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The slowly signaling G protein-biased CB_2 cannabinoid receptor agonist LY2828360 suppresses chemotherapy-induced neuropathic pain with sustained efficacy and attenuates morphine tolerance and dependence

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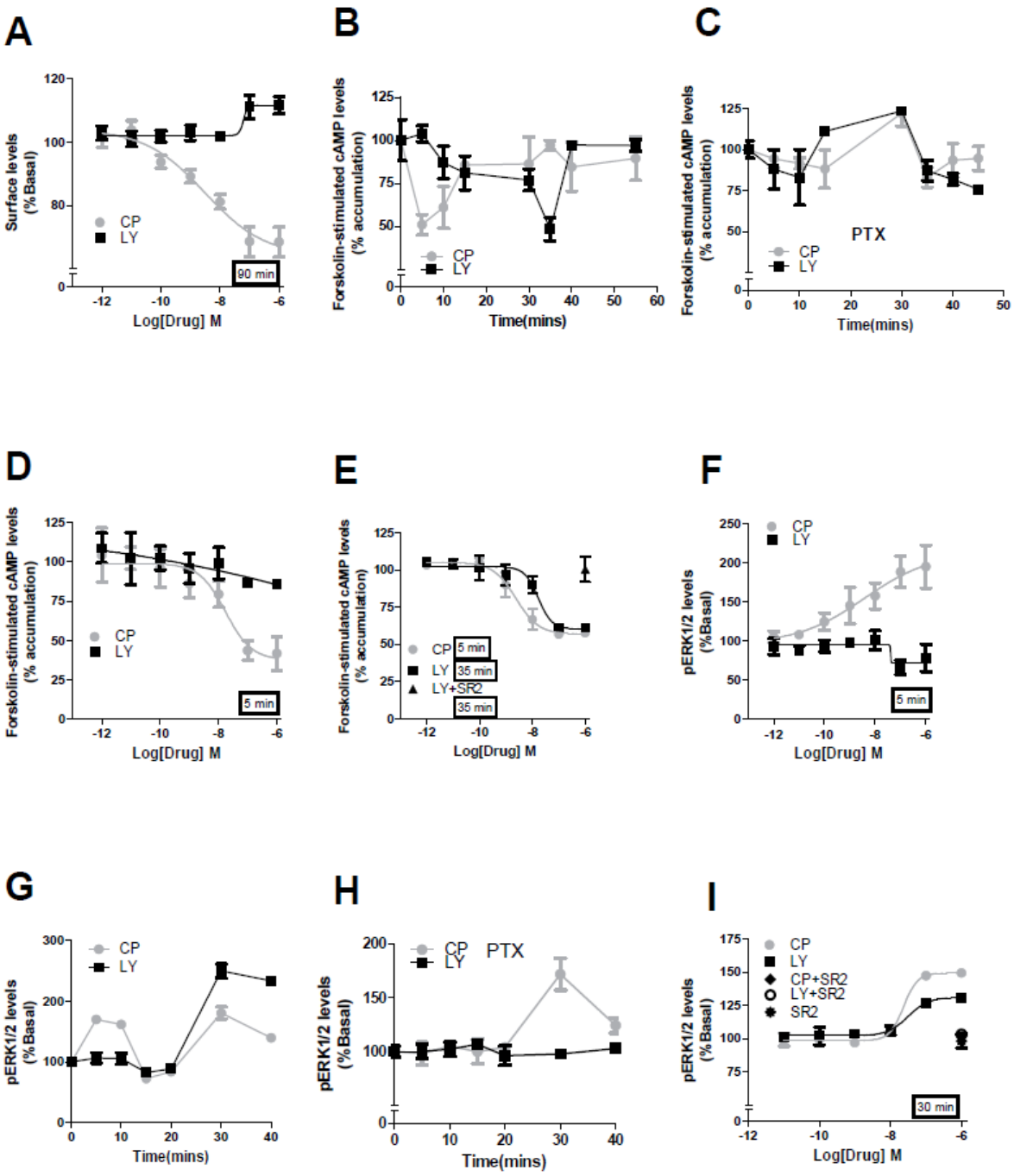
Supplemental Figure legends

