Effects of glucose and its metabolites on calcium-induced mitochondrial permeability transition

Jan Škrha Jr., Juraj Gáll, Richard Buchal, Eva Sedláčková, Jan Pláteník

Institute of Medical Biochemistry, First Faculty of Medicine, Charles University in Prague, Czech Republic

Introduction
Diabetes mellitus represents one of the leading causes of morbidity and mortality worldwide. Mitochondrial production of reactive oxygen species (ROS) has recently emerged as a critical factor in the pathogenesis of long-term diabetic complications. According to the unifying mechanisms of these complications proposed by M. Brownlee [1], hyperglycemia-induced overproduction of ROS in mitochondria leads to inhibition of glyceroldehyde 3-phosphate dehydratase, and subsequent accumulation of upstream glycolytic intermediates activates all the known major pathways of hyperglycemic tissue damage, namely, the polypeptide, protein kinase C activation, hexoseamine pathway and production of the advanced glycation end products (AGEs).

Figure 1: The scheme of glycolysis, mitochondrial overproduction of reactive oxygen species and major pathways of hyperglycemic damage

In this scenario, however, there is no obvious reason why increased intracellular glucose oxidation per se should lead to overproduction of mitochondrial ROS. We consider possible involvement of the mitochondrial permeability transition (MPT).

MPT phenomenon consists of rearrangement of certain inner mitochondrial membrane proteins, creating a "megachannel" that allows passage of some mitochondrial proteins and collapses the proton motive force. The MPT pore opening is triggered by calcium, and modulated by numerous other agents: e.g. it is potentiated by mitochondrial depolarization, phosphatase, and ROS, but inhibited by low matrix pH, adenine nucleotides, and cyclosporine A. Transient "flickering" of the MPT pore is likely to play a role in cellular calcium homeostasis and calcium signaling, while a long-lasting MPT causing mitochondrial swelling, outer membrane rupture and release of cytochrome c has been widely implicated in various modes of cell death, especially necrosis.

Results
During the whole measurement, Glic 6-P maintained mitochondrial potential with succinate (+tutonate), as detected with the JC-1 probe. (Fig. 2)

Addition of 20 µM calcium chloride consistently induced rapid swelling of mitochondria, completely inhibited by cyclosporine A (Fig. 3 A), which qualifies the phenomenon as the genuine MPT. The initial swelling rate (30-120 sec. after Ca addition) was significantly slower with 30 µM glucose, 30 nM Glic 1-P and 6 nM MGO, but significantly faster in the presence of 30 mM Fru 6-P (Fig. 4 A). Unlike the other effective metabolites, the 30 mM glucose rather delayed the onset of MPT in response to calcium (Fig. 4 B).

Glic 6-P can regulate the MPT pore through binding to hexokinase or glucokinase that physically associate with the outer mitochondrial membrane in many cell types, but not in the liver - isolated rat liver mitochondria were devoid of glucokinase, which is in agreement with absent effects of Glic 6-P in our experiments. (Fig. 5)

Induction of MPT by calcium resulted in transient increase in H2O2 release that roughly coincided with the course of swelling and was not markedly affected by Glic 1-P (Fig. 6). In the presence of MGO the basal rate of ROS production appeared higher, but was comparable to control after correction for generation of ROS by MGO without mitochondrial, probably by redox cycling the traces of transition metals (Fig. 6 B).

Conclusions
• Methyglyoxal (6 mM), glucose 1-P (30 mM) and glucose 30 (mM) significantly inhibit calcium-induced MPT. In case of glucose, the effect can be described as a delayed onset of MPT.
• On the other hand, fructose 6-P (30 mM) significantly accelerates the calcium-induced MPT.

Discussion
To the best of our knowledge, the effects of glucose on the MPT of isolated normal mitochondria have not been studied. The delayed MPT in the presence of glucose we observed fits well to the work of Kristal et al. [2], where the mitochondria isolated from liver of streptozotocin-treated rats displayed also a delayed induction of MPT by calcium phosphate, which correlated with hyperglycemia of the animals. It is explicable by the reduced knowledge, our effects of glucose on the MPT of isolated normal mitochondria have not been studied. The delayed MPT in the presence of glucose we observed fits well to the work of Kristal et al. [2], where the mitochondria isolated from liver of streptozotocin-treated rats displayed also a delayed induction of MPT by calcium phosphate, which correlated with hyperglycemia of the animals. It is explicable by the reduced knowledge, glucose can also influence the mitochondrial calcium uniporter directly. The observed opposing effects of Glic 1-P and Fru 6-P on the MPT are novel, albeit of uncertain physiological significance as the 30 mM concentrations of these intermediates appear quite unlikely in vivo.

Glic 6-P is likely to affect the pore opening. As an example we reported as potent MPT inhibitor by Irwin et al. [3]. From all the compounds we tested, MGO appears as the most potent inhibitor of MPT as described by Speer [4]. Although MPT has been widely implicated in cell death, it is also involved in physiological calcium signaling where its inhibition would not be beneficial. The role of MPT is of particular relevance in long-term diabetic complications. Although MGO has been shown to act as metabolite and antioxidant in diabetics, it is yet to be discovered whether it is also involved in the pathophysiology of diabetes.

Figure 2: Effect of glucose, fructose, and phosphate on calcium-induced mitochondrial membrane potential of isolated rat liver mitochondria

References

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Corresponding author: Phone +420-224 964 275; Fax +420-224 964 280; jabed@f1.mff.cuni.cz