Reply to Rutter *et al.*: The roles of cytosolic and intramitochondrial Ca^{2+} and the mitochondrial Ca^{2+}-uniporter (MCU) in the stimulation of mammalian oxidative phosphorylation

DOI 10.1074/jbc.RL120.014342

Frank N. Gellerich[1,2,*], Marten Szibor[3,4], Zemfira Gizatullina[1,2], Volkmar Lessmann[5,6], Michael Schwarzer[4], Torsten Doenst[4], Stefan Vielhaber[1,7], and Wolfram S. Kunz[8]

From the [1]Department of Neurology, Otto-von-Guericke-University, Magdeburg, Germany, the [2]Leibniz-Institute for Neurobiology, Magdeburg, Germany, the [3]Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland, the [4]Department of Cardiothoracic Surgery, Jena University Hospital, Jena, Germany, the [5]Institute of Physiology, Otto-von-Guericke-University, Magdeburg, Germany, the [6]Center for Behavioral Brain Sciences (CBBS), Magdeburg, Germany, the [7]German Center for Neurodegenerative Diseases, Magdeburg, Germany, and the [8]Institute of Experimental Epileptology and Cognition Research, University of Bonn, Bonn, Germany

Each model used in the work referred to by Rutter *et al.* (1) addressed certain aspects of mitochondrial biology, and together, they fully support the conclusions made. Please note that we describe Ca^{2+}-mediated regulation of oxidative phosphorylation (OXPHOS) fluxes (2, 3) and do not question Ca^{2+}-responsiveness of pyruvate dehydrogenase enzyme activity (4). To address concerns such as those raised by Rutter *et al.* (1), we studied glutamate/malate-dependent OXPHOS in the absence of exogenous pyruvate in mitochondria, omitted pyruvate from cell experiments, and implemented the working rat heart model perfused by Krebs–Henseleit (glucose) buffer. This unequivocally demonstrates in a broad range of models that MAS (malate-aspartate shuttle) inhibition induces a state of mitochondrial pyruvate starvation (3).

An unresolved observation is that mitochondria of MCU knockout mice show negligible activity of Ca^{2+}-uptake (5), which we confirm (3). We attributed this activity to residual expression of wild-type *Mcu* transcripts (3) as the result of a rare event of gene-trap excision during mRNA splicing, since this activity was sensitive to ruthenium red, an inhibitor of the MCU. Besides, please also note the low MCU Ca^{2+} affinity (6). *In vivo*, the endoplasmic reticulum is thought to facilitate the generation of microcompartments of high Ca^{2+} concentration to allow Ca^{2+} uptake *via* MCU (6). This mechanism is compromised in MCU knockout mice and can be ruled out in isolated mitochondria. Thus, our data support the notion that, depending on tissue, model system and pathophysiological status, a combination of mechanisms (*e.g.*, mitochondrial gas pedal and MCU) control OXPHOS substrate supply.

Notably, the viability of MCU knockout mice (3, 5), albeit living in a laboratory cage, indicates that MCU-dependent pathways are dispensable for a sedentary life. It remains interesting to elucidate, however, why MCU-dependent activation of matrix dehydrogenases is indispensable for high activity states (7) and how this may allow stressful life in the wild.

* For correspondence: Frank N. Gellerich, Frank.Gellerich@med.ovgu.de.

Conflict of interest—The authors declare that they have no conflicts of interest with the contents of this article.

References

1. Rutter, G. A., McCormack, J. G., Halestrap, A. P., and Denton, R. M. (2020) The roles of cytosolic and intramitochondrial Ca^{2+} and the mitochondrial Ca^{2+}-uniporter (MCU) in the stimulation of mammalian oxidative phosphorylation. *J. Biol. Chem.* **295,** 10506–10506
2. Gellerich, F. N., Gizatullina, Z., Trumbekaite, S., Korzeniewski, B., Gaynutdinov, T., Seppet, E., Vielhaber, S., Heinze, H.-J., and Striggow, F. (2012) Cytosolic Ca^{2+} regulates the energization of isolated brain mitochondria by formation of pyruvate through the malate-aspartate shuttle. *Biochem. J.* **443,** 747–755 CrossRef Medline
3. Szibor, M., Gizatullina, Z., Gainutdinov, T., Endres, T., Debska-Vielhaber, G., Kunz, M., Karavasili, N., Hallmann, K., Schreiber, F., Bamberger, A., Schwarzer, M., Doenst, T., Heinze, H.-J., Lessmann, V., Vielhaber, S., *et al.* (2020) Cytosolic, but not matrix, calcium is essential for adjustment of mitochondrial pyruvate supply. *J. Biol. Chem.* **295,** 4383–4397 CrossRef Medline
4. McCormack, J. G., Halestrap, A. P., and Denton, R. M. (1990) Role of calcium ions in regulation of mammalian intramitochondrial metabolism. *Physiol. Rev.* **70,** 391–425 CrossRef Medline
5. Pan, X., Liu, J., Nguyen, T., Liu, C., Sun, J., Teng, Y., Fergusson, M. M., Rovira, I. I., Allen, M., Springer, D. A., Aponte, A. M., Gucek, M., Balaban, R. S., Murphy, E., and Finkel, T. (2013) The physiological role of mitochondrial calcium revealed by mice lacking the mitochondrial calcium uniporter. *Nat. Cell Biol.* **15,** 1464–1472 CrossRef Medline
6. Carafoli, E. (2012) The interplay of mitochondria with calcium: an historical appraisal. *Cell Calcium* **52,** 1–8 CrossRef Medline
7. Wu, Y., Rasmussen, T. P., Koval, O. M., Joiner, M.-L. A., Hall, D. D., Chen, B., Luczak, E. D., Wang, Q., Rokita, A. G., Wehrens, X. H. T., Song, L.-S., and Anderson, M. E. (2015) The mitochondrial uniporter controls fight or flight heart rate increases. *Nat. Commun.* **6,** 6081 CrossRef Medline