Elucidation of the mode of action of TMEM16A modulators and their effects on vascular tone

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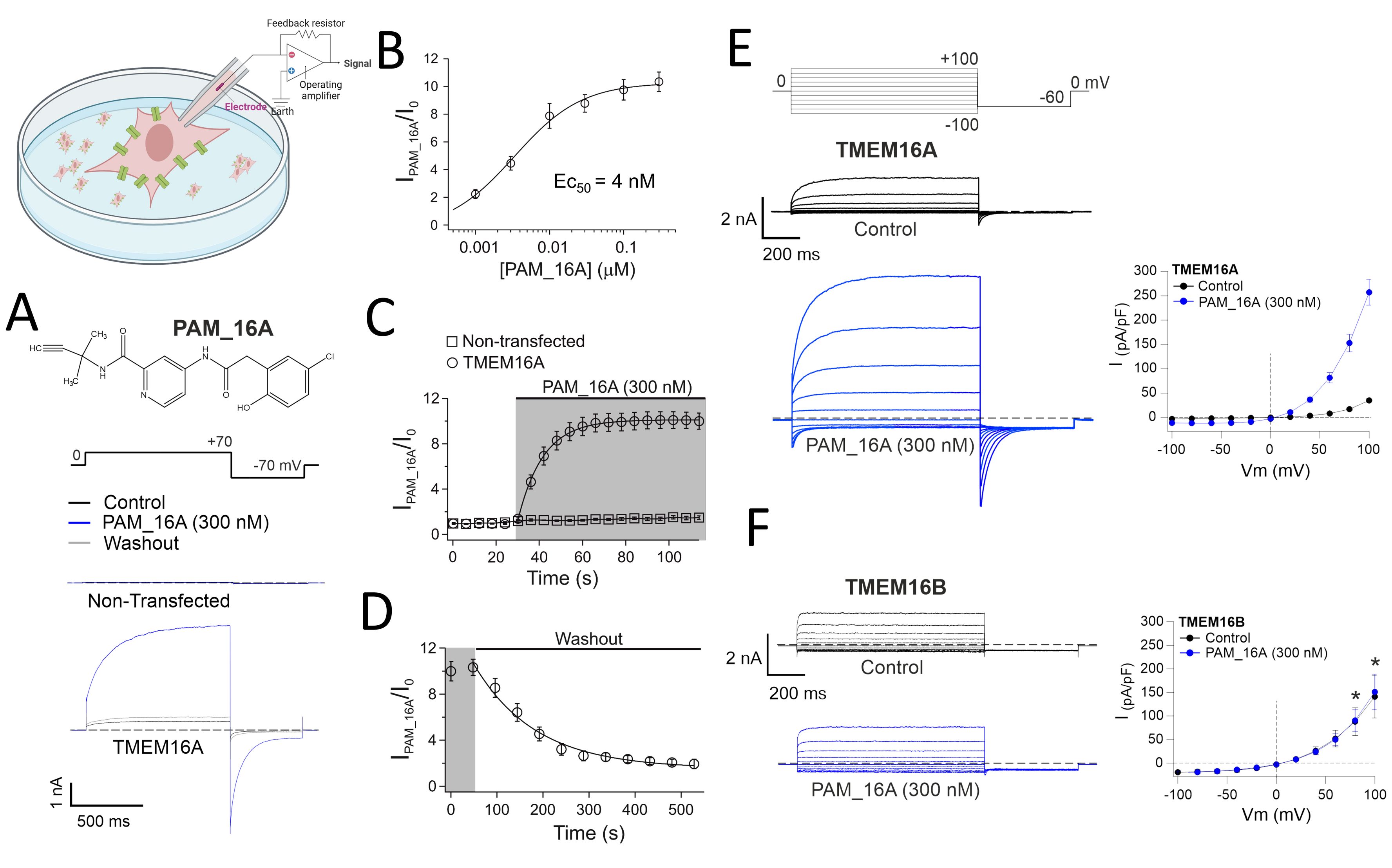
Introduction

