

UV light phototransduction depolarizes human melanocytes

Nicholas W Bellono and Elena Oancea*

Department of Molecular Pharmacology, Physiology and Biotechnology; Brown University; Providence, RI USA

Keywords: melanocyte, ultraviolet, phototransduction, calcium signaling, UV

Exposure of human skin to low doses of solar UV radiation (UVR) causes increased pigmentation, while chronic exposure is a powerful risk factor for skin cancers. The mechanisms mediating UVR detection in skin, however, remain poorly understood. Our recent studies revealed that UVR activates a retinal-dependent G protein-coupled signaling pathway in melanocytes. This phototransduction pathway leads to the activation of transient receptor potential A1 (TRPA1) ion channels, elevation of intracellular calcium (Ca^{2+}) and rapid increase in cellular melanin content. Here we report that physiological doses of solar-like UVR elicit a retinal-dependent membrane depolarization in human epidermal melanocytes. This transient depolarization correlates with delayed inactivation time of the UVR-evoked photocurrent and with sustained Ca^{2+} responses required for early melanin synthesis. Thus, the cellular depolarization induced by UVR phototransduction in melanocytes is likely to be a critical signaling mechanism necessary for the protective response represented by increased melanin content.

Introduction

Melanocytes are neural crest derived cells that migrate during development to the skin, ear, brain, and heart.^{1–5} All of these melanocytes share the ability to produce melanin and, in the case of skin, to transfer it to neighboring keratinocytes.⁶ Melanin production is essential for melanocyte function; defects in this process can lead to skin pigmentation disorders, increased susceptibility to melanoma, as well as visual and hearing defects.^{7,8} Unlike the skin of most other vertebrates, human skin contains melanocytes within the basal epidermal layer that are responsible for the skin's pigmentation response to solar UV radiation (UVR). Several functional ion channels are expressed at the plasma membrane of human epidermal melanocytes (HEMs).^{9–12} Changes in melanocyte plasma membrane potential can control cellular functions such as migration, proliferation and morphology.¹³

UVR, a constant presence in our environment, has damaging effects on human skin, causing skin cancers and photoaging.¹⁴ Exposure to low doses of UVR stimulates a protective pigmentation response mediated by melanocytes present in the epidermis. UVR causes DNA damage in the epidermis and results in melanin synthesis in melanocytes and transfer to neighboring keratinocytes, leading to increased skin pigmentation within one day after exposure.¹⁵ Considering the time course and activation mechanism of the delayed pigmentation response, it is likely that other signaling pathways in skin contribute to earlier UVR detection to initiate protection prior to DNA damage.

We recently found that human epidermal melanocytes (HEMs) have a retinal-dependent and G protein-mediated

UVR-activated pathway that leads to a rapid intracellular Ca^{2+} response and activation of an ionic current, suggesting the presence of a phototransduction cascade in melanocytes.^{12,16} We found that UVR activates transient receptor potential A1 (TRPA1) ion channels in the plasma membrane, which, in response to prolonged UVR stimuli, mediate sustained Ca^{2+} responses necessary for early increases in cellular melanin content.^{12,16}

How does TRPA1 mediate the long-lasting, sustained Ca^{2+} response in response to a transient stimulus? Here we show that physiological UVR doses induce a retinal-dependent membrane depolarization in HEMs. We hypothesize that the change in membrane potential delays the inactivation of UVR-activated TRPA1 channels, thus allowing Ca^{2+} influx for an extended period of time. Our findings demonstrate that the equivalent of seconds to minutes of sun exposure depolarizes melanocytes and that melanocyte depolarization contributes to the elevated intracellular Ca^{2+} required for early melanin synthesis.

Results and Discussion

Our recent work showed that exposure of cultured primary human epidermal melanocytes (HEMs) to UVR with spectral characteristics (90% UVA and 10% UVB) similar to solar UVR leads to activation of TRPA1 ion channels.¹² Because opening of TRPA1, a nonselective cation channel, allows primarily Ca^{2+} and Na^+ ions to enter the cell at resting membrane potentials, we investigated whether the UVR-mediated activation of TRPA1 leads to a change in membrane potential that might regulate downstream events.

*Correspondence to: Elena Oancea; Email: elena_oancea@brown.edu
Submitted: 05/17/13; Revised: 06/05/13; Accepted: 06/07/13
<http://dx.doi.org/10.4161/chan.25322>

