the catecholamines, amino acids, and curcumin significantly shift iron oxidation potentials and stabilize \( \text{Fe}^{3+} \) over \( \text{Fe}^{2+} \) and \( \text{Cu}^{2+} \) over \( \text{Cu}^{+} \), a factor that may prevent metal redox cycling in vivo. These results highlight the disparate antioxidant activities of neurotransmitters, drugs, and supplements and highlight the importance of considering metal binding when identifying antioxidants to treat and prevent neurodegenerative disorders.

Introduction

Redox-active metal ions, primarily \( \text{Fe}^{2+} \) and \( \text{Cu}^{+} \), generate damaging hydroxyl radical (\( \cdot \text{OH} \)) in the presence of hydrogen peroxide (H\(_2\)O\(_2\); Fig. 1), a byproduct of cellular respiration.\(^{1,2}\) Hydroxyl radical can cause lipid and protein oxidation,\(^{3,4}\) and DNA damage by metal-generated \( \cdot \text{OH} \) can lead to neurodegeneration. Iron and copper redox cycling (Fig. 1) and the resulting DNA damage can lead to apoptosis\(^{5,6}\) and has been linked to neurodegenerative diseases such as Parkinson’s,\(^{7,8}\) Huntington’s,\(^{9,10}\) and Alzheimer’s diseases.\(^{9,10}\) Increased brain iron levels are found in Parkinson’s disease,\(^{9,14}\) and labile (non-protein bound) copper concentrations in the brains of Alzheimer’s disease patients are approximately seven times higher than normal.\(^{15}\) Serum levels of labile copper are inversely related to cognitive function, whereas levels of protein-bound copper are not,\(^{16}\) also emphasizing the importance of labile metal ions. As a result, in addition to antioxidants, metal chelators have been widely examined to treat or ameliorate neurodegeneration.\(^{17-19}\)

This evidence for misregulation of labile metal concentrations in neurodegenerative diseases highlights the need to understand interactions of endogenous neurotransmitters, as well as neurological drugs and supplements, with iron and copper. Two classes of neurotransmitters are examined in this work: the catecholamines dopamine (DA), epinephrine (EP), and norepinephrine (NE), and the amino acid neurotransmitters glycine (Gly), glutamate (Glu), and 4-aminobutyrate (GABA) (Fig. 2).

![Fig. 1 Iron- and copper-mediated hydroxyl radical generation and biological redox cycling.](image-url)
Dopamine plays a primary role in motor function, binge eating tendencies, and is proposed to play a role in depression. It is also responsible for controlling mood and can produce the DNA damage product 8-hydroxydeoxyguanosine in the presence of Cu2+. Epinephrine (also known as L-adrenaline) helps control motor behaviour and regulate emotional stress. Norepinephrine (also known as noradrenaline) regulates both anxiety and depressive mood disorders. Dopamine is the most biologically abundant catecholamine, with concentrations in the human and mouse brain around 8 μM. Epinephrine and norepinephrine are also in the low micromolar range in rat brain (1–2 μM). Gly and GABA are inhibitory neurotransmitters responsible for motor function, and Glu is an excitatory neurotransmitter. Poor glycine and GABA regulation can decrease rapid eye movement sleep and cause epilepsy. Elevated levels of glutamate are observed in patients with mania and diminished levels are observed in patients with depression. In patients with depression, glutamate in the presence of labe copper decreases cortical excitability. Glutamate levels in the brains of cats, swine, and rats range from 1–20 μM, and glycine levels in cat brain and human blood plasma are around 0.5 and 0.2 μM, respectively. GABA, however, typically is found in low micromolar concentrations (1.8 mM) in the human brain.

In addition to their signaling functions, catecholamines and amino acid neurotransmitters are known antioxidants. Catecholamines are potent radical scavengers, but under some conditions, they can increase DNA damage in the presence of copper and iron. In the presence of Cu2+, DA and EP both promote DNA damage due to their ability to participate in copper redox cycling, with DA producing substantially more damage in the presence of copper.

Gly is also a potent radical scavenger, and glycine–iron supplementation has been reported to increase superoxide dismutase and catalase enzyme production in vivo, an effect that may be due to radical scavenging properties, iron binding, or both. Glu inhibits lipid peroxidation in vivo. Alone, GABA does not promote DNA damage, but in the presence of Cu2+, DNA damage has been observed.

In addition to endogenous neurotransmitters, drugs such as L-dihydroxyphenylalanine (L-DOPA) and dietary supplements such as curcumin (Fig. 2) have also been investigated for their antioxidant and metal binding properties in the treatment of neurodegenerative diseases. Brain concentrations of L-DOPA have been measured up to 12 μM in rats after chronic administration. Similar to dopamine, L-DOPA can promote DNA damage in the presence of Fe2+ and Cu2+ in addition to its radical scavenging abilities. Curcumin is the active compound in the herbal remedy and spice turmeric (Curcuma longa) and has a range of biological properties, including radical scavenging, anti-inflammatory activity, and suppression of Aβ-protein aggregation in animal studies of Alzheimer’s disease. In the mouse brain, curcumin concentrations of approximately 3 μM have been reported upon oral administration, and concentrations as low as 1 μM have been reported to prevent Aβ aggregation and to promote its disaggregation in vitro.