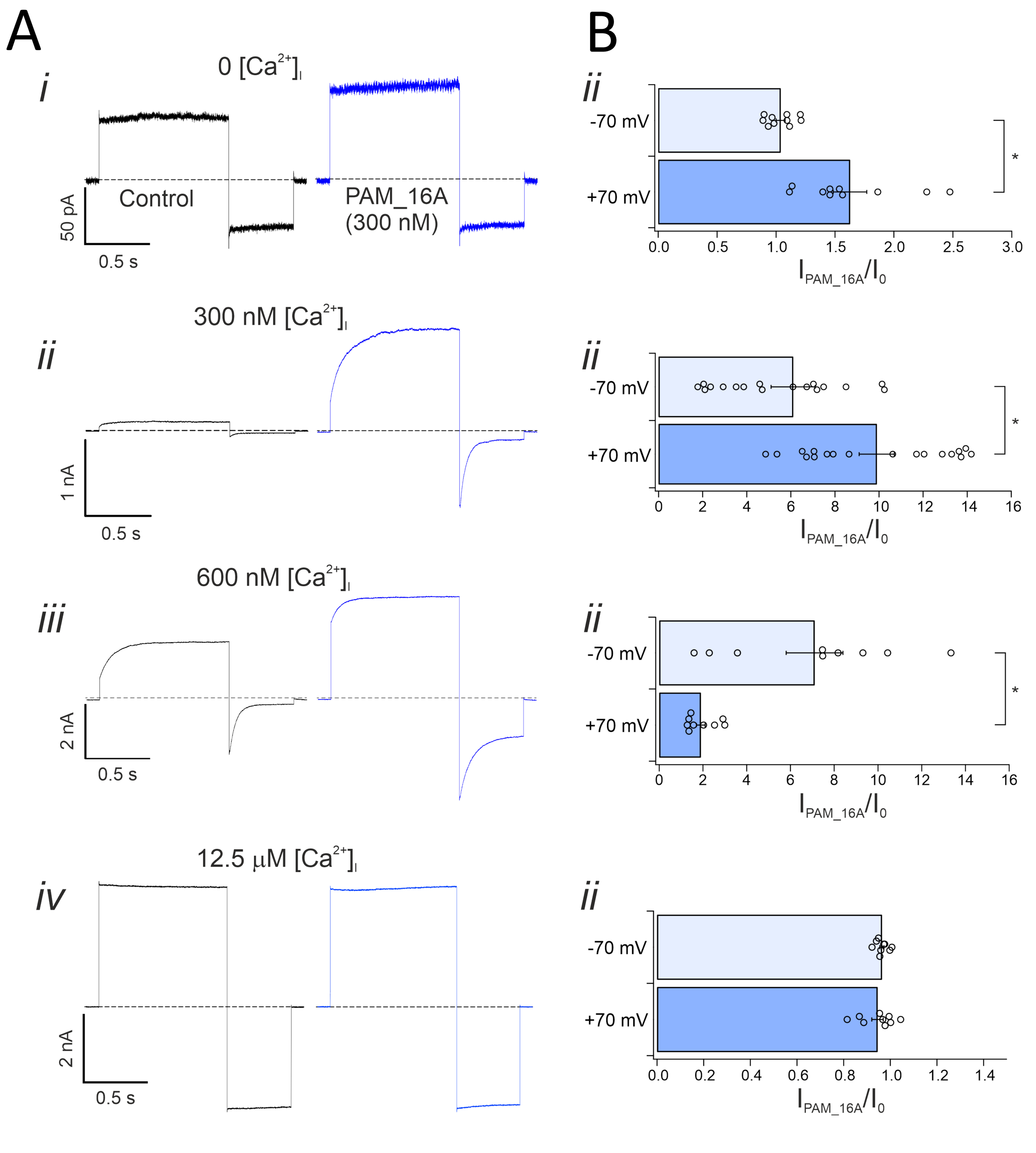
- The TMEM16A **calcium-activated chloride channel (CaCC) constitutes a key depolarising mechanism in contractile vascular cells.**
- The channel is activated in response to a rise in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) following agonist binding to G_q-protein coupled receptors (G_qPCR); this results in Cl^- efflux, membrane depolarization and ultimately SMC and pericyte contraction (1, 2).
- The channel is a proposed therapeutic target for diseases of impaired vascular tone including **stroke, hypertension** and **vascular dementia**; however, the mechanisms of modulation of the channels by synthetic inhibitors and activators are still incompletely understood.
- Here, we provide a functional characterization of a recently identified activator of the TMEM16A channel (Compound 1 from Maas et al (2021), patent WO2021/014168) (3), termed **"PAM_16A"** in this study. We discover that PAM_16A is a **gating modifier of the TMEM16A channel** with the potential of enhancing the extent of TMEM16A activation during G_qPCR stimulation.
- We further study the selectivity of PAM_16A alongside that of a range of structurally unrelated synthetic modulators of the channel.

