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Biogenic amines and apoptosis: Minireview article

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Summary. The programmed cell death is a very complex mechanism involving many factors, among them the intracellular concentration of biogenic amines (BA) appears to be important for apoptosis triggering. The mitochondrial damage is imputable to hydrogen peroxide and aldehydes, produced by amine oxidases (AO)-mediated oxidation of BA. On the other hands, the apoptosis protection observed by high BA concentration appears to be related to their scavenger effect of ROS and/or their interaction with membrane pores. Also monoamine oxidase (MAO) inhibitors, like propargylamines, preserve the mitochondria integrity by inhibiting MAO and therefore the production of H_2O_2 and aldehydes and, as cations, by regulating membrane pores, like BA.

As general conclusion, apoptosis is protected by high concentration of BA and/or other cations while it is favoured by ROS produced by AOs or other mechanisms.

Keywords: Amine oxidases – Apoptosis – Biogenic amines – Hydrogen peroxide – Aldehydes – Mitochondria

Biogenic amines (BA), including polyamines, monoamines and histamine, appear to be deeply involved in the mechanism of apoptosis progression.

It is well known that the programmed cell death is a very complex mechanism involving many factors, among them the intracellular concentration of BA appears to be important for apoptosis triggering. The present review will demonstrate that many apparently contradictory results, reported in the literature, can be explained by the observation that high intramitochondrial concentration of BA appear to be protecting and low damaging features.

The content of BA in the cytoplasm can be changed by activation or overexpression of the enzymes responsible for their synthesis, resulting in increased BA levels, or by similar effects on catabolic enzymes, which reduce BA content. These variations can exert different effects on mitochondrial permeability transition (MPT), which is strictly related to apoptosis and/or necrosis. The MPT refers to a dramatic increase in unspecific inner membrane permeability in mitochondria in the presence of supraphysiological Ca^{2+} concentration and of a wide variety of inducing agents. The BA are oxidized by both cytosolic and mitochondrial amine oxidases (AOs). Since the reaction products of BA oxidation are involved in the induction and/or amplification of MPT, the/some function of the AOs responsible of BA oxidation, shall be briefly summarized.

The AOs are enzymes widely distributed among all living organisms: (microorganisms, plants and mammals) (Mondovì, 1985) and represent a class of enzymes heterogeneous in structure and substrate oxidation mechanism. Mono, di and polyamines, as well as several N-acetyl amines, are oxidatively deaminated by AOs in a reaction consuming O_2 and H_2O and producing the corresponding aldehyde, NH_3 and H_2O_2.

On the basis of the nature of the prosthetic group contained in the enzyme molecule, at first two classes of AOs can be described: a) containing FAD and b) having copper and an organic cofactor as prosthetic group, which has been identified as 6-hydroxydopa quinone (TPQ) (Janes et al., 1990; Klinman et al., 1991). The last one are also called, mainly by pharmacologists or clinicians, "SSAO", i.e. semicarbazide-sensitive amine oxidases.

Thus, the first distinction for an adequate nomenclature appears to be that between FAD (FAD-AOs) and copper-

dependent amine oxidases (Cu-AOs). The FAD-dependent AOs are then sub-classified as monoamine oxidase (MAO) and polyamine oxidase (PAO). The general equation of amines oxidation by AOs is the following:

i) cleavage at a primary amino group, (terminal oxidation reaction)

$$E_{ox} + R{-}CH_2{-}NH_3^+ \rightarrow E_{red}{-}NH_3^+ + RCHO$$
$$E_{red}{-}NH_3^+ + O_2 \rightarrow E_{ox} + NH_4^+ + H_2O_2 \quad (1)$$

E_{ox} = oxidized enzyme, E_{red} = reduced enzyme

ii) cleavage at a secondary amino group (interconversion reaction)

$$R_1{-}CH_2{-}NH{-}CH_2{-}R_2 + O_2 + H_2O \rightarrow R_1{-}CHO + H_2N{-}CH_2{-}R_2 + H_2O_2 \quad (2)$$

Eq. (1) concerns copper-AOs and MAOs and Eq. (2) PAOs. PAOs split the C–N bond between the aminopropyl residue and the secondary amino group to produce spermidine from N^1-acetylspermine and putrescine from N^1-acetylspermidine. Sometimes also some copper AO is classified as PAO.

The function of AOs in living organisms is so far not completely understood but, it is certainly concerned with the biogenic amines metabolism and therefore involved in essential processes such as the cell growth and differentiation, the regulation of neurotransmitters, the metabolism of histamine, the balance of polyamines pool. It follows that many neurological disorders, allergic disease and a tumor progression bear with these enzymes.

The oxidation products of AOs are cytotoxic and are involved on the inhibition of cell growth and proliferation. Cytotoxicity is essentially related to hydrogen peroxide and aldehydes generated by AO-mediated oxidation of BA. Aminoaldehydes interact with DNA (Eilon and Bachrach, 1969; Bachrach and Eilon, 1967).