Due to the established link between increased labile metal concentrations and neurodegenerative disease, it is critical to understand the interactions of neurotransmitters, drugs, and supplements with iron and copper. The eight compounds in Fig. 2 have well-known metal binding groups: the catechols of the catecholamines, the amine and carboxylic acid groups of the amino acids, and the keto-hydroxy group of curcumin. The studies described in this paper examine and compare the metal-binding mechanisms for antioxidant behaviour of these compounds by quantifying their ability to prevent iron- and copper-mediated DNA damage. Cyclic voltammetry (CV) studies are also conducted to compare DNA damage prevention with oxidation potentials, and iron and copper binding to these compounds is also directly examined using UV-vis spectroscopy. Together, these results provide a comprehensive understanding of metal binding as an essential antioxidant mechanism for catecholamine and amino acid neurotransmitters, L-DOPA, and curcumin, results that will improve the understanding of neurodegenerative disease development and treatment.

**Results and discussion**

**Prevention of metal-mediated DNA damage**

Iron-mediated DNA damage prevention by the six neurotransmitters, L-DOPA, and curcumin was quantified using a plasmid DNA damage assay; this method has been used previously to determine polyphenol DNA damage inhibition by Fe2+ (2 μM) and H2O2 and were carried out at pH 6 to prevent iron precipitation. Normal labile iron concentrations have been measured to be 1–15 μM in human cells, so these conditions mimic expected biological iron concentrations.

The gel image in Fig. 3A illustrates that dopamine prevents DNA damage by Fe2+/H2O2 with a maximum DNA damage prevention of 92% at 200 μM, the highest concentration tested. Graphing DNA damage inhibition vs. DA concentration affords a dose–response curve used to determine the concentration at which 50% of DNA damage prevention is observed (IC50). For dopamine, this IC50 value is 18 ± 1 μM (Fig. 3B and Table 1). Epinephrine also behaves as an antioxidant to prevent iron-mediated DNA damage, with a maximum percent damage inhibition of 97% at 50 μM and an IC50 value of 4.6 ± 0.01 μM (Table 1). L-DOPA has an IC50 value of 6.9 ± 0.3 μM. Norepinephrine has the lowest IC50 value of the catecholamines at 3.2 ± 0.2 μM and prevents 87% of DNA damage at 100 μM.
Although curcumin has a metal-binding keto-hydroxy group rather than a catechol group, its IC$_{50}$ for prevention of iron-mediated DNA damage is similar to those of the catecholamines (6.2 ± 0.2 μM; Table 1). In all cases, the antioxidant effects of these compounds are near or below their biological concentrations (1–12 μM).

In contrast, the amino acid neurotransmitters GABA and glutamate have no effect on iron-mediated DNA damage (Table 1). Glycine is a weak antioxidant, preventing only 41% of iron-mediated DNA damage at 10,000 μM, well above biological concentrations. Clearly, catecholamines are significantly more effective than amino acid neurotransmitters in preventing iron-mediated DNA damage. All gel images, IC$_{50}$ plots, and data tables for the DNA damage assays are provided in the ESI (Fig. S1–S10 and Tables S1–S26).

![Image](https://via.placeholder.com/150)

**Fig. 3** (A) Gel electrophoresis image of dopamine (DA) DNA damage prevention with Fe$^{2+}$ (2 μM) and H$_2$O$_2$ (50 μM). Damaged (nicked) plasmid DNA is in the top band; undamaged (supercoiled) DNA is in the bottom band. Lanes: MW = 1 kb ladder; 1: plasmid (p); 2: p + H$_2$O$_2$; 3: p + H$_2$O$_2$ + 200 μM DA; 4: p + H$_2$O$_2$ + Fe$^{2+}$; 5–18: lane 5 + 0.01, 0.1, 1, 2, 5, 10, 25, 50, 100, and 200 μM, respectively. (B) Dose–response curve for dopamine inhibition of iron-mediated DNA damage. Data are the average of three trials with standard deviations.

To establish whether iron chelation is required for DNA damage prevention, similar DNA damage assays were conducted using Fe(EDTA)$^{2-}$ instead of FeSO$_4$. Fe(EDTA)$^{2-}$ damages DNA in the presence of H$_2$O$_2$ (Fig. S4A†, Lane 4), but adding Fe(EDTA)$^{2-}$ as the iron source prevents iron binding by the compounds in Table 1. With Fe(EDTA)$^{2-}$, DA prevents no DNA damage up to 80 μM (Table S10†). Similarly, NE and L-DOPA also show no DNA damage prevention with Fe(EDTA)$^{2-}$ up to the highest catecholamine concentration tested (200 μM). EP prevents substantially less damage under these conditions: 28% compared to 97% DNA damage inhibition at 50 μM for Fe(EDTA)$^{2-}$ and Fe$^{2+}$, respectively. With Fe(EDTA)$^{2-}$, curcumin prevents only 38% damage at 75 μM compared to 98% with Fe$^{2+}$ as the iron source. In all cases, significantly less DNA damage inhibition is observed with Fe(EDTA)$^{2-}$ compared to Fe$^{2+}$ as the iron source, and the small amount of DNA damage prevention observed for EP and curcumin in the presence of Fe(EDTA)$^{2-}$/H$_2$O$_2$ is likely due to an alternate antioxidant mechanism, such as reactive oxygen species scavenging.

