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REPLY TO AHLUWALIA ET AL.:

Contributions of melatonin receptors are tissue-dependent

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In our recent publication (1), we advance the previous findings regarding melatonin type 1 (MT_1) receptor in brain mitochondria. We demonstrate that melatonin is synthesized in brain mitochondria and signals through the MT_1 receptor located in the outer mitochondrial membrane (MM). We further show that melatonin binds mitochondria MT_1, which in turn activates inhibitory Gi proteins in the intermembrane space and inhibits stress-mediated cytochrome *c* release (1, 2). We have exclusively worked with neurons or brain tissue, and have therefore not performed experiments in other cell types at this stage.

In the letter from Ahluwalia et al. (3), the authors propose that melatonin receptors, especially MT_2, are located in MMs of gastric endothelial cells (GEC). Their data expand the knowledge of melatonin receptors beyond brain and neuronal mitochondria. Furthermore, the authors find that melatonin increases both MT_1 and MT_2 protein levels in GEC cell mitochondria (3). In a previous study we demonstrated that melatonin administration inhibited the mutant huntingtin-induced reduction of MT_1 protein and mRNA, but not MT_2 protein and mRNA in the R6/2 murine brain (2), suggesting that dysfunctional melatonin signaling through MT_1 in the murine brain may be relevant to neurodegeneration and may be amenable to modulation by exposure to exogenous melatonin. In our more recent study (1), we found that luzindole, which inhibits MT_1 and MT_2, decreased melatonin's ability to inhibit cytochrome *c* release while the MT_2 receptor-specific antagonist, 4P-PDOT, was unable to do so. These data indicate that luzindole antagonized melatonin's inhibitory effect on cytochrome *c* release due to its inhibition of MT_1 and not MT_2 in the brain.

These findings by Ahluwalia et al. (3) and our studies (1, 2, 4) indicate that the contribution of melatonin receptors and its signaling pathways can presumably be cell- and tissue-dependent. However, it must be pointed out that caution needs to be taken when interpreting data obtained using MT_1 antibodies. Extensive MT_1 antibody validation data in our laboratory and by others (5) suggest that none of the currently commercially available MT_1 antibodies that we tested are specific to the MT_1 protein. Thus, work showing localization of endogenous melatonin receptors using antibody-based methods must be validated by including robust controls, such as tissues from knockout MT_1 and MT_2 mice to demonstrate antibody reliability.

1 Suofu Y, et al. (2017) Dual role of mitochondria in producing melatonin and driving GPCR signaling to block cytochrome c release. *Proc Natl Acad Sci USA* 114:E7997–E8006.
2 Wang X, et al. (2011) The melatonin MT1 receptor axis modulates mutant Huntingtin-mediated toxicity. *J Neurosci* 31:14496–14507.
3 Ahluwalia A, Brzozowska IM, Hoa N, Jones MK, Tarnawski AS (2018) Melatonin signaling in mitochondria extends beyond neurons and neuroprotection: Implications for angiogenesis and cardio/gastroprotection. *Proc Natl Acad Sci USA*, 10.1073/pnas.1722131115.
4 Cecon E, Oishi A, Jockers R (August 17, 2017) Melatonin receptors: molecular pharmacology and signalling in the context of system bias. *Br J Pharmacol*, 10.1111/bph.13950.
5 Jockers R, Maurice P, Boutin JA, Delagrange P (2008) Melatonin receptors, heterodimerization, signal transduction and binding sites: what's new? *Br J Pharmacol* 154:1182–1195.

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The authors declare no conflict of interest.

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