Prostaglandin D₂ production in FM55 melanoma cells is regulated by α-melanocyte-stimulating hormone and is not related to melanin production

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Abstract: This study shows that prostaglandins in human FM55 melanoma cells and epidermal melanocytes are produced by COX-1. Prostaglandin production in FM55 melanoma cells was unrelated to that of melanin suggesting that the two processes can occur independently. a-Melanocyte-stimulating hormone, which had no effect on melanin production in FM55 cells, stimulated PGD₂ production in these cells without affecting PGE₂. While cAMP pathways may be involved in regulating PGD₂ production, our results suggest that a-MSH acts independently of cAMP, possibly by regulating the activity of lipocalin-type PGD synthase. This a-MSH-mediated effect may be associated with its role as an immune modulator.

Key words: α-melanocyte-stimulating hormone – liquid chromatography electrospray tandem mass spectrometry – melanogenesis – pigment cells – prostaglandin D₂

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Background

Melanocytes produce melanin and have a role in skin pigmentation (1,2). Cutaneous prostaglandins, such as PGE₂ and PGF₂α, may act as mediators in this process because they increase melanocyte dendricity and melanogenesis (3,4). It has been suggested that these prostaglandins arise from keratinocytes (4) but it is possible that they are also produced by melanocytes acting as autocrine factors in the pigmentedary response.

However, prostaglandins have a wide range of biological activities, and while some such as PGE₂ act as pro-inflammatory mediators (5), others such as PGD₂ down-regulate immune responses (6). PGD₂ is formed by prostaglandin D synthase (EC 5.3.99.2) (PGDS), an isoform of which, lipocalin-PGDS (L-PGDS), is expressed in pigment cells (7). Expression of L-PGDS is dependent upon microphthalmia-associated transcription factor (MITF) (7) which is activated via cAMP and is involved in regulating melanogenesis (1,8,9). Thus, it is possible that PGD₂ production is associated with melanogenesis, and the two processes have a common regulatory pathway. α-Melanocyte-stimulating hormone (α-MSH) regulates melanogenesis via the cAMP-coupled melanocortin 1 receptor (MC1R) expressed on melanocytes (10–12). This peptide is also a potent immunomodulator through its effects on MC1R expressing immune cells such as monocytes and macrophages (13). Because melanocytes are immunocompetent (14–16), they might also mediate immunomodulatory actions of α-MSH. Their production of prostaglandins and, specifically, PGD₂ could therefore be associated with this function.

Questions addressed

Is the production of prostaglandins related to that of melanin in pigment-producing cells, and is it regulated by α-MSH?

Experimental design

Prostaglandins were measured in melanin-producing FM55 human melanoma cells (17) and in human epidermal melanocytes. FM55 cells were used as a model system to
examine the relationship between prostaglandin and melanin production and the effect of α-MSH. Because their MC1R does not couple to cAMP FM55 cells do not produce melanin in response to α-MSH (18). Their use therefore allowed the possibility of dissociating prostaglandin production from that of melanin.

The lightly pigmented FM55 cells were established from metastatic melanoma nodules (Dr AF Kirkyn, Danish Cancer Society, Copenhagen, Denmark). Human epidermal melanocytes were isolated from skin samples obtained with local ethics committee approval and informed consent from donors undergoing elective plastic surgery. Cell culture (19,20), eicosanoid analysis (21), stimulation and measurement of melanin (20,22) and COX-1/-2 protein expression (5) were performed as published; L-PGDS was measured using an immunometric kit (Appendix S1).

Results

PGD2 and PGE2 were the major prostaglandins identified in human epidermal melanocytes and FM55 melanoma cells (Fig. 1a, b). Lipidomic analysis did not confirm production of PGF2α by FM55, as previously reported using a less specific radiometric approach (17). Western blotting analysis revealed that FM55 cells and melanocytes expressed the constitutive isoform of cyclooxygenase (COX-1) but not the inducible isoform COX-2 (Fig. 1c, d).

Increasing melanin production in FM55 had no effect on prostaglandin production (Fig. 2a, b); when prostaglandins were stimulated with arachidonic acid, melanin production was not affected (23 ± 6 and 24 ± 4 µg melanin/mg cell protein, before and after treatment, respectively).

α-MSH had no effect on PGE2 production in FM55 cells but increased PGD2 and PGD1 with no effect on melanin (Fig. 2c). 3-Isobutyl-1-methylxanthine (IBMX), which raises cAMP production may be via a cAMP independent pathway as indicated by the dotted line. AA, arachidonic acid; PGH2, prostaglandin H2; MC1R, melanocortin 1 receptor; MITF, microphthalmia-associated transcription factor.

**Figure 2. Prostaglandin (PG) and melanin production in FM55 human melanoma cells. The effect of NH4Cl (10 mM) and l-tyrosine (400 µM) on (a) melanogenesis and (b) prostaglandin production. (c) The effect of α-Melanocyte-stimulating hormone (α-MSH) (10–8 M) and IBMX (10–4 M) on cell number, levels of melanin, PGD1, PGD2 and PGE2, following 48-h treatment. Dose-dependent effect of α-MSH (10–10–10–8 M) on (d) PGD1 and PGD2 production and (e) lipocalin-prostaglandin D synthase expression. Data expressed as mean ± SEM of n = 3 independent experiments. *P < 0.05, **P < 0.01 and ***P < 0.001 comparing data to control (CTR). (f) Schematic outline of the major signalling pathways involved in melanin and PGD2 production. In FM55 cells, the MC1R does not couple to cAMP as indicated by the cross. As a consequence, α-MSH fails to stimulate melanin production, and the regulation of PGD2 production may be via a cAMP independent pathway as indicated by the dotted line. AA, arachidonic acid; PGH2, prostaglandin H2; L-PGDS, lipocalin-prostaglandin D synthase; COX-1/-2, cyclooxygenase 1/-2; MITF, microphthalmia-associated transcription factor.

