



Tyrosine Kinase Dependent Suppression Of Homomeric Kv1.1, 1.2, And Heteromeric Kv1.1/1.2 Channels: Implications For Alzheimer'S Disease

D. Jamshidi, J. Hyatt, A. Khan, T. Yaklin, J. Farley

Indiana University Bloomington
Program in Neuroscience



Introduction

The roles of Aβ in Alzheimer's disease (AD) pathogenesis include disruption of Ca²⁺ homeostasis, synaptic communication, and synaptic/neuronal plasticity underlying learning & memory. How these abnormalities arise is not fully understood. One explanation of the link between neuronal dysfunction and amyloid metabolism is the "dysregulated calcium hypothesis" of AD. During aging, in vulnerable individuals, declines in cognition may result from Ca²⁺ signal remodeling, that is then exacerbated by activation of amyloidogenic pathways in AD (Khachaturian, 1984; Berridge, 2010).

Our lab has focused on the potential involvement of voltage-gated potassium channels (VGKCs) in these processes. VGKCs are activated by membrane depolarization (e.g., action potentials) and regulate Ca²⁺ influx. Inhibition of VGKCs can lead to larger and longer action potentials that may result in synapto-/neuro-toxicity and excess glutamate release (from excitatory neurons), as well as postsynaptic depolarization-enhanced excitotoxicity. We've previously shown that Aβ peptides rapidly suppress homomeric Kv1.1, Kv1.2, and heteromeric Kv1.1/1.2 channel activities in *Xenopus* oocytes: ~50% suppression of currents within 30 min of exposure to Aβ (1 μM). For all Kv1.x channels we've examined to date, Aβ-suppression was Ca²⁺- and PP2B-dependent, being greatly reduced (~ 50%) by BAPTA-AM or Cyclosporine A (CsA; PP2B inhibitor). Aβ -based suppression of Kv1.x channels was also found to be critically dependent on (endogenous) protein tyrosine kinase (PTK) activity, being completely abolished by the broad spectrum PTK inhibitor genistein. PTKs may be involved in synaptic plasticity, neuronal hyperexcitability, and memory via tyrosine phosphorylation pathways. These pathways may also be altered in the presence of toxic Aβ-oligomers, potentially causing internalization of Kv channels and reduced surface expression. However, it is not clear whether the PTK-modulation involves direct phosphorylation of the channels and which PTK(s) are implicated.

Here we considered the role of a key tyrosine residue in Kv1.2 and the PTK PYK2, which is potentially involved in synaptic plasticity and AD. Residue Y132 in the tetramerization domain was mutated to F. The resulting macroscopic currents showed only ~17% suppression post Aβ(1-42) exposure compared to WT channels (~ 50%), with no obvious effects on baseline amplitude, kinetics, or voltage dependency. Additionally, we found that co-expression of exogenous PYK2 with Kv1.2 channels, and activation of PYK2 by PMA, profoundly suppressed WT Kv1.2 currents (as first shown by Lev et al., *Nature*, 1995), but not Y132F mutant currents. Further, Aβ(1-42)-suppression was occluded by exogenous PYK2 activated by PMA. Our results suggest that Aβ(1-42)-alone suppression occurs in part through activation of endogenous PYK2 and involves phosphorylation of Y132.

Methods

Xenopus laevis oocytes were purchased from Ecocyte Bioscience (Austin, TX) and incubated in 1X ND96-PK (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 5 mM HEPES, 2.5 mM sodium pyruvate, 50 μg/μL Kanamycin, pH 7.5) supplemented with 5% horse serum. cDNA plasmids containing the Kv1.1 and Kv1.2 genes were constructed and used as templates for *in vitro* transcription. The Kv1.2 Y132F mutant cRNA was generated using a PCR based site-directed mutagenesis kit (New England Biolabs).

Each oocyte was microinjected with ~1-5 ng of Kv1.1 or Kv1.2 cRNA, approximately 2-3 days after the initial oocyte harvest. Heteromeric-channel expressing oocytes were injected with equal volumes of Kv1.1 and Kv1.2 cRNA, diluted to inject ~0.5 ng of each cRNA. Oocytes were maintained in 1X ND96-PK for 24 hours before electrophysiology experiments were conducted. All recordings obtained at room temperature (~19-20° C).

Human recombinant Aβ (1-42) [Peptide] was dissolved in DMSO, "aged" as in previous work, aliquoted, and stored at -20°C until use. For all experiments utilizing Aβ (1-42), 5μl was added to a bath solution for a total concentration of 1μM. Application of equimolar DMSO was used as a solvent control.

Stock solutions of BAPTA-AM, CsA, and Genistein were made up using DMSO and diluted with 1X ND96-PK to the appropriate working solution (50μM, 10μM, and 100μM respectively) with the final concentration of DMSO being < 0.1% in all cases. Oocytes were incubated in BAPTA-AM for 60 minutes, or overnight for CsA and Genistein. Electrophysiology experiments were conducted immediately following the end of the incubation and wash cycles to remove excess compound.