These results establish iron binding as the primary mechanism for antioxidant activity of the catecholamines and curcumin, similar to results observed for hydroxychromones and other catechol compounds.63,70 As demonstrated for other catechol and gallol compounds,60,63 there is a strong correlation between log IC$_{50}$ value vs. catecholamine first phenolic hydrogen pK$_a$ (R$^2$ = 0.95; graph in Fig. S11A†), indicating that deprotonation and iron binding is critical for antioxidant activity.

DNA damage assays were also performed to determine the ability of the neurotransmitters and related compounds to prevent copper-mediated DNA damage. For these experiments, Cu$^{2+}$ (6 μM) and ascorbic acid (1.25 equiv) were combined to generate Cu$^{+}$ in situ prior to adding the tested compound and DNA. Under these conditions, the amino acids Glu and Gly prevent significantly more copper-mediated DNA damage (IC$_{50}$ values of 15 and 35 μM, respectively; Fig. 4) than L-DOPA (IC$_{50}$ = 73.1 μM). Curcumin also prevents copper-mediated DNA damage with an IC$_{50}$ value of 30.8 μM. EP and NE prevent no copper-mediated DNA damage, and DA has been previously reported to be a pro-oxidant under these conditions, increasing DNA damage from 0.2–2000 μM,68 consistent with reports of DA cytotoxicity in copper-rich systems.71

To establish copper binding as an antioxidant mechanism, DNA damage assays were also conducted with [Cu(bpy)$_2$]$^{2+}$/H$_2$O$_2$ (bpy = 2,2′-bipyridine), similar to the previously-described...
experiments with Fe(EDTA)$^{2-}$. Under these conditions, Glu, Gly, and l-DOPA does not prevent copper-mediated DNA damage up to 150 μM, and curcumin prevents little copper-mediated DNA damage up to 50 μM, the highest concentration tested, indicating that copper binding is the primary antioxidant mechanism for copper-mediated DNA damage. Using [Cu(bpy)$_3$]$^{2+}$ instead of Cu$^+$ as the copper source also inhibited the pro-oxidant activity observed for dopamine.$^{68}$ The effects observed for copper-mediated DNA damage prevention are generally at higher concentrations than those observed for iron system, but they remain in the low micromolar range.

Importantly, the different classes of neurotransmitters have very different abilities to inhibit iron- and copper-mediated DNA damage.

**Electrochemistry of neurotransmitters, l-DOPA, and curcumin with and without iron**

Because the biological generation of hydroxyl radical can only occur in a limited electrochemical window (approximately $-0.324$ V to $0.460$ V vs. NHE$^{2-}$), altering metal redox potentials through neurotransmitter binding may prevent metal redox cycling and subsequent DNA damage (Fig. 1). Cyclic voltammetry (CV) was therefore performed with the eight compounds in Table 1 with and without addition of 1 equiv Fe$^{2+}$ or Cu$^{2+}$.

![Fig. 4 Gel electrophoresis image of glycine (Gly) with Cu$^+$ (6 μM) and H$_2$O$_2$ (50 μM). Damaged (nicked) plasmid DNA is in the top band; undamaged (supercoiled) DNA is in the bottom band. Lanes: MW = 1 kb ladder; 1 = plasmid DNA (p); 2 = p + H$_2$O$_2$; 3 = p + 200 μM glycine + H$_2$O$_2$; and 4 = p + Cu$^+$ + H$_2$O$_2$; lanes 5–9: p + Cu$^+$ + H$_2$O$_2$ + 1, 2, 5, 10, 25, 50, 100, 150, and 200 μM glycine, respectively.](Image)

**Table 2** Electrochemical data vs. NHE for the neurotransmitters, l-DOPA, and curcumin with and without addition of 1 equiv Fe$^{2+}$ or Cu$^{2+}$

