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Molecular mechanisms of cellular injury produced by neurotoxic amino acids that generate reactive oxygen species

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Summary. There is now strong experimental evidence that the basic precursors for the synthesis of catechol(amine) and indolamine neurotransmitters, tyrosine and tryptophan can act as generators of ROS (reactive oxygen species): peroxides, superoxide and peroxyradicals. The consequences of free radicals formation from precursors during oxidative degradation process, their possible participation in electron transfer/addition reactions and chain processes involving cell antioxidant defense system were presented and discussed. Although the generation of neurotoxic ROS by tyrosine and tryptophan is accepted to occur in the presented model systems, doubts can exist as to the situation *in vivo*, which may be completely different and remain to be explored. The relevance of the present findings with regard to a variety of neurological diseases cannot be ignored.

Keywords: Tyrosine – Tryptophan – Oxidative degradation – Chain processes – Scavenging – Cellular injuries analysis

Neurons transmit impulses by release of neurotransmitters, which are reacumulated by presynaptic nerves or destroyed after use (Fig. 1). Catecholamine (CA) neurotransmitters represent a universally distributed neurochemical systems in the brain.

The biochemical toxicology of semiquinones and quinones, the products of their enzymatic or non-enzymatic degradation has been an object of very intense research over the past 20 years (Kalyanaraman et al., 1985; Bindoli et al., 1992; Metodiewa and Dunford, 1993). Generation of reactive oxygen species (ROS) is an unavoidable byprocess of CAs one – and two electron reactions. Under conditions of oxidative stress, endogenous antioxidative defences are usually inadequate for scavenging them completely (Metodiewa and Dunford, 1993; Winterbourn and Metodiewa, 1994; Halliwell, 1996), so that ongoing oxidative damage to DNA, lipids, proteins and other molecules leads to the secondary overproduction of ROS as a risk factor for neurodegenerative changes.

It could partially explain the observed neuronal lesioning in certain neurological and psychiatric disorders (AD-PDs). The concentration of brain CAs is directly related to their biosynthesis from precursors (Fig. 1): the first and rate-limiting enzyme TH (tyrosine hydroxylase) has received the greatest attention. It was proposed (Zigmond et al., 1989) that CAs may act as feedback inhibitors of TH, and more recently – as feedback inducers of the enzyme (Stull and Jacovitti, 1996).

Recent discoveries (Jin et al., 1993; Pichorner et al., 1995; Giebauß et al., 1996) that the basic precursors; tyrosine and tryptohan, can act as generators of ROS (organic peroxides, $O_2^{\cdot-}/HO_2^{\cdot}$, $RO_2^{\cdot}$) or as their effective scavengers raise the question about their participation in a variety of pathological events. The consequences of free radical(s) formation from precursors (Metodiewa and Dunford, 1993; Jin et al., 1993; Pichorner et al., 1995; Giebauß et al., 1996; Winterbourn et al., 1997), their participation in electron transfer/addition reactions, formation of ROS and modification of dopamine transporter function by produced ROS (Berman et al., 1996) remain to be explored.

It has been hypothesized that ROS play a major role in the progressive and selective loss of nigrostratial dopaminergic neurons, but the exact molecular mechanisms are still unknown. It remains puzzling that for many substances, where a definite influence of the precursor free radical reaction(s) is evident, the physological relevance has not been considered.