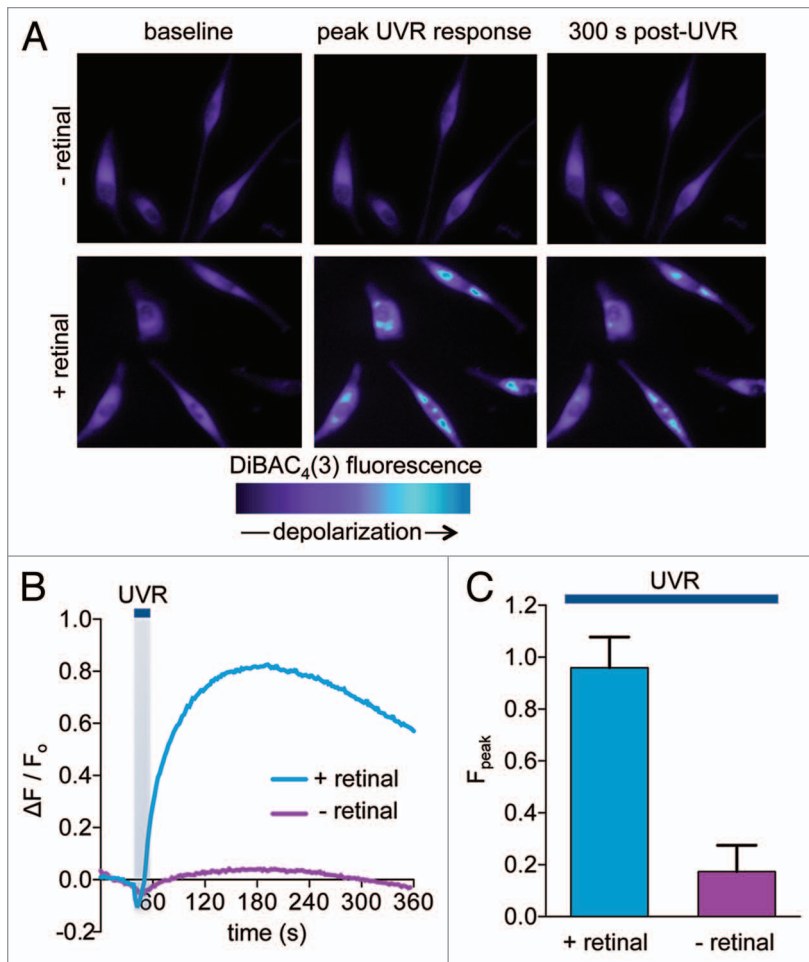


Figure 1. UVR induces a retinal-dependent increase in the fluorescence of the voltage-sensitive dye DiBAC4(3). **(A)** Pseudochrome fluorescence images of human epidermal melanocytes (HEMs) loaded with DiBAC4(3). HEMs preincubated with all-*trans* retinal (lower images) or vehicle (upper images) were imaged before (baseline, left images) or after exposure to 240 mJ/cm² (12-sec exposure to 20 mW/cm²) UVR. The images (peak UVR response, middle) were recorded 120 sec after exposure, at the peak UVR-induced DiBAC4(3) fluorescence response and at 300 sec post-UVR (300 sec post-UVR, right images). **(B)** Relative changes in the DiBAC4(3) fluorescence intensity of representative HEMs preincubated with retinal (+ retinal) or vehicle (– retinal) and stimulated with UVR (240 mJ/cm²), as a function of time. **(C)** Mean amplitudes (F_{peak}) of DiBAC4(3) relative fluorescence intensity of HEMs preincubated with retinal or vehicle and stimulated with 240 mJ/cm² UVR. Bars represent average \pm SEM. $F_{\text{peak}} = 0.96 \pm 0.11$ for retinal, $F_{\text{peak}} = 0.17 \pm 0.10$ for vehicle, $p < 0.0006$, $n = 6$ experiments per condition.

UVR exposure causes retinal-dependent cellular depolarization. To measure UVR-induced changes in HEM membrane potential we used the voltage sensitive dye bis-(1,3-dibutylbarbituric acid) [DiBAC4(3)] and monitored the fluorescence intensity as a function of time of HEMs loaded with DiBAC4(3) and exposed to solar-like UVR doses equivalent to 240 sec of full sun exposure¹⁶ (240 mJ/cm²). An UVR-induced increase in fluorescence intensity was observed only when cells were preincubated with all-*trans* retinal (12 μ M) (Fig. 1A, top panels vs. bottom panels), suggesting that UVR elicits a retinal-dependent membrane depolarization in HEMs (Fig. 1A–C). The change in DiBAC4(3) fluorescence that reflects the retinal-dependent

depolarization was sustained, persisting for longer than 300 sec (Fig. 1A and B).

To our knowledge, direct measurements of HEM membrane potentials have not yet been reported. To further investigate the UVR-induced depolarization, we directly measured membrane potential (V_m) using whole-cell current clamp recordings with an internal solution containing physiological ionic concentrations. Current clamp measurements determined that unstimulated HEMs have a resting membrane potential of about -40 mV that is not altered by retinal preincubation ($V_{\text{rest}} = -40.14 \pm 1.94$ mV, $n = 7$ in the absence of retinal and $V_{\text{rest}} = -39.85 \pm 1.27$ mV, $n = 11$ in cells preincubated with retinal). UVR exposure did not significantly change V_{rest} in the absence of retinal (Fig. 2A, –retinal). However, in retinal-treated HEMs, UVR exposure induced a depolarization that became measurable during the UVR pulse, reached its maximum value within 20 sec after UVR exposure ($\Delta V_m = 16.5 \pm 1.06$ mV), and then slowly repolarized to V_{rest} after more than 1 min following UVR stimulation (Fig. 2A and B).

Because during voltage clamp recordings UVR exposure of HEMs leads to retinal-dependent TRPA1 activation,¹² we tested the contribution of TRPA1 ionic currents to the observed depolarization, using the specific TRPA1 antagonist HC-030031 (HC). In the presence of 100 μ M HC, 240 mJ/cm² UVR did not elicit a retinal-dependent depolarization in HEMs (Fig. 2A and B). Our data suggest that TRPA1 activation is necessary for the UVR-induced depolarization.