Standard two microelectrode voltage-clamp (TEVC) methods were used to evoke and measure Kv1.1, Kv1.2, or Kv1.1/Kv1.2 currents. Oocytes were clamped at a holding potential of -60 mV throughout, except when brief step depolarizations were used to evoke Kv1.1, Kv1.2 or Kv1.1/Kv1.2 currents. The stimulation protocol, given every 5 min for 30 min, entailed depolarizing steps (380 msec) in increments of +10 mV, from -70 mV to +20 mV.

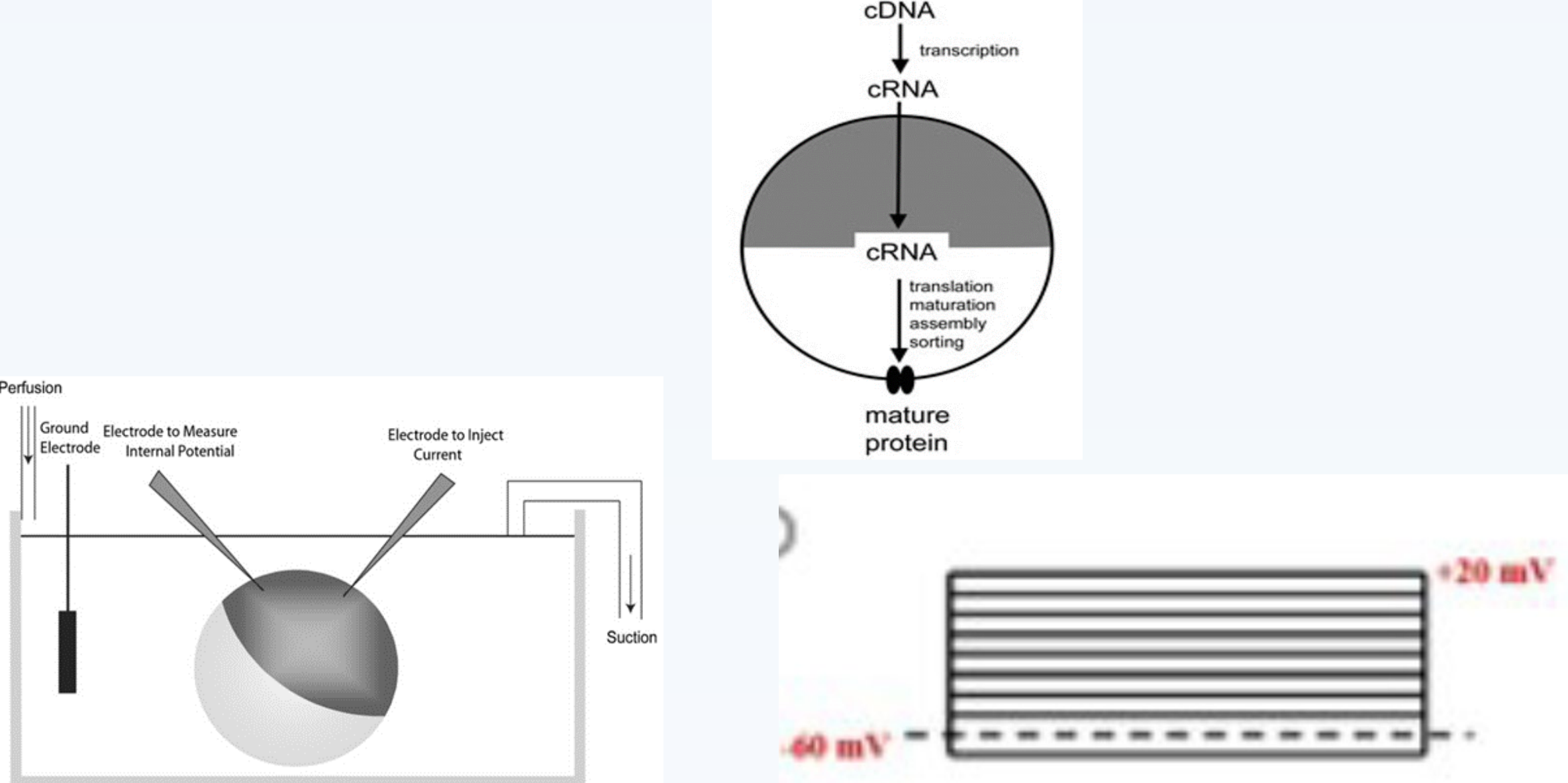
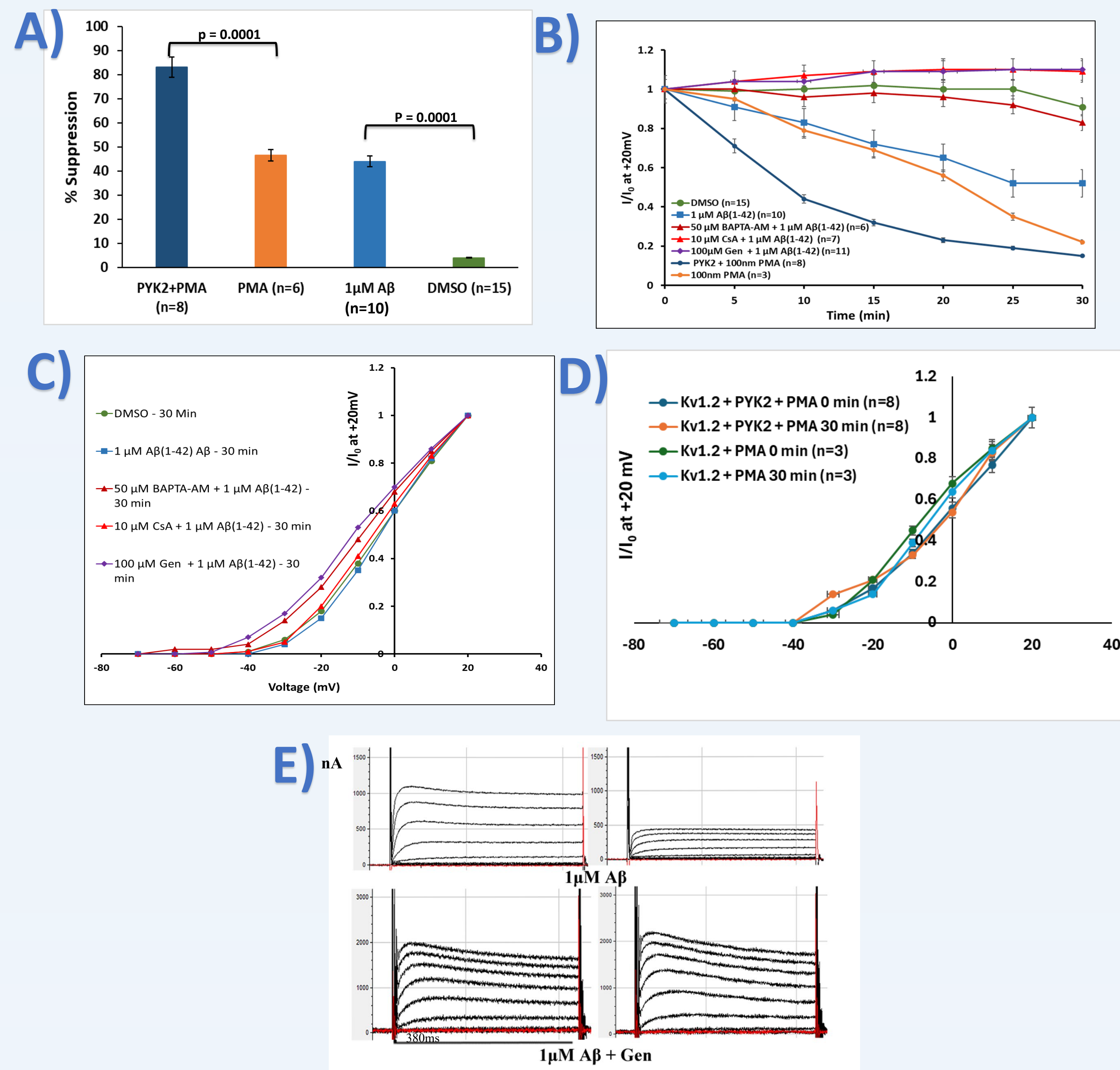
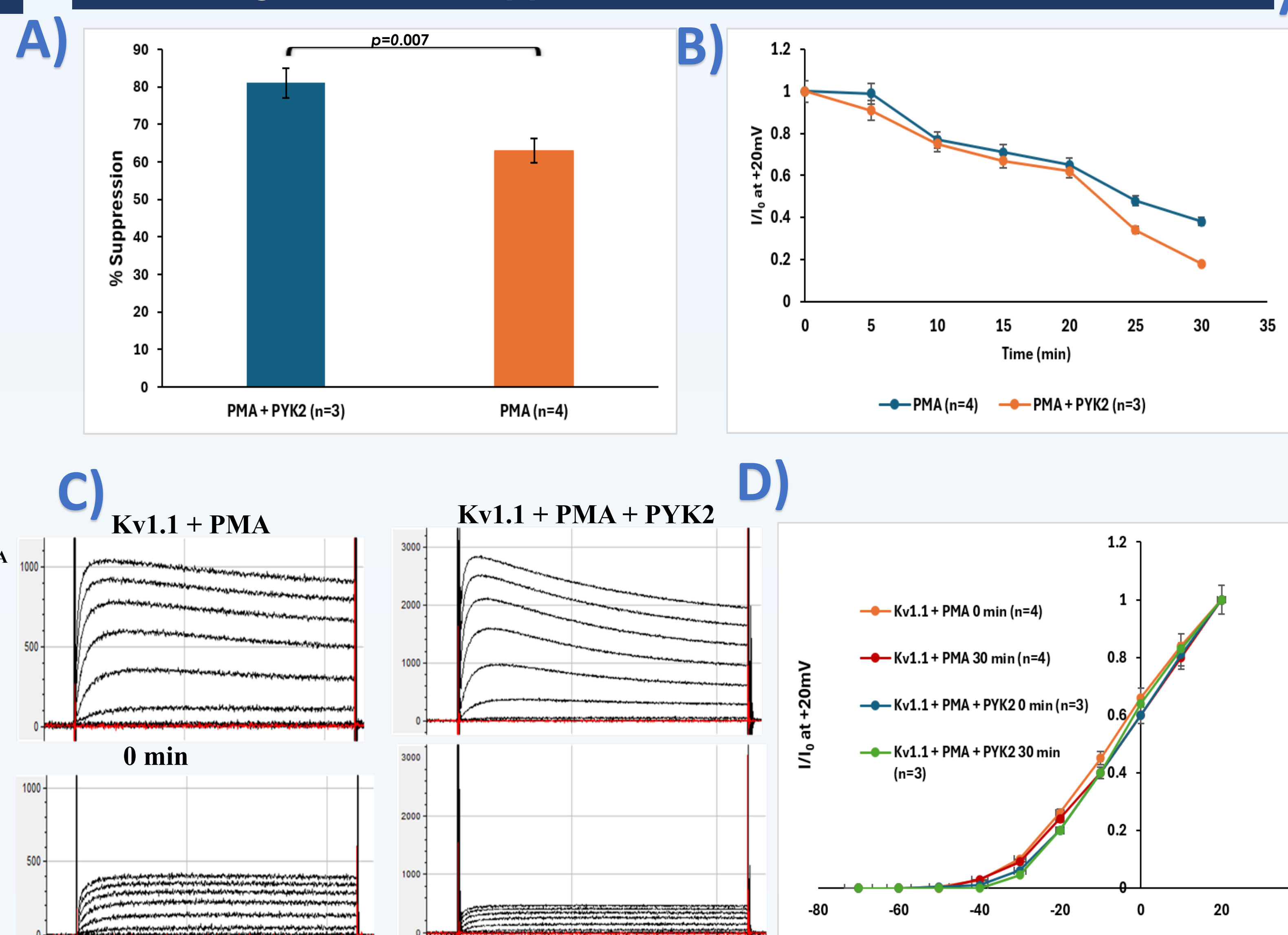