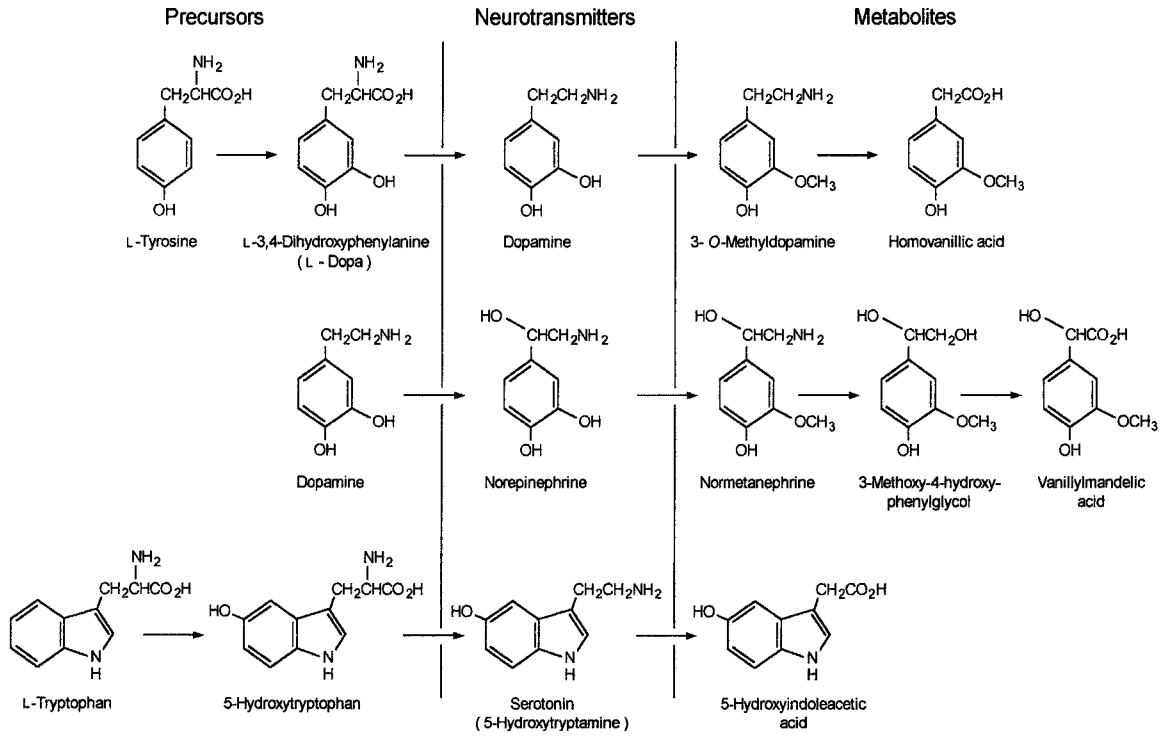

Fig. 1. Chemistry of neurotransmission

The purpose of this article is to focus on the dual role of tyrosine and tryptophan as substrates for neurotransmitters synthesis/regulation and generators/scavengers of free radicals and ROS.

As is shown in Fig. 2 (Pichorner et al., 1995), one-electron oxidation of tyrosine by heme ferryl ions (FeO^{3+} and FeO^{2+}) resulted in high reactive tyrosyl (phenoxyl) radical formation (TyrO$^{\cdot}$), which can cause lipid peroxidation and crosslinkink of protein(s) tyrosyl residues in situ. TyrO$^{\cdot}$ are scavenged very efficently by GSH (reduced glutathione), thereby generating highly reactive glutathionyl radicals and $O_2^{\cdot -}$ (Winterbourn and Metodiewa, 1994; Jin et al., 1993). This process leads to oxygen – dependent chain oxidation/depletion of GSH, the major antioxidant of neuronal cells. Tyrosyl radicals have been shown to generate another potentially injurious oxidant, TyrOOH (tyrosine hydroperoxide), by scavenging of $O_2^{\cdot -}$ (Fig. 2). The formation of TyrOOH has been observed previously (Jin et al., 1993) as a product of one-electron oxidation of tyrosine and reaction of radiolytically formed radical with $O_2^{\cdot -}$ as shown in Fig. 3. It was discovered (Jin et al., 1993) that $O_2^{\cdot -}$ reacts with tyrosyl radicals by addition rather than by electron transfer, as was suggested previously (Butler et al., 1988). Formed TyrOOH (Fig. 3, products 6 and 7) (Jin et al., 1993) are also observed after enzymatic (peroxidatic) oxidation of tyrosine (c.f. Fig. 2, left, and Fig. 3) (Pichorner et al., 1995).