UVR photocurrent inactivation is dependent on V_m . Because TRPA1-mediated UVR photocurrents are outward rectifying (Fig. 2C), depolarization will have little effect on current amplitudes near physiological potentials. However, previous work showed that depolarized membrane potentials alter TRPA1-mediated inactivation kinetics in response to chemical agonists.¹⁷ To determine whether sustained depolarization of HEMs might similarly alter the kinetics of TRPA1-mediated currents, we monitored the retinal-dependent UVR-induced current as a function of time at hyperpolarized vs. depolarized potentials (Fig. 2C, inset).

At a constant negative potential ($V_m = -60$ mV), UVR evoked an inward photocurrent that inactivated shortly after the stimulus ceased, with the time constant $\tau_{\text{off}} = 18$ sec (Fig. 2C, middle trace). In contrast, at a constant positive potential ($V_m = +40$ mV), the same UVR dose evoked an outward current that persisted for many seconds after the stimulus, inactivating very slowly and often incompletely ($\tau_{\text{off}} > 150$ sec, Fig. 2C, bottom trace). Thus, the time course of the UVR photocurrent inactivation in HEMs is dependent on membrane potential: currents inactivated relatively quickly and completely at a hyperpolarized

potential, and slowly and often incompletely under strong depolarization (Fig. 2C and D).

UVR-induced depolarization contributes to sustained Ca^{2+} responses. We previously found that in HEMs UVR induced a TRPA1-mediated sustained Ca^{2+} response that was critical for cellular melanin increase.¹² To investigate whether the observed UVR-induced membrane depolarization can account for the persistent Ca^{2+} response, we performed Ca^{2+} imaging with the fluorometric Ca^{2+} indicator Fluo-4, while simultaneously monitoring the membrane potential. HEMs loaded with Fluo-4 and preincubated with retinal were stimulated with 240 mJ/cm^2 UVR in whole-cell current clamp configuration (Fig. 3A and B). The membrane potential depolarized by ~ 10 mV and remained elevated for more than 200 sec (Fig. 3B, top panel). The intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_{\text{ic}}$) of the monitored cell, as measured by the relative change in fluorescence intensity, increased with a similar time course and decreased very slowly after reaching the peak (Fig. 3B, bottom panel). We then monitored $[\text{Ca}^{2+}]_{\text{ic}}$ while maintaining the membrane potential constant near the resting potential ($V_m = -40$ mV) (Fig. 3C). The time course and amplitude of the increase in $[\text{Ca}^{2+}]_{\text{ic}}$ was similar to that seen in the current clamp experiment, but after reaching a peak, the $[\text{Ca}^{2+}]_{\text{ic}}$ decreased more rapidly (Fig. 3D, bottom panel).

To compare Ca^{2+} responses under the two recording conditions, we measured the change in fluorescence intensity at the peak (F_{peak}) and 100 sec after the peak ($F_{\text{peak}+100}$) (dashed lines in Fig. 3B and D, bottom panels). The average UVR-induced peak Ca^{2+} responses (F_{peak}) were very similar when the membrane potential was allowed to depolarize (current clamp) and when it was maintained constant (voltage clamp). In contrast, sustained Ca^{2+} responses ($F_{\text{peak}+100}$) were reduced when the cell was voltage clamped at -40 mV, when compared with current clamp conditions (Fig. 3E, light blue bars). To directly compare the sustained Ca^{2+} responses, we represented the ratio $F_{\text{peak}+100}/F_{\text{peak}}$ and found that it was reduced by $\sim 51\%$ in voltage clamp, as compared with the current clamp configuration (Fig. 3F), suggesting that membrane depolarization contributes to the persistent Ca^{2+} response.

Our results show that UVR phototransduction in HEMs leads to persistent membrane depolarization, which delays the inactivation of TRPA1-mediated photocurrents, thus allowing for sustained Ca^{2+} responses. In whole-cell voltage clamp experiments, inactivation of agonist-induced TRPA1 currents occurs within seconds,^{17,20} the time-course of this inactivation is modulated by Ca^{2+} via an unknown mechanism,^{17,20} and has also been reported to depend on membrane potential.¹⁷ UVR-induced activation of TRPA1 ion channels is associated with an initial transient increase in intracellular Ca^{2+} mediated by both opening of the TRPA1 channels and by release from internal stores,¹² which could regulate TRPA1 channel inactivation by directly binding