**Table 1.** Dose–response of α-MSH to melanin and PGD2 production in FM55 human melanoma cells. The effect of α-MSH (10–8 M) on cell number, levels of melanin, PGD1, PGD2 and PGE2, following 48-h treatment. Dose-dependent effect of α-MSH (10–10–10–8 M) on (d) PGD1 and PGD2 production and (e) lipocalin-prostaglandin D synthase expression. Data expressed as mean ± SEM of n = 3 independent experiments. *P < 0.05, **P < 0.01 and ***P < 0.001 comparing data to control (CTR). (f) Schematic outline of the major signalling pathways involved in melanin and PGD2 production. In FM55 cells, the MC1R does not couple to cAMP as indicated by the cross. As a consequence, α-MSH fails to stimulate melanin production, and the regulation of PGD2 production may be via a cAMP independent pathway as indicated by the dotted line. AA, arachidonic acid; PGH2, prostaglandin H2; L-PGDS, lipocalin-prostaglandin D synthase; COX-1/-2, cyclooxygenase 1/-2; MITF, microphthalmia-associated transcription factor.

| α-MSH (log M) | Melanin (µg/mg protein) | PGD1 (pg/mg protein) | PGD2 (pg/mg protein) |
|--------------|------------------------|----------------------|----------------------|
| 0            | 13.7 ± 1.2             | 27 ± 5               | 10 ± 11              |
| 10 M         | 170 ± 36               | 307 ± 54             | 80 ± 25              |
| 100 M        | 9.5 ± 2.2              | 39 ± 41              | 109 ± 75             |

**Figure 1.** Sample profile of prostaglandins (PG) produced by human epidermal melanocytes F39 (a) and human melanoma FM55 (b) under resting conditions (Control) and following treatment with arachidonic acid (AA) (10 µM for 24 h). Expression of COX-1 and COX-2 proteins in human epidermal melanocytes F39 (c) and human melanoma FM55 cells (d) assessed by Western blotting analysis. MW: molecular weight markers; Lane 1: COX-1; Lane 1: COX-1+ COX-1 blocking peptide; Lane 3: COX-2; Lane 4: COX-2-positive control using FM3 hamster melanoma cells. Note: each antibody (i.e. COX-1 and COX-2) was independently carried on its own lane of the same gel. Data shown as mean ± SEM of n = 3 independent experiments. *P < 0.05 and **P < 0.005, comparing data to control.
Our findings indicate that a comitant stimulation on PGD1 but lack of effect on PGE2, the level of L-PGDS (Fig. 2f). This is supported by the concomitant stimulation on melanin (10) and NO (15). As with many of the properties of pigment-producing cells, a monophasic response curve similar to that observed for melanin (10) and NO (15). With many of its actions, it seems that α-MSH is a modulator rather than an outright stimulator.

PGD2 is a major prostaglandin in both epidermal melanocytes and FM55 cells. α-MSH modulated the production of PGD2 in a concentration-dependent manner in FM55, producing a bell-shaped dose response curve similar to that observed for melanin (10) and NO (15). With many of its actions, it seems that α-MSH is a modulator rather than an outright stimulator.

α-MSH may act specifically to regulate PGD2 synthesis at the level of L-PGDS (Fig. 2f). This is supported by the concomitant stimulation on PGD1 but lack of effect on PGE2, indicating that α-MSH is not acting at the level of COX. Our findings indicate that α-MSH may affect the activity, but not expression of L-PGDS. Expression of L-PGDS is upregulated by MITF (7), which is under the control of the cAMP signalling pathway (23). This would explain the increase in PGD2 production observed in response to IBMX-dependent increased cAMP. It is unlikely that α-MSH acts in this way because the MCIR on FM55 cells does not couple to cAMP (18). We therefore propose that α-MSH acts independently of cAMP and activates L-PGDS rather than inducing its expression. Further studies using human epidermal melanocytes and melanoma cells with different degrees of pigmentation are needed to elucidate this effect of α-MSH and determine whether it is a common property of pigment-producing cells.

PGD2 can inhibit growth of human melanoma cells (24) and loss of L-PGDS expression may be important in allowing the tumor to avoid immune surveillance (25). The fact that PGD2 is a product of immune cells, such as Langerhans cells, mast cells and macrophages (26) emphasizes its importance as an immunomodulator. Melanocytes are another potential source of cutaneous PGD2; this together with the regulation of PGD2 production by α-MSH underlines their importance as mediators of immune responses in the skin.

Conclusion

It has been suggested that prostaglandins have a role in the pigmentary response (3,4). However, we found no such association between prostaglandin and melanin production in FM55 melanoma cells. This dissociation was further demonstrated in experiments with α-MSH. Although this peptide is melanogenic in human melanocytes via the MCIR (10,11), it fails to have this effect in FM55 cells (18), as confirmed here, yet it increased prostaglandin production. Thus, it would seem that in FM55 cells prostaglandins are produced as part of some non-pigmentary function.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Materials and methods.

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