Figure 1. Ca²⁺- & PTK-Dependent Suppression of Kv1.2 by Aβ



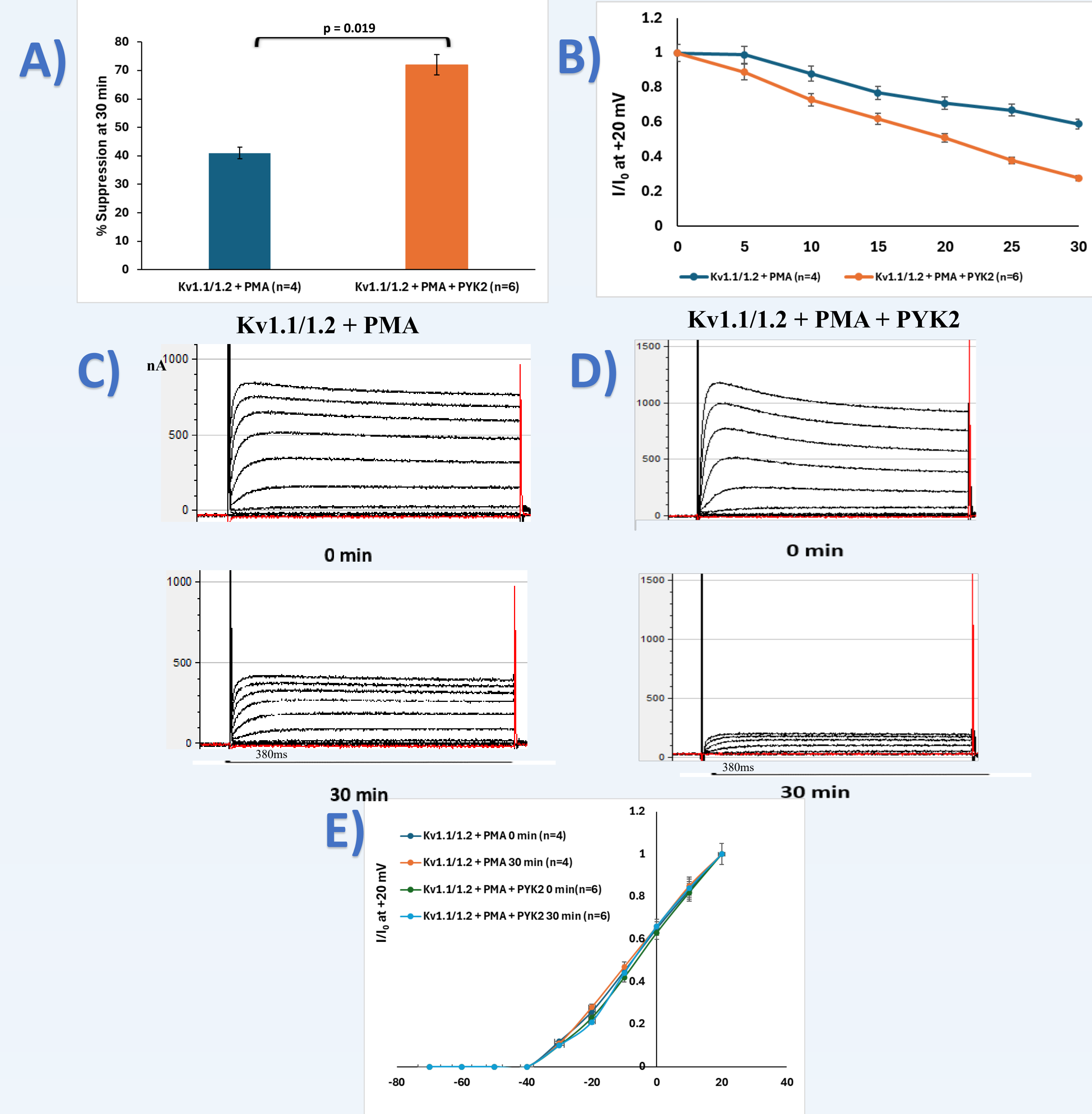
Aβ(1-42) suppression of macroscopic Kv1.x currents is PTK- and Ca²⁺-dependent. **A)** 1μM Aβ(1-42) and 100nM PMA produced significant suppression of current in Kv1.2 expressing oocytes at 30 min (p<0.0001) compared to solvent control application. Co-expression of PYK2 resulted in greater suppression when stimulated with PMA compared to without (p=0.0002). Peak currents, and their suppression, were measured at +20 mV. In this and all subsequent figures, the data plotted are: arithmetic means ± s.e.m.s. **B)** Time-dependent development of changes in peak current (measured at +20 mV). Aβ(1-42)-exposed oocytes treated with BAPTA-AM, Cyclosporine A, or Genistein (a broad-spectrum PTK inhibitor), showed significant reductions in Aβ-suppression with little systematic change in baseline currents. There were no obvious effects of these compounds alone (in the absence of Aβ) on the macroscopic currents (not shown). **C) & D)** Current-Voltage relationships of homomeric Kv1.2 for indicated treatment conditions. Current-Voltage relationships for oocytes exposed to BAPTA-AM and CsA in **C)** showed slight shifts in voltage-dependence of current activation to more hyperpolarized potentials. These do not explain the suppressions. No obvious changes were produced by PMA with or w/o exogenous PYK2 in **D)**. **E)** Representative Kv1.2 current traces from individual oocytes just prior to exposure (left) to 1μM Aβ(1-42) and after 30 min (right).