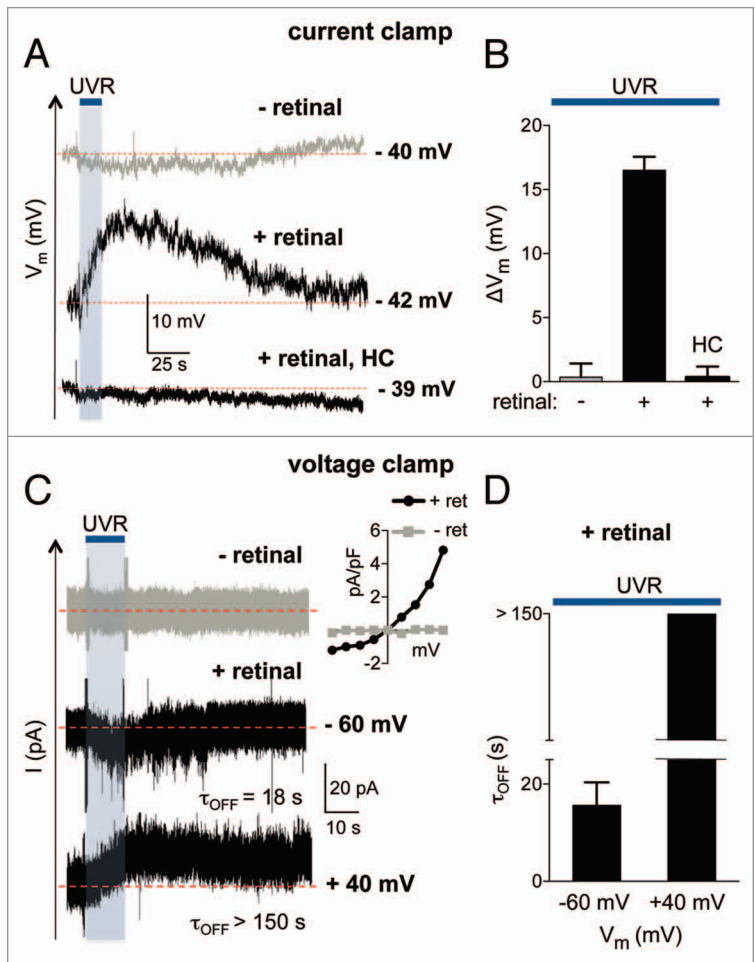


Figure 2. UVR exposure leads to cellular depolarization and sustained photocurrents. (A) In current clamp experiments, exposure of a representative HEM in whole-cell configuration to 240 mJ/cm^2 UVR induced a retinal-dependent cellular depolarization that was abolished in the presence of the TRPA1 antagonist HC-030031 (HC, 100 μM). (B) The maximal change in mean membrane potential (ΔV_m) elicited by UVR was significant only when HEMs were preincubated with retinal, but not in the absence of retinal. The retinal-dependent depolarization was abolished in the presence of HC-030031 (HC, 100 μM). $\Delta V_m = 16.5 \pm 1.06$ mV for retinal, 0.35 ± 1.05 mV in the absence of retinal and 0.33 ± 0.84 mV when cells were treated with HC and retinal. $n = 6$ –11 cells per condition. $p < 0.0001$ for retinal vs. no retinal and for retinal vs. HC. (C) The retinal-dependent UVR (240 mJ/cm^2) photocurrent of a representative HEM inactivated completely with a time constant (τ_{OFF}) of 18 sec when the voltage was clamped at hyperpolarized potentials (-60 mV). In voltage-clamp recordings at a sustained depolarized potential ($+40$ mV), the UVR photocurrent inactivated incompletely, with a time constant (τ_{OFF}) > 150 sec. No photocurrents were measured in response to UVR in the absence of retinal. Inset: Current-voltage relationship of the UVR-activated whole-cell current, obtained using a voltage step protocol (-80 mV to $+80$ mV in 20 mV increments). (D) The average time constant (τ_{OFF}) for the decay of UVR photocurrents was $\tau_{\text{OFF}} = 15.62 \pm 4.72$ sec at -60 mV, while at $+40$ mV the current decay was incomplete, with a $\tau_{\text{OFF}} > 150$ sec. $n = 5$ cells per condition.

to the cytosolic region of the channel^{21,22} or via an indirect mechanism. At resting membrane potential the initial Ca^{2+} response decays with a $\tau_{1/2} < 100$ sec (Fig. 3D). We discovered, however, that simultaneous with the UVR-induced Ca^{2+} response, melanocytes exhibit a shift in their membrane potential toward positive values (Fig. 3B), which correlates with slower inactivation