<table>
<thead>
<tr>
<th>Compound</th>
<th>$E_{p1}$ (V)</th>
<th>$E_{p2}$ (V)</th>
<th>$\Delta E$ (V)</th>
<th>$E_{1/2}$ (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DA (pH 6.0)</td>
<td>$-0.265, 0.115$</td>
<td>$-0.208, 0.246$</td>
<td>$0.057, 0.131$</td>
<td>$-0.237, 0.181$</td>
</tr>
<tr>
<td>DA (pH 7.0)</td>
<td>$-0.335, 0.058$</td>
<td>$-0.182, 0.244$</td>
<td>$0.153, 0.186$</td>
<td>$-0.259, 0.151$</td>
</tr>
<tr>
<td>DA + Fe$^{2+}$ (pH 6.0)</td>
<td>$-0.273^a, 0.100$</td>
<td>$0.362^a$</td>
<td>$0.635^a$</td>
<td>$0.045^a$</td>
</tr>
<tr>
<td>DA + Cu$^{2+}$ (pH 7.0)</td>
<td>$-0.600, -0.235^a, 0.097$</td>
<td>$-0.032^a, 0.184$</td>
<td>$0.203^a, 0.087$</td>
<td>$-0.134^a, 0.141$</td>
</tr>
<tr>
<td>EP (pH 6.0)</td>
<td>$-0.195$</td>
<td>$-0.147, 0.307$</td>
<td>$0.048$</td>
<td>$-0.171$</td>
</tr>
<tr>
<td>EP (pH 7.0)</td>
<td>$-0.275$</td>
<td>$-0.216, 0.210$</td>
<td>$0.059$</td>
<td>$-0.246$</td>
</tr>
<tr>
<td>EP + Fe$^{2+}$ (pH 6.0)</td>
<td>$-0.287^a$</td>
<td>$-0.147^a, 0.426^a$</td>
<td>$0.713^a$</td>
<td>$0.070^a$</td>
</tr>
<tr>
<td>EP + Cu$^{2+}$ (pH 7.0)</td>
<td>$-0.291^a$</td>
<td>$-0.231, 0.239$</td>
<td>$0.060$</td>
<td>$-0.203$</td>
</tr>
<tr>
<td>NE (pH 6.0)</td>
<td>$-0.184, 0.121$</td>
<td>$-0.120^a, 0.290$</td>
<td>$0.064, 0.169$</td>
<td>$-0.152, 0.206$</td>
</tr>
<tr>
<td>NE (pH 7.0)</td>
<td>$-0.297$</td>
<td>$-0.133, 0.283$</td>
<td>$0.164$</td>
<td>$-0.215$</td>
</tr>
<tr>
<td>NE + Fe$^{2+}$ (pH 6.0)</td>
<td>$-0.193^a, 0.135$</td>
<td>$-0.118^a, 0.385^a$</td>
<td>$0.578^a$</td>
<td>$0.096^a$</td>
</tr>
<tr>
<td>NE + Cu$^{2+}$ (pH 7.0)</td>
<td>$-0.278^a$</td>
<td>$0.198$</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Gly + Fe$^{2+}$ (pH 6.0)$^f$</td>
<td>$-0.251^a$</td>
<td>$0.208^a$</td>
<td>$0.459^a$</td>
<td>$0.022^a$</td>
</tr>
<tr>
<td>Gly + Cu$^{2+}$ (pH 7.0)$^f$</td>
<td>$-0.196^a$</td>
<td>$-0.096^a$</td>
<td>$0.459^a$</td>
<td>$100^a$</td>
</tr>
<tr>
<td>Glu + Fe$^{2+}$ (pH 6.0)$^f$</td>
<td>$-0.227^a$</td>
<td>$0.251^a$</td>
<td>$0.478^a$</td>
<td>$0.012^a$</td>
</tr>
<tr>
<td>Glu + Cu$^{2+}$ (pH 7.0)$^f$</td>
<td>$-0.190^a$</td>
<td>$0.053^a$</td>
<td>$0.243^a$</td>
<td>$-0.069^a$</td>
</tr>
<tr>
<td>GABA + Fe$^{2+}$ (pH 6.0)$^f$</td>
<td>$-0.238^a$</td>
<td>$0.213^a$</td>
<td>$0.451^a$</td>
<td>$-0.013^a$</td>
</tr>
<tr>
<td>GABA + Cu$^{2+}$ (pH 7.0)$^f$</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>l-DOPA (pH 6.0)</td>
<td>$-0.415$</td>
<td>$-0.123^b, 0.333$</td>
<td>$0.358$</td>
<td>$-0.292$</td>
</tr>
<tr>
<td>l-DOPA (pH 7.0)</td>
<td>$-0.458^a, 0.222$</td>
<td>$-0.126^b, 0.333$</td>
<td>$0.332, 0.111$</td>
<td>$-0.292, 0.278$</td>
</tr>
<tr>
<td>l-DOPA + Fe$^{2+}$ (pH 6.0)</td>
<td>$-0.236^a$</td>
<td>$0.953^a, 0.375^a$</td>
<td>$0.306^a$</td>
<td>$0.070^a$</td>
</tr>
<tr>
<td>l-DOPA + Cu$^{2+}$ (pH 7.0)</td>
<td>$-0.595, -0.196^a, 0.136$</td>
<td>$0.223$</td>
<td>$0.087$</td>
<td>$0.180$</td>
</tr>
<tr>
<td>Cur (pH 6.0)</td>
<td>$-0.729, 0.103^b$</td>
<td>$0.517$</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cur (pH 7.0)</td>
<td>$-0.756, 0.026$</td>
<td>$0.202, 0.439$</td>
<td>$0.228$</td>
<td>$0.088$</td>
</tr>
<tr>
<td>Cur + Fe$^{2+}$ (pH 6.0)</td>
<td>$-0.217^b, 0.135$</td>
<td>$0.250^a$</td>
<td>$0.467^a$</td>
<td>$0.017^a$</td>
</tr>
<tr>
<td>Cur + Cu$^{2+}$ (pH 7.0)</td>
<td>$-0.592, -0.169^a, 0.065^b$</td>
<td>$-0.073^a, 0.117^b$</td>
<td>$0.096^a, 0.052$</td>
<td>$-0.121^a, 0.091$</td>
</tr>
<tr>
<td>FeSO$_4$ (pH 6.0)</td>
<td>$0.037^a$</td>
<td>$0.39, 0.57^a$</td>
<td>$-0.443^a$</td>
<td>$-0.259^a$</td>
</tr>
<tr>
<td>CuSO$_4$ (pH 7.0)</td>
<td>$-0.144^a$</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

$^a$ Metal-based potential. $^b$ Weak wave. $^c$ Gly, Glu, and GABA alone show no electrochemical activity at pH 6.0 or 7.0.
cycling between their oxidized semiquinone and quinone forms. At pH 6.0, the oxidation wave (Ep) for DA observed at 0.246 V results in a two-electron catecholamine o-quinone product (Table 2), and the reduction wave (Ep) observed at the most positive voltage (0.115 V) is due to a two-electron reduction back to the parent compound. The second redox couple at pH 6.0 for DA at lower potentials (~0.265 and ~0.208 V) can be assigned to a reversible, two-electron oxidation of the cyclised catecholamine to the cyclised o-quinone. Formation of the semiquinones is not observed under these conditions. It is well-documented that redox potentials of catecholamines are pH dependent: as pH increases, the oxidation and reduction potentials shift toward more negative voltages as shown in Table 2.

