



The Drug Salicylate as an Activator of AMP-Activated Protein Kinase (AMPK) in the Heart and its Possible Cardioprotective Effects Against Ischemia/Reperfusion Injury

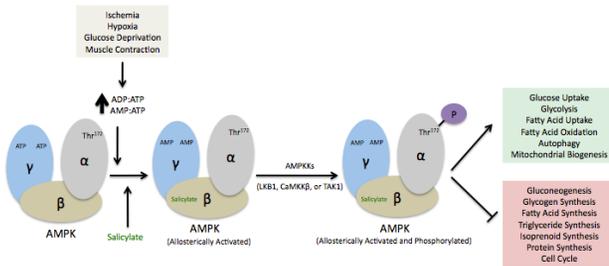


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Introduction

AMP-Activated Protein Kinase (AMPK) is an enzyme that modulates and senses cellular energy status. Activation of AMPK in the heart has been shown to protect against myocardial infarction, and therefore interest has grown about pharmacological activators of AMPK as possible treatments for ischemic heart disease. Recent research has demonstrated the drug salicylate activates AMPK in liver and colorectal cancer cells. The goal of our study is to determine if salicylate activates AMPK in the heart, and to determine if salicylate is cardioprotective against ischemia/reperfusion injury by augmenting AMPK signaling.



Methods

In vivo dose- and time-dependent activation of AMPK

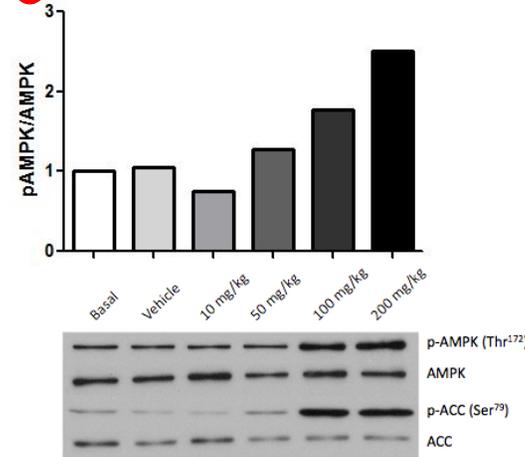
C57BL/6J mice were anesthetized and given tail vein injections of salicylate (or vehicle). For dose-dependence the hearts were collected 30 minutes after drug administration and analyzed by Western Blot for protein levels. For time-dependence the mice were given a dose of 100 mg/kg salicylate and the hearts were collected and analyzed for protein levels at the given times after drug administration.

In vivo regional ischemia/reperfusion and myocardial infarct size measurement

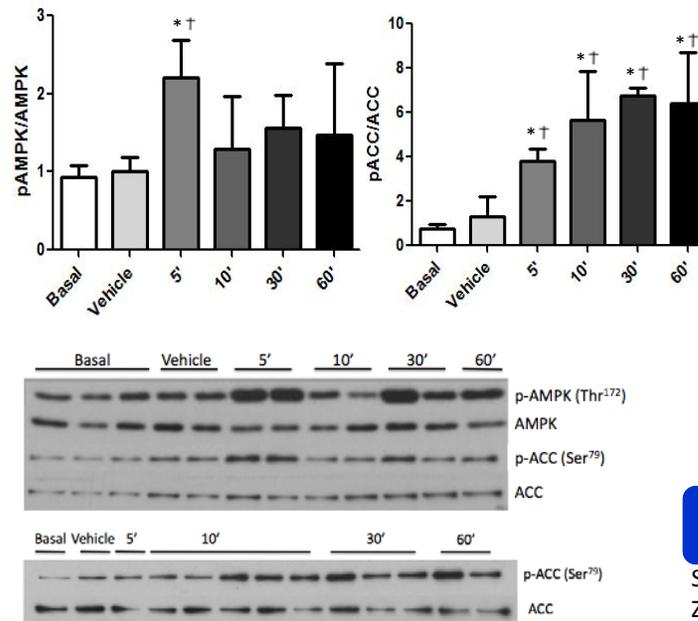
C57BL/6J mice were anesthetized, intubated, and ventilated with a respirator (Harvard apparatus, Holliston, MA). After left lateral thoracotomy, the left anterior descending coronary artery (LAD) was occluded for 20 minutes with an 8-0 nylon suture and reperused for 4 hours. Vehicle or salicylate was administered via tail vein injection 5 minutes before reperfusion. ECGs confirmed ischemic hallmark ST-segment elevation during coronary artery occlusion. At reperfusion conclusion hearts were excised for dual staining. Non-necrotic tissue in the ischemic region was stained red by 2,3,5-triphenyltetrazolium (TTC) and non-ischemic regions were stained blue with Evan's blue dye. Hearts were fixed and sectioned into 1 mm slices, photographed using a Lexica MZ95 microscope. Myocardial infarct size was calculated as the ratio of myocardial necrosis to ischemic area at risk (AAR) percentage.

Results

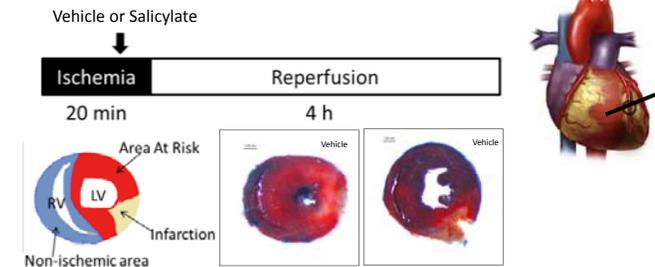
1 Salicylate dose-dependently activates AMPK in vivo



2 Salicylate time-dependent AMPK activation is less clear



3 In vivo regional ischemia/reperfusion and myocardial infarct size measurement



Conclusions and Future Directions

•Salicylate shows promise to activate AMPK in the heart in basal conditions in a dose-dependent manner but more samples are needed to confirm
•Salicylate appears to show significant allosteric activation of AMPK within five minutes of administration, however, the time-dependence data is unclear and will need more samples and consistency before a conclusion can be drawn
•Future experiments include:

1. Increase sample size of dose- and time-dependence AMPK activation by salicylate
2. Vehicle collection at 5, 10, and 60 minutes in addition to 30 minutes
3. Positive control (A-769662)
4. Determine if salicylate reduces myocardial infarction from ischemia/reperfusion injury
5. Determine if salicylate's cardioprotective effects are mediated by AMPK or its other pathways (use AMPK KD mice, β_1 KO mice, Western Blot analysis of AMPK activation in the ischemia/reperfusion model heart)

Acknowledgments

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