Stimulation of Tyrosinase by Dihydroxy Phenyl Derivatives

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Isoprenaline (0.3 mM) moderately increased the tyrosinase activity of cultured human melanoma cells, while another β-adrenoceptor agonist, terbutaline (1–3 mM) and a catechol compound, dopac (0.1–0.3 mM) induced a several fold increase in the enzyme activity. Isoprenaline (0.3–1 mM) and dopac (0.3–1 mM) also exerted pronounced toxic effects on the cells. The data suggest: 1) a possible role for β-adrenoceptors in the regulation of human melanogenesis; 2) two different ways of action for isoprenaline in inducing tyrosinase elevation; 3) the possible usefulness of dopac as a chemotherapeutic agent. ***Key words: Melanoma cells; β-adrenoceptor agonists; Isoprenaline; Terbutaline; Dopac; Theophylline***

(Accepted May 22, 1989.)

Acta Derm Venereol (Stockh) 1989; 69: 521–524.

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The tyrosinase activity of the melanocytes can be increased by several substances (e.g. cholera toxin, prostaglandin E_1, theophylline, MSH) which raise the intracellular levels of cAMP. β-Adrenoceptors also operate through cAMP-dependent mechanisms, and indeed, pigment cells in lower vertebrates are known to be controlled by both β- and α-adrenoceptor agonists (1). Recently, murine hair follicular melanocytes have been reported to possess β-adrenoceptors, (2), while the iridal melanocytes of rabbits were proved to be under α-adrenergic control (3).

In a previous experiment we observed a 4–7-fold increase in the adenylate cyclase activity in homogenates of human melanoma cells after treatment with isoprenaline (4). The present data demonstrate the effect of this drug on the tyrosinase activity of the human melanoma cell line IGR 1. We also investigated the effect of another dihydroxy phenylamine, terbutaline, that has β-adrenoceptor stimulating properties without being a catechol. Furthermore, since the tyrosinase activity of amelanotic Bomirski hamster melanoma cells has been reported to be increased by L-dopa (5), dopac, a catechol compound without β-adrenergic effects was also tested. Theophylline was used as the control substance of our model system.

MATERIAL AND METHODS

Terbutaline sulfate was kindly supplied by Draco Co. (Lund, Sweden), (–)-isoprenaline HCl was purchased from Sigma Chemical Co. (St. Louis, Mo., USA). Dopac and theophylline were obtained from Labkemi AB (Göteborg, Sweden) and Serva Feinbiochemica (Heidelberg, West Germany), respectively.

The test substances were added to the melanoma cell cultures 2 days after replating. Tyrosinase was extracted from the cells after a 48-h treatment with the drugs by adding 3 ml 0.2% Triton-X in 0.5 M phosphate buffer, pH 7.4. Determinations of the tyrosinase activity (6) were performed in aliquots quantifying the amount of 5-S-L-cysteinyl-L-dopa specifically produced when 2 mM D, L-dopa was incubated with 3 mM L-cysteine in 0.5 M phosphate buffer, pH 7.4, maintained at 37°C and gently areated. The contents of 5-S-L-cysteinyl-L-dopa were determined by HPLC with electrochemical detection. The quantity of the formed 5-S-L-cysteinyl-D-dopa gave information on the non-specific dopa oxidation. Protein was analysed *ad modum* Lowry et al. (7), the vitality of the cells was determined by the trypan blue exclusion test.

RESULTS AND DISCUSSION

The tyrosinase activity of the untreated cells was 13.0 ± 3.4 nmol/mg protein·min (mean ±S.D. of all the control flasks). This is lower than the value (39 ± 7.1 nmol/mg protein·min) found in the same melanoma cell line in a previous experiment (8). However, the number of seeded cells (0.75×10^6 versus $1.5 \cdot 10^6$ cell/flask) also differed on the two occasions. The basal tyrosinase activity in the Cloudman S91 melanoma cells has also been reported to be dependent on the density of cells in the culture (9). The proportion of dead cells among the adhered cells was about the same (7–10%) in both the control and test flasks.