Both the acyclic and cyclised o-quinone redox couples are observed at pH 6.0 for NE, but the acyclic quinone reduction is not observed for NE or EP at pH 7.0. In comparing the voltammograms of the four catecholamine compounds, dopamine is the only compound that has two clear reduction and oxidation potentials at both pH 6.0 and pH 7.0, and 1-DOPA shows four clear waves only at pH 7. Bian et al. identified two distinct redox couples for norepinephrine at 400 µM using a modified glassy carbon electrode; when a unmodified glassy carbon electrode was used, only one redox couple and one oxidation wave were observed. Thus, additional redox waves for EP, NE, and 1-DOPA may be detectable at higher concentrations.

Curcumin is also electrochemically active at pH 7 under these conditions (Table 2), with one reversible oxidation of a phenolate group (Ep) due to Fe3+/2+ reduction and one broad oxidation wave around 0.39–0.57 V (Fig. 5A) due to Fe2+/3+ oxidation, similar to CV for FeSO4 reported by Nemtoi and coworkers at pH 4.82. Upon adding 1 equiv compound to FeSO4 at pH 6.0 and waiting 10 min, the cyclic voltammograms for iron with each of the eight compounds are similar in both shape and redox potentials to those of FeSO4 alone (Fig. 5B). The observed potentials are similar in both shape and redox potentials to those of FeSO4 alone (Fig. 5B), suggesting that the observed waves are from iron-based reactions. The Fe3+/2+ reduction potentials (E1/2) for the tested compounds with iron are in the range ~0.13 to 0.096 V and are shifted from those of iron alone by ~0.272 to ~0.163 V. These iron reduction potentials are substantially lower than for iron alone, indicating that the stability of Fe3+ increases upon binding to the compounds, as would be expected for iron-based reactions. The Fe3+/2+ reduction potentials (E1/2) for the tested compounds with iron are in the range ~0.13 to 0.096 V and are shifted from those of iron alone by ~0.272 to ~0.163 V. These iron reduction potentials are substantially lower than for iron alone, indicating that the stability of Fe3+ increases upon binding to the compounds, as would be expected for iron-based reactions. The Fe3+/2+ oxidation potentials are ~0.249 to 0.460 V, and the Fe2+/3+ oxidation potentials are ~0.324 V to 0.460 V. Since stabilization of Fe3+ increases upon binding to the compounds, as would be expected for iron-based reactions. The Fe3+/2+ oxidation potentials are ~0.249 to 0.460 V, and the Fe2+/3+ oxidation potentials are ~0.324 V to 0.460 V. Since Fe3+ generates damaging hydroxyl radical (Fig. 1), stabilization of Fe3+ relative to Fe2+ will inhibit iron-mediated DNA damage. However, changes in iron redox potentials in the presence of the catecholamines, amino acids, and curcumin show little correlation with their ability to prevent iron-mediated DNA damage, suggesting that factors other than thermodynamic stability also must be taken into account.

In the cyclic voltammogram of CuSO4 alone (pH 7), an Ep due to Cu2+/Cu+ reduction is observed at ~0.144 V. When Gly, Glu, and L-DOPA, and curcumin are combined with 1 equiv Cu2+, all the voltammograms show decreases in this copper-based reduction potential ranging from ~0.235 to ~0.169 V, representing shifts of ~0.091 to ~0.025 V compared to CuSO4 alone. Similar to the iron studies, these results indicate that interaction of these compounds with copper stabilizes Cu2+ relative to Cu+, as expected upon interaction with compounds containing hard oxygen or nitrogen donor ligands. Identifiable copper-based waves are not observed for the compounds that do not prevent copper-mediated DNA damage (EP, NE, GABA). As for iron, however, the observed stabilization of Cu2+ does not correlate well to the prevention of copper-mediated DNA damage, although it likely is a contributing factor in antioxidant activity.

It is also important to note that while copper interactions with catecholamines and curcumin may stabilize Cu2+ over Cu+, it is well known that these compounds also can readily oxidize to form radical semiquinone species with concomitant reduction of Cu2+ to DNA damaging Cu+. Dopamine has also been reported to reduce Fe3+ to Fe2+ and cause DNA damage, but to a much lesser extent than with copper, a fact that may be responsible for its higher IC50 value for prevention of iron-mediated DNA damage compared to the other catecholamines. In addition, under the conditions of these experiments, all iron and copper redox potentials in the presence of the tested compounds are within the range of biological hydroxyl radical formation (~0.324 V to 0.460 V). Since stabilization of Fe3+ or Cu2+ does not directly correlate with prevention of iron- or copper-mediated DNA damage for the compounds, factors in
Addition of Fe\(^{2+}\) to DA, EP, NE, and L-DOPA results in Fe\(^{2+}\) oxidation as determined from the UV-vis spectra (Fig. 6A). The absorbance changes upon Fe\(^{2+}\) addition (Fig. S22†), so iron oxidation cannot be determined using this method. Upon Cu\(^{+}\) addition, however, the UV-vis spectra for Gly and Glu show a new band at \(\lambda_{\text{max}}\) 231 and 234 nm, respectively (Fig. S25†), arising from \(\sigma(O\text{-carboxylate}) \rightarrow \text{Cu}^{2+}\) LMCT transitions, indicating that Cu\(^{+}\) is oxidized to Cu\(^{2+}\) upon binding. GABA shows no significant absorbances upon copper addition, consistent with its lack of copper-mediated DNA damage prevention.

