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Tyrosinase: a pleiotropic regulator by means of tyrosine level control and melanogenesis

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Biologia. — *Tyrosinase: a pleiotropic regulator by means of tyrosine level control and melanogenesis.* Nota di MICHELE MIRANDA (*) e DARIO BOTTI (*), presentata (**) dal Socio S. RANZI.

RIASSUNTO. — Viene presentato un modello nel quale la tirosinasi occupa un ruolo centrale, come possibile regolatore della catecolaminosintesi, dell'assemblaggio dei microtubuli, della sintesi di tiroxina, della glicolisi e del ciclo dei pentosi fosfati, mediante una auto modulazione basata sul livello di tirosina e sulla produzione di melanina. Sono discusse possibili applicazioni del modello a condizioni normali e patologiche, come la fenilchetonuria, il parkinsonismo, la proliferazione dei melanociti e la rigenerazione della pelle (in condizioni fisiologiche o dopo irradiazione U.V.). Sono anche discusse brevemente le implicazioni sistemiche del modello.

Melanins (eu and phaeomelanins) are widespread animal pigments occurring in many body regions in the different species: skin, brain, eye, ear, kidney, adrenals, leptomeninges, endothelia, liver, hair and feathers [1–4]. During early development many species show melanin throughout the embryo.

Melanogenesis is carried out by the action of tyrosinase (o-monophenol-3, 4-dihydroxyphenylalanine: oxygen oxidoreductase EC 1.14.18.1) on tyrosine or L-3,4-dihydroxyphenylalanine. This enzyme is compartmented in the melanosomes contained in the melanocytes [5–7]. These cells are of neuroectodermic origin and share a common origin with other chromatophores; all the pigment-containing organelles, according to Bagnara *et al.* [8], originate from a common precursor organelle. Melanosomes can be transferred from melanocytes to adjacent cells, i.e. keratinocytes, which are ultimately lost by skin desquamation [9]. *In vivo*, melanosomes are degraded within lysosomes [10–12], but the question has been highly debated due to their strong resistance to chemical attack [13].

Many roles have been attributed to melanins: mimetism and adaptation [14], energy transduction by phonon-electron coupling [15], protection from unwanted metal ions [16], protection from radiation and sink for free radicals [15, 17]. Many pathological conditions are associated with defective melanogenesis or melanin: phenylketonuria [18], parkinsonism [19, 20], otopathies and retinopathies [20] and the De Sanctis–Cacchionne syndrome [20]. Indeed, the intermediates of melanin synthesis have been demonstrated to be highly cytotoxic against proto and eukaryotic cells [20–29].

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Nagatsu *et al.* [19] have demostrated that melanin is an activator of brain and adrenal tyrosine hydroxylase and an inhibitor of liver phenylalanine hydroxylase, which is also inhibited, under some conditions, by tyrosine [30]. In addition, it has been recently found [31] that L-epinephrine activates *Bufo bufo* embryos and Harding-Passey melanoma tyrosinases, which are in activated by L-DOPA oxidation products, and interact with indole, melatonin, tryptamine, while the same substances and tryptophan inhibit mushroom tyrosinase [32].

On the basis of the literature cited we shall discuss here a model according to which tyrosinase, by lowering the tyrosine level at the cellular and possibly at the systemic level [33–35], and producing melanin, may influence several different organism processes. From this point of view an important finding is the tyrosylation of α-tubulin by tyrosyl-tubulin ligase [36, 37].

