On the Putative Mechanism of Induction and Regulation of Melanogenesis by L-tyrosine

JEFF HOWE, ROBERT COSTANTINO and ANDRZEJ SLOMINSKI

Department of Microbiology and Immunology, Albany Medical College, Albany and H. W. Toolan Institute for Medical Research, Bennington, USA

The stimulation of melanogenesis by L-tyrosine in hamster melanoma is several-fold higher than that by norepinephrine, epinephrine, clonidine and isoproterenol and absent in the case of tyramine dopamine and phenylephrine. Therefore, the melanogenic effect of L-tyrosine in hamster melanoma follows a different pathway than that linked to the activation of dopaminergic and adrenergic receptors.

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A. Slominski, Department of Microbiology and Immunology, Albany Medical College, 47 New Scotland Avenue, Albany, NY 12208, USA.

L-tyrosine is a precursor for proteins, tyramine, catecholamines, melanin and thyroid hormones (1). In some pigmentary systems, L-tyrosine, besides its function as a precursor to melanin, can also act as an inducer and a regulator of the melanogenic apparatus (2–6). In cultured melanoma cells, L-tyrosine is converted to melanin, and can also be metabolized to catecholamines (5, 7). In addition, it has been reported that activation of adrenergic receptors can stimulate melanogenesis (8). We therefore decided to test whether the regulatory role of L-tyrosine in melanin synthesis is specific for this amino acid, or follows the pathway linked to the activation of dopaminergic or adrenergic receptors. As an experimental model for these studies, we used Bomirski amelanotic hamster melanoma cells, in which L-tyrosine can act as an inducer and regulator of the melanogenic apparatus (2, 6, 9, 10). The effect of L-tyrosine was found to be dose dependent (2), and apparently unrelated to pathways linked to cyclic AMP, cyclic GMP, or $InsP_3$ (11).

MATERIALS AND METHODS

L-tyrosine, tyramine, L-dopa, dopamine, L-phenylephrine, clonidine, (-)isoproterenol, (-)epinephrine, (±)norepinephrine were obtained from Sigma. L-(ring-3,5-^{3}H)-tyro-

Table I. *The melanogenic response to L-tyrosine, biogenic amines and synthetic adrenergic agonists*

Addition to culture medium	Melanin production	Stimulation of tyrosinase activity
L-tyrosine	****	****
Tyramine	–	–
Dopamine	–	–
Norepinephrine	+	+
Epinephrine	–	+
Phenylephrine	–	–
Clonidine	–	(+)
Isoproterenol	–	(+)

Melanin production was judged by the color of cell pellets: white,- ; dark, + ; jet black, ****.
Tyrosinase activity represents tyrosine hydroxylase activity of tyrosinase.

sine (50.0 Ci/mmol, New England Nuclear) was from Du Pont, and Ham's F-10 medium, antibiotics and newborn calf serum were from Gibco.

Monolayer cultures of the AbC1 subline of the Bomirski Ab amelanotic melanoma were grown in low-tyrosine Ham's F-10 medium, containing newborn calf serum (10%), streptomycin (11 μg/ml), and penicillin (100 U/ml) (11). For the experiments, cells were inoculated into 25 cm^2 flasks (5×10^5 cells/flask) in 5 ml Ham's F-10 medium plus 10% newborn calf serum. After 24 h, fresh medium was supplied and the cells were incubated in the presence or absence of 200 μM L-tyrosine, L-phenylephrine, clonidine, isoproterenol, epinephrine, norepinephrine, dopamine, tyramine, for the following 72 h. The medium was changed daily.

Tyrosine hydroxylase activity of tyrosinase was assayed by the Pomerantz method with 0.5 μCi of ^{3}H-L-tyrosine plus 100 μM of unlabelled L-tyrosine and 100 μM L-dopa as a co-factor in 0.1 M sodium phosphate buffer, pH 6.8, at 37°C for 1 h as previously described (9). Tyrosinase activity was expressed in cpm of tritiated water produced during 1 h of incubation per 1 mg protein. The protein content was estimated with the aid of a Bio-Rad protein assay kit. Statistical analysis was done using independent Student's *t*-test.