1. Effects of PAM_16A on heterologous TMEM16A currents



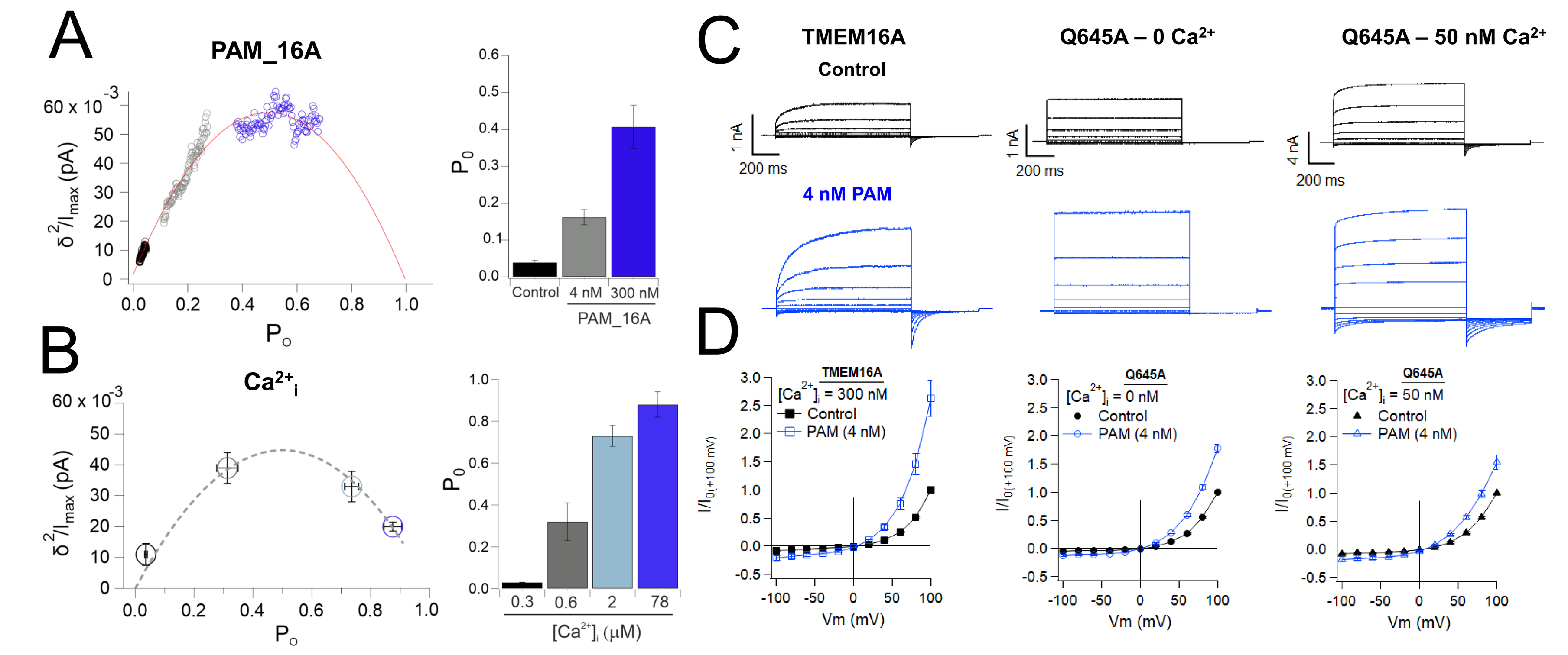
A. Whole-cell currents recorded from HEK-293T cells expressing TMEM16A or non-transfected cells. PAM_16A (300 nM) was applied as indicated. **B.** Mean whole-cell TMEM16A current recorded at + 70 mV and in the presence of various [PAM_16A] (n=10 in each case). **C.** Time-course of TMEM16A current activation in response to PAM_16A (300 nM) and **D.** wash out. **E.** Whole-cell TMEM16A currents recorded in response to the stimulation protocol shown in the upper panel and in the absence of presence of PAM_16A (n=10-12). **F.** Whole-cell TMEM16A currents recorded in response to the stimulation protocol shown in the upper panel and in the absence of presence of PAM_16A (n=10-14).

