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In Vitro Preliminary Evidences on the Antioxidant Properties of Biogenic Amines

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ABSTRACT

Antioxidant properties of the principal biogenic amines were determined *in vitro* by four analytical methods—Folin Ciocalteu, DPPH, enzymatic and inhibition of lipid peroxidation—in order to avoid possible measuring-method linked mistakes. Different results are obtained, depending on the parameters that each of them measures. The combination of the data indicates that all examined amines show antioxidant characteristics: in particular, tyramine, serotonin, L-norepinephrine, (-)-epinephrine and dopamine owing to their (poly)phenolic structure too, while aliphatic polyamines-spermine, spermidine, putrescine and cadaverine-histamine, melatonin and tryptamine appear to act specifically on the oxygen-consuming species involved in the lipid peroxidation of polyunsaturated fatty acids.

Keywords: Biogenic Amines; Antioxidants; Lipid Peroxidation; DNA Protection; Membrane Protection

1. Introduction

Biogenic amines constitute a large group of naturally occurring and biologically active compounds containing one or more amino groups, produced by decarboxylation of amino acids.

Biogenic amine can be split into two physiologically distinct groups:

1) Monoamines, containing one amino group, sometime derivatized, connected to an aromatic ring by a two-carbon chain, which acts as neuromodulators or neurotransmitters [1]; their reduced level appears to be the cause of neurodegenerative diseases [2];

2) Polyamines, characterized by two or more amino groups placed in a linear aliphatic chain, involved in physiological processes such as cell growth and differentiation [3]; their polycationic nature at neutral pH induces electrostatic interactions with negative charges on nucleic acids and phospholipids, stabilizing the structure of chromosomes and membranes [4-6].

Some papers have been reported on the possible antioxidant role of singular biogenic amines, while no systematic investigation on their antioxidant properties *in vitro* or *in vivo* has been published.

Catecholamines and their metabolites appear to play a key role in the redox balance for the formation of new synapses and the removal of old ones [7], while melatonin and its metabolites are involved in the reduction of oxidative stress (antioxidant cascade of melatonin) [8].

Between polyamines, it has been found that spermine acts as free radicals scavenger in nucleous, mitochondria and brain [9-11] and a radical scavenging activity on lipoperoxidation *in vivo* [12] or on methylmethacrylate polymerization [13] by spermine and spermidine was tested.

In order to contribute to individuating a possible role of biogenic amines on the protection against ROS (reactive oxygen species) injury, we studied *in vitro* the antioxidant properties of twelve of these compounds by applying four different assays, to avoid possible measuring-method linked mistakes. Besides, cyclic voltammetry measurements, to verify the ability of biogenic amines to easily give electrons to oxidant species, were also carried out.

2. Materials and Methods

2.1. Chemicals

All chemicals, of the highest available quality, were obtained from Sigma Chemical Co. (St. Louis, USA); ABIP (2,2'-azobis[2'-(2-imidazolin-2-yl)propane] dihydrochlo-

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ride) was obtained form Wako Chemicals (Germany). The aqueous solutions were prepared with quality milliQ water. Each experiment was in triplicate.

2.2. Experimental Procedures

The ability of amines to prevent linoleic acid (LA) peroxidation was studied in sodium dodecyl sulfate (SDS) micelles. As previously reported [14], the amines antioxidant capacity was calculated as the concentration that halves the rate of oxygen consumption due to the peroxidation process and it is expressed as inhibitory concentration (IC_{50}).

2,2-Diphenyl -1-picrylhydrazyl (DPPH) radical scavenging ability assay is based on the capacity of an antioxidant to scavenge the stable free radical DPPH [15,16] and the results are expressed as catechin equivalent (CE). Whole reducing capacity by Folin Ciocalteu assay and Total Phenolics Content (TPC) determination by enzymatic method were carried out spectrophotometrically, according to the procedure previously quoted [16] and the results are expressed as catechin equivalent (CE).