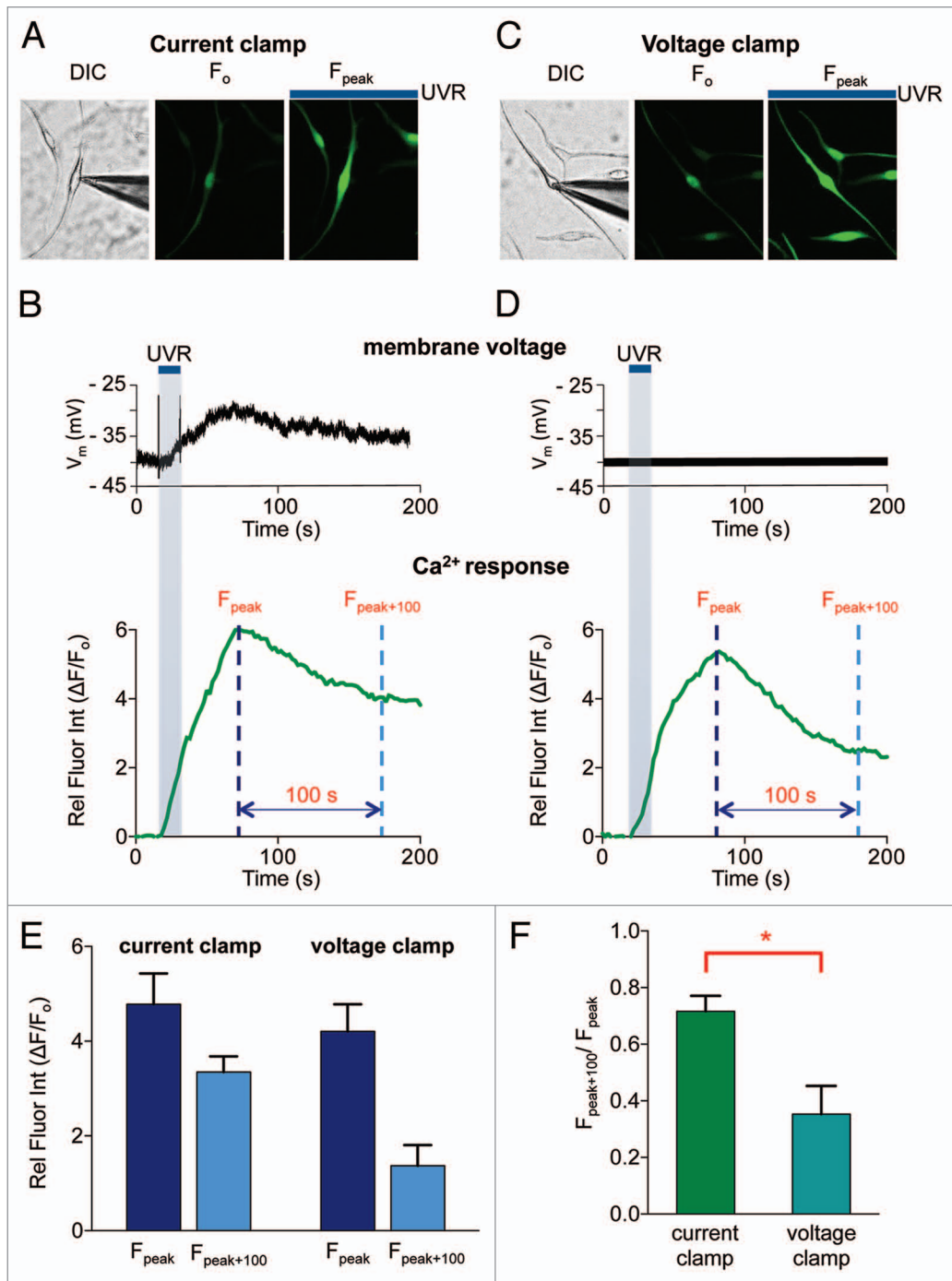


Figure 3. For figure legend, see page 247.

of TRPA1 channels, in a similar fashion as reported for agonist-dependent activation of TRPA1.¹⁷ When membrane depolarization occurs, the initial transient Ca^{2+} response is followed by a persistent response, which is required for the melanin increase induced by UVR in melanocytes.¹²

What conductances mediate the UVR-induced depolarization? Blocking TRPA1 precludes depolarization, suggesting that TRPA1 is required. The relatively small inward currents through TRPA1 channels (Fig. 2C) alone might not account for the

measured depolarization; other channels activated downstream of UVR-signaling could contribute to voltage changes. The initial depolarization caused by cation flux through TRPA1 could activate voltage-gated Na^+ or Ca^{2+} channels that would further depolarize the cell. Despite being non-excitable cells, primary human melanocytes might express voltage-gated Na^+ channels⁹ and Ca^{2+} channels,²³ but the characterization and function of these channels in melanocytes remains unknown. Alternatively, other Ca^{2+} -dependent mechanisms could contribute to the

Figure 3 (See previous page). UVR-induced depolarization of HEMs modulates Ca^{2+} responses. **(A)** Images of a representative HEM loaded with the Ca^{2+} indicator Fluo-4 and stimulated with 240 mJ/cm^2 UVR. In whole-cell current clamp experiments the fluorescence intensity of the recorded HEM (DIC, left image and F_{or} , middle image) increased in response to UVR (F_{peak} , right image). **(B)** In current clamp conditions the time course of the UVR-induced change in membrane voltage (top panel) and intracellular Ca^{2+} measured by the relative fluorescence intensity of the cell ($\Delta F/F_{\text{or}}$) (lower panel) were measured simultaneously for the HEM shown in **(A)**. **(C)** Images of a representative HEM in whole-cell voltage clamp experiments near the resting membrane potential of HEMs (-40 mV) loaded with the Ca^{2+} indicator Fluo-4 (DIC, left image, and F_{or} , middle image). UVR stimulation (240 mJ/cm^2) leads to increased fluorescence intensity of the recorded HEM and neighboring cells (F_{peak} , right image). **(D)** The membrane potential of the representative HEM shown in **(C)** was maintained at -40 mV (top panel). The Ca^{2+} response, as measured by the change in relative fluorescence intensity ($\Delta F/F_{\text{or}}$), as a function of time was determined in response to UVR stimulation (240 mJ/cm^2) (lower panel). **(E)** Comparison of the peak (F_{peak}) and delayed ($F_{\text{peak}+100}$) Ca^{2+} responses elicited by UVR (240 mJ/cm^2) when the membrane was allowed to depolarize (current clamp) or was maintained constant (voltage clamp). The peak Ca^{2+} responses were not affected by membrane potential (dark blue bars, $n = 5$ cells per condition) and were reduced 100 sec after the peak response ($F_{\text{peak}+100}$) (light blue bars). $F_{\text{peak}} = 4.78 \pm 0.07$ for current clamp and 4.07 ± 0.46 for voltage clamp. $F_{\text{peak}+100} = 3.35 \pm 0.33$ for current clamp and 1.37 ± 0.44 for voltage clamp. $n = 5$ cells per condition. **(F)** The ratio of the peak and delayed Ca^{2+} responses ($F_{\text{peak}+100}/F_{\text{peak}}$) measured under current and voltage clamp conditions, respectively, reflects that the concentration of intracellular Ca^{2+} decays more rapidly under voltage clamp conditions than in current clamp, when the membrane is allowed to depolarize. $(F_{\text{peak}+100})/F_{\text{peak}} = 0.72 \pm 0.05$ for current-clamp and 0.35 ± 0.10 for voltage-clamp. $n = 5$ cells per condition. $p < 0.03$.