Microinjection of AOs in chick embryo fibroblasts by the use of Rous Sarcoma virus provoked cell death, while in non transformed cells the damage was reversible (Bachrach et al., 1987). Probably, the lethal effect on tumor cell was imputable to higher concentration of polyamines, and, as a consequence, higher cytotoxic products.

It is a matter of fact that bovine serum AO, added in the incubation mixture of cultured hamster fibroblast in the presence of added polyamines, shows a cytotoxic effect.

The cytotoxity induced by bovine serum AO appears to be imputable both to hydrogen peroxide and aminoaldehydes: it is inhibited by catalase during the first 20 min of incubation (Agostinelli et al., 1991), while, at longer times of incubation, aldehyde dehydrogenase protected the cells (Averill-Bates DA et al., 1994). A complete protection was observed in the presence of catalase and aldehyde dehydrogenase.

Immobilized pig kidney diamine oxidase injected into the peritoneal cavity of swiss mice 24 hr after a viable intraperitoneal transplantation of Ehrlich ascite cells remarkably inhibited tumor growth (Mondovì et al., 1982). These results may suggest a possible use of AOs in cancer therapy (Mondovì et al., 1994).

The results reported above strongly indicate the importance of H_2O_2 and aldehyde in the apoptosis progression. The catabolites can be produced directly in mitochondria, into the cells or, alternatively, in the proximity of the cells. In the former case, they are oxidation products of monoamines by mitochondrial monoamine oxidases (MAO). In the latter, extramitochondrial Cu and FAD-AO are responsible of the BA oxidation although it is not definitively established whether these enzymes are localized in or out the mitochondria (Maccarrone et al., 2001; Bieganski et al., 1983). The direct production of H_2O_2 and/or aldehydes into the mitochondria appears to be very harmful for the cells. In fact, cytofluorimetric studies allowed detecting $\Delta\psi$ (mitochondrial membrane potential), so called JC1 (Malorni et al., 1998) that after 48 hours starvation, M14 (human melanoma) cells showed a marked increase of mitochondrial membrane depolarization. This effect was consistently reduced in the presence of pargyline and clorgyline, well known MAO A inhibitors showing also cell protection.

These conclusions are in agreement with Hu and Pegg (1997) and Maccarrone et al. (2001) on the hindering of apoptosis by AO inhibitors.

The MAO inhibitors protect the cells also by the means of other mechanisms. It is matter of fact, in any case, that H_2O_2 and aldehydes produced "*in situ*" appears to play a major role on triggering apoptosis. The involvement of MAO substrates in the mitochondrial membrane transition is clearly demonstrated by a dose-dependent effect of tyramine, a good substrate of both MAO A and B, on the induction of mitochondrial swelling, (accompanied by $\Delta\Psi$ collapse) which occur after 20 min of incubation as a function of the tyramine concentration (Marcocci et al., 2001; Marcocci et al., 2002). The maximal effect of tyramine on mitochondrial swelling was observed at 500 μM, while at higher concentration the effect is gradually lost and becomes ineffective at 2 mM tyramine. A dose-dependent effect of tyramine on the rate of H_2O_2

production was also observed. These data indicate that tyramine acts as an inhibitor of MPT at high concentration. Therefore a possible effect as free radical scavenger by tyramine cannot be ruled out (Yen and Hsieh, 1997). Other possible mechanisms should be discussed later. A dose-dependent inhibition of mitochondrial swelling induced by 100 μM Ca^{++} was observed with a complete blockage at 2 mM tyramine concentration and a block of $\Delta\Psi$ collapse, cation efflux and oxidation of polynucleotides, all events connected with mitochondrial swelling. Completely different results were observed at low doses of tyramine. In fact, the addition of 100 μM tyramine into the incubation mixture induces a matrix swelling demonstrated by an absorbance decrease. Similar effects were obtained with other substrates of MAO A and B, like octopamine and benzylamine. Catalase inhibits mitochondrial swelling determined by 100 μM tyramine demonstrating that H_2O_2 produced during the oxidation of tyramine by MAO is involved in MPT induction. However the inhibition by catalase is incomplete, indicating that also hydroxyphenylacetaldehyde (HPA) produced during tyramine oxidation contributes to the phenomenon. Hydrogen peroxide, 100 μM, induces a change of the absorbance of mitochondrial suspension to a lesser extent than 100 μM tyramine. On the other hand 100 μM HPA induces a very limited change in absorbance. It is interesting to note that when both H_2O_2 and HPA are added to the incubation mixture a synergistic effect was observed. The effect of 100 μM tyramine are completely prevented in the presence of 50 μM clorgyline or 500 μM pargyline, without any effect on Ca^{++} transport. Same results were obtained with deprenyl, a MAO B inhibitor. The inhibitory effect of clorgyline and pargyline on mitochondrial functions do not parallel the effects of the enzyme, pointing to an additional mechanism of MAO inhibitors, different from that ascribable to the enzymatic oxidation products of tyramine on MPT. The mitochondrial swelling induced by 100 μM Ca^{++} is inhibited by clorgyline or pargyline also in the absence of tyramine.