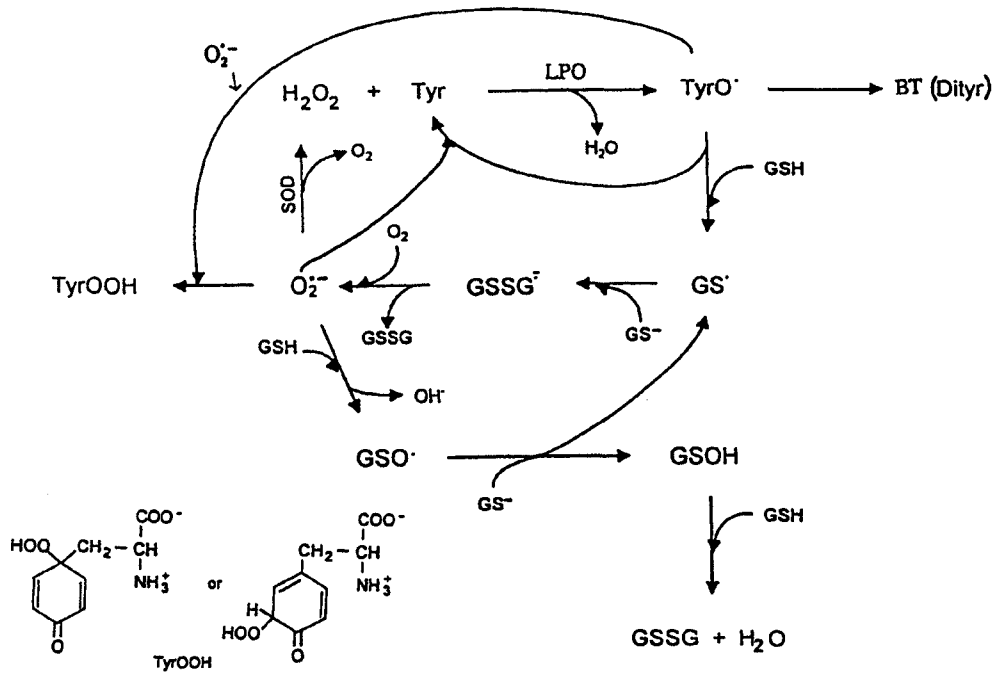

Fig. 2. Generation of superoxide ($O_2^{\cdot -}$), tyrosyl radical (*TyrO*$^{\cdot}$), tyrosine – hydroperoxide(s) (*TyrOOH*), glutathionyl radical (*GS*$^{\cdot}$), sulfinyl radical (*GSO*$^{\cdot}$), diglutathionyl anion radical (*GSSG*$^{\cdot -}$), oxidized glutathione (*GSSG*) and dityrosine (*BT*) after peroxidatic oxidation of tyrosine (*Tyr*) in the presence of reduced glutathione (*GSH*) (Pichorner et al., 1995)

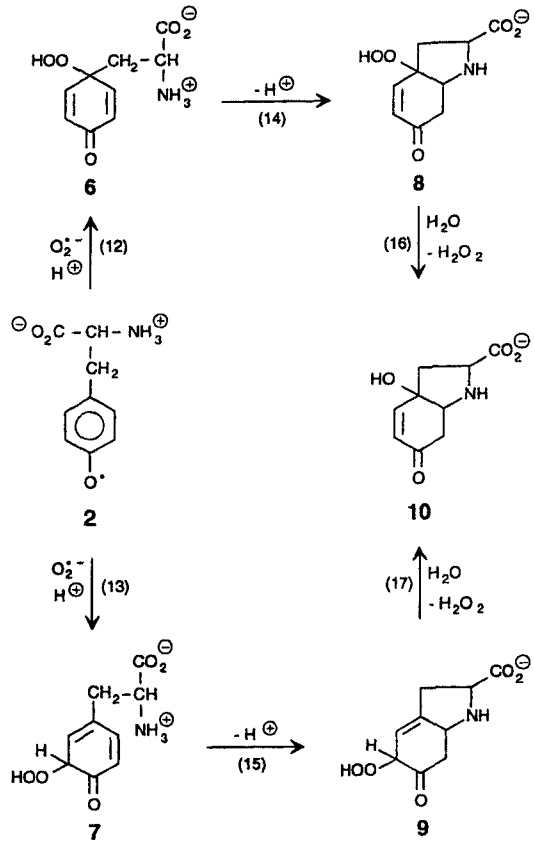

Fig. 3. The superoxide ($O_2^{\cdot -}$) reactions with radiolytically generated tyrosyl radical ($TyrO^{\cdot}$) and tyrosine – hydroperoxide(s) formation (Jin et al., 1993)