2. Effects of intracellular Ca^{2+} on the activation of TMEM16A by PAM_16A



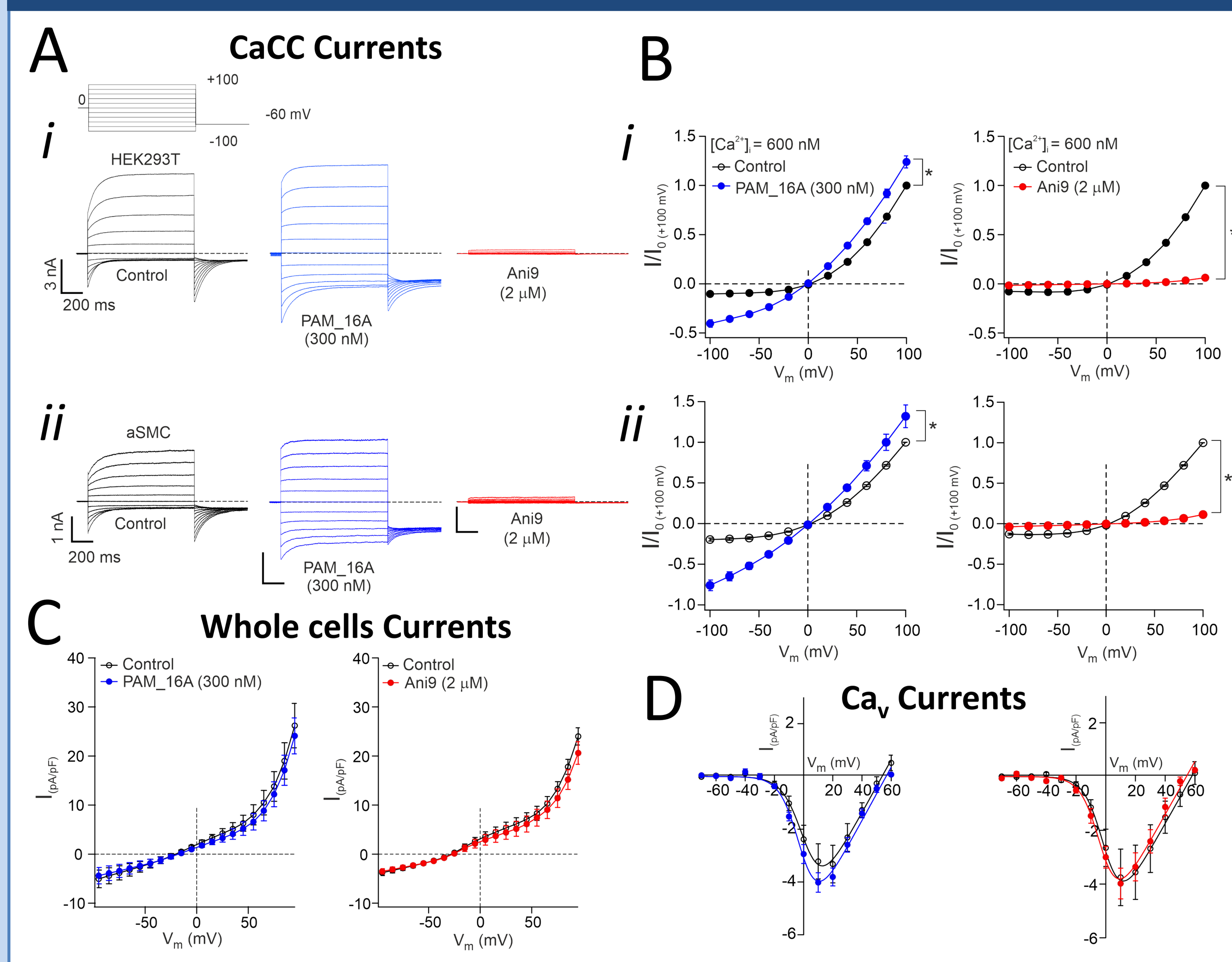
A. Whole-cell currents recorded from HEK-293T cells expressing TMEM16A. $[Ca^{2+}]_i$ was (i) 0, (ii) 300 nM, (iii) 600 nM or 12.5 μM (iv). PAM_16A (300 nM) was applied as indicated. **B.** Mean "rectification index" (ratio of current measured at +70 and -70 mV) for currents measured in various $[Ca^{2+}]_i$ as indicated.

