Mitochondrial inhibition and oxidative production differs between pharmacologic models of Parkinson’s disease
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Mitochondrial dysfunction is now clearly implicated in Parkinson’s disease (PD) pathogenesis. This is evidenced by the fact that mitochondrial inhibitors cause PD-like symptoms in rodent models and humans (1, 2). Inhibition of mitochondrial function may be caused by increased reactive oxygen species (ROS) production. Additionally, increased ROS production may be caused by inhibition of mitochondrial function. The cause/effect relationship is unclear.

To determine if PD-like symptoms are caused by a common mechanism in pharmacologic models of PD, cultured, intact, dopaminergic cells were treated with the PD mimetics 1-methyl-4-phenylpyridinium (MPP+), rotenone, 6-hydroxydopamine (6-OHDA), and paraquat. We hypothesized that there would be no direct relationship between inhibition of mitochondrial function and increased production of ROS in these models of PD.

Methods

Model PD Compounds

Figure 1. Seahorse XF24 Analysis technology utilized in the study of in vitro PD model compounds. Model PD Compounds

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Figure 2. Seahorse XF24 technology platform. The Seahorse XF24 platform is a high-throughput platform that measures metabolic activity and oxygen consumption in real time for up to 96 samples in parallel. The system measures oxygen consumption rates, extracellular acidification rates, and extracellular pH over extended time periods against each other for the analysis of the experiment. Aromatic acidification is representative of a shift in glycolytic metabolism in response to MPP+ and paraquat. For clarity, the ESI baseline measurements and statistical significance levels are shown from the mean ± SD (n=3 per treatment group).

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Results

Figure 3: MPP+ and rotenone inhibit mitochondrial function and stimulate a shift in metabolic profile compared to untreated controls. For clarity, the ESI baseline measurements and statistical significance levels are shown from the mean ± SD (n=3 per treatment group). For clarity, only one probe was expressed in response to MPP+ and paraquat.

Conclusions

In preliminary experiments, MPP+ (150 µM), rotenone (1 µM), and 6-OHDA (100 µM) decreased mitochondrial oxygen consumption within 4 h, while paraquat (500 µM) modestly stimulated basal oxygen consumption. Further analysis revealed that the decrease in oxygen consumption by MPP+, rotenone, and 6-OHDA was due to inhibition of ATP-linked oxygen consumption. Rotenone inhibited mitochondrial function within minutes of treatment, whereas treatment with MPP+ required 3 h to achieve maximal inhibition. 2-hydroxyethidium (2-OH-E+), the specific product of the reaction of HE and superoxide, was increased 2-fold as compared to control in cells treated with 150 µM MPP+ or 1 µM rotenone, but not in cells treated with 6-OHDA (100 µM).

Taken together, these studies demonstrate for the first time that ROS production and mitochondrial dysfunction are not mechanistically linked in models of PD.

References


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