Although TyrOOH formation in well defined model systems has been described (Jin et al., 1993; Pichorner et al., 1995), the physiological consequences of this discovery have not been considered. Being a metastable oxidant (Jin et al., 1993) TyrOOH is capable of diffusion from its site of formation. As other peroxides TyrOOH should react with transition metal(s), promote lipid peroxidation of cell membrane(s) and damage DNA and proteins (Pichorner et al., 1995; Winterbourn et al., 1997). The formation of TyrOOH is favored over BT (dityrosine) formation when tyrosyl radical and $O_2^{\cdot -}$ are both generated (Fig. 2 and 3). It is known and widely accepted that initiation and propagation of endoperoxide(s) formation may create a slow free-radical chain process, leading to oxidative breakdown of the essential cell macromolecules. The lipoidal framework of neural cell membranes is most susceptible. Because formation of TyrOOH has been postulated to occur from peroxidatic oxidation of tyrosine (Pichorner et al., 1995; Winterbourn et al., 1997), it is of paramount importance to reflect, that presence of a brain peroxidase, similar to MPO (myeloperoxidase) was demonstrated in substantia nigra, putamen and caudate nucleus (Adams and Odunze, 1991). Under oxidative stress, where increased turnover of

dopamine (MAO activity) leads to an increase in H_2O_2 production, the peroxidative degradation of tyrosine could proceed very efficiently. However, GSH-transferase(s) can also function as a non-selenium dependent peroxidase(s) (Adams and Odunze, 1991). Similarly peroxidatic activity of the membrane-associated PGHS (prostaglandin synthase) cannot be ignored (Metodiewa and Dunford, 1993).

Tryptophan, the basic precursor of indoleamine neurotransmitter(s) synthesis can act as an $^1O_2$ quencher (Singh et al., 1984) (Fig. 4, left). Products of this reaction are TrypOOH (tryptophan-hydroperoxides A and B). The scavenging of OH by tryptophan also results in TrypOOH formation (Fig. 4, right, A and E) (Singh et al., 1984). Because the ·OH can add to four positions in the benzene ring and two in the hetero-ring a large number of organic hydroperoxides can be formed. However ·OH addition to the benzene ring is required for 5-HT(5-hydroxytryptophan) formation (Fig. 1, left, and Fig. 4, right) (Singh et al., 1984). It must be noted that the semiquinoneimine radical(s), formed by the peroxidatic oxidation of 5-HT are extremely toxic and undergo a rapid polymerization to melanin-like products and ROS (Metodiewa and Dunford, 1993; Perez-Reyes and Mason 1981). The physiological relevance and/or implication of these observations remains to be determined. Recently, the formation of carbon ($Tryp^{\cdot}$) and peroxyl ($TrypO_2^{\cdot}$) radicals and TrypOOH (Type A, Fig. 4, left and Fig. 5) was reported (Giebauß et al., 1996). According to the scheme in Fig. 5, the initiating species might be Cu^+, $TrypO_2^{\cdot}$ or both, being strong prooxidant species. However, almost all effects due to ROS actions *in vivo* depend on transition metals, which are thought to produce radicals via Fenton-type reactions. At any place in the neural cell, where metals are present and to which hydroperoxide(s) can diffuse, radical reactions and chain processes can again be initiated.