Figure 2. PYK2 suppresses homomeric Kv1.1 channels



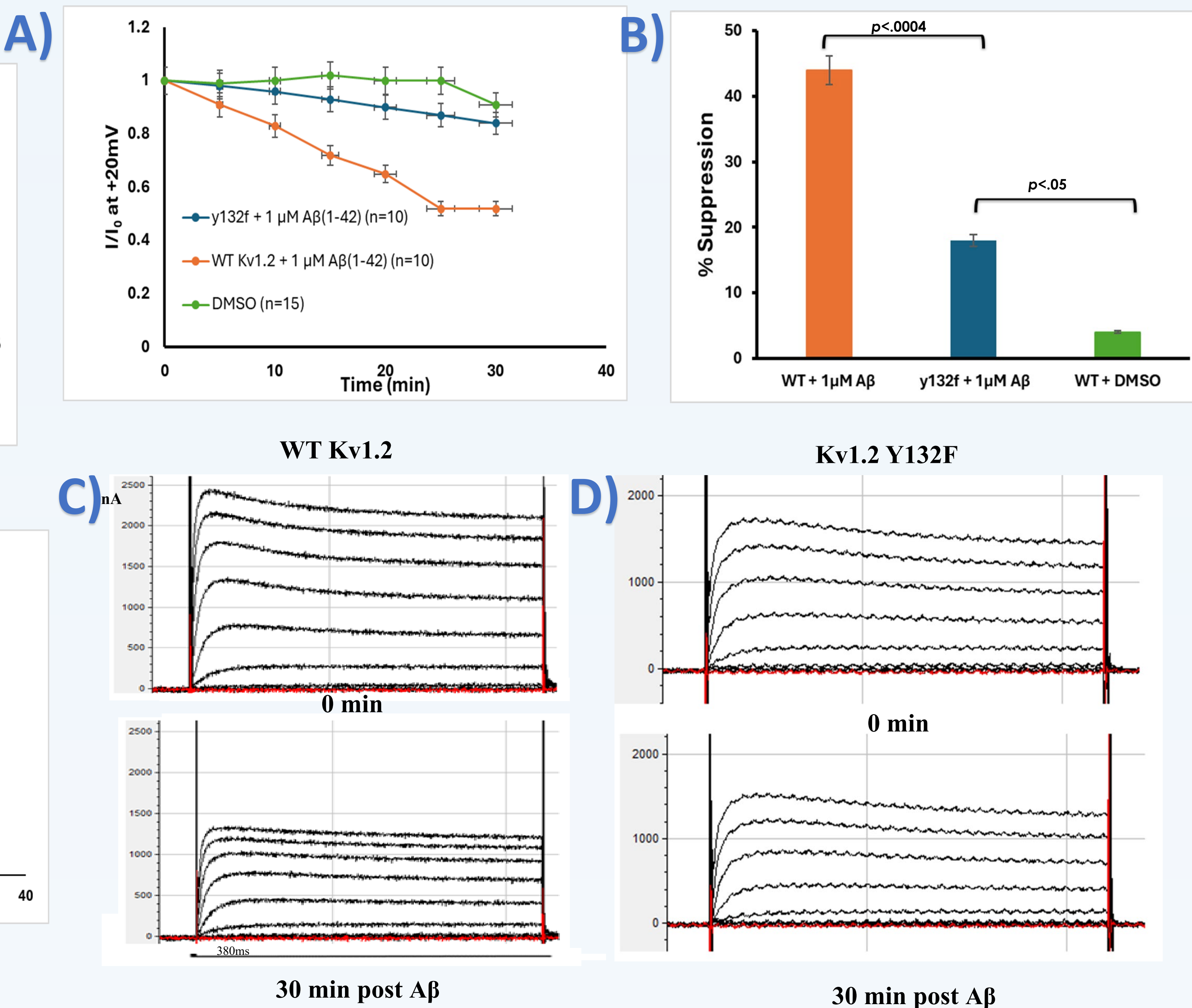
Co-expression of PYK2 with homomeric Kv1.1 results in greater suppression when stimulated by PMA compared to PMA-stimulation alone in *X. laevis* oocytes. **A)** Suppression of macroscopic Kv1.1 current (at +20 mV) by PMA was significantly greater when PYK2 was co-expressed with PYK2 than when stimulated by PMA alone at 30 minutes post PMA application (p=0.007, t=4.27, df=5). **B)** Time-dependent development of changes in peak Kv1.1/1.2 current (at +20mV). **C)** Representative Kv1.1 current traces from oocytes just prior to exposure (top) to 5μM PMA alone and 30 min after **D)** when co-expressed with PYK2. **E)** Current-Voltage relationship of homomeric Kv1.1 currents for indicated treatment conditions at 0 and 30 minutes. Neither PMA alone, nor when combined with PYK2, had an obvious effect on voltage dependency.