The keto-hydroxy moiety of curcumin is known to bind metal ions, and curcumin strongly binds Fe\(^{2+}\), Fe\(^{3+}\), and Cu\(^{2+}\). Examination of the antioxidant behaviour of curcumin analogues with fewer than two phenol groups show that both the keto-hydroxy and the phenol groups are necessary for antioxidant activity. Upon addition of Fe\(^{2+}\) to curcumin, the UV-vis spectrum shows a new LMCT absorbance at \(\sim\)510 nm (Fig. S18D and E†), indicating iron oxidation to Fe\(^{3+}\) upon binding. After addition of Cu\(^{+}\) to curcumin, the UV-vis LMCT absorbance at \(\sim\)480 nm also indicates binding to Cu\(^{2+}\), not Cu\(^{+}\) (Fig. S26†), as previously reported for curcumin–Cu\(^{2+}\) complexes. Since curcumin, catecholamine, and amino acid binding promotes oxidation of Fe\(^{2+}\) and Cu\(^{+}\), this is likely one factor for their prevention of metal redox cycling in vivo and observed antioxidant activity.

**Kinetics of iron oxidation**

Stabilization of Fe\(^{3+}\), as observed from the electrochemical studies with the neurotransmitters, L-DOPA, and curcumin, can prevent iron redox cycling and generation of hydroxyl radical, but kinetic factors must also be considered in understanding antioxidant activity. Previous studies have determined a direct correlation between rate of iron oxidation upon catechol or hydroxycromone binding and prevention of iron-mediated DNA damage. To determine whether this relationship also holds for the four catecholamines and curcumin, their rates of Fe\(^{2+}\) oxidation were measured at a 3 : 1 iron-to-compound ratio required for maximal coordination. Upon addition of EP (435 \(\mu\)M) to Fe\(^{2+}\) (145 \(\mu\)M) in a 3 : 1 ratio at pH 6, the increase in the Fe\(^{2+}\)–EP LMCT band at 583 nm was measured for 30 min (Fig. 6A). The initial rate for Fe\(^{2+}\)–EP oxidation, 0.382 min\(^{-1}\), was determined from the slope of the best-fit line through the initial linear section of this kinetic curve (Fig. 6B, Table 3). Kinetic data were also acquired for varying EP-to-iron ratios (1 : 1 to 5 : 1) and the graph of Fe\(^{2+}\) oxidation rate vs. EP to iron (Fig. 6A, Table 1). These LMCT bands do not increase significantly beyond a ligand-to-iron ratio of 3 : 1, indicating formation of complexes of the expected FeL\(_3\) stoichiometry under these conditions. Catecholate binding to iron is known to accelerate Fe\(^{2+}\) to Fe\(^{3+}\) in the presence of \(O_2\).
Table 3  Catecholamine, l-DOPA, and curcumin Fe²⁺ oxidation rates at a 3:1 ligand to Fe²⁺ ratio and pKₐ values

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rate (min⁻¹)</th>
<th>IC₅₀ with Fe²⁺ (μM)</th>
<th>pKₐ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine (DA)</td>
<td>0.353 ± 0.009</td>
<td>18 ± 1</td>
<td>8.90¹⁰²</td>
</tr>
<tr>
<td>Epinephrine (EP)</td>
<td>0.382 ± 0.004</td>
<td>4.6 ± 0.1</td>
<td>8.50¹⁰³</td>
</tr>
<tr>
<td>Norepinephrine (NE)</td>
<td>0.404 ± 0.006</td>
<td>3.2 ± 0.2</td>
<td>8.4¹⁰⁴</td>
</tr>
<tr>
<td>l-DOPA</td>
<td>0.369 ± 0.003</td>
<td>7.0 ± 0.5</td>
<td>8.72¹⁰⁵</td>
</tr>
<tr>
<td>Curcumin</td>
<td>0.352 ± 0.008</td>
<td>6.2 ± 0.2</td>
<td>8.3¹¹⁰</td>
</tr>
</tbody>
</table>

*a* Rate and IC₅₀ values are averages of three trials with standard deviations. *b* Rate data for curcumin were collected at a concentration five times lower than for DA, EP, NE, and l-DOPA.

Fig. 7  Graph of Fe²⁺ oxidation rate vs. epinephrine to iron ratios indicating that Fe²⁺ oxidation has first-order dependence upon epinephrine concentration (y = 0.197 x − 0.1771, R² = 0.984). Data are the average of three trials with standard deviations.

