The mitochondrial complex one inhibitor R419 potently stimulates AMPK in dorsal root ganglion neurons and reduces incision-evoked pain in vivo

Theodore Price, Galo Mejia, Marina Asiedu, Yasunori Hibi and Gregory Dussor
The University of Texas at Dallas and Riget Pharmaceuticals

Adenosine monophosphate activated protein kinase (AMPK) is a ubiquitous protein kinase that negatively regulates anabolic pathways such as mechanistic target of rapamycin (mTOR) and mitogen activated protein kinase (MAPK). AMPK activity can be augmented by increasing AMP levels in cells, by positive allosteric modulation or by protection from hypophosphorylation. R419 is a prototypical AMPK activator that interferes with mitochondrial complex one to raise AMP levels in cells and activate AMPK. R419 is a potent complex one inhibitor that has been proposed to activate AMPK in somatic cells leading to both metabolic and cellular effects. We tested whether R419 can activate AMPK in dorsal root ganglion (DRG) neurons and if the compound modulates pain hypersensitivity in vivo. Other lines of evidence suggest that AMPK activation can alleviate pain and pain hypersensitivity but potent complex one inhibitors like R419 have not been tested in this context. We find that R419 stimulates AMPK activity and blocks MAPK activity in DRG neurons at concentrations as low as 300nM but has little effect on mTOR signaling. With similar potency, R419 inhibits nascent protein synthesis and induces p body formation in DRG neurons, two signaling events that are induced by other AMPK activators. Moreover, R419 reduces the excitability of DRG neurons exposed to nerve growth factor (NGF) and stimulated with slowly depolarizing ramp currents. Finally we tested if R419 influences NGF- or incision-evoked mechanical hypersensitivity and hyperalgesia priming in vivo. R419 co-treatment with NGF blocked mechanical hypersensitivity and hyperalgesic priming in a dose-dependent fashion. R419 treatment by local injection at the time of plantar incision and 24 hrs after also attenuated incision-evoked mechanical hypersensitivity and completely blocked hyperalgesic priming. Pharmacokinetic data from hindpaw samples indicate that R419 has a tissue half-life of approximately 1 or 2 hrs. We conclude that R419 potently activates AMPK in DRG neurons resulting in decreased MAPK activity and cellular excitability and that R419 reduces pain hypersensitivity and the transition to a chronic pain-like state in vivo.

Conclusions
1) R419 specifically inhibits complex one to activate AMPK, resulting in ERK inhibition and reduced nascent protein synthesis in DRG neurons.
2) R419 inhibits NGF-induced pain in vivo and reduces nociceptor excitability in vitro.
3) R419 has a short tissue half-life in vivo but still reduces incision-induced pain hypersensitivity and hyperalgesic priming.

We conclude that R419 is a promising AMPK activator for the alleviation of post surgical pain via a local mechanism of action.

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