LETTER

Melatonin signaling in mitochondria extends beyond neurons and neuroprotection: Implications for angiogenesis and cardio/gastroprotection

Amrita Ahluwalia[a,1], Iwona M. Brzozowska[b,1], Neil Hoa[a], Michael K. Jones[a,c], and Andrzej S. Tarnawski[a,c,2]

We read with interest the paper of Suofu et al. (1), demonstrating that neuronal mitochondria produce melatonin, which upon binding to its melatonin type 1 (MT_1) receptor on the mitochondrial membrane (MM) inhibits cytochrome *c* release, caspase activation, and apoptosis. We commend the authors on their thorough investigation but wish to point out some major questions: (*i*) Is the presence of the MT_1 receptor in mitochondria limited to the neuronal cells or may it also apply to other cells, for example, endothelial cells that are components of blood vessels and are critical for delivery of oxygen and nutrients to all tissues, vessels' regeneration, and angiogenesis? (*ii*) Is the MT_2 receptor expressed on mitochondria and what is its role?

Melatonin is abundantly expressed beyond the neural system; for example, in gastrointestinal tissues, including the stomach, where it has been shown to protect gastric mucosa against stress, ischemia, and nonsteroidal antiinflammatory drug-induced injury, and to accelerate ulcer healing (2–6). We recently demonstrated that both MT_1 and MT_2 are expressed on the MM of gastric endothelial cells (GECs); MT_1 was expressed in MM while MT_2 was expressed in the nucleus and MM (Fig. 1). However, in contrast to the study of Suofu et al. (1), we found that treatment of GECs with exogenous melatonin (10 μM) increased by 1.7-fold ($P < 0.001$) MM potential, which drives ATP synthesis (7). Moreover, treatment of GECs with exogenous melatonin increased the MM expression of MT_1 and MT_2 by 2.5- and 1.6-fold, respectively, and increased in vitro angiogenesis (new blood vessel formation) by 1.4-fold ($P < 0.001$). It should be noted that the angiogenic response to melatonin was independent of either cell proliferation or apoptosis, leading us to speculate that, at least in GECs, melatonin signaling via mitochondria extends beyond cellular protection. Although, we have not yet determined the relative contributions of MT_1 vs. MT_2 in the angiogenic response of GECs to melatonin signaling, our finding that exogenous melatonin increased the MM expression of both MT_2 and MT_1 is at least indicative that both receptors are involved. Suofu et al. (1) did not assess the relative MM expression levels of MT_1 vs. MT_2, but rather used the MT_2 selective inhibitor, 4P-PDOT, as evidence that melatonin signaling in neuronal mitochondria is mediated exclusively through MM-expressed MT_1. Nevertheless, luzindole, which did prevent melatonin from blocking Ca^{2+}-mediated cytochrome *c* release, has a much greater affinity for MT_2 vs. MT_1. Moreover, the possibility that MT_2 plays a "decoy" role, akin to Flt1 in VEGF signaling, cannot be excluded. In such a scenario, MT_2 may play a more subtle role in regulating (e.g., dampening) melatonin signaling. On the other hand, the role of MT_2 in mitochondrial signaling by melatonin may be restricted to certain cell types (e.g., endothelial cells but not to brain neurons). The work of Suofo et al. (1) demonstrates novel melatonin signaling in neuronal mitochondria. Our work uncovered that this mechanism operates in endothelial cells that are major components of all blood vessels. We further suggest that melatonin signaling in mitochondria may also be applicable to other physiological processes, such as angiogenesis, gastroprotection, cardioprotection, and aging (3–6, 8, 9).

Acknowledgments

This work was supported by Merit Review Award I01 BX000626-05A2 from the US Department of Veterans Affairs Biomedical Laboratory Research and Development Service (to A.S.T.).

[a]Medical and Research Services, Veterans Affairs Long Beach Healthcare System, Long Beach, CA 90822; [b]Department of Anatomy, Jagiellonian University Medical College, 31-008 Cracow, Poland; and [c]Department of Medicine, University of California, Irvine, CA 92617

Author contributions: A.A. and A.S.T. designed research; A.A. and A.S.T. performed research; A.A., I.M.B., N.H., M.K.J., and A.S.T. analyzed data; and A.A., I.M.B., N.H., M.K.J., and A.S.T. wrote the paper.

The authors declare no conflict of interest.

[1]A.A. and I.M.B. contributed equally to this work.

[2]To whom correspondence should be addressed. Email: atarnawski@yahoo.com.

Published online February 9, 2018.

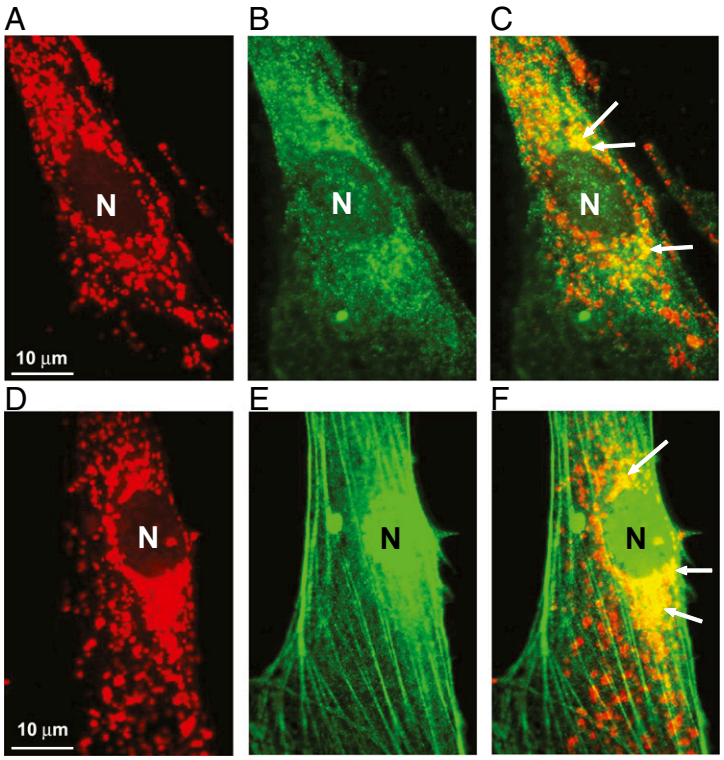

Fig. 1. Localization of melatonin receptors MT_1 and MT_2 in MM of gastric endothelial cells (GECs). (*A*) Staining of mitochondria using MitoTracker, a fluorescent dye that stains mitochondria (red) in a manner dependent on mitochondrial membrane potential. (*B*) Immunofluorescence staining for MT_1 in GECs (green). (*C*) Overlay of MitoTracker and MT_1 immunostaining images showing localization of MT_1 in MM as yellow/orange staining (arrows). (*D*) Staining of mitochondria using MitoTracker (red). (*E*) Immunofluorescence staining for MT_2 in GECs (green); please note strong MT2 expression in the nucleus (N). (*F*) Overlay of MitoTracker and MT_2 immunostaining images showing localization of MT_2 in MM as yellow/orange staining (arrows).

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