Conclusions

Catecholamine and amino acid neurotransmitters behave differently as antioxidants, and the results of DNA damage assays, UV-vis spectroscopy, and cyclic voltammetry studies all point to metal interactions as a primary factor for the observed antioxidant activity. The catecholamines DA, EP, NE, and l-DOPA, as well as curcumin, prevent Fe²⁺ mediated DNA damage at low micromolar concentrations, whereas the amino acids Gly, Glu, and GABA prevent little or no DNA damage under the same conditions. In contrast, Gly and Glu are more effective at preventing copper-mediated DNA damage. In all cases, iron or copper binding is the primary antioxidant mechanism, and DNA damage prevention is observed at or near biological concentrations of these compounds.

Cyclic voltammetry of the catecholamines, amino acids, and curcumin with iron show that binding of these compounds lowers iron and copper oxidation potentials. UV-vis spectroscopy studies also indicate that the catecholamines and curcumin bind Fe²⁺ and promote oxidation to Fe³⁺, corresponding to their abilities to prevent iron-mediated DNA damage. In addition, the IC₅₀ values for prevention of iron-mediated DNA damage correlate strongly to phenolic pKₐ and rate of iron oxidation. UV-vis studies with Glu, Gly, and curcumin show that these compounds oxidize Cu⁺ to Cu²⁺, in accordance with their ability to prevent copper-mediated DNA damage. These findings emphasize the general importance of metal binding on the antioxidant behaviour of neurotransmitters and related compounds, a factor that should be strongly considered when developing treatments for Alzheimer’s, Parkinson’s, and other neurodegenerative diseases.
Experimental procedures

General

Water was purified using a Barnstead NANO Pure Diamond Life Science (UV/UF) water deionization system (Barnstead International). Dopamine hydrochloride, 98.5% (Alfa Aesar), l-adrenaline (TCI America), l-noradrenaline bitartrate (TCI America), glycine (J.T. Baker), dl-glutamic acid (TCI Tokyo Kasei), 4-amino-n-butric acid (TCI America), curcumin (MP Biomedicals), 2-(N-morpholino)ethanesulfonic acid (MES; Calbiochem), 3-(N-morpholino)propanesulfonic acid (MOPS; Alfa Aesar), KNO₃ fine crystal (Mallinckrodt Chemicals), FeSO₄·7H₂O (Acros Organics), CuSO₄·5H₂O (Fisher), and l-(+)-ascorbic acid, ACS, 99+% (Alfa Aesar), sodium bitartrate (Electron Microscopy Sciences), Chelex 100 resin (Sigma-Aldrich), and disodium dihydrogen ethylenediaminetetraacetate (EDTA; TCI America) were all used as received.

To dissolve epinephrine in buffered solutions, 1 M HCl was added as needed (up to 50 μL/50 mL for a 400 μM solution) until dissolved while maintaining a final pH of 6.0 or 7.0. To prevent oxidation of epinephrine in solution, samples were prepared daily and stored in the dark on ice prior to use. To dissolve curcumin, a small amount of MES or MOPS buffer (2.0 mL at 10 mM; pH 6.0 or 7.0, respectively) was added to curcumin and then a small amount of pure ethanol was added (0.05 M in final stock solution). This buffer/ethanol solution was then adjusted to a basic pH (~10) by adding less than 50 μL of a 1 M NaOH solution to 50 mL curcumin solution (identifiable by development of a dark red colour) and then adjusted to pH 6.0 or 7.0 by slowly adding MES or MOPS buffer at low concentrations (10–45 mM) to yield a brilliant yellow solution. Ethanol concentrations in curcumin solutions are accounted for in the gel electrophoresis experiments. All experiments with norepinephrine bitartrate were also performed with sodium tartrate as a control. To avoid metal contamination, all microcentrifuge tubes were washed with 1 M HCl, rinsed in deionized H₂O, and dried prior to use. Buffered solutions were treated with Chelex resin (2.4 g/80 mL buffer) for 24 h prior to use.

Transfection, amplification, and purification of plasmid DNA

Plasmid DNA (pBSSK) was purified from E. coli strain DH1 using a PerfectPrep Spin kit (Fisher). The plasmid DNA was dialyzed at 4 °C against EDTA (1 mM) and NaCl (50 mM) for 24 h and then against NaCl (130 mM) for 24 h to remove metal ions. For all experiments, the absorbance ratios of DNA solutions were A₂₅₀/A₂₆₀ ≤ 0.95 and A₂₆₀/A₂₈₀ ≥ 1.8.

Gel electrophoresis DNA damage assays

For each reaction, reagents were added in the following order to achieve the given final concentrations in a final volume of 10 μL: deionized H₂O, MES buffer (10 mM, pH 6.0 to ensure Fe²⁺ solubility), NaCl (130 mM), 100% ethanol (10 mM), neurotransmitter, l-DOPA, or curcumin in the indicated concentration, and Fe²⁺ (2 μM). Iron solutions were immediately prepared from solid FeSO₄·7H₂O prior to each experiment. This mixture was allowed to stand at room temperature for 5 min, followed by addition of plasmid DNA (0.1 pmol in 130 mM NaCl). After an additional 5 min, H₂O₂ (50 μM) was added to initiate DNA damage. After 30 min, EDTA (50 μM) was used to stop the reaction, and loading dye (2 μL) was added to give a final volume of 12 μL. In all cases, the concentrations of FeSO₄, Fe(EDTA)²⁻, CuSO₄, and Cu(bpy)²⁺ were adjusted to achieve ~90% DNA damage so that damage inhibition between the tested compounds could be reliably compared.