observed depolarization. While TRPA1 contributes to both the initial transient and sustained phase of the Ca^{2+} response,¹² membrane depolarization only contributes to the persistent Ca^{2+} response (Fig. 3B–F). Depolarization cannot occur in the absence of TRPA1, but TRPA1 will open in the absence of depolarization, as shown in voltage clamp experiments.

Short-term depolarization of excitable cells is a commonly used mechanism for intra- and intercellular signaling. Melanocytes have not been shown to function as excitable cells; persistent depolarization of human melanocytes with a depolarizing agent reportedly altered cellular morphology,¹³ but the physiological significance of this finding is not clear. Here we provide evidence that physiological doses of UVR are able to transiently depolarize human melanocytes in order to modulate the Ca^{2+} responses that lead to increased cellular melanin. Based on our results, we propose that UVR exposure leads to TRPA1-dependent depolarization, which acts to delay TRPA1 inactivation, leading to the sustained Ca^{2+} responses necessary for melanin synthesis (Fig. 4A and B).

Materials and Methods

UVR stimulation. UVR stimulation was performed as previously described,^{12,16} using a Mercury-Xenon Arc lamp (Newport) with filtered output (90% UVA and 10% UVB) comparable to solar UVR. Each 1 sec of experimental irradiance of 10 mW/cm^2 equates to 10-sec exposure to bright solar UVR (with a UV index of 10). Light was filtered using 280-nm long-pass (LP)/400-nm short-pass (SP) filters for UV radiation (UVR).

Electrophysiology. Electrophysiological experiments were performed as previously described.¹² All-*trans* retinal was stored, solubilized, and applied as previously described.¹⁶ All experiments were performed under dimmed or infrared illumination. Whole-cell patch clamp recordings were carried-out using micropipettes with 3–6 $\text{M}\Omega$ resistance at room temperature using an EPC 10 amplifier (HEKA Instruments Inc.) with PatchMaster software (HEKA Instruments Inc.), filtered at 2.9 kHz

