FEBS Letters 455 (1999) 364–366 FEBS 22339

Metabolism of progesterone to DOC, corticosterone and 18OHDOC in cultured human melanoma cells

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Received 11 May 1999

Abstract We are now showing that cultured human melanoma cells can synthesize steroids such as corticosterone from progesterone or deoxycorticosterone. Corticosterone production is strongly responsive to deoxycorticosterone substrate addition (12-fold increase), but unresponsive to the adrenal stimulating factors ACTH and angiotensin II. This is the first demonstration that skin cells (malignant melanocytes) have the capability to synthesize 11-deoxycorticosterone, corticosterone, and 18-hydroxydeoxycorticosterone.

Key words: Steroid; Melanoma cell; Corticosterone; 11-Deoxycorticosterone; Aldosterone; 18-Hydroxydeoxycorticosterone; Progesterone

1. Introduction

The adrenal gland is the main site of mineralo- and glucocorticosteroid production [1]. The gonads represent additional classical endocrine organs that can produce corticosteroids, but in smaller quantities [1]. However, recent evidence has shown that corticosteroids can also be produced at extraendocrine sites [2–4]. Production of corticosterone and aldosterone has been already documented in the brain [2] and vascular system [3], while production of 11-deoxycortisol and 11-deoxycorticosterone was demonstrated in lymphocytes [4].

In the adrenal gland, the most important regulator of glucocorticoid biosynthesis and release to the systemic circulation is pituitary adrenocorticotropin (ACTH) that derives from the precursor proopiomelanocortin (POMC) protein, after stimulation by corticotropin releasing hormone (CRH) [1]. Mineralocorticoid production is, in contrast, controlled by the renin-angiotensin system through its effector angiotensin II (A-II) [1]. Skin, the largest body organ, also has the capability to produce CRH and POMC derived peptides, perhaps as part of a local defense against stressful stimuli [5–7]. Moreover, we reported that human skin cells express the ACTH receptor gene (MC2), and produce mRNAs for three obligatory enzymes of steroid synthesis, the cytochromes P450scc (CYP11A1), P450c17 (CYP17) and P450c21 (CYP21A2)[8]. This raised the question on whether selected corticosteroids could be produced locally in the skin (cf. 6, 8). Therefore, we investigated if a human melanoma line, shown to express CRH, POMC, CYP17 and CYP21A2 genes, could metabolize progesterone and deoxycorticosterone substrates to biologically active corticosteroids.

2. Materials and methods

2.1. Materials

^{14}C-progesterone (^{14}C-PROG), ^{3}H-progesterone (^{3}H-PROG) and ^{3}H-deoxycorticosterone (^{3}H-DOC) were from NEN life Sciences (Boston, MA, USA). ACTH-1–24 was purchased from Organon (West Orange, NJ, USA). (Sar^{1})angiotensin II (A-II), progesterone (PROG), deoxycorticosterone (DOC), corticosterone, 18-hydroxydeoxycorticosterone (18OHDOC) and aldosterone (Aldo) were from Sigma Chemical Company (St. Louis, MO, USA). Ham F-10, fetal bovine serum, Hank's balanced salt solution (HBSS), antibiotic antimycotic mixture were purchased from GIBCO (Grand Island, NY, USA).

2.2. Cell culture

Semi-confluent cultures of the human melanoma SK-MEL188 cells were maintained serum at 37°C in the presence of 5% CO_2 in Ham F-10 medium with added antibiotics and 10% fetal bovine, as described previously [9,10]. Media were changed every 48 h.

For experimental treatment, melanoma cells were plated in 6-well dishes, grown near confluence and the medium was replaced by Ham's F-10 without serum. The cells were then incubated for 24 h in serum free medium containing 1 μM DOC, in the presence or absence of A-II (10^{-7} M) or ACTH-1–24 (10^{-8} M). The media were collected and assayed for aldosterone and corticosterone using an ELISA as described previously [3,11].