Gel electrophoresis was run on a 1% agarose gel in TAE buffer for 30 min at 140 V to separate the nicked (damaged) and supercoiled (undamaged) forms of the plasmid DNA. Gels were then stained for 5 min using ethidium bromide and washed for an additional 10 min in deionized H₂O before imaging under UV light. The intensities of the damaged and undamaged DNA gel bands were quantified using UVIproMW software (Jencons Scientific Inc., 2003). Ethidium stains supercoiled DNA less efficiently than nicked DNA, so supercoiled DNA band intensities were multiplied by 1.24 prior to comparison. Intensities of the nicked and supercoiled bands were normalized for each lane so that % nicked + % supercoiled = 100%. Sodium tartrate prevented only 11.4% of DNA damage at 200 μM (Table S9†). The highest concentration of norepinephrine tested, so norepinephrine DNA damage prevention percentages are uncorrected.

For gel electrophoresis experiments with Fe(EDTA)³⁻ as the iron source, Fe(EDTA)²⁻ (400 μM) was added in place of FeSO₄ and experiments were performed as described for the Fe²⁺/H₂O₂ studies.

Gel electrophoresis experiments with Cu²⁺/H₂O₂ were performed in MOPS buffer (10 mM, pH 7.0) with addition of CuSO₄·5H₂O (6 μM) and ascorbic acid (7.5 μM final concentration to reduce Cu²⁺ to Cu⁺ in situ) in place of FeSO₄ in MES buffer. Tartrate showed no inhibition of copper-mediated DNA damage under these conditions (Table S22†).

For gel electrophoresis experiments with [Cu(bpy)]²⁺ as the copper source, a solution of [Cu(bpy)]²⁻ (50 μM) in place of CuSO₄ was combined with ascorbic acid (62.5 μM) and experiments were performed as described for the Cu²⁺/H₂O₂ studies.

Electrochemical studies

Cyclic voltammetry (CV) measurements for neurotransmitters, l-DOPA, and curcumin alone (380 μM) and with 1 equiv FeSO₄ or CuSO₄ (380 μM each, final concentrations) were measured using a CH Electrochemical Analyzer (CH Instruments, Inc.). All experiments were externally referenced to ferricyanide (0.358 V) and all samples were allowed to complete one full ±1 V cycle prior to data collection. Experiments with FeSO₄ and CuSO₄ were performed in MES buffer (64 mM, pH 6.0) or MOPS buffer (64 mM, pH 7.0, respectively, with KNO₃ as a supporting electrolyte (64 mM). After Fe²⁺ or Cu²⁺ addition, samples were allowed to stand for 10 min prior to data collection. Prior to analysis, all solutions were deoxygenated by bubbling with argon for 45 min; during analysis, samples were blanketed with N₂. For cyclic voltammetry, the samples were cycled between −1.0 V and 1.0 V vs. Ag/AgCl/3 M KCl (+210 mV vs. NHE) using a glassy carbon working electrode and a platinum counter-electrode at a scan rate of
Iron and copper binding by UV-vis spectroscopy

All cuvettes were washed in 6 M HCl for at least 30 min, thoroughly rinsed with deionized water, and dried to avoid metal contamination. Samples were prepared using fresh FeSO₄·7H₂O (145 μM) or CuSO₄·5H₂O (145 μM) with 1.25 equiv ascorbate in buffered solutions (10 mM MES at pH 6.0 for Fe²⁺ studies or 10 mM MOPS at pH 7.0 for Cu²⁺ studies) with neurotransmitter or L-DOPA (145 μM). Concentrations were final concentrations in a 3 mL volume. For higher ligand-to-metal ratios, the compound concentration was increased as indicated, keeping the metal concentration at 145 μM. UV-vis spectra were acquired after 10 min on an Agilent 8453 or a Shimadzu UV-3101PC spectrophotometer. Due to the high absorbance of curcumin, concentrations of curcumin (29–145 μM) and FeSO₄ (29 μM) or CuSO₄ (29 μM) were lower to avoid saturation. Upon Fe²⁺ addition, the UV-vis spectra of sodium tartrate showed only a slight absorbance (<0.1 AU) from 240–400 nm, making the contribution of tartrate to noradrenaline + Fe²⁺ UV-vis spectra negligible (Fig. S23A†). No absorbances were observed in the UV-vis spectra of tartrate +Cu²⁺ (Fig. S22B†).

Kinetics of iron oxidation

Solutions of FeSO₄ (145 μM) and the catecholamines (435 μM) in MES buffer (50 mM, pH 6.0) were combined at room temperature and kinetic data were measured at the λmax for each Fe³⁺ complex (Table 1) for 30 min. Iron oxidation kinetics for curcumin, concentrations of curcumin (29–145 μM) and FeSO₄ (29 μM) or CuSO₄ (29 μM) were lower to avoid saturation. Upon Fe²⁺ addition, the UV-vis spectra of sodium tartrate showed only a slight absorbance (<0.1 AU) from 240–400 nm, making the contribution of tartrate to noradrenaline + Fe²⁺ UV-vis spectra negligible (Fig. S23A†). No absorbances were observed in the UV-vis spectra of tartrate +Cu²⁺ (Fig. S22B†).

Notes and references

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