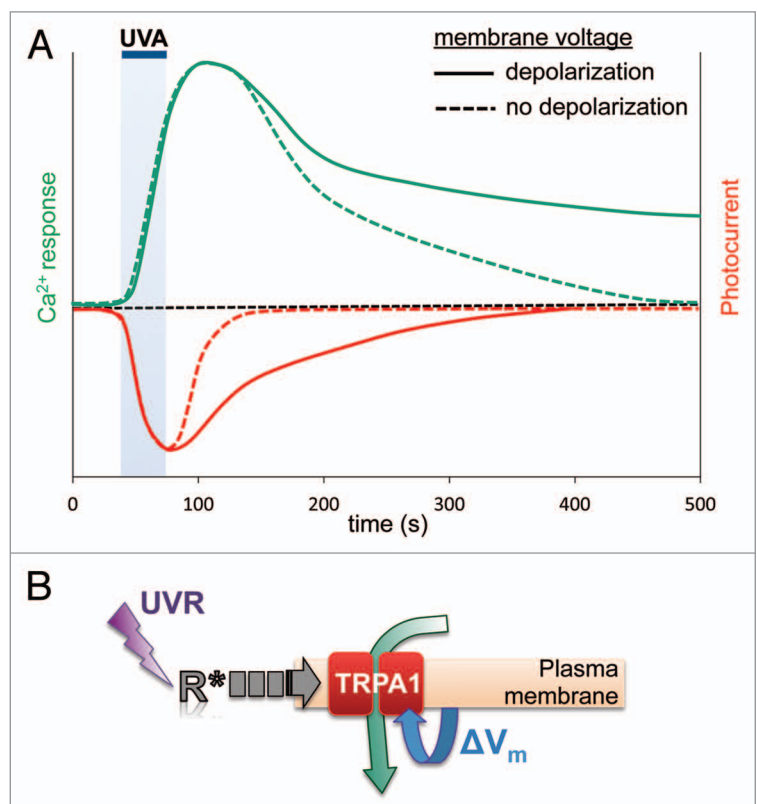


Figure 4. Membrane voltage modulates UVR phototransduction in human melanocytes. **(A)** Graphic representation of typical UVR-induced Ca^{2+} and photocurrent responses when the plasma membrane is allowed to depolarize or maintained at constant V_m (no depolarization). UVR exposure leads to HEM depolarization, which delays inactivation of UVR photocurrents (red traces), resulting in prolonged Ca^{2+} influx and increased duration of Ca^{2+} responses (green traces). Under voltage clamp, in the absence of membrane depolarization, UVR photocurrents inactivate faster and Ca^{2+} responses decay more rapidly. **(B)** Schematic representation of the proposed membrane voltage regulation of the UVR phototransduction pathway in human melanocytes. UVR activates a retinal-dependent G protein-coupled receptor, which initiates a signaling cascade to activate TRPA1. TRPA1 activation depolarizes the plasma membrane to modulate TRPA1-mediated UVR photocurrents by delaying channel inactivation. Prolonged photocurrents lead to the persistent Ca^{2+} responses required for early melanin synthesis.

and digitized at 20 kHz. Modified Ringer's extracellular solution contained (in mM): 150 NaCl, 1.8 CaCl₂, 1.2 MgCl₂, 10 D-glucose, 25 HEPES; pH 7.4, 310 mOsm/L. Voltage clamp recording internal pipette solution contained (in mM): 140 CsCl, 1 MgCl₂, 4 MgATP, 10 EGTA, 10 HEPES; pH 7.2, 290 mOsm/L. Current clamp recording internal pipette solution contained (in mM): 120 K-gluconate, 4 NaCl, 6 Na-gluconate, 2 MgATP, 0.02 EGTA, 10 HEPES; pH 7.2, 290 mOsm/L. Current clamp pipette solution was used for simultaneous voltage clamp and Ca²⁺ imaging experiments. Whole-cell current values were plotted as a function of time and fitted with a single-exponential function in Prism 6 (GraphPad) to calculate the time constants of inactivation.

Membrane potential measurement and Ca²⁺ imaging. Image series were acquired at 2-sec intervals and the fluorescence intensity in a region of each cell was measured as a function of time using NIS Elements software (Nikon). The data were then analyzed with MatLab (MathWorks) and plotted using Prism 6 software. For membrane potential measurements, cells were incubated for 20 min in Ringer's solution containing 5 μM DiBAC4(3) (Molecular Probes) and 250 μM sulfinpyrazone to prevent loss of DiBAC4(3) from cells. Fluorescent calcium imaging was performed using the fluorometric calcium indicator

Fluo-4 AM (Invitrogen/Molecular Probes). Cells were incubated for 20 min in Ringer's solution containing 2 μM Fluo-4 AM and 250 μM sulfinpyrazone, then washed and imaged at room temperature. DiBAC4(3) and Fluo-4 fluorescence intensities were quantified as $\Delta F/F_o(t) = [F_{cell}(t) - F_{baseline}]/F_{baseline}$. UVR-induced sustained Ca²⁺ responses were measured 100 sec after peak responses and quantified as $(F_{sustained} - F_o)/(F_{peak} - F_o)$.

Statistical analyses. Numerical data are mean ± SEM and p-values were calculated using the two-tailed Student's t test and considered significant when p < 0.05. n refers to the number of cells for electrophysiology and Ca²⁺ imaging experiments and the number of independent experiments for membrane potential imaging.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This work was supported by grants from Brown University (to EO) and a National Science Foundation Graduate Research Fellowship (to NWB). We thank Dr Anita Zimmerman, Dr Julie Kauer and Valerie Yorgan for helpful discussion and Sarah Pierce for assistance with experiments.