2.3. Identification of radioactive metabolites

Labeling with radioactive steroid substrates and identification of metabolites followed the methodology described previously [3,11]. Briefly, mixtures of ^{14}C-PROG (1 μCi), ^{3}H-PROG (25 μCi) or ^{3}H-DOC (25 μCi), with unlabeled PROG or DOC, respectively, to the final concentration of 1 μM, were added to the culture medium. After 24 h, media were collected, extracted using a C18 Bond Elut solid phase extraction column (Varian Corporation, Palo Alto, CA, USA). Steroids were eluted with ethyl acetate and evaporated in siliconized tubes to which the unlabeled standards dissolved in ethanol were added; in the experiments of incubation with ^{3}H-PROG, unlabeled steroids were not added and the extract was used directly for RP-HPLC. The steroid mixtures were spotted onto a Whatman LK5F TLC plate and developed in chloroform/methanol/water at ratios 100/5/0.1 for DOC and 100/3/0.1 for PROG. The radioactive products from the progesterone incubation were visualized by autoradiography using Kodak LE intensifying screen after 3 weeks exposure at −70°C. The unlabeled standards were visualized under UV light.

For reversed phase high-performance liquid chromatography (RP-HPLC) the radioactive products from the tritiated precursors that migrated in the TLC at the positions corresponding to Aldo, DOC, corticosterone and 18OHDOC standards were eluted with isopropanol [3,11]. The eluted fractions were evaporated and suspended in methanol water and subjected to RP-HPLC separation on Hypersil column (250×4.6 mm; 5 μm particle size; Alltech, Deerfield, IL, USA). Separation was performed using a 49–56% methanol elution gradient over 75 min and fractions were collected every minute and counted. The measured radioactivity was then matched to the UV

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PII: S0014-5793(99)00889-3

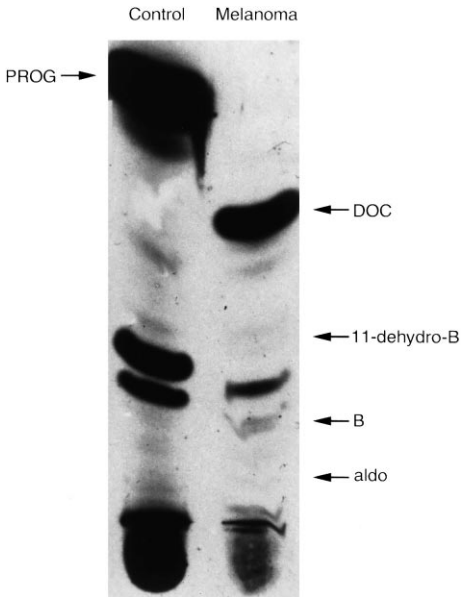

Fig. 1. TLC separation of the products of ^{14}C-progesterone metabolism by melanoma cells after 24 h of incubation. PROG, progesterone; DOC, 11-deoxycorticosterone; B, corticosterone; Aldo, aldosterone; 11-dehydro-B, 11-deoxydrocorticosterone.

peaks of unlabeled standards. In the case of incubation with ^{3}H-PROG (1 μM), the fractions were also assayed for corticosterone using an ELISA as detailed previously [3,11].

3. Results and discussion

Our previous molecular analyses documented that the SK-Mel188 melanoma cells express CYP17 and CYP21A2 genes [8]. Specifically, the predicted 158 bp product from exon 1 of CYP17, and the 306 bp product corresponding to the spliced mRNA fragment from the exons 9 and 10 of CYP21A2 were detected by RT-PCR followed by Southern blot hybridization to the oligonucleotide probe containing the sequence in the amplified fragment [8]. To test whether this expression is accompanied by the actual synthesis of corticosteroids, we initially incubated melanoma cells with ^{14}C-PROG. The autoradiogram from the cell culture incubated with ^{14}C-PROG showed rapid substrate metabolism, that resulted in complete disappearance from the culture media by 24 h (Fig. 1). The autoradiography of the TLC separated products also showed several radioactive products; the predominant steroids migrated at the same rate as the deoxycorticosterone (DOC) and corticosterone (B) standards (Fig. 1). Aldosterone production was below the level of detectability under tested conditions.

