EXTENDED ABSTRACT

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KATP Channels Openers Are Capable of Brain Mitochondrial KATP Channel Opening on Nanomolar Scale Independent of MgATPase Activity

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Abstract

The abundance of mitochondrial ATP-dependent potassium channels (mKATP channels) in neurons implies important physiological role of ATP-sensitive potassium transport in nervous tissues. As it was shown by numerous studies, in CNS mKATP channel was a promising target for the treatment of metabolic stress conditions and neurodegenerative diseases (Busija et al., 2004; Correja et al., 2011). Protection of nervous tissues by mKATP channels openers - diazoxide, pinacidil, nicorandil (KCOs) largely is based on bioenergetic effects of mKATP channels opening, which estimation in brain mitochondria is required for the effective application of KCOs under pathophysiological conditions.

Complex alteration of mitochondrial bioenergetics (ROS production, Ca2+ transport and ATP synthesis) caused by the activation of energy-dissipating potassium cycle and so-called mild uncoupling, in turn is dependent on the sensitivity of mKATP channels to KCOs. Of mKATP channels openers, pinacidil and diazoxide are most widely used ones, but several off-target concentration dependent effects of both drugs were reported, such as inhibition of respiratory complexes I by pinacidil and II by diazoxide, and the inhibition of ATP synthase by diazoxide (Coetzee, 2013). It was remarkable that all reported off-target effects were caused by high micromolar concentrations of KCOs.

From the literature, KATP channels are octameric complexes, which possess four K+ conductant (Kir in sarcolemmal KATP channels) and four receptor subunits (SUR). KATP channels opening by KCOs require the binding of the drugs to the SUR subunit of KATP channels. From the studies on sarcolemmal KATP channels, it is generally known that SUR subunit possesses intrinsic MgATPase activity. So, in numerous studies on isolated mitochondria, it was generally assumed that the presence of Mg2+ and ATP was required for mKATP channel opening by KCOs. However, literary data obtained on isolated mitochondria were controversial, and in heart mitochondria the activation of ATP-sensitive K+ transport in the absence of MgATP as well was reported.

Recently (Akopova et al., 2020) we have shown that native liver mKATP channel was activated by diazoxide on sub-micromolar scale in the absence of MgATP, which implies that MgATPase activity was not a prerequisite for mKATP channel activation by diazoxide. So, the aim of this work was to study the effect of diazoxide and pinacidil on mKATP channel activity in isolated brain mitochondria in the absence and the presence of MgATP.

Methods: The study was conducted on isolated rat brain mitochondria. Potassium transport and mKATP channel activity were assessed indirectly using light scattering technique.

Results: Without MgATP, we obtained strong evidence of high sensitivity of brain mKATP channel to diazoxide and pinacidil with full activation at <0.5 μM of the drugs. Neither Mg2+, nor ATP alone affected the channels affinity to the drugs, but MgATP shifted it to micromolar concentration level, which agreed with literary data. By our earlier estimations (Akopova et al., 2014), in native brain mitochondria V0 of ATP-sensitive K+ transport constituted ~40 nmol K+·min−1·mg−1. As we have found in this work, KCOs increased mKATP channel activity by ~1.5-2 times, so that V0 of ATP-sensitive K+ transport when activated by KCOs could reach up to ~80 nmol K+·min−1·mg−1.

To ascertain full channel activation without MgATP, it was specifically blocked by MgATP with consequent activation by KCOs in micromolar concentrations range, 30 μM. Conventional blocking of the activated channel by glibenclamide and 5-hydroxydecanoate (Jaburek et al., 1998) gave the same estimate of maximal channel activity proving KCOs' ability to elicit full activation on nanomolar scale without MgATP, thus independent of MgATPase activity.

One crucial issue of our work was molecular identification of mitochondrial ATP-sensitive K+ transport by pharmacological means. From the studies on sarcolemmal (Foster and Coetzee, 2016) and mitochondrial KATP channels (Garlid et al., 1996; Jaburek et al., 1998), native mKATP channel is blocked by MgATP, and activated mKATP channel is blocked by KATP channels blockers (glibenclamide and 5-hydroxydecanoate) in the presence of MgATP. So, to prove identity of mKATP channel on pharmacological level, we developed an approach of combined blocking of ATP sensitive K+ transport by MgATP and specific blockers of KATP channels glibenclamide and 5-HD. As we observed, in the presence of MgATP, no additional blocking of both native and activated ATP-sensitive K+ transport by either glibenclamide or 5-HD was observed. When mKATP channel was activated in the presence of MgATP, the same blocking effect of glibenclamide and 5-HD was obtained, which proved pharmacological identity of ATP-sensitive K+ transport studied in our work with mKATP channel activity. Thus, the properties of ATP-sensitive K+ transport studied in this work could be ascribed to the same molecular entity known as 'mKATP channel', however, based on the present knowledge, mitochondria can possess more than one type of ATP-sensitive K+ conductance (Foster et al., 2012; Paggio et al., 2019)..

Discussion: While it is generally assumed that pharmacological mKATP channels openers (KCOs) require MgATPase activity for mKATP channel opening, our data obtained on liver (Akopova et al., 2020) and brain mitochondria showed that MgATPase activity was dispensable for mKATP channels activation by diazoxide and pinacidil. Based on our experiments we came to the following conclusions: 1) high sensitivity of mKATP channels to KCOs with full channel activation at <0.5 μM of the

drugs independent of MgATPase activity is one common property of native mKATP channels; 2) neither Mg2+, nor ATP alone affect the mKATP channels affinity to KATP channels openers, but the presence of MgATP shifts it to much higher micromolar concentration level; 3) native mKATP channel can comprise the sites with high affinity to diazoxide and pinacidil screened by the binding of MgATP.

Obtained results indicate novel common features in the mechanism of native mKATP channel activation by pharmacological openers and allow us hypothesize that under ATP deficiency, which is a hallmark of many pathophysiological conditions, affinity of mKATP channel to KCOs can increase several times, which might explain high efficiency of these drugs for neuroprotection.