Spectrophotometric measurements were recorded on a UV-VIS Shimadzu UV-1800 instrument equipped with a temperature controlled quartz cell. The measures of oxygen consumption were carried out by an oxygen Microelectrodes MI-730 microelectrode connected to a potentiostat Amel 559.

Cyclic voltammetry measurements were carried out in a solution containing 1 mM amine in 50 mM sodium phosphate, pH 7.4, and 50 mM potassium chloride.

A potentiostat μAutolab type III equipped with three electrodes (vitreous graphite as working electrode, platinum as counterelectrode and SCE as reference electrode) was used and the data collected by GPES Autolab software.

3. Results

The histograms of **Figure 1** show the results, expressed as catechin equivalent, obtained applying Folin, DPPH and enzymatic method to the examined biogenic amines.

It appears that spermine, spermidine, putrescine, cadaverine, *i.e.* linear aliphatic polyamines, do not show reducing activity or electron scavenging ability; furthermore no response to the enzymatic assay was found, because lacking of phenolic structure.

On the contrary, tyramine, serotonin, norepinephrine, epinephrine and dopamine, all containing phenolic functional groups, give significative results for all three assays, with the exception of tyramine for which negative appears the DPPH assay. This is not surprising on the light of above reported considerations. Another exception is the positive response of tryptamine to the Folin test. In fact, from the chemical point of view, this last molecule appears very similar to histamine that gives negative result.

Data obtained measuring the inhibition of lipid peroxidation appear sensitively different. In fact, **Figure 2** shows high inhibition values also when aliphatic polyamines were used, as well as histamine, tryptamine and melatonin show. It is interesting to observe the almost

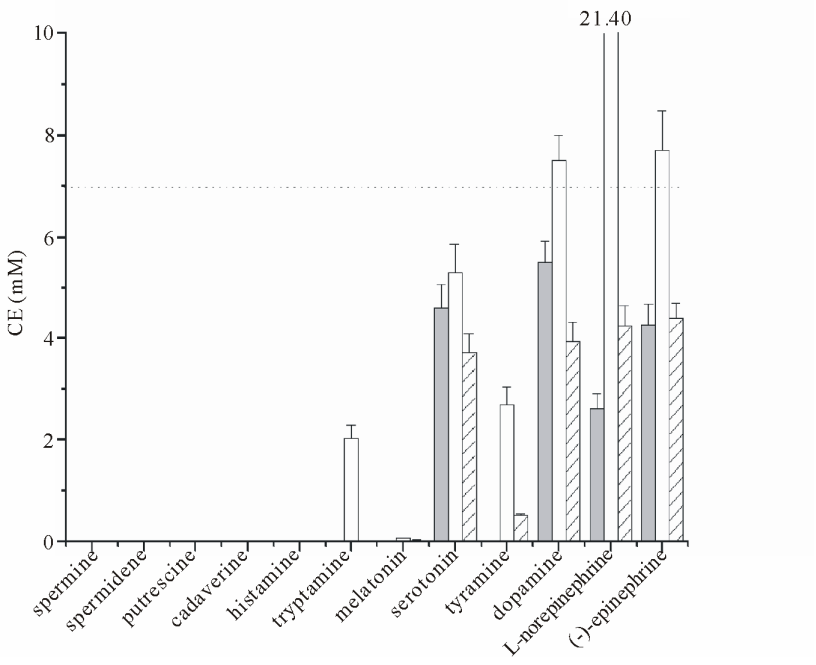

Figure 1. Histograms, expressed as catechin equivalent, of the results obtained applying; ▩ DPPH, □ Folin and ▨ Enzymatic method to the examined polyamines.