To characterize further this process we used ^{3}H-DOC as the substrate (Figs. 2 and 3). The fractions eluted from the TLC plates were separated by RP-HPLC. Radioactive peaks with

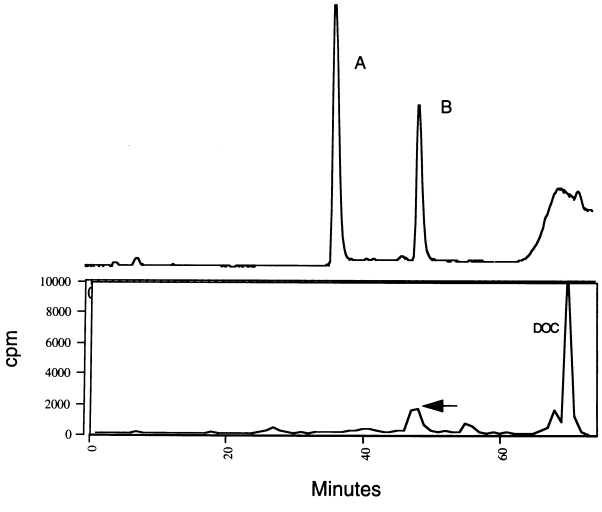

Fig. 2. RP-HPLC identification of ^{3}H-corticosterone as metabolite of ^{3}H-DOC. Upper panel: UV detection of 11-dehydrocorticosterone (A) and corticosterone (B) standards. Lower panel: Radioactivity in eluted fractions with arrow indicating ^{3}H-corticosterone peak.

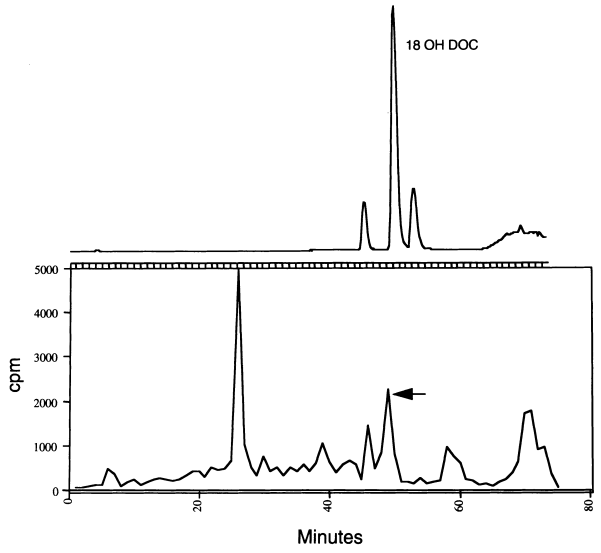

Fig. 3. RP-HPLC identification of ^{3}H-18OHDOC as metabolite of ^{3}H-DOC. Upper panel: UV detection of 18OHDOC standard. Lower panel: Radioactivity in eluted fractions with arrow indicating ^{3}H-18OHDOC peak.

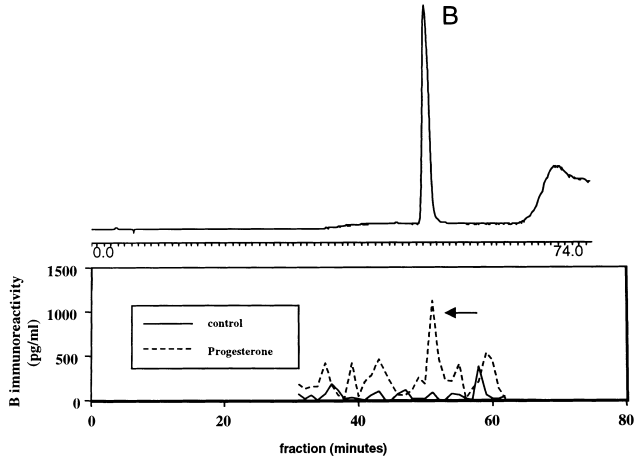

Fig. 4. ELISA identification of corticosterone (B) in RP-HPLC separated fractions. Upper panel: UV detection of corticosterone (B) standard. Lower panel: Amount of immunoreactive corticosterone (arrow) in media from human melanoma cells incubated with progesterone. Control represents media from cells cultured without progesterone added.

