Resveratrol engages AMPK to attenuate ERK and mTOR signaling in sensory neurons and inhibits incision-induced acute and chronic pain

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Abstract

Background

Despite advances in our understanding of basic mechanisms driving post-surgical pain, treating incision-induced pain remains a major clinical challenge. Moreover, surgery has been implicated as a major cause of chronic pain conditions. Hence, more efficacious treatments are needed to inhibit incision-induced pain and prevent the transition to chronic pain following surgery. We reasoned that activators of AMP-activated protein kinase (AMPK) may represent a novel treatment avenue for the local treatment of incision-induced pain because AMPK activators inhibit ERK and mTOR signaling, two important pathways involved in the sensitization of peripheral nociceptors.

Results

To test this hypothesis we used a potent and efficacious activator of AMPK, resveratrol. Our results demonstrate that resveratrol profoundly inhibits ERK and mTOR signaling in sensory neurons in a time- and concentration-dependent fashion and that these effects are mediated by AMPK activation and independent of sirtuin activity. Interleukin-6 (IL-6) is thought to play an important role in incision-induced pain and resveratrol potently inhibited IL-6-mediated signaling to ERK in sensory neurons and blocked IL-6-mediated allodynia in vivo through a local mechanism of action. Using a model of incision-induced allodynia in mice, we further demonstrate that local injection of resveratrol around the surgical wound strongly attenuates incision-induced allodynia. Intraplantar IL-6 injection and plantar incision induces persistent nociceptive sensitization to PGE2 injection into the affected paw after the resolution of allodynia to the initial stimulus. We further show that resveratrol treatment at the time of IL-6 injection or plantar incision completely blocks the development of persistent nociceptive sensitization consistent with the blockade of a transition to a chronic pain state by resveratrol treatment.

Conclusions

These results highlight the importance of signaling to translation control in peripheral sensitization of nociceptors and provide further evidence for activation of AMPK as a novel treatment avenue for acute and chronic pain states.

Incision associated with surgery causes acute pain and surgery has been identified as a potential major cause of chronic pain conditions. Between 10 and 50% of patients develop chronic pain following surgical procedures and despite improvements in post-surgical pain treatment strategies, the incidence of moderate to severe pain after surgery is still high in several patient populations. Moreover, the exact mechanisms involved in the development of persistent pain following surgery have not been elucidated. Interleukin 6 (IL-6), a pro-inflammatory cytokine, is a significant mediator of nociceptive plasticity in pre-clinical pain models and is implicated in several human pain conditions. IL-6 levels in serum and skin around the incision increase significantly in patients immediately after surgery and circulating IL-6 levels are proportional to the extent of tissue injury during an operation, rather than being proportional to the duration of the surgical procedure itself. Although these reports are suggestive of involvement of IL-6 in post-surgical pain, the precise mechanisms by which IL-6 drives post-surgical pain are poorly understood.

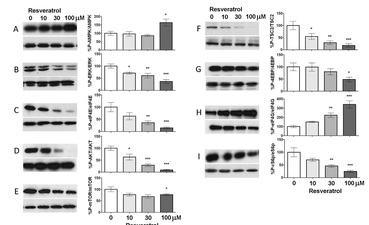
Recently we demonstrated that IL-6 causes induction of nascent protein synthesis in primary afferent neurons and their axons which can contribute to increased nociceptive sensitivity. We have also shown that AMP-activated protein kinase (AMPK) activators reverse mechanical allodynia in neuropathic pain models and that these compounds negatively regulate protein synthesis in sensory afferents. AMPK, the energy sensor of the cell, is a heterotrimeric Ser/Thr protein kinase activated by alterations in cellular AMP: ATP ratio. Once activated, AMPK inhibits ATP consuming anabolic processes such as protein translation. AMPK activation achieves these effects largely through inhibition of mammalian target of rapamycin (mTOR) signaling but AMPK activation has also been linked to inhibition of mitogen activated protein kinase (MAPK) signaling.

HYPOTHESIS - Activation of the AMPK signaling pathway may represent a novel pharmacological mechanism for the treatment of post-surgical pain.

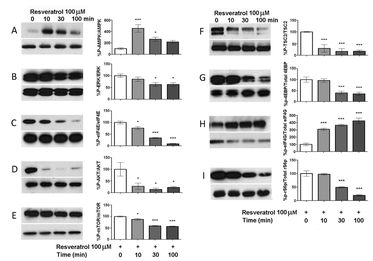
Results

Resveratrol suppresses signaling to translation machinery in sensory neurons

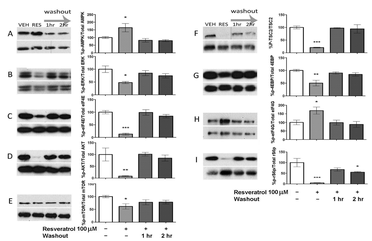
1.1 Resveratrol suppresses ERK and mTOR signaling in sensory neurons in a concentration-dependent manner.



