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Research report

Metformin prevents cerebellar granule neurons against glutamate-induced neurotoxicity

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ABSTRACT

Metformin, a wildly used drug for type 2 diabetes, has recently been proven to protect a variety of cells from stress including stroke. Glutamate is an excitatory neurotransmitter that contributes to excitatory neuronal damage involved in stroke and neurodegenerative disorders. In this study, we demonstrated that pretreatment of rat cerebellar granule neurons (CGN) with metformin greatly enhanced cell viability against glutamate-induced neurotoxicity. Metformin significantly attenuated neuronal apoptosis in glutamate-treated CGN by reducing cytochrome c releasing, caspase-3 activation and phosphorylation of MAP kinases. Our results suggested that metformin was able to directly inhibit glutamate induced excitotoxicity in neurons and might be beneficial to patients suffered from stroke and neurodegenerative disorders.

1. Introduction

Metformin is a biguanide compound commonly used to treat type 2 diabetes. This compound activates cellular energy sensor adenosine monophosphate kinase (AMPK) and subsequently inhibits the mammalian target of rapamycin (mTOR) pathway that leads to inhibition of energy genesis, protein synthesis and cell proliferation (Rena et al., 2013). Recently, it was demonstrated that chronic, but not acute metformin treatment was associated with a lower risk of stroke and this protection was independent of its glucose-lowering effect (Li et al., 2010; UK Prospective Diabetes Study (UKPDS) Group, 1998). Although AMPK was reported to play a role in metformin-induced endothelial protection (Eriksson and Nystrom, 2014), it remained unknown how chronic treatment of metformin protected brains against ischemic injury and whether metformin was able to directly protect neurons.

Glutamate is the principal excitatory neurotransmitter in the brain and can also be an excitotoxic agent in inappropriate conditions such as excessive release or reduced clearance (Jacintho and Kovacic, 2003). Excitotoxicity, induced by excessive activation of glutamate receptors, has been suggested to underlie the neuronal death in stroke, traumatic brain injury, and neurodegenerative disorders (Lai et al., 2014). Previous studies indicated that excitotoxicity induced by overstimulation of glutamate receptors occurred via prolonged rise in intracellular Ca^{2+} (Montal, 1998) and caspase 3 activation (Du et al., 1997). Glutamate also induced phosphorylation of p38 and JNK in different types of neurons (Wang et al., 2004b; de Groot and Sontheimer, 2011) and both active JNK and p38 mediated caspase 3-independent excitotoxicity in cultured cerebellar granule neurons (CGN) (Ferrer et al., 2001). In the present study we found that pretreatment of metformin was able to attenuate glutamate-induced apoptosis in CGN by blocking glutamate-induced caspase 3 activity and phosphorylation of JNK or p38. Our data strongly suggested that metformin could directly protect CGN against glutamate-induced neuronal apoptosis by inhibiting either caspase 3-dependent or −independent JNK/p38 pathways.

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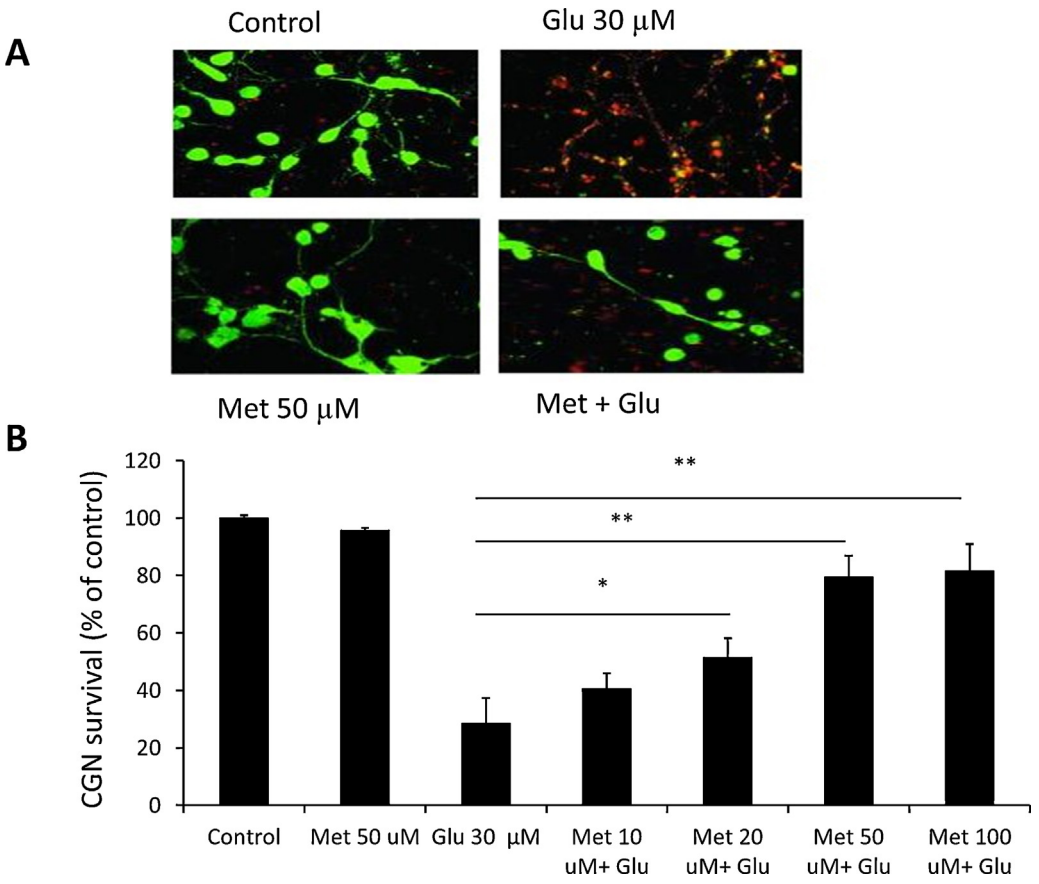