Table 1
Production of corticosterone and aldosterone by melanoma cells incubated in serum free media for 24 h

Addition to the media	Aldosterone (pg/well)	Corticosterone (pg/well)
No addition	8.46 ± 3.48	44.4 ± 4.92
A-II	5.16 ± 1.62	61.8 ± 11.4
ACTH-1–24	5.46 ± 2.88	49.8 ± 2.4
DOC	3.84 ± 1.2	540 ± 156
DOC plus A-II	6.48 ± 2.04	294 ± 174
DOC plus ACTH-1–24	7.49 ± 1.92	492 ± 234

The data represent mean ± S.E. ($n = 4$).

retention times corresponding to those of corticosterone (B) and 18OHDOC were then identified (Figs. 2 and 3); in contrast, at the elution time of aldosterone, only background radioactivity was seen. Thus, both TLC and RP-HPLC analyses showed that melanoma cells can selectively transform ^{14}C-PROG or ^{3}H-DOC to corticosterone (B) and 18OHDOC radioderivatives.

Finally, we used ^{3}H-PROG as the substrate (Fig. 4). The fractions eluted from TLC plates were again separated by RP-HPLC, divided and submitted separately for liquid scintillation spectroscopy and ELISA assay for corticosterone. The liquid scintillation spectroscopy showed again the radioactive peak corresponding to elution time of DOC and a small radioactive peak corresponding to the elution time of corticosterone (not shown). The collected fractions were analyzed thereafter by ELISA that showed corticosterone (B) immunoreactivity at the same elution time as the corticosterone standard (Fig. 4). Thus, this assay confirmed that melanoma cells transform PROG to corticosterone (B).

The accumulation rate of corticosterone and aldosterone was then measured by ELISA using specific antibodies against those steroids (Table 1). Because each confluent well from a 6-well plate contains approximately 10^6 human melanoma cells, the reported net production/well would correspond to the activity of approximately 10^6 of human melanoma cells. The concentration of corticosterone in conditioned serum free media from the melanoma cells cultured in the presence of 1 μM DOC was 540 pg/well, compared to only 44.4 pg/well in control media (no addition of DOC) (Table 1). Thus, supplying cells with metabolic precursor (DOC) significantly stimulated corticosterone production (12 times) and had no effect on aldosterone production (Table 1). In this context, the prevailing concentrations of corticosterone and aldosterone in media without DOC may either represent low basal endogenous production of those compounds or residual background levels of exogenous corticosteroids that were not completely washed-out after change from serum containing to serum free media (see Section 2). Incubation of cells for 24 h with the tested concentrations of A-II and ACTH-1–24 did not affect significantly corticosterone or aldosterone production (Table 1). This suggests that production of corticosterone by melanoma cells is autonomous from the exogenous supply of the normal adrenal steroidogenic stimulants A-II and ACTH-1–24.

In summary, complementing and extending our previous molecular studies on cutaneous expression of hypothalamic pituitary adrenal axis components [8], we now document that melanoma cells can metabolize rapidly and efficiently the steroid progesterone, e.g. the progesterone substrate was consumed entirely within 24 h of incubation (Fig. 1). The intermediates of this metabolism include DOC, corticosterone (B) and 18OHDOC. Possible clinico-pathological and physiological implications of those findings are multiple. For example, because of the immunosuppressive properties of corticosterone [12], the steroid may provide a melanoma strategy that allows evasion from immune surveillance by the host. At the cellular level, corticosterone could have intra- or paracrine actions; perhaps attenuating CRH and POMC peptides production. It is already known that these peptides are produced by melanoma cells, and that at least CRH production is inhibited by exogenous dexamethasone [10,13–16]. Finally, this is the first demonstration that cells of skin origin, malignant melanocytes, can produce corticosteroids, providing a strong background for further testing the hypothesis that, as part of local defense mechanism against stress skin cells can also produce corticosteroids [7,8]. Alternatively, these compounds may be utilized as immunosuppressors by malignant cutaneous cells.

Acknowledgements: The work was supported by grants from the National Science Foundation (#IBN-9604364) to A.S. and Medical Research Funds from the Department of Veterans Affairs and HL 27255 from the National Institutes of Health to C.E.G.-S.

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