The phosphodiesterase inhibitor theophylline (0.3–3 mM) induced a 7-fold elevation in the tyrosinase activity (Fig. 1). After a 48-h treatment with 0.3, 1 and 3 mM concentrations the protein values were

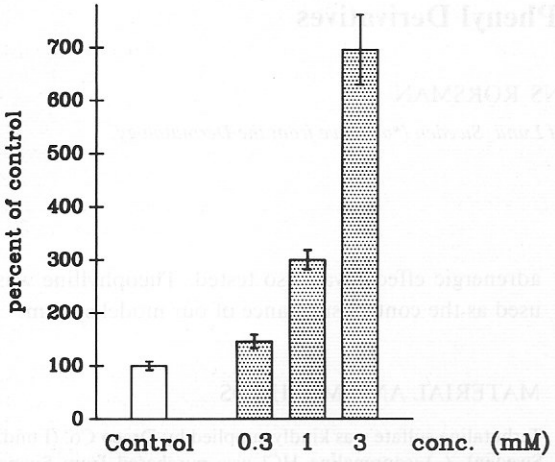

Fig. 1. Effect of theophylline on the tyrosinase activity of the melanoma cells. Values are expressed as a percentage of the controls (mean ± S.D. of $n = 12$, 9, 12 and 9 samples in order of the columns).

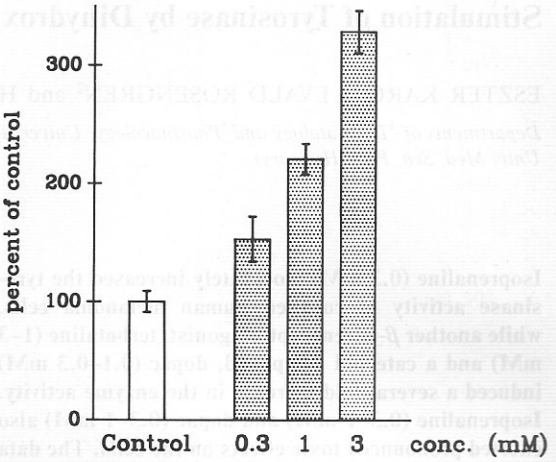

Fig. 3. Effect of terbutaline on the tyrosinase activity of the melanoma cells. Values are expressed as a percentage of the controls (mean ± S.D. of $n = 10$, 10, 10, and 8 samples in order of the columns).

98 ± 3%, 84 ± 4% and 72 ± 4% of the controls, respectively. Both the induction of melanogenesis and inhibition of cell proliferation are probably cAMP related effects of the substance (10), though other mechanisms have also been suggested (11).

Isoprenaline (0.3 mM) induced a moderate rise in the enzyme activity to 129 ± 13% of the controls (Fig. 2). An increase in the tyrosinase level induced by cAMP elevating agents can be detected only after 9–12 h of continuous stimulation (12). Catechols are readily oxidizable compounds and the moderate effect of isoprenaline may most probably be ascribed to this phenomenon. Furthermore, the oxidation of the catechols produces semiquinones and quinones toxic to the cells. Indeed, in a pilot study we have found the 1 mM concentration of isoprenaline to kill all the cells in the cultures. Pronounced cell loss (to 46 ± 9% of the controls), as indicated by the protein value, could be observed even with 0.3 mM concentration. By further reduction of the dose (0.1 mM) this toxic effect disappeared (the protein value was 96 ± 3%).