Fig 1. Metformin blocked glutamate-induced cerebellar granule neurons (CGNs) death. CGNs were pretreated with 10, 20, 50, and 100 μM metformin (Met) for 6 h, then exposed to 30 μM glutamate (Glu) for 18 h. Viable CGNs were visualized as fluorescein (green) positive cells. Dead cells were visualized as red positive cells. (B) Metformin protected against glutamate-induced CGN death in a concentration-dependent fashion with an EC50 of approximately 50 μM. Bars represent the mean ± SEM of quadruplicate wells from a single experiment repeated three times with similar results (Control set to 100%, Student *t*-test, n = 3, $^{*}p < 0.05$, $^{**}p < 0.01$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

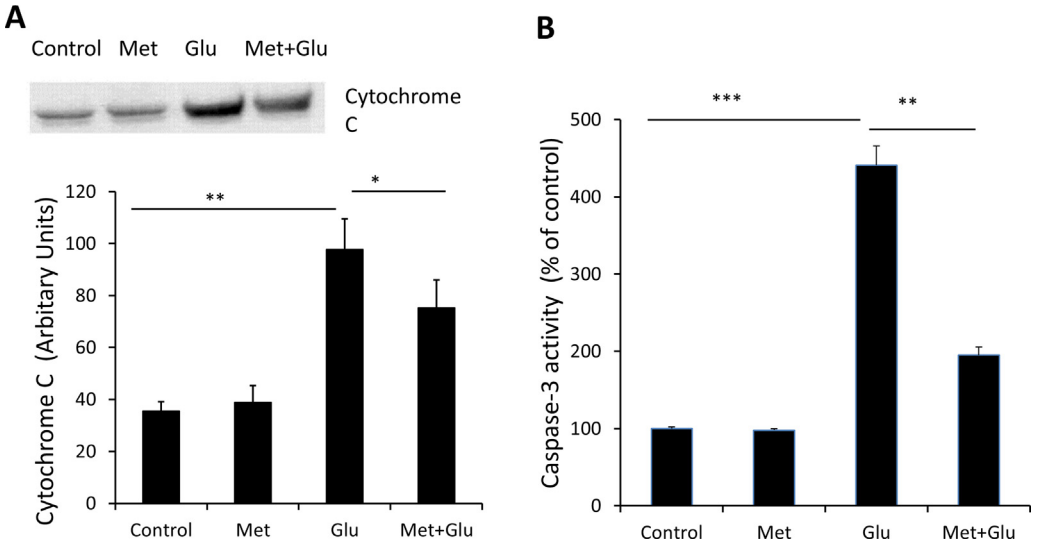

Fig. 2. (A). Metformin inhibited glutamate-induced caspase-3 activation. CGN was pretreated with 50 μM metformin for 6 h, and then co-incubated with 30 μM glutamate for 18 h. Caspase-3 activity was examined after 24 h metformin incubation. Caspase-3 activity in Control was set to 100%. The data represent the mean ± SEM of triplicate determinations from a representative experiment repeated at least three times with similar results ($^{*}p < 0.05$ by Student's *t*-test). (B). Metformin pretreatments attenuate glutamate-induced caspase-3 activation in vitro. caspase-3 protease activity in cultured CGNs is induced by glutamate. The data represent the mean ± SEM of triplicate determinations from a representative experiment repeated at least three times with similar results (Student *t*-test, n = 3, $^{**}p < 0.01$).