3. PAM_16A is a TMEM16A channel gating modifier



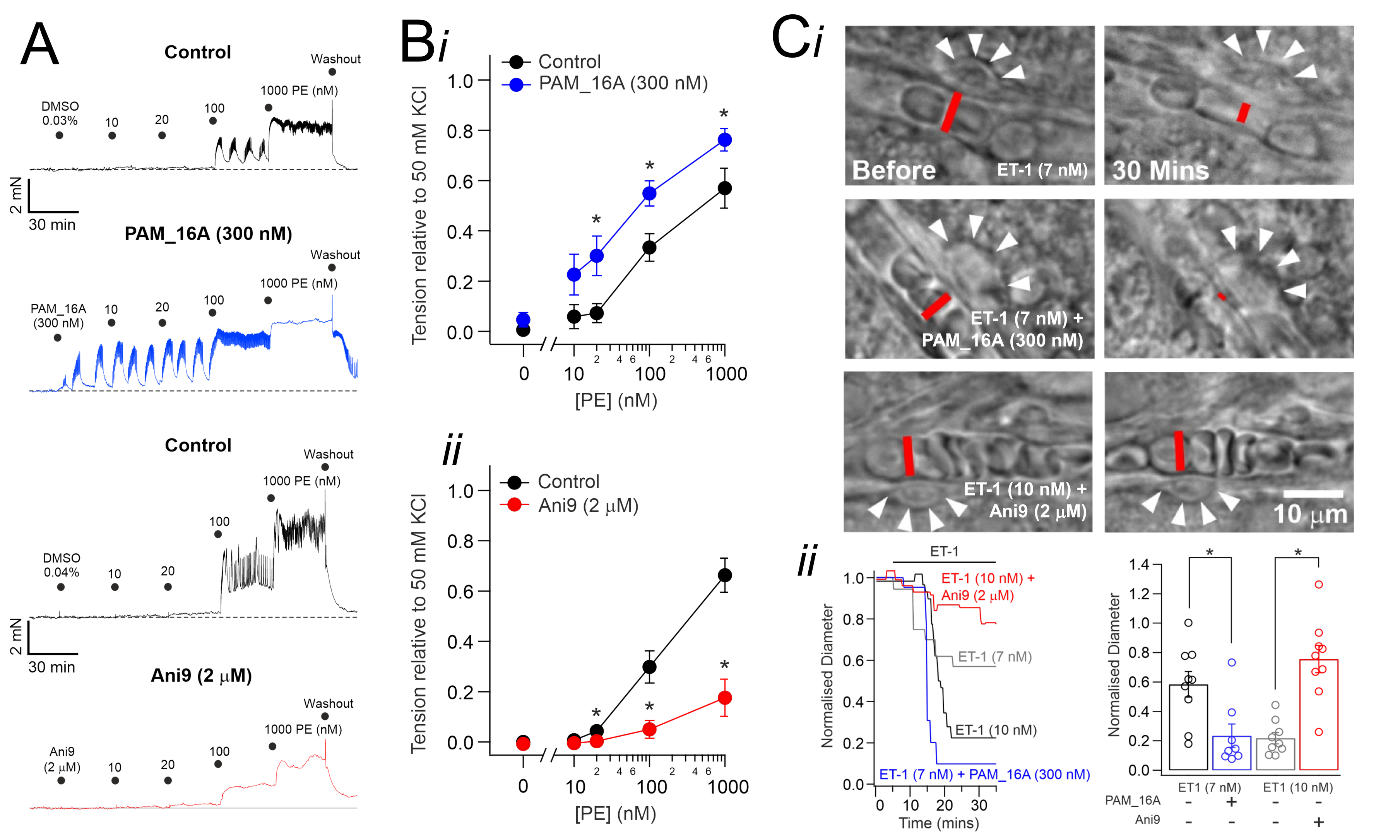
A. Stationary noise analysis of the whole-cell TMEM16A current performed in the absence or presence of various [PAM_16A] **B.** Non-stationary noise analysis of TMEM16A current measured in inside-out recordings in various $[Ca^{2+}]_i$. **C.** Whole-cell currents recorded from HEK-293T cells expressing TMEM16A, or TMEM16-Q645A channels in the absence or presence of $[Ca^{2+}]_i$. **D.** Mean TMEM16A current versus V_m relationship for wild type and mutant channels assessed in the absence or presence of PAM_16A (300 nM) (n=7-9).