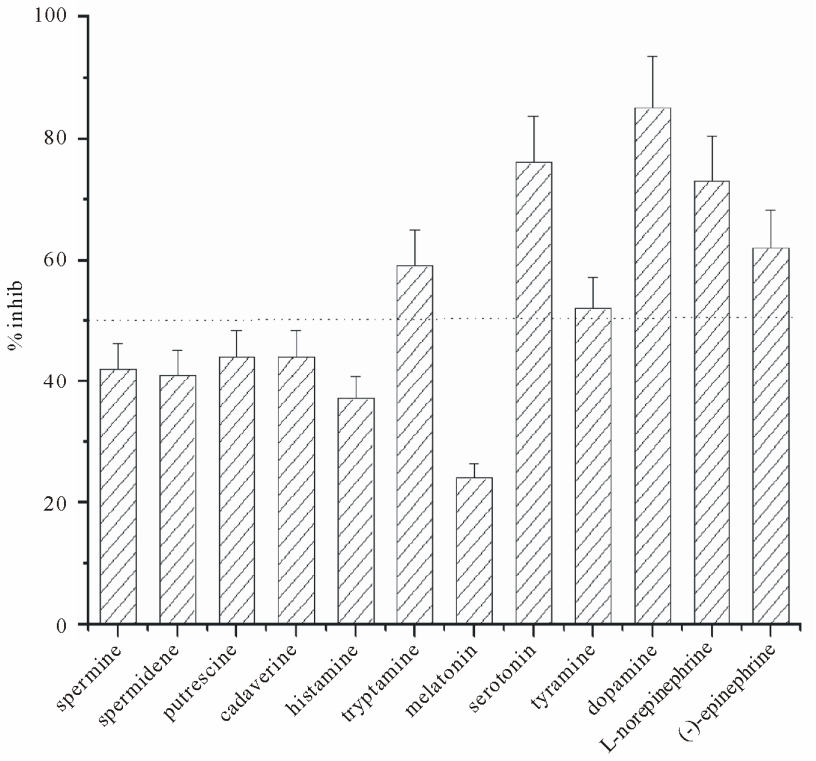

Figure 2. Inhibition of oxygen consumption (%) due to the peroxidation process at 2 μM concentration of biogenic amines.

equal value of aliphatic polyamines, which ranges around 43%.

High inhibition values, more than 50% and until 85% were obtained for other phenol based biogenic amines, as expected.

The results obtained by this last method, even if it is more laborious and time consuming, appear more reliable, because the assay, more than others, mimes the efficacy of an antioxidant compound to prevent oxidative damage on lipoproteins or cell membrane by ROS injury [17].

The experimental data here obtained confirm this assumption and demonstrate that usual methods (Folin, DPPH quenching, etc.), generally adopted to investigate antioxidant properties of molecules, are not reliable for their chemical and mechanicistic limits with respect to the complexity of whole reactions involved in the ROS injury.

In fact, aliphatic polyamines, besides histamine and tryptamine, show high inhibition (ranging from about 40 to 60%) of lipid peroxidation at very low concentrations (2 μM), but give negative responses to other assays here used. This means that these amines are not susceptible by Folin reagent oxidation, electron transfer from DPPH radical, and not involved in the enzymatic reaction because lacking of phenolic structure essential for the enzymatic coupling reaction [16]. Moreover, triazolopyridines derivatives, which do not contain (poly)phenolic groups but primary and tertiary amino groups, similarly to the examined biogenic amines, appear to acts directly and indirectly as an efficient ROS scavenging *in vivo*, as found by measuring the concentration of malondialdehyde by paraquat-induced lipid peroxidation [18].

To verify the ability of biogenic amines to easily give electrons to oxidant species, cyclic voltammetry measurements were carried out. The data of **Table 1** show that polyamines spermine, spermidine, cadaverine e putrescine are characterized by a very high discharge potential (≥1 V), while lower values (ranging from 0.196 V of dopamine to 0.805 V of tryptamine) were recorded for all others amines.