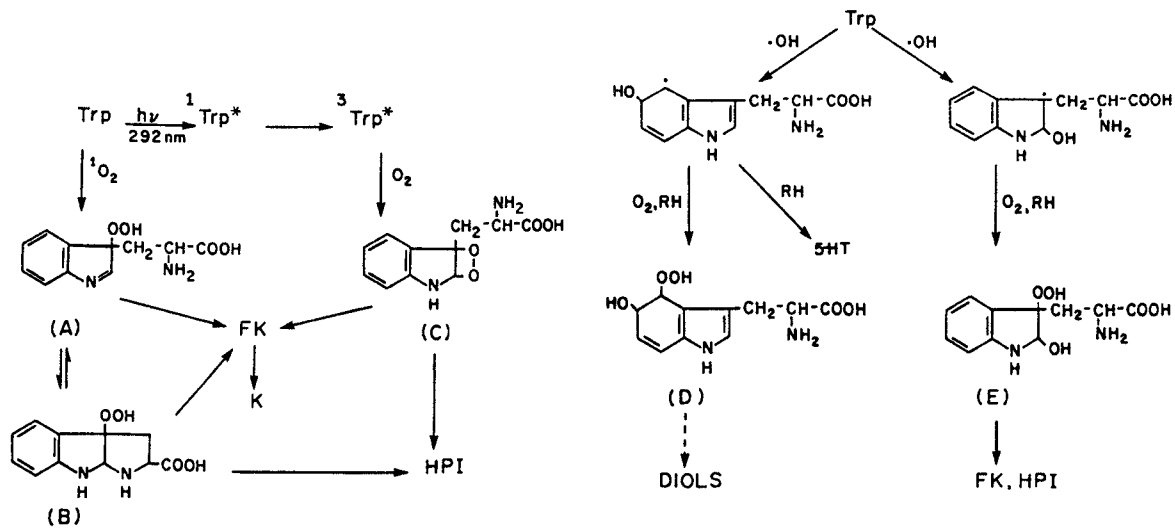

Fig. 4. The reaction of singlet oxygen ($^1O_2$) and OH with tryptophan and tryptophan-hydroperoxide(s) formation (Singh et al., 1984)

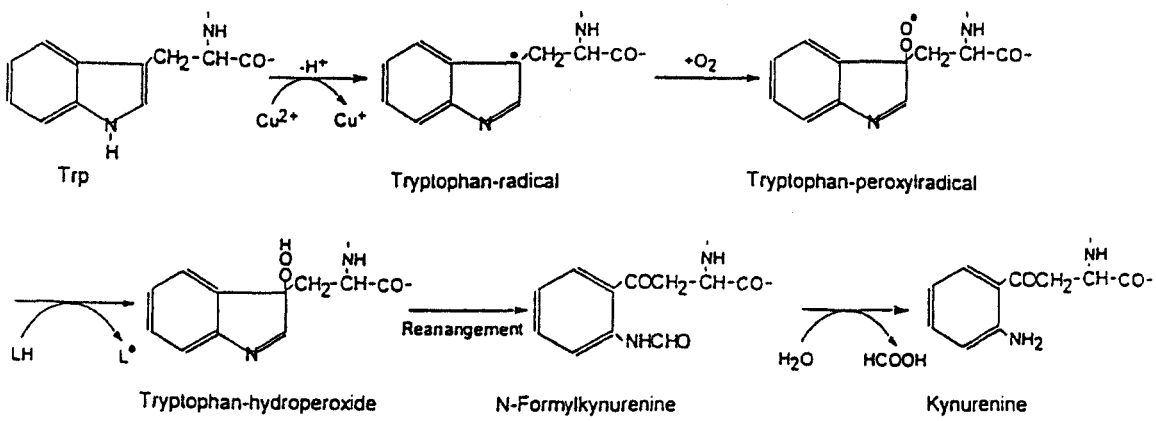

Fig. 5. Tryptophan (carbon) radical (Tryp$^{\bullet}$), tryptophan-peroxyl radical (TrypO$_2^{\bullet}$) and tryptophan-hydroperoxide (TrypOOH) formation in metal-catalyzed one-electron oxidation of tryptophan (Giebauß et al., 1996)

Although the generation and/or scavenging of neurotoxic free radicals and ROS by tyrosine and tryptophan clearly occurs *in vitro* (Metodiewa and Dunford, 1993; Jin et al., 1993; Pichorner et al., 1995; Giebauß et al., 1996; Winterbourn et al., 1997), there is some question about the situation *in vivo*. Cellular injury depends on many factors, including the cellular level of tyrosine and tryptophan, the oxygen tension, cell redox status, and the cellular defense system. The brain level of GSH for preventing dopamine-induced cell death (Offen et al., 1996), the role of PRS (proximal regulatory system) in modulating ROS production and enzyme(s) regulation (Niwa et al., 1996) and the status of repair processes are of vital importance. Intraneuronal activity of NOS (nitric oxide synthase) and RNS (reactive nitrogen species) interactions with ROS are other elements to be taken into account (Van Norby et al., 1997). Exposure of cells to organic hydroperoxide(s) also lead to depletion of ATP (via depletion of NAD^+), deficiency of mitochondrial activity, crucial changes in neuronal metabolism, and loss of cell viability.

At present it is still not possible to give an exact and complete description of all the possible reactions within neural cell(s) simply by projecting data that have been accumulated in model systems. An enormous amount of additional investigation is needed to determine whether tyrosine and tryptophan oxidative degradations produce neurotoxic actions and/or chemically relevant effects in humans.

Acknowledgement

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