Highly potent agonists reveal Protease Activated Receptor Type 2 (PAR_2)-dependent hyperalgesic priming relying on central trkB/aPKC maintenance mechanisms

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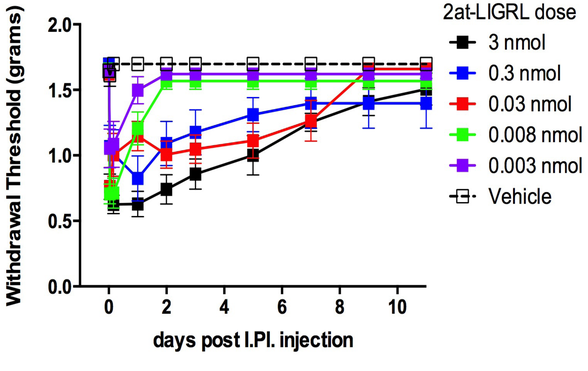
Abstract

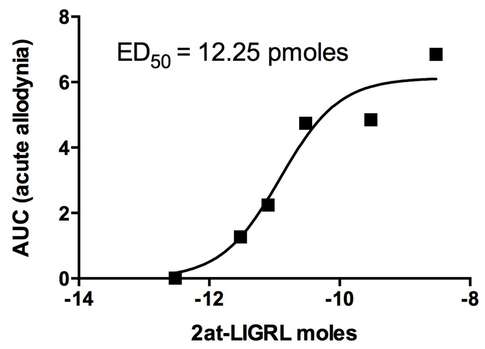
Protease Activated Receptor Type 2 (PAR2) is a G-protein coupled receptor (GPCR) containing a tethered ligand in the N-terminal domain that is exposed upon protease digestion of the N-terminal domain. This peptide sequence, SLIGRL in rodents, has served as a basis for peptide ligand discovery at the native receptor capable of bypassing proteolytic cleavage of the N-terminal domain. We have developed a wide range of highly potent and efficacious agonists to probe PAR2 function in vitro and in vivo. PAR2 is thought to play an important role in inflammatory- and cancer-evoked pain based on studies using PAR2-/- mice. Recently hyperalgesic priming has emerged as important model system for probing plasticity in the nociceptive system. We have shown that the maintenance of hyperalgesic priming evoked by a single injection of interleukin-6 relies on a dorsal horn signaling axis involving Brain Derived Neurotrophic Factor (BDNF) signaling via trkB to atypical PKC (aPKC). Here we have tested the hypothesis that specific activation of PAR2 should be capable of evoking hyperalgesic priming. We have further tested whether the maintenance of this priming involves a BDNF/trkB/aPKC signaling axis. We find that intraplantar injection of the potent and specific PAR2 agonist, 2-aminothiazol-4-yl-LIGRL-NH2 (2at-LIGRL), evokes a long-lasting acute allodynia (EC50 ~ 0.03 nmoles) that is followed by a profound hyperalgesic priming to subsequent prostaglandin E2 (PGE2) injection. The pro-allodynic effect of 2at-LIGRL is completely absent in PAR2-/- mice as is hyperalgesic priming. Hence, stimulation of PAR2 is sufficient to evoke hyperalgesic priming in mice. We then asked if the maintenance of this hyperalgesic priming can be reversed by inhibition of BDNF/trkB/aPKC signaling. Systemic dosing with the trkB antagonist ANA-12 (0.5 mg/kg) following the resolution of acute 2at-LIGRL-induced allodynia inhibited priming precipitated by PGE2 injection into the hindpaw. Likewise, injection of the aPKC inhibition, ZIP, into the lumbar spinal cord completely reversed the maintenance of priming over the same time course. Hence, PAR2 activation is sufficient to evoke hyperalgesic priming. Moreover, the maintenance of this primed state is dependent on a CNS BDNF/trkB/aPKC signaling axis suggesting a generalized role for this signaling pathway in maintenance of hyperalgesic priming.

Results

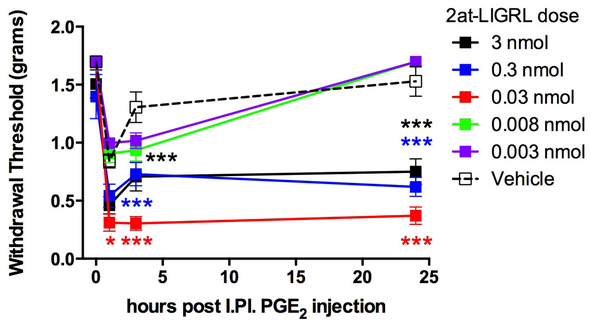
The potent PAR_2 agonist 2-aminothiazole-LIGRL (2at-LIGRL) induces mechanical allodynia in mice