Figure 3. PYK2 suppresses heteromeric Kv1.1/1.2 Channels



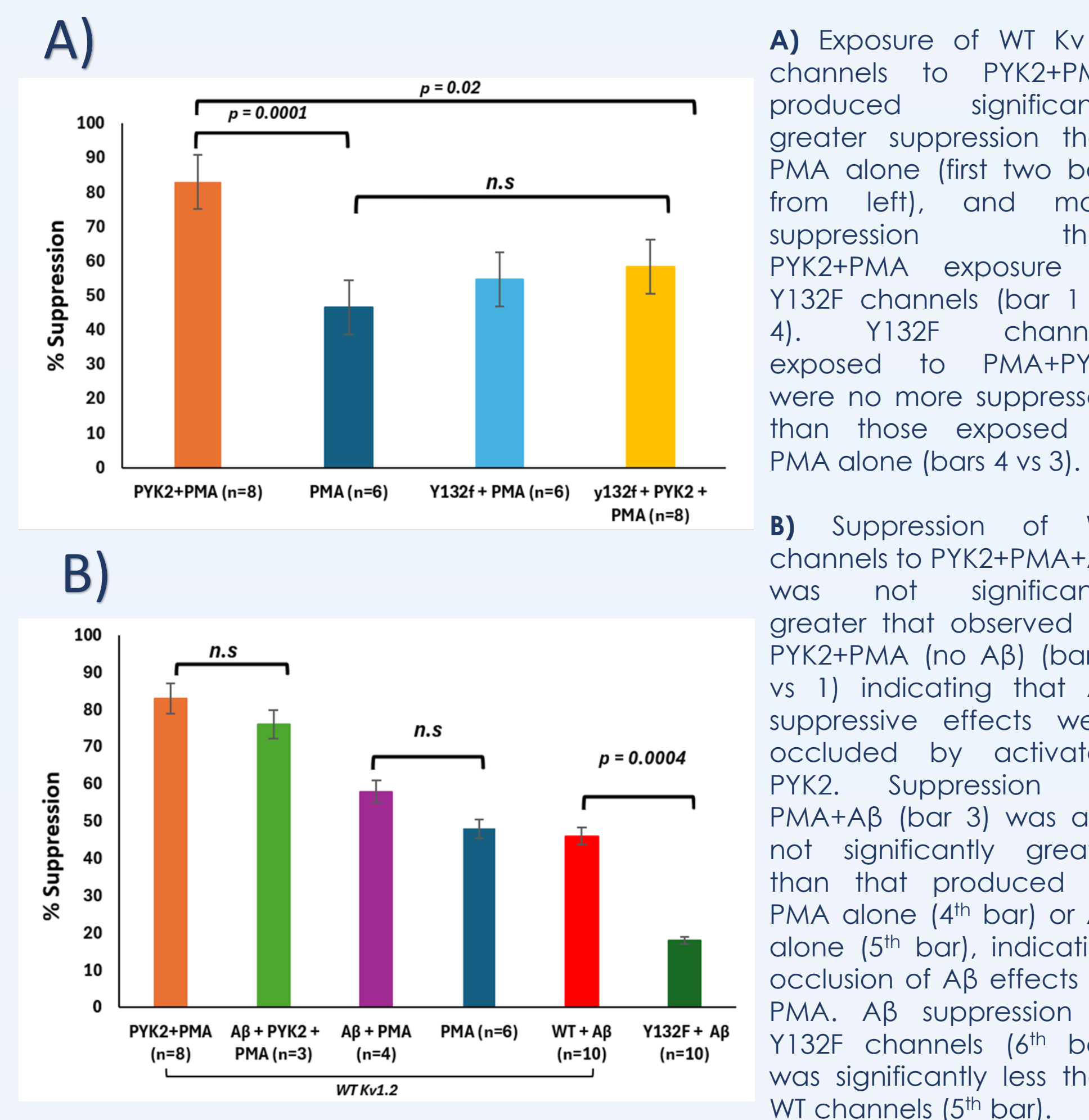
Co-expression of PYK2 with heteromeric Kv1.1/1.2 results in greater suppression when stimulated by PMA compared to PMA by itself in *X. laevis* oocytes. **A)** Suppression of macroscopic Kv1.1/1.2 current by PMA was significantly greater when co-expressed with PYK2 than when stimulated by PMA alone at 30 minutes post PMA application (p=0.0192, t=2.9, df=8). **B)** Time-dependent development of changes in peak Kv1.1/1.2 current (at +20mV). **C)** Representative Kv1.1/1.2 current traces from oocytes just prior to exposure (top) to 5μM PMA alone and 30 min after **D)** when co-expressed with PYK2. **E)** Current-Voltage relationship of heteromeric Kv1.1/1.2 currents for indicated treatment conditions at 0 and 30 minutes. No obvious changes in voltage-dependency were produced by PMA-stimulation with or w/o exogenous PYK2.