THE MODEL AND ITS APPLICATIONS

In Fig. 1 a model is presented, according to which tyrosinase reaction is a possible regulator of catecholaminosynthesis, microtubule assembly, thyroxine synthesis, glycolysis and the pentose phosphate cycle, by a self modulation which is based on tyrosine level and melanin production. The model shows that tyrosine is a substrate shared by tyrosinase, tyrosine hydroxylase and tyrosyl-tubulin ligase. The tyrosine pool is mainly replenished by food and tissue proteins and phenylalanine hydroxylase reaction. The block of the latter reaction in the liver results in phenylketonuria, due to a systemic increase of phenylpyruvate and phenylalanine, which are competitive inhibitors of key steps in hexoses metabolism [38]. Phenylalanine also induces phenylalanine hydroxylase [39, 40]. According to other authors hyperphenylalaninemia may affect myelination of the central nervous system and protein synthesis [41]. According to the model also the catecholamine level should be affected, since the lowering of the tyrosine pool reduces substrate availability for tyrosine hydroxylase and tyrosinase reactions. As a consequence both melanin and catecholamines will decrease and, since tyrosine hydroxylase is activated by melanin [19] and tyrosinase is activated by L-epinephrine [31], a vicious circle will be produced, with progressively greater damage to catecholamine-dependent cells. Moreover, Boylen and Quastel [18] found that L-epinephrine production from tyrosine was inhibited *in vitro* by phenylpyruvate and suggested that this may be responsible for the low plasma L-epinephrine concentration of phenylketonurics.

When phenylalanine hydroxylase is not limiting the tyrosine pool is increased by phenylalanine and this will result in a reciprocal activation of tyrosinase by catecholamines and tyrosine hydroxylase by melanin. As a net result the tyrosine pool will be normalized due to the lowering of tyrosine concentration, which can modulate phenylalanine hydroxylase [30].

The model, in the species which have melanized liver, kidneys and adrenals, suggests that the inhibition exerted by melanin on phenylalanine hydro-

xylase [19] may modulate hexoses metabolism by regulating phenylalanine and phenylpyruvate levels; on the other hand a tyrosine pool decrease will lower melanin and catecholamine production, so removing phenylalanine hydroxylase inhibition and promoting the increase of hexose breakdown. Catecholamine concentration will increase, due to the increase of the tyrosine pool, exerting the well-known effect on hexose mobilization from glycogen.