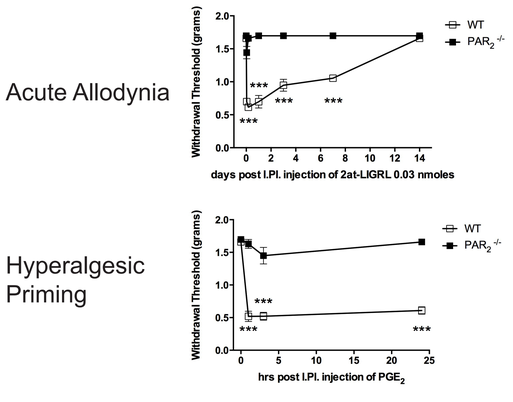
2at-LIGRL ED_{50} is in the low picomole range



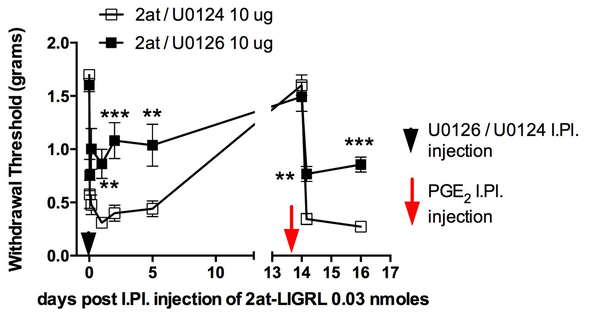
2at-LIGRL induces hyperalgesic priming to subsequent PGE_2 exposure



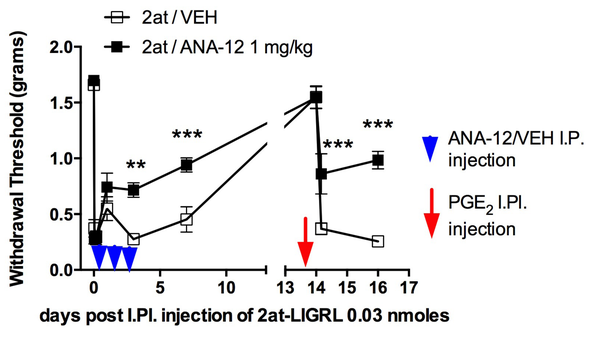
2at-LIGRL effects are PAR_2-dependent



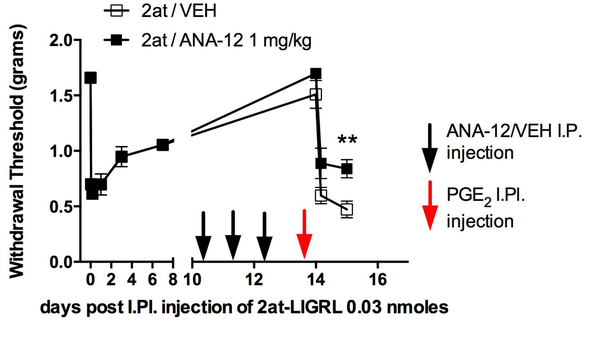
PAR_2-mediated allodynia and hyperalgesic priming initiation depends on peripheral ERK activity



Blockade of TrkB receptors with ANA-12 attenuates initation of PAR_2-mediated allodynia and hyperalgesic priming



Blockade of TrkB receptors with ANA-12 interferes with the maintenance of PAR_2-mediated hyperalgesic priming



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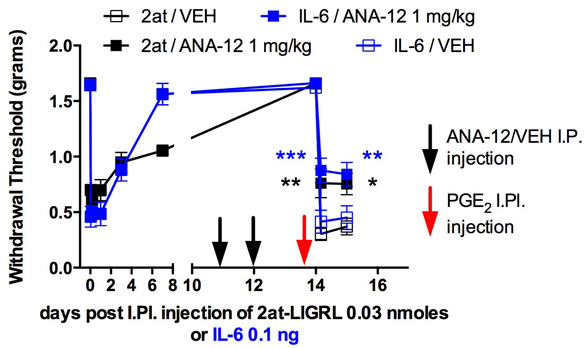
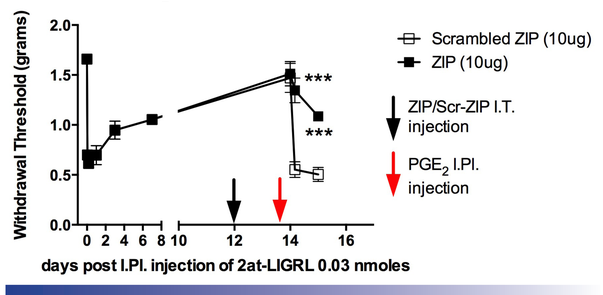
Blockade of TrkB receptors with ANA-12 interferes with the maintenance of PAR_2-and IL-6-mediated hyperalgesic priming to a similar extent



The aPKC inhibitor ZIP reverses the maintenance of PAR_2-mediated hyperalgsic priming



Conclusions

- PAR_2 activation is sufficient to induce hyperalgesic priming
- PAR_2-mediated allodynia is ERK-dependent
- Blockade of TrkB receptors blunts PAR_2-mediated effects suggesting a CNS role for BDNF in PAR_2-induced pain plasticity
- Inhibition of spinal aPKCs with ZIP reverses hyperalgesic priming for multiple priming stimuli

Aknowledgements

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