**Gαq coupled AT1R is indispensable for MAPK activation in arterial smooth ‎muscle cell**

Mitogen activated protein kinases (MAPK) including ERK1/2 have been linked to cardiovascular diseases including hypertension when challenged ‎with mitogens such as angiotensin II (AngII)‎ [[1](#_ENREF_1)]. We have previously elucidated the mechanisms underpinning angiotensin type 1 (AT1R) mediated cell proliferation in rat arterial smooth muscle cells [[2](#_ENREF_2)]. Moreover, the majority of early ERK phosphorylation (≤2 min) has been shown to be induced via the G protein-dependent pathway, while sustained activity (>5 min) is regulated by arrestin-dependent pathway in overexpressed model cell systems [[3](#_ENREF_3)]. However, the spatiotemporal control of MAPKs including ERK1/2 phosphorylation remains undeciphered at endogenously expressed AT1 receptor in the vasculature. Therefore, we sought to identify the cut off time point between G protein-dependent and arrestin- dependent pathways in rat aortic smooth muscle cells (RASM) via the inhibition of Gαq using YM-254890 (‎selective Gαq inhibitor). Agonist-driven ERK phosphorylation was determined via standard western blotting techniques ‎using a specific anti-phospho-p44/42 MAPK (ERK1/2) (Thr202/Tyr204) antibody. To ensure that all samples contained the same ‎levels of protein, membranes were washed and re-probed for total ERK immunoreactivity, ‎using an anti-ERK antibody. Protein expression was quantified by densitometry with the use of Image J (version 1.51, ‎National Institutes of Health, Bethesda, MD).‎ Stimulation of RASM with 100nM Ang II induced rapid increases in ERK phosphorylation peaking at 5 min‎, followed by a sustained phase of signalling over the 30 min experimental time-course (basal 1022‎±‎477, 5 min 10404‎±‎730, p<0.001,‎ 30 min 6398±1224, p<0.01, one-way ANOVA, Dunnett’s *post-hoc* test, mean ± SEM‎, n=4-5)‎. Pre-incubation with YM-254890 (1µM, 15 min) ‎virtually abolished peak 5 min (from 10404‎±‎730 to 937‎±‎384, p<0.001, two-way ANOVA, Sidak’s *post hoc* test; mean ± SEM‎, n=4-5) and the sustained phase of AngII-stimulated ERK phosphorylation (from 7206‎±1300 to 475±201, p<0.001, two-way ANOVA, Sidak’s *post hoc* test; mean ± SEM‎, n=4-5)‎. Smilairly, stimulation of RASM cells with TRV055 caused time-dependent increases in pERK ‎immunoreactivity which peaked between 2 and 5 mins, and gradually declined between 15 and 30 mins (basal 2208‎±‎ 499, 5 min 9660±1908, p<0.05‎ ,30 min 3159‎±‎1072, one-way ANOVA, ‎Dunnett’s *post-hoc* test; mean ± SEM‎, n=4)‎. Peak (5 min) TRV055-stimulated ERK ‎phosphorylation (from 9660±1908 to 891‎±‎518, two-way ANOVA, Sidak’s *post hoc* test; mean ± SEM‎, n=4‎), and sustained phase >5 min was completely suppressed following pre-incubation with YM-254890. Application of TRV027 triggered slow peak increases in ERK phosphorylation at 5 min and returned to the basal after 15 min ‎(basal 2133‎±‎843, 5 min 7410±656, p<0.001‎,30 min 1964‎±‎604, one-way ANOVA, ‎Dunnett’s *post-hoc* test; mean ± SEM‎, n=4‎)‎. YM-254890 pre-treatment attenuated the peak (5 min) TRV027 stimulated pERK activation (from 7410±656 to 3701±404, p<0.001‎, ‎two-way ANOVA, Sidak’s *post hoc* test; mean ± SEM‎, n=4‎). Together, these results suggest that TRV027 is a partial agonist in comparison to AngII and TRV055. Furthermore, it seems that arrestin recruitment likely requires Gq activation in RASM as ERK phosphorylation was completely abolished when RASM were pre-incubated with YM-254890, and stimulated by either full or biased G protein agonists. Hence, our next step is to deplete arrestin2/3 to determine the divergent point between G protein and arrestin signalling as such signalling pathway outcomes are crucial in health and disease.

**References**

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