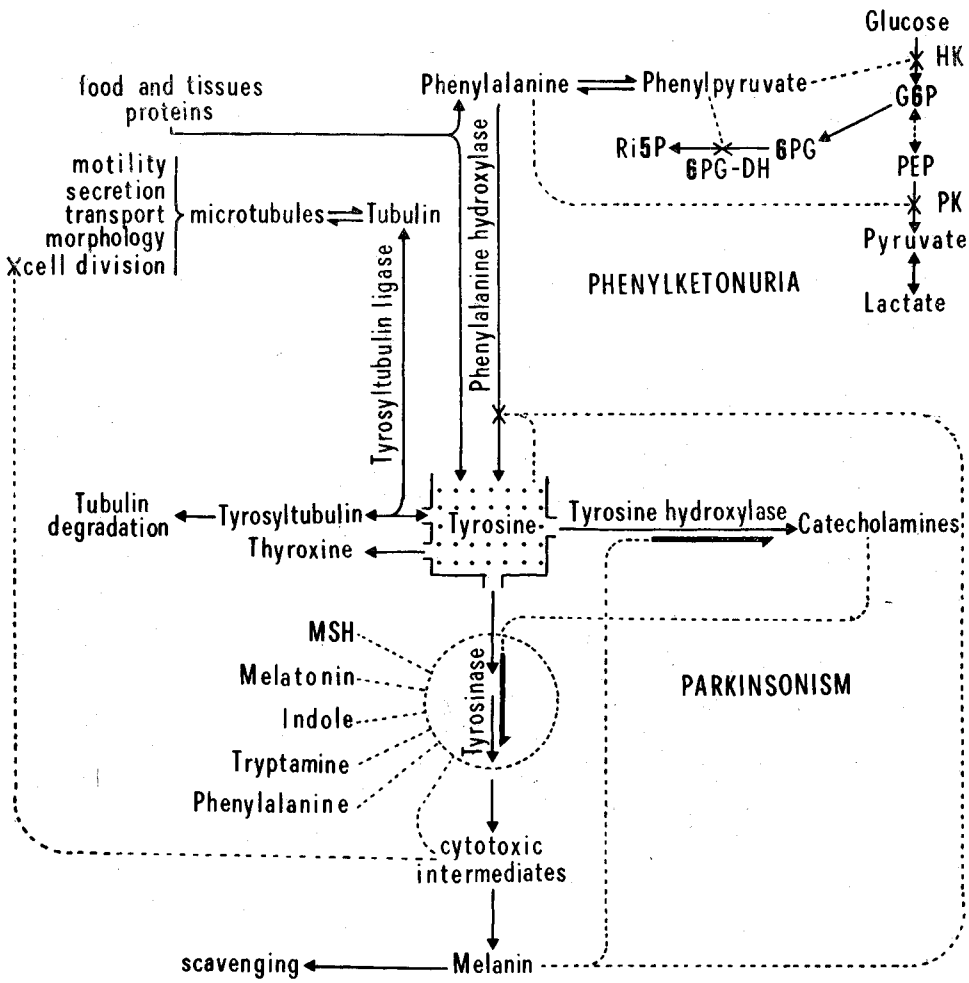

Fig. 1 - Model of the pleiotropic control exerted by tyrosinase on some biological processes. Activations are shown by thick arrows, inhibitions by crosses.

In sequence, higher phenylalanine concentration, higher tyrosine concentration, higher melanin production, higher catecholaminosyntheis and then a higher hexose mobilization to counteract the competitive inhibition by phenylalanine and phenylpyruvate on hexoses metabolism. Thus viewed, also the competitive inhibition of tyrosinase by phenylalanine [42] must be considered as

one of the factors affecting the tyrosine pool. The system in Fig. 1 should respond to an increase of phenylalanine concentration with an increased phenylalanine hydroxylase synthesis [29, 40], except in phenylketonurics, with the inhibition of tyrosinase by phenylalanine [42] and with the increase of tyrosine level, which results in an inhibition of phenylalanine hydroxylase [30] and in a consequently reduced level of tyrosine.

The model can also be applied to parkinsonism, the essential lesion of which is an idiopathic degeneration of nigro-striatal dopaminergic neurons and other pigmented areas in the brain, with a preferential destruction of melanin-containing cells [20]. The cellular toxicity of melanin *per se* has been suggested [20] to be responsible for pathological conditions such as parkinsonism and some age-dependent destruction of pigmented tissues in the eye and middle ear (*stria vascularis*). Also, it has been suggested [20] that the preferential destruction of pigmented cells may be due to a non radiative energy transfer which should produce cytotoxic agents. As reported above, melanin synthesis intermediates are highly cytotoxic, but when they are bound to proteins cytotoxicity is highly reduced [26]. According to Nagatsu *et al.* [19] the main lesion in parkinsonism may be the loss of tyrosine hydroxylase activation by melanin, due to the disappearance of melanin in nigro-striatal neurones, and this, on the other hand, may be dramatized, according to the model, by the reduced activation of tyrosinase by catecholamines. In fact the monoamines, in particular norepinephrine, are central nervous system neurotransmitters or modulators, and the unilateral lesion of the *locus coeruleus*, and the resultant norepinephrine depletion, influences cortical oxidative metabolism *in situ* [43]. In conclusion, according to the model, tyrosinase reaction self regulates via catecholaminosynthesis and melanin synthesis. This mechanism may be regarded as an oscillating regulator.