4. Ani9 and PAM_16A are selective TMEM16A modulators



(A,B) Whole-cell TMEM16A currents recorded from (*i*) HEK293T cells expressing TMEM16A transfected or (*ii*) isolated rat aortic SMCs, in the presence or absence of PAM_16A or Ani9 as indicated. **(C,D)** Selective effects of PAM_16A and Ani9 on whole-cell currents in isolated rat aortic SMCs.

5. Effects of pharmacological modulation of the TMEM16A channel on isolated rat aortic rings and on cortical pericytes



Effects of PAM_16A and Ani9 on the responses of aortic rings to phenylephrine (PE), and cortical pericytes to endothelin-1 (ET-1). **(A)** Representative isometric tension recordings obtained from rat aortic rings. [PE] was applied as indicated. **(B)** Mean tension *versus* [PE] relationships in the presence of (***i***) PAM_16A, or (***ii***) Ani9. **(C)** (***i***) The constriction induced by ET-1 (7 nM) on the capillary adjacent to pericyte somata in rat brain slices is potentiated by PAM_16A (300 nM) and relaxed by Ani9 (2 μM). (***ii***) Representative measurements of capillary diameter normalised to baseline diameter, in response to ET-1 (7 nM) with and without PAM_16A, and ET-1 (10 nM) with and without the TMEM16A inhibitor Ani9.

Conclusions

- **PAM_16A is potent and selective TMEM16A activator**
- PAM_16A potentiates the response of the TMEM16A channel to GqPCR activation.
- TMEM16A may constitute a **therapeutic target** in the treatment of disorders of altered vascular tone.

References

1. Ji, Q. et al. (2019) J. Cell Physiol. **236** (6):7856-7873. **2.** Agostinelli, E. et al. (2022) Int. J. Mol. Sci. **23** (3): 1580. ***3.*** Compound 1 from Maas et al (2021), patent WO2021/014168