RESULTS AND DISCUSSION

Table I summarizes the changes in melanin synthesis and tyrosinase activity in hamster amelanotic melanoma cells after addition to the culture medium of L-tyrosine, tyramine, dopamine, norepinephrine, epinephrine, phenylephrine, clonidine or isoproterenol at final concentrations of 200 μM. The inductory effect on melanin synthesis was very strong for L-tyrosine, comparatively low for norepinephrine and absent for tyramine, dopamine, epinephrine, phenylephrine (α_1-adrenergic agonist), clonidine (α_2-adrenergic agonist) and isoproterenol (mixed beta-adrenergic agonist). The stimulatory effect on tyrosinase activity was high for L-tyrosine, comparatively low for norepinephrine, epinephrine, clonidine and isoproterenol, and absent for tyramine, dopamine and phenylephrine (Figs. 1 and 2). The described effect of epinephrine, norepinephrine and isoproterenol is consistent with the results reported by the Rorsman group (8). In several other experiments the effects of norepinephrine and epinephrine on tyrosinase activity were lower than those presented in the Fig. 1, or even absent, in contrast to L-tyrosine which consistently stimulated tyrosinase activity. In addition, we found that the supply of dopamine to

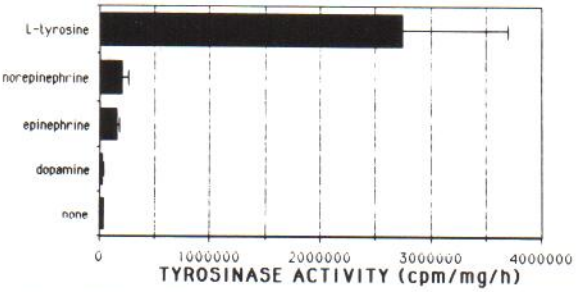

Fig. 1. Effects of L-tyrosine and catecholamines on tyrosinase activity. Tyrosine hydroxylase activity of tyrosinase in cell extracts is expressed as cpm of tritiated water produced during 1 hour of incubation per 1 mg protein. The data represent the mean ± SE from one representative experiment, performed in triplicate. *Ordinate*: addition to the culture medium.

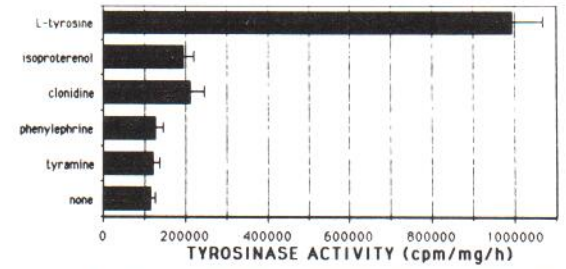

Fig. 2. Effects of L-tyrosine, tyramine and alfa- and beta-adrenergic agonists on tyrosinase activity. Tyrosine hydroxylase activity of tyrosinase in cell extracts is expressed as cpm of tritiated water produced during 1 hour of incubation per 1 mg protein. The data represent the mean of two separate experiments performed in quadriplicate (n=8). *Ordinate*: addition to the culture medium. Differences between tyrosinase activity for control cells versus tyramine- or phenylephrine-treated cells were statistically non-significant ($p > 0.5$), and for control cells versus clonidine- or isoproterenol-treated cells, were statistically significant ($0.025 > p > 0.01$).

the cells cultured in the presence of L-tyrosine inhibits melanin synthesis (not shown), which is consistent with already reported results showing that dopamine can inhibit melanogenesis (12).

In previous studies we obtained evidence that L-tyrosine and the product of its hydroxylation, L-dopa, can induce and regulate the melanogenic apparatus in hamster melanoma cells via distinct, but overlapping pathways (2, 9, 10). L-tyrosine is a precursor of several biogenic amines. It can be metabolized via decarboxylation to tyramine or via hydroxylation to dopa and by further enzymatic reactions including side-chain decarboxylation, side-chain hydroxylation, and N-methylation to dopamine, norepinephrine and epinephrine, respectively (1). The last two catecholamines act as physiological agonists for adrenergic receptors, which have recently been proposed to be involved in the positive regulation of melanogenesis (12).

This raises the question whether the induction and regulation of melanogenesis by L-tyrosine is caused by its metabolic products tyramine and catecholamines and/or can be mediated by a direct activation of adrenergic receptors by L-tyrosine. The results presented above – in addition to those reported previously which showed no or a little effect of D-isomers of tyrosine and dopa, L-phenylalanine, L-tryptophan and L-valine on melanogenesis (9) – provide evidence that the induction and regulation of melanogenesis by L-tyrosine is specific for this amino acid and follows a different pathway than that linked to the activation of dopaminergic and adrenergic receptors.

Recently, we proposed that the in vivo regulation of the melanogenic apparatus by L-tyrosine and L-dopa may be mediated via specific receptors for those two bioregulatory molecules (13). Following this line of thought, one could speculate that norepinephrine, epinephrine, isoproterenol and clonidine may act as partial agonists of putative L-tyrosine and -dopa receptors. This could explain their low but significant stimulatory effect on melanogenesis.

In conclusion, we provide evidence that in one well-characterized rodent pigment cell line the induction and stimulation of melanogenesis by L-tyrosine follows a specific pathway for this amino acid that is not linked to the conversion of L-tyrosine to tyramine and catecholamines, nor the activation of alfa- or beta-adrenergic receptors.

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