Persistent Signaling by Thyrotropin-Releasing Hormone Receptors Correlates with G Protein and Receptor Levels

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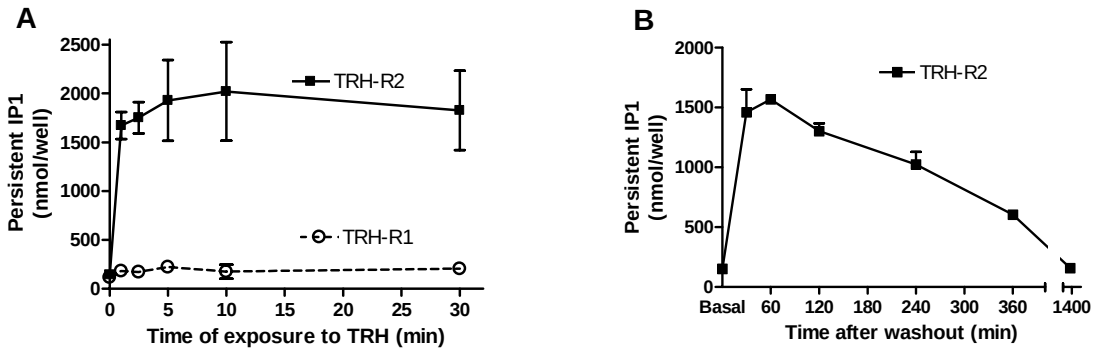
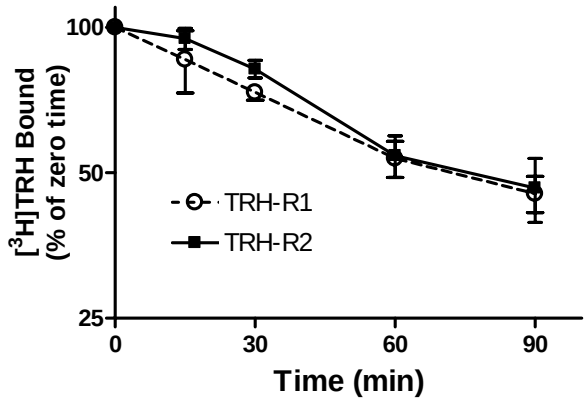


Supplementary Figure 1. IP1 Persistent Signaling in HEK-EM293 Stably Expressing TRH-R2 or TRH-R1

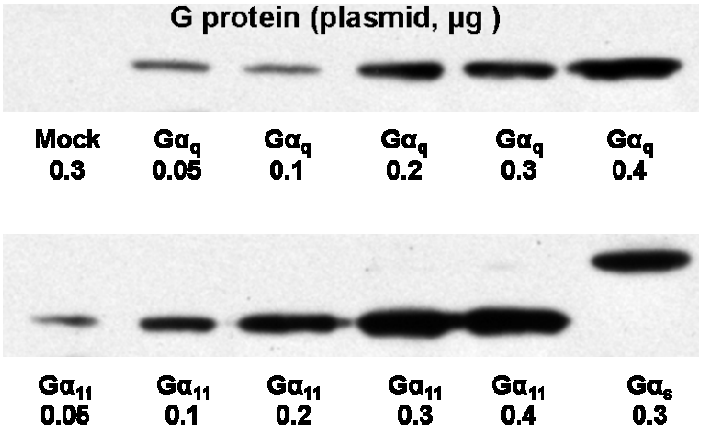
A. Time of Acquisition of Persistent Signaling. TRH-R1 or TRH-R2-expressing cells were incubated without or with 1 μM TRH for the indicated pretreatment times at 37C. After 0 (control), 1, 2.5, 5, 10 or 30 min of exposure to TRH ("pretreatment"), the cells were washed and then incubated in HBSS/HEPES for 60 min ("washout"). Thereafter, the cells were incubated in HBSS/HEPES with LiCl. After additional 30 min "production", the incubations were stopped and IP1 levels were measured. The data are from three experiments with duplicate samples and are presented as mean±SE.

B. Time of Decay of Persistent Signaling. TRH-R2-expressing cells were incubated without or with 1 μM TRH at 37C. After 30 min pretreatment, the cells were washed and then incubated in HBSS/HEPES for 60 min ("washout"). At the times indicated after washout, the buffers were aspirated and the cells were incubated in HBSS/HEPES with LiCl for 30 min ("production"). The incubations were stopped and IP1 levels were measured. The data are from three experiments with duplicate or triplicate samples and are presented as mean±SE.



Supplementary Figure 2. Rates of [^{3}H]TRH Dissociation from HEK-EM293 Stably Expressing TRH-R1 or TRH-R2.

TRH-R1 and TRH-R2-expressing cells were incubated with 10 nM [^{3}H]TRH for 1 h at 37C, washed with ice cold acetic acid buffer, pH 2.8, incubated in HBSS/HEPES at 37C for 0, 15, 30, 60, and 90 min and then washed with ice cold HBSS and solubilized. Total binding was measured in the absence of unlabeled TRH and nonspecific (NS) binding in the presence of 10 μM unlabeled TRH. Specific binding was calculated as a percentage of zero time. The bars are the mean±range of duplicate measurements in three experiments.



Supplementary Figure 3. Levels of Protein Expression of $G\alpha_s$, $G\alpha_q$ or $G\alpha_{11}$ Transfected in HEK-EM293 Cells Stably Expressing TRH-Rs.

Cells were transfected with the indicated amounts of Glu-Glu-epitope-tagged $G\alpha_s$, $G\alpha_q$ or $G\alpha_{11}$. After 48 h, the cells were washed, lysed and probed for G protein content using antibody against Glu-Glu internal epitope tag in Western blot analysis.