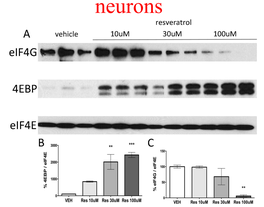
1.2 Suppression of ERK and mTOR signaling by resveratrol is time dependent.



1.3 Suppression of ERK and mTOR signaling by resveratrol is reversible



1.4 Resveratrol suppresses eIF4F complex formation in sensory neurons

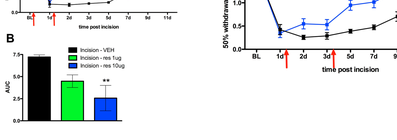


Resveratrol - mediated inhibition of ERK and mTOR does not require Sirt1

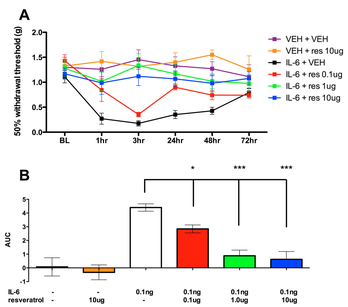
Antibody	Vehicle	nicotinamide 10 mM + resveratrol 100 μM	resveratrol 100 μM
p-AMPK/AMPK	100 ± 7.3	199.8 ± 28.1	222.4 ± 40.0
p-ERK/ERK	100 ± 11.8	41.8 ± 8.5 **	47.2 ± 8.8 **
p-eIF4E/eIF4E	100 ± 5.4	27.6 ± 4.7 ***	30.2 ± 6.2 ***
p-AKT/AKT	100 ± 6.2	11.6 ± 3.1 ***	12.5 ± 2.4 ***
p-mTOR/mTOR	100 ± 4.4	70.6 ± 4.4 *	62.9 ± 6.4 **
p-TSC2/TSC2	100 ± 8.3	26.6 ± 4.2 ***	36.5 ± 5.2 ***
p-4EBP/4EBP	100 ± 2.3	38.5 ± 3.0 ***	37.9 ± 7.2 ***
p-rS6p/rS6p	100 ± 7.0	33.9 ± 5.9 ***	36.5 ± 6.8 ***

Antibody	Vehicle	CAY10602 20 μM	CAY10602 60 μM
p-AMPK/AMPK	100 ± 13.6	95.0 ± 14.4	107.3 ± 16.6
p-ERK/ERK	100 ± 6.50	117.2 ± 5.91	126.4 ± 3.32 *
p-eIF4E/eIF4E	100 ± 13.0	105.9 ± 17.1	102.0 ± 17.0
p-AKT/AKT	100 ± 11.8	105.8 ± 5.0	107.2 ± 7.5
p-mTOR/mTOR	100 ± 8.8	103.8 ± 17.0	125.7 ± 18.5
p-TSC2/TSC2	100 ± 3.3	97.8 ± 6.8	115.1 ± 5.2
p-4EBP/4EBP	100 ± 8.3	126.2 ± 11.1	122.9 ± 8.5
p-eIF4G/eIF4G	100 ± 14.1	76.2 ± 13.8	109.0 ± 16.7
p-rS6p/rS6p	100 ± 12.5	120.3 ± 6.8	134.0 ± 10.5

Resveratrol blocks IL-6 induced signaling in sensory neurons

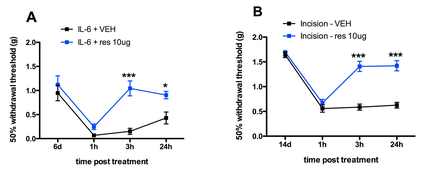


Resveratrol blocks IL-6 induced acute allodynia in a dose dependent manner

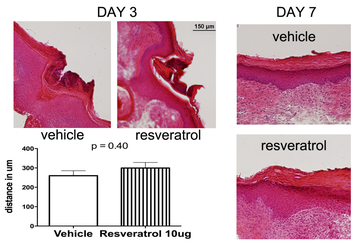


Resveratrol inhibits allodynia in a mouse model of post-surgical pain

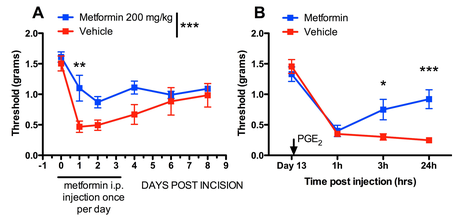
Resveratrol blocks IL-6- and plantar incision-induced persistent sensitization



Resveratrol has no effect on wound healing



Metformin treatment inhibits post-surgical allodynia and the transition to chronic pain



Conclusions

- AMPK activators reduce allodynia resulting from incision
- AMPK activators block IL-6-induced signaling
- AMPK activators attenuate hyperalgesic priming provoked by surgical incision
- Collectively, these findings suggest that AMPK activators might be used clinically for the treatment of post-surgical pain with potential beneficial effects on development of chronic pain

Aknowledgements

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