AN APPLICATION TO MICROTUBULE ASSEMBLY AND SKIN CELL REGENERATION

The recent discovery of tyrosyltubulin ligase, which reversibly tyrosylates the glutamate or glutamine C-terminal of α-tubulin, suggests the possible implication of tyrosine in the regulation of microtubule assembly-disassembly [36, 37]. In fact it has been shown that the maximum of tyrosyltubulin ligase activity during the cell cycle corresponds closely to the maximum of tubulin degradation, suggesting that tyrosylation may be a signal for tubulin degradation [36]. Tubulin assembly-disassembly affects many cellular processes: cell division organelle transfer, cell motility, morphology, secretion, transport [36]. From this point of view, the regulation of tyrosine endocellular and tissue concentration may assume a very important role in the control of microtubule assembly-disassembly; as a consequence the regulation of tyrosinase activity is of paramount importance. An application of the model in Fig. 1 to melanocyte proliferation and skin regeneration (physiological or

post U.V. irradiation) is presented here, based on tyrosinase activation or expression, by MSH or U.V., involving microtubules.

a) It was demonstrated that after skin U.V. irradiation tyrosine concentration in the blood decreases as skin pigmentation increases [33, 34]. It was found that L-DOPA was formed in the skin from tyrosine under U.V. irradiation [35, 44].

b) Tyrosinase activation by U.V. has been recently discussed [45]. Pathak *et al.* [46] suggested that U.V. irradiation promotes melanocyte proliferation and tyrosinase activity increase [47–49]. Morpurgo *et al.* [45] presented a model to explain tyrosinase activation by U.V., based on a U.V. induced expression of MSH receptors in the melanocyte cell membrane, paralleled by tyrosinase autoinduction and cell proliferation.

On the ground of the literature reported above we suggest a possible mechanism by which melanocyte proliferation and skin regeneration may result from U.V. irradiation. According to the authors cited, MSH-sensitive melanocytes (U.V. sensitized or not) express higher tyrosinase levels; tyrosinase, as indicated in Fig. 1, is sensitive to its reaction products, especially indole intermediates of melanin synthesis, which affects the enzyme from Vertebrates as well as mushroom tyrosinase [31, 32]. The activated or inhibited enzyme may be the modified tyrosinase inducer postulated by Morpurgo *et al.* [45]. As a consequence of the increased tyrosinase activity, the melanocyte tyrosine pool is depleted, and tubulin cannot be tyrosylated, so that the equilibrium of tubulin is shifted toward microtubule assembly which may result in cell division and melanosome tranfer to keratinocytes [50]. Moreover, the pumping of tyrosine into melanin might lower the tyrosine pool also in the germinative layer, stimulating, also here, microtubule assembly and mitosis. Obviously microtubule assembly may be influenced according to the model wherever tyrosinase competes with tyrosyltubulin ligase for tyrosine, i.e. nerve cells, hair, feathers, etc.

The widespread melanin content during early development in many animals, i.e. Amphibia, may be correlated with the preservation of the tubulin pool from a tyrosylation stimulated degradation [36], while mitoses are occurring very fast.

Of course an exact knowledge of tyrosine cellular levels and tyrosyltubulin ligase V_{max} and K_m is required to test the feasibility of the model. As reported above, the tyrosine blood level is significantly influenced by U.V. induced melanogenesis and, consequently, transport rate into the cells may be affected.

Possible pleiotropic systemic action of melanogenesis

When the melanogenesis distribution of the various types of cells in the different species is better elucitated, also some possible systemic actions of this process may be tested. For instance skin, liver, and kidney melanogenesis in Amphibia may regulate blood tyrosine level and this may result

in a pleiotropic control of microtubule assembly, catecholaminosynthesis and hexoses metabolism (see Fig. 1). Due to the sensitivity of tyrosinase to L-epinephrine [31], this hormone may contribute to a systemic regulation of tyrosinase activity. Obviously, at the systemic level tyrosine scavenging by the liver transaminative-oxidative pathway must also be considered, as well as cell compartmentation for both tyrosine and L-epinephrine, in the sites of production and release and, finally, in the sites of uptake and accumulation. In fact, we know that tyrosine as well as L-DOPA are specifically transported into melanocytes and in melanomas the transport is increased [21, 23, 27, 29].

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