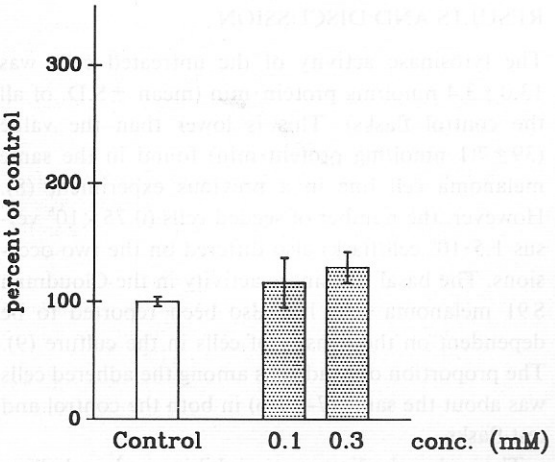

Fig. 2. Effect of isoprenaline on the tyrosinase activity of the melanoma cells. Values are expressed as a percentage of the controls (mean ± S.D. of $n = 10$, 9 and 5 samples in order of the columns).

The resorcinol compound terbutaline is more stable to oxidation than are catechols, and indeed, this substance (0.3, 1, 3 mM) increased the tyrosinase activity up to about 3-fold of the control value in a dose-dependent manner (Fig. 3). The protein values were 96 ± 2%, 98 ± 3% and 92 ± 3%, respectively.

The stimulating effect of the two β-receptor agonist compounds, isoprenaline and terbutaline constitutes further evidence of the possible role of β-receptors in the regulation of human melanogenesis. However, the effect of isoprenaline on tyrosinase may be mediated through some other type of mechanisms, too, since the other catechol compound studied, dopac (0.1, 0.3 mM) also induced a pronounced and dose-dependent increase in the tyrosinase activity (Fig. 4). According to Slominski et al. (13) L-dopa has little or no effect on levels of free intracellular cyclic nucleotides in the Bomirski amelanotic melanoma system. This may also be the case for dopac though the action mechan-

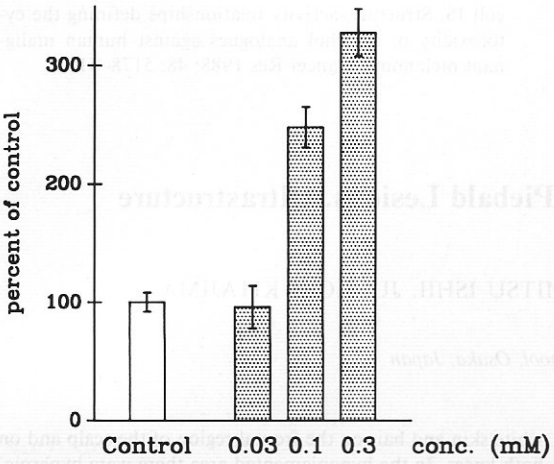

Fig. 4. Effect of L-dopac on the tyrosinase activity of the melanoma cells. Values are expressed as a percentage of the controls (mean ± S.D. of $n = 10$ samples).

ism of this latter substance is still to be elucidated. Besides stimulating the tyrosinase activity, dopac also exerted toxic effects on the human melanoma cells. In a pilot study 1 mM concentration of the drug killed about 90% of the cells. In the present experiments the amount of adhered cells according to the protein values was 97 ± 3%, 108 ± 4% and 58 ± 7% of the controls after the treatment at 0.03, 0.1 and 0.3 mM concentrations.

Previously, Wick (14) showed that catecholic compounds related to dopa and dopamine possess significant antitumour activities against melanoma cells, both in vitro and in vivo. Catechols also have systemic toxicity that may be due to their auto-oxidation. Since tyrosinase is unique to the melanocyte, a rational approach to develop anticancer drugs for melanomas is to apply catechol analogues that are activated by this enzyme. And indeed, several catechol compounds have been shown to be effective antitumour agents, probably with fewer side effects than dopa and dopamine (15). Since the cytotoxic effect of these agents is dependent upon their intracellular activation by tyrosinase, tumour cells with low melanogenic activity are less susceptible to them.

Dopac, which, in the presence of L-cysteine, can be converted to 5-S-L-cysteinyl-dopac by tyrosinase and subsequently be co-polymerized, stimulates its own intracellular activation by elevating the tyrosinase level. Thus it may also prove to be toxic to melanoma cells with originally low melanogenic activity.