References

1. Zecca L, Tampellini D, Gatti A, Crippa R, Eisner M, Sulzer D, et al. The neuromelanin of human substantia nigra and its interaction with metals. *J Neural Transm* 2002; 109:663-72; PMID:12111458; <http://dx.doi.org/10.1007/s007020200055>
2. Brito FC, Kos L. Timeline and distribution of melanocyte precursors in the mouse heart. *Pigment Cell Melanoma Res* 2008; 21:464-70; PMID:18444965; <http://dx.doi.org/10.1111/j.1755-148X.2008.00459.x>
3. Yajima I, Larue L. The location of heart melanocytes is specified and the level of pigmentation in the heart may correlate with coat color. *Pigment Cell Melanoma Res* 2008; 21:471-6; PMID:18627529; <http://dx.doi.org/10.1111/j.1755-148X.2008.00483.x>
4. Tachibana M. Sound needs sound melanocytes to be heard. *Pigment Cell Res* 1999; 12:344-54; PMID:10614574; <http://dx.doi.org/10.1111/j.1600-0749.1999.tb00518.x>
5. Kawakami A, Fisher DE. Key discoveries in melanocyte development. *J Invest Dermatol* 2011; 131(E1):E2-4; PMID:22094402
6. Costin GE, Hearing VJ. Human skin pigmentation: melanocytes modulate skin color in response to stress. *FASEB J* 2007; 21:976-94; PMID:17242160; <http://dx.doi.org/10.1096/fj.06-6649rev>
7. Hearing VJ. Biogenesis of pigment granules: a sensitive way to regulate melanocyte function. *J Dermatol Sci* 2005; 37:3-14; PMID:15619429; <http://dx.doi.org/10.1016/j.jdermsci.2004.08.014>
8. Plonka PM, Passeron T, Brenner M, Tobin DJ, Shibahara S, Thomas A, et al. What are melanocytes really doing all day long...? *Exp Dermatol* 2009; 18:799-819; PMID:19659579; <http://dx.doi.org/10.1111/j.1600-0625.2009.00912.x>
9. Ekmechag B, Persson B, Rorsman P, Rorsman H. Demonstration of voltage-dependent and TTX-sensitive Na(+)-channels in human melanocytes. *Pigment Cell Res* 1994; 7:333-8; PMID:7886006; <http://dx.doi.org/10.1111/j.1600-0749.1994.tb00636.x>
10. Oancea E, Vriens J, Brauchi S, Jun J, Splawski I, Clapham DE. TRPM1 forms ion channels associated with melanin content in melanocytes. *Sci Signal* 2009; 2:ra21; PMID:19436059; <http://dx.doi.org/10.1126/scisignal.2000146>
11. Stanis H, Stark A, Kilch T, Schwarz EC, Müller CS, Peinelt C, et al. ORAI1 Ca(2+) channels control endothelin-1-induced mitogenesis and melanogenesis in primary human melanocytes. *J Invest Dermatol* 2012; 132:1443-51; PMID:22318387; <http://dx.doi.org/10.1038/jid.2011.478>
12. Bellono NW, Kammel LG, Zimmerman AL, Oancea E. UV light phototransduction activates transient receptor potential A1 ion channels in human melanocytes. *Proc Natl Acad Sci U S A* 2013; 110:2383-8; PMID:23345429; <http://dx.doi.org/10.1073/pnas.1215555110>
13. Blackiston D, Adams DS, Lemire JM, Lobikin M, Levin M. Transmembrane potential of GlyCl-expressing instructor cells induces a neoplastic-like conversion of melanocytes via a serotonergic pathway. *Dis Model Mech* 2011; 4:67-85; PMID:20959630; <http://dx.doi.org/10.1242/dmm.005561>
14. Heck DE, Gerecke DR, Vetrano AM, Laskin JD. Solar ultraviolet radiation as a trigger of cell signal transduction. *Toxicol Appl Pharmacol* 2004; 195:288-97; PMID:15020191; <http://dx.doi.org/10.1016/j.taap.2003.09.028>
15. Cui R, Widlund HR, Feige E, Lin JY, Wilensky DL, Igras VE, et al. Central role of p53 in the sun-tan response and pathological hyperpigmentation. *Cell* 2007; 128:853-64; PMID:17350573; <http://dx.doi.org/10.1016/j.cell.2006.12.045>
16. Wicks NL, Chan JW, Najera JA, Ciriello JM, Oancea E. UVA phototransduction drives early melanin synthesis in human melanocytes. *Curr Biol* 2011; 21:1906-11; PMID:22055294; <http://dx.doi.org/10.1016/j.cub.2011.09.047>
17. Nagata K, Duggan A, Kumar G, Garcia-Añoveros J. Nociceptor and hair cell transducer properties of TRPA1, a channel for pain and hearing. *J Neurosci* 2005; 25:4052-61; PMID:15843607; <http://dx.doi.org/10.1523/JNEUROSCI.0013-05.2005>
18. Wang YY, Chang RB, Allgood SD, Silver WL, Liman ER. A TRPA1-dependent mechanism for the pungent sensation of weak acids. *J Gen Physiol* 2011; 137:493-505; PMID:21576376; <http://dx.doi.org/10.1085/jgp.201110615>
19. Jordt SE, Bautista DM, Chuang HH, McKemy DD, Zygmunt PM, Högestätt ED, et al. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature* 2004; 427:260-5; PMID:14712238; <http://dx.doi.org/10.1038/nature02282>
20. Wang YY, Chang RB, Waters HN, McKemy DD, Liman ER. The nociceptor ion channel TRPA1 is potentiated and inactivated by permeating calcium ions. *J Biol Chem* 2008; 283:32691-703; PMID:18775987; <http://dx.doi.org/10.1074/jbc.M803568200>
21. Doerner JF, Gisselmann G, Hatt H, Wetzel CH. Transient receptor potential channel A1 is directly gated by calcium ions. *J Biol Chem* 2007; 282:13180-9; PMID:17353192; <http://dx.doi.org/10.1074/jbc.M607849200>
22. Zurborg S, Yurgionas B, Jira JA, Caspani O, Heppenstall PA. Direct activation of the ion channel TRPA1 by Ca²⁺. *Nat Neurosci* 2007; 10:277-9; PMID:17259981; <http://dx.doi.org/10.1038/nn1843>
23. Das A, Pushparaj C, Bahi N, Sorolla A, Herreros J, Pamplona R, et al. Functional expression of voltage-gated calcium channels in human melanoma. *Pigment Cell Melanoma Res* 2012; 25:200-12; PMID:22260517; <http://dx.doi.org/10.1111/j.1755-148X.2012.00978.x>