Figure 4: Kv1.2 Y132F Currents Show Greatly Reduced Aβ-Suppression



Kv1.2 Y132F resists suppression by Aβ (1-42). **A)** Time-dependent changes in Kv1.2 Y132F peak currents at +20 mV for each treatment are shown. **B)** Suppression values for WT or mutant Kv1.2 currents 30 min post Aβ(1-42) or DMSO application. Y132F Kv1.2 currents (n=10) showed only ~ 17% suppression compared to the ~ 45% suppression of WT channels [t(18)= 4.30, p<.0004] or the ~3% shown by DMSO controls. **C)** Kv1.2 Y132F current traces from an individual oocyte prior to (top) and 30 min post (bottom) exposure to 1μM Aβ(1-42). On average, baseline Y132F channel currents did not differ significantly in amplitudes, kinetics, or voltage-dependencies compared to WT controls.

Figure 5: PYK2 or PMA Occlude Aβ-Suppression

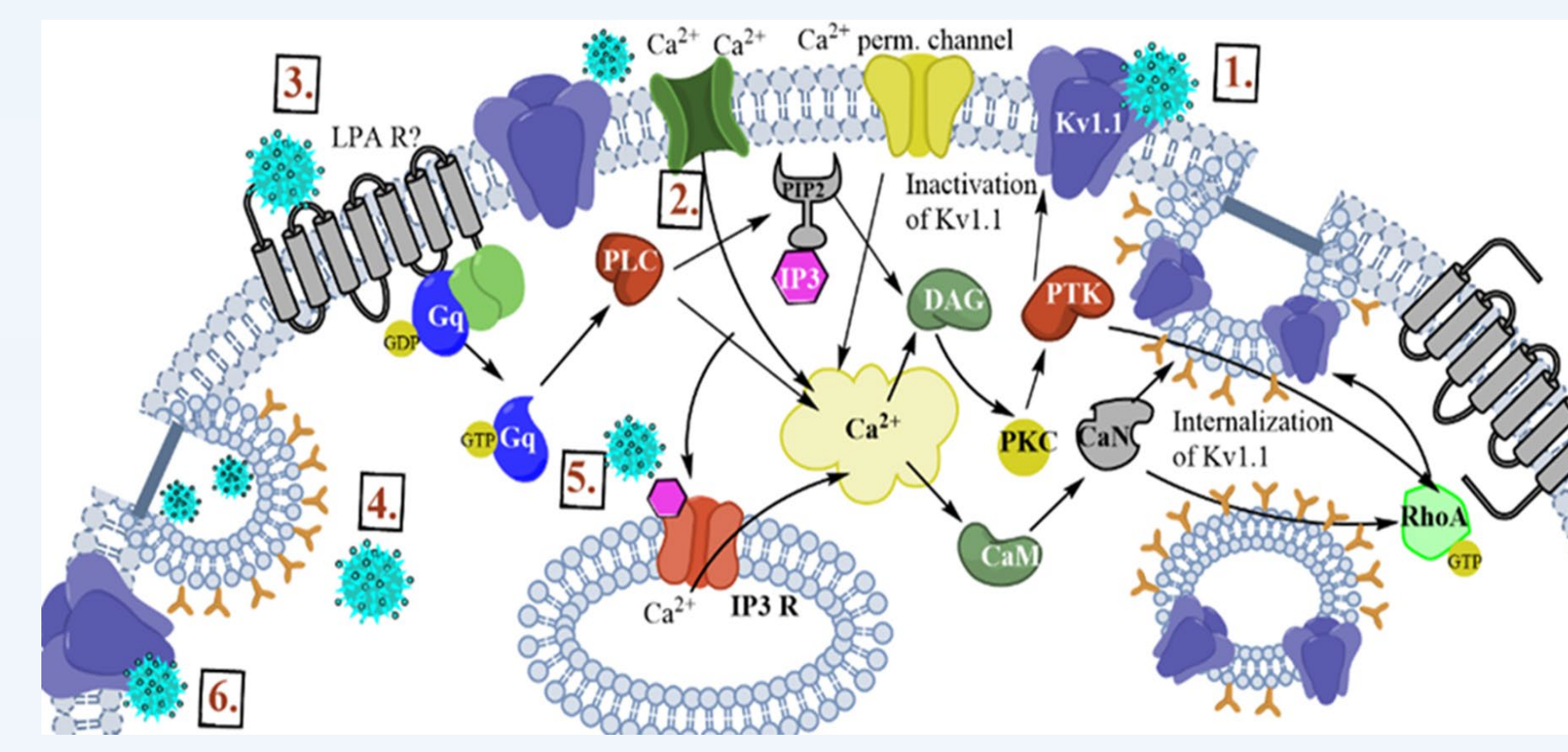


A) Exposure of WT Kv1.2 channels to PYK2+PMA produced significantly greater suppression than PMA alone (first two bars from left), and more suppression than PYK2+PMA exposure of Y132F channels (bar 1 vs 4). Y132F channels exposed to PMA+PYK2 were no more suppressed than those exposed to PMA alone (bars 4 vs 3).

B) Suppression of WT channels to PYK2+PMA+Aβ was not significantly greater than PYK2+PMA (no Aβ) (bar 2 vs 1) indicating that Aβ suppressive effects were occluded by activated PYK2. Suppression by PMA+Aβ (bar 3) was also not significantly greater than that produced by PMA alone (4th bar) or Aβ alone (5th bar), indicating occlusion of Aβ effects by PMA. Aβ suppression of Y132F channels (6th bar) was significantly less than WT channels (5th bar).

Potential Mechanisms of Aβ-Suppression