ACKNOWLEDGEMENTS

This investigation was supported by grants from the Swedish Cancer Society (project 626-B89-17XB), the Swedish Medical Research Council, the Walter, Ellen and Lennart Hesselman Foundation for Scientific Research, the Edvard Welander Foundation for Scientific Research, the Thure Carlsson Foundation for Scientific Research, The Crafoord Foundation for Scientific Research, the donation funds of the University Hospital at Lund, and the donation funds of the Faculty of Medicine, University of Lund.

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Acquired Pigmented Macules in Human Piebald Lesions. Ultrastructure of Melanocytes in Hypomelanotic Skin

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Two cases of piebaldism are reported. The first patient was a 9-month-old girl with inborn hypopigmented areas on the frontal region of the scalp and both knees. There were no melanocytes in the lesions. In the second case, we observed the patient from 2 months of age for a period of 9 years. Many hyperpigmented spots appeared on the hypomelanotic areas on the frontal region of the scalp, abdomen and both knees. Electronmicroscopic examinations of the hypomelanotic skin disclosed an area with regularly distributed melanocytes as well as an area with no melanocytes. Most of the melanosomes were ellipsoidal and lamellar. They were in stage II to III, which signified delayed pigmentation. Hyperpigmented spots were slightly enlarged following PUVA treatment.

(Accepted April 10, 1989.)

Acta Derm Venereol (Stockh) 1989; 69: 524–527.

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Piebaldism is a rare, autosomal dominant inherited disorder, characterized by inborn hypopigmented skin and hair. The lesions are usually found on the forehead, ventral aspect of the trunk, and the extremities. Accepted knowledge of this disease is that white macules and hair do not change throughout the life time and that there are no melanocytes in the affected skin (1). Herein, we report 2 cases of piebaldism, in one of which we found the spontaneous appearance of hyperpigmented spots and the presence of well-distributed melanocytes in piebald skin.

CASE REPORTS

Case 1

A 9-month-old girl was brought to the dermatological department of Osaka City University Hospital with a problem of white skin and hair on the frontal region of the scalp and on both knees. In the hypopigmented area there were hyperpigmented spots. No macroscopic changes of the hypomelanotic skin were seen during next 3 years. An electronmicroscopic study failed to detect any melanocytes in the hypomelanotic skin.

Case 2

A full-term female baby was noted at birth to have depigmented lesions on the forehead, abdomen and knees. Six months later, in the absence of therapy, pigmented macules appeared on the knees (Fig. 1). Over the next few years, the pigmented macules gradually increased in size and number (Fig. 2). Light brownish macules also appeared on the depigmented lesions on the abdomen and forehead.

An electronmicroscopic study of hypopigmented skin in the left knee disclosed regularly distributed melanocytes (seven melanocytes out of 50 basal keratinocytes). Most of the melanosomes were in stage II to III (Fig. 3), and a small number of stage IV melanosomes were recognized in some of the dendrites. They were ellipsoidal in shape and had regular lamellar structures, but a few were irregular in shape and had irregular melanization. Several melanosomes were found disintegrating in a limiting membrane in one of the dendrites

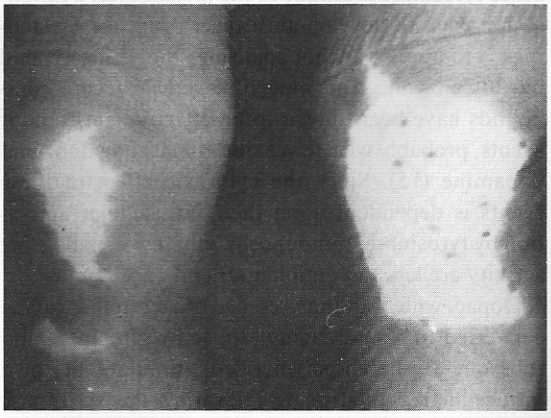

Fig. 1. Case 2 (6 months old). Pigmented macules appeared on the leukoderma, there being no such spots at birth.