All these results indicate that aliphatic polyamines, histamine, tryptamine and, to some extent, melatonin, can inhibit lipid peroxidation only interacting directly via chemical mechanism with oxygen consuming species, that is towards ROS or ROS-induced species of the same type and structure of those forming in the lipid peroxidation of membranes. Hydroxyl radicals, reactive species for which efficient scavenging properties for these amines were found [9-10,12], can not be invoked in this case because these radicals apparently are not directly formed in the lipid peroxidation mechanism. Moreover, indications about the scavenging activity of polyamines towards alkyl or peroxy radicals derived from polyunsaturated fatty acids were suggested by indirect measurements [13].

Table 1. Discharge potential vs SCE of biogenic amines. A vitrous graphite as working electrode was used.

Amine	E(V)
spermine	≥1
spermidine	≥1
putrescine	≥1
cadaverine	≥1
histamine	0.765 ± 0.001
tryptamine	0.805 ± 0.003
melatonin	0.680 ± 0.001
serotonin	0.430 ± 0.005
tyramine	0.692 ± 0.006
dopamine	0.196 ± 0.008
L-norepinephrine	0.302 ± 0.007
(-)-epinephrine	0.311 ± 0.007

4. Discussion

As previously reported [14], no analytical method can give a reliable measure of the antioxidant efficacy of a molecule, because of the different parameters that each assay measures and the manifold types of reactions involved in the ROS damage. In fact, biogenic amines show very different chemical structures and can be assembled into three groups.

Besides primary amine group, they are characterized respectively: 1) by aliphatic chain spaced or ended by other secondary or primary amine groups respectively (spermine, spermidine, putrescine, cadaverine); 2) by a terminal imidazole (histamine) and indole group (tryptamine, melatonin and serotonin); 3) by phenolic (tyramine and serotonin again) or polyphenolic (L-norepinephrine, (-)-epinephrine, dopamine) function.

For this reasons in this work, multiple tests, representing various redox mechanisms, have been used as the best strategy for stating an empirical and approximate scale of effectiveness. In fact, the well-known Folin-Ciocalteu assay is a general measure of antioxidant's reducing capacity and it is largely a specific because correlated only to the redox potential of the chemical species involved; DPPH based method measures the ability of a molecule to scavenge the stable free radical DPPH, but this last compound may be inert to many antioxidant and the reaction could not go to completion, as found for some phenolic derivatives [19]; enzymatic method, we proposed, determines phenolic structures owing to peroxidise specificity [16]. In this paper, in addition to these analytical methods, the measures of the inhibitory capacity of ABIP-induced lipid peroxidation and of the electrochemical ability to give electrons to oxidative species are also carried out.

The experimental data indicate that all examined biogenic amines show antioxidant properties; in particular, tyramine, serotonin, L-norepinephrine, (-)-epinephrine and dopamine for their (poly)phenolic structure too, while aliphatic polyamines, histamine, tryptamine and melatonin appear to act specifically on the oxygen consuming species involved in the lipid peroxidation of polyunsaturated fatty acids in biological systems.

Considering that spermine is coupled by electrostatic bonds to phosphate groups of nucleic acids and membrane phospholipids, stabilizing helical and bilayer structures, these results suggest that spermine and probably other polyamines also could carry out a protective action of nucleotide bases and acyl chains of phospholipids against ROS injuries. Moreover, it is necessary to take into consideration that polyamines oxidation, consequent their protective action, decreases their stabilizing role towards the above reported structures.

Furthermore, catecholamines, owing to effective catechol group which can oxidatively convert to the corresponding o-quinones, can perform a protective function towards synaptic vesicles and, in particular, their phospholipid moiety from ROS induced peroxidation, but their loss contribute to neurodegenerative disorder, notably Parkinson's disease [2]. Moreover, quinone structure can easily react with primary amine groups of biogenic amines or proteins to form Schiff base.

Further investigations to explain a possible chemical mechanism justifying the antioxidant properties of biogenic amines are in progress.

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