S1: LY2828360 displays a delayed signaling profile at human CB2 receptors. A) In HEK cells stably expressing human CB2 receptors, LY2828360 failed to internalize the receptor; B) In a forskolin-stimulated cAMP time course assay, CP55940 (1 μM) inhibited cAMP accumulation at 5 minutes, while LY2828360 (1 μM) displayed a similar efficacy only after 35 minutes of agonist incubation; C) Pertussis toxin pretreatment abolished this effect at all the time points tested/examined for both drugs; D) After 5 minutes of treatment, CP55940 was potent and efficacious in inhibiting forskolin-stimulated cAMP accumulation while LY2828360 had no effect; E) After 35 minutes, LY2828360 was a potent and efficacious agonist in inhibiting forskolin-stimulated cAMP accumulation and this inhibition was completely blocked by a CB2 receptor antagonist, SR144528; F) In the pERK1/2 assay, CP55940, at 5 min time point, was potent and efficacious in increasing ERK1/2 phosphorylation, while LY2828360 was ineffective; G) Examination of a time course of ERK1/2 phosphorylation revealed that LY2828360 (1 μM) increased ERK1/2 phosphorylation after 30 minutes, but not at 5 minutes. In contrast, CP55940 (1 μM) efficaciously increased ERK1/2 phosphorylation after 5, 10, and 30 minutes; H) Pertussis toxin treatment abolished ERK1/2 phosphorylation after treatment with LY2828360 (1 μM), while CP55940-stimulated phosphorylation of pERK1/2 after 30 minutes was retained; I) LY2828360- and CP55940-stimulated phosphorylation of ERK1/2 was completely blocked by SR144528 (1 μM) (SR2). Forskolin-stimulated cAMP assays were performed in duplicates. All other assays were performed in triplicates. All experimental data were plotted and analyzed using GraphPad Prism 4.

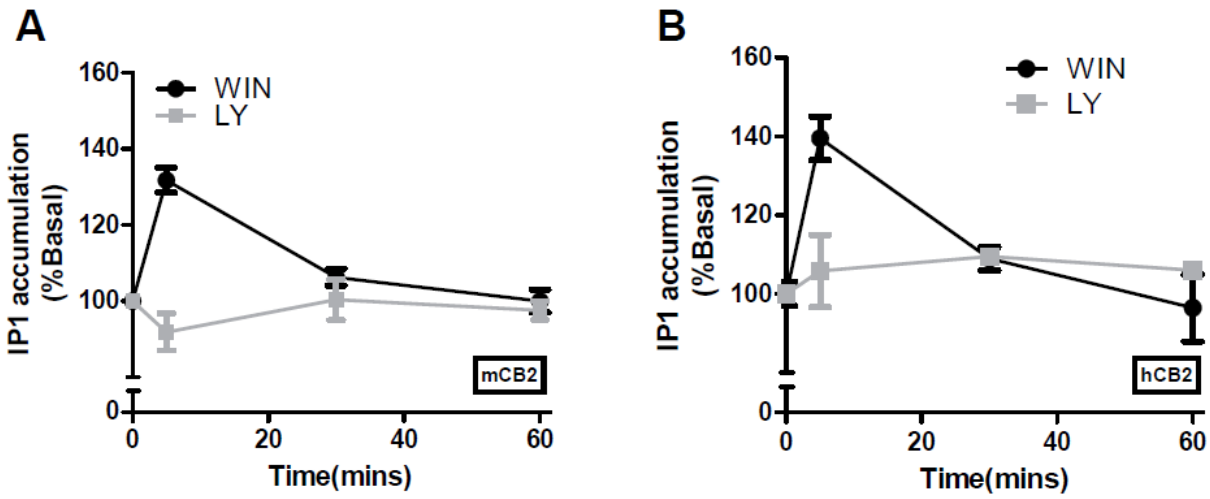
S2: LY2828360 failed to affect IP1 levels through either mouse or human CB2 receptors. WIN55212-2 increased IP1 accumulation after 10 minutes by either mouse pr human CB2 receptors. Assays were performed using HEK cells stably expressing mouse or human CB2 receptors. IP1 assays were performed in triplicates and the data were plotted and analyzed using GraphPad Prism 4.

Supplemental Figures

S1



S2


A
160
140
120
100
0
IP1 accumulation (%Basal)
WIN
LY
mCB2
0
20
40
60
Time(mins)
B
160
140
120
100
0
IP1 accumulation (%Basal)
WIN
LY
hCB2
0
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40
60
Time(mins)