The effect of deprenyl on menadione-induced MPT appear to confirm this hypothesis, taking into account that MAO B is not involved in the process of MPT induction by menadione because its oxidative catabolism is not MAO-catalyzed (De Marchi et al., 2003). As mentioned above, MPT is strictly associated with apoptosis by the opening of a proteinaceous pore, mediated by the presence of superphysiological Ca^{++} concentration or by pro-oxidant agents. The opening of the pore causes unspecific solute traffic across the mitochondrial membrane which leads the bioenergetic collapse of the sub-cellular particle. Rat liver mitochondria in the presence of 100 μM menadione and 50 μM Ca^{++} exhibit a decrease of optical density at 540 nm, indicating the swelling of mitochondria, a collapse of $\Delta\Psi$ and an endogenous cation efflux.

In the presence of Ca^{++}, menadione induces also a decrease of mitochondrial fluorescence indicating a $NAD^+/NADP^+$ pool oxidation, and cytochrome c release, distinctive of the mitochondrial damage. All these events are completely blocked by cyclosporin A and deprenyl, thus independently from MAO products.

The MPT is the result of the oxidative stress caused by the semiquinone radicals generated by the interaction of menadione with the respiratory chain. The semiquinone radical produces, in a redox cycle, superoxide radical by transfer of electron to O_2. Superoxide and its derivatives are able to form hydroxyl radicals $OH^{\cdot}$, that are very toxic for the cells. Moreover, the oxidation state of pyridine nucleotides accounts for the oxidative stress induced by menadione. Early studies indicate that two sites are involved in the MPT induction and modulation. The first site called "S" has been identified as a membrane dithiol, whose oxidation appears to be controlled by reactive oxygen species (ROS) generated by pro-oxidant. The second site called "P" is chemically unidentified and its mechanism of action is unknown.

Taking into account all the results described above, deprenyl and other propargylamines should protect the cells from MPT by two different mechanisms: first the inhibition of H_2O_2 and aldehyde production as a consequence of MAOs activity inhibitor. The second mechanism appears to be related to the structure of deprenyl. This compound contains a tertiary amino and an acetylene group and it is stereospecific because of the presence of a chiralic carbon. The presence of a tertiary amino group, with a pKa of about 9.2, makes the molecule of deprenyl almost fully protonated at physiological pH and acts as a monovalent cation by inhibiting MPT as polyamines do. This effect can be explained by the interaction of biogenic amines with most K^+ channels (Liepins et al., 1989; Pearson and Nichols, 1998; Weiger and Hermann, 1994). Probably the transition pore has preserved structural features of the archetypal K^+ channels (Halestrap and Davidson, 1990) that allow interaction with protonated compounds, in this case deprenyl.

It should be pointed out that serum amine oxidase modulates K^+ channels currents (Wu et al., 1996). A relationship should be noticed between ceruloplasmin and BSAO on the modulation of membrane properties of neuroblastoma cells. However, these copper proteins show opposite

effect on neuronal K^+ channels. Whereas ceruloplasmin alone inhibits K^+ channel current acting as an endogenous depolarizing factor, BSAO enhances K^+ in a time-dependent manner. The modulation of K^+ channel currents could be related to possible BSAO and ceruloplasmin interactions with relevant binding proteins producing different second messenger. Otherwise BSAO and ceruloplasmin may directly bind to K^+ channel proteins opening or closing the channels. Heat denaturated BSAO has not effect at all on membrane. These results suggest a possible binding of AO on mitochondrial membranes, thus regulating the concentration of BA and their oxidation products. This could explain the double mechanism of protection/damage considering that AOs is able to control BA, H_2O_2, and aminoaldehydes concentration.

In other word, a high BA level protects the membrane by the means of their interaction as cations with the mitochondrial membrane by regulating the K^+ pore opening and/or effect as ROS scavenger. Their oxidation products, instead, cause damage on the membranes, as menadione, by producing H_2O_2 and O_2 active species.

Propargylamines preserve the mitochondria integrity by two different mechanisms, in agreement to their action: inhibition of MAO activity and therefore control of H_2O_2 production and, as cations, regulation of K^+ pores, like BA.

Otherwise, both propargylamine and BA at high concentration should protect mitochondria by their anti-oxidative properties (Yen and Hsieh, 1997; Kitani et al., 2001). As a consequence, apoptosis is protected by high concentration of BA and/or other cations while it is favoured by ROS produced by AOs or other product like menadione.

Recently it has also been observed that a deprenyl structurally related propargylamine, rasagiline, prevent apoptosis by inhibiting the MPT by a stereochemical mechanism (Akao et al., 2002; Marujama et al., 2000).

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