MOLECULAR PHARMACOLOGY

Thyrotropin Causes Dose-dependent Biphasic Regulation of cAMP Production Mediated by Gs and Gi/o Proteins

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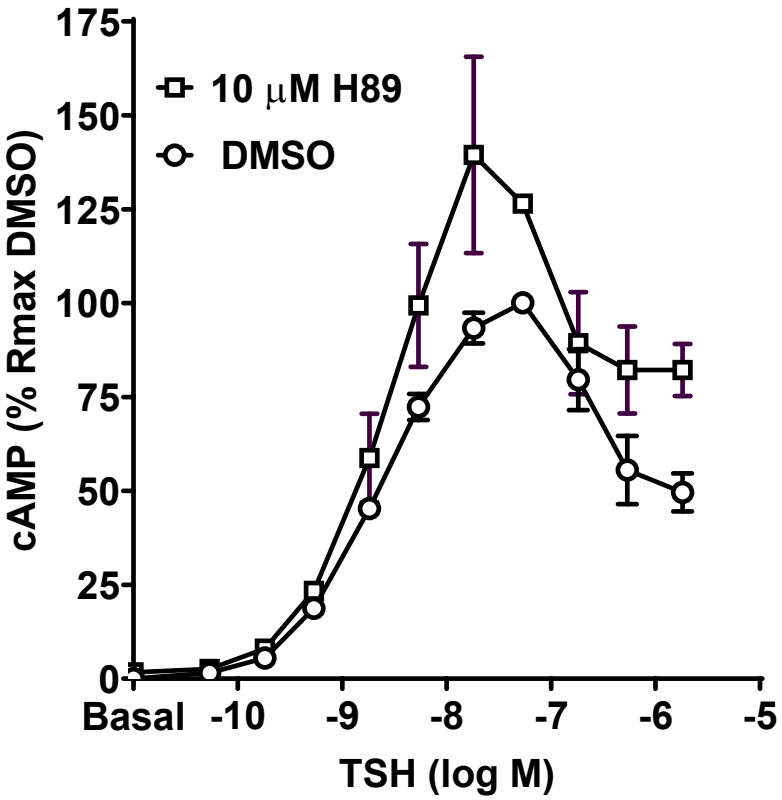
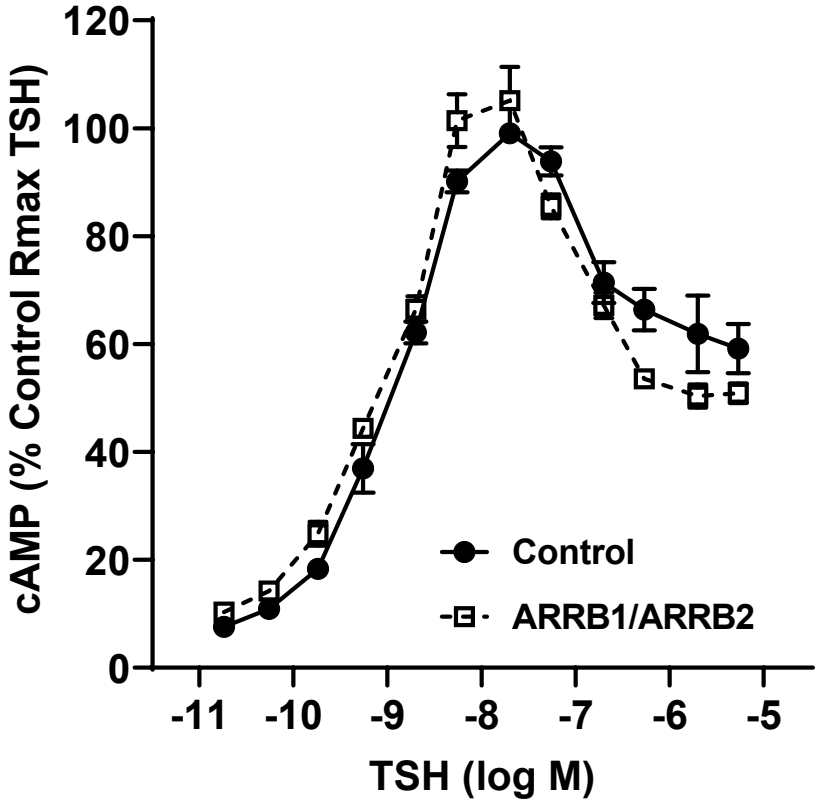


Supplemental Figure 1

Heterologous desensitization by protein kinase A (PKA) does not affect biphasic cAMP dose response to TSH. HEK-TSHR cells were preincubated with either HBSS/HEPES alone or HBSS/HEPES containing 10 μM of the PKA inhibitor H-89 for 15 minutes a 37 ºC. The supernatant was removed, and subsequently, cells were incubated in HBSS/HEPES/1 mM IBMX containing increasing doses of bTSH (0 to 100 mU/ml/1.8 μM) with or without 10 μM H-89 for 60 minutes at 37 ºC. Afterwards, the cells were lysed, and cAMP production was measured. The data represent the mean±SEM of triplicate measurements in 2 experiments.



Supplemental Figure 2

Homologous desensitization does not play a role in the biphasic cAMP response. Co-knockdown of β-arrestin 1 and β-arrestin 2 does not affect the decrease in cAMP at high TSH doses. HEK-TSHR cells were co-transfected with ARRB1 (β-arrestin 1) and ARRB2 (β-arrestin 2) siRNA or transfected with non-