Aβ (1-42) [represented in turquoise] may cause suppression of Kv1.1 and Kv1.2 by: 1) binding to distinct extracellular regions of the channel, which prevents normal gating/activation by membrane depolarization, 2) forming Ca²⁺ mega pores that allow large influxes of Ca²⁺ which then cause subsequent activation of Ca²⁺-dependent signaling pathways [such as PTKs (like PYK2), PKC, and PP2B], that modulate Kv1.1, Kv1.2, and Kv1.1/1.2 channels, 3) acting on endogenous GPCRs in oocytes that couple to intracellular Ca²⁺ release [e.g., lysophosphatidic acid (LPA) R], even when applied extracellularly. Aβ may enter the cytoplasm of the oocyte through 4) the native endocytotic/pinocytotic systems of the oocyte, which could then 5) activate Ca²⁺-signaling pathways and affect channel activity via phosphorylation/ dephosphorylation, or 6) directly bind to the intracellular side of the Kv1.x channels (e.g., peptide-peptide interactions) and affect channel activity independent of phosphorylation/dephosphorylation. Our previous work has provided evidence for most of the above.



Conclusions

Our previous and present results suggest that Aβ and PMA suppression of WT homomeric Kv1.2 and heteromeric Kv1.1/1.2 is mediated by one or more PTKs since these effects are blocked by genistein. Here we show PYK2, a known PTK, that suppresses Kv1.2, also suppresses heteromeric Kv1.1/1.2 channels when stimulated by PMA. We also show Y132 in WT Kv1.2 channels is a critical mediator of Aβ-, PMA- and activated PYK2 suppression.

Suppression of Kv1.x channels by Aβ, both pre- and post-synaptically, can be expected to lead to: 1) excessive Ca²⁺ influx, resulting in supranormal glutamate release from excitatory neurons, 2) widespread disruption of Ca²⁺ homeostasis and excitotoxicity. These may lead to hyperexcitability and synapto- and neuro-toxicity. These effects may contribute to early AD progression and phenotypic markers. Activation of PYK2 may lead to internalization and reduction of surface expression of Kv1.x channels. Based on these findings, Kv1.x family members may represent novel and druggable molecular targets for therapeutic intervention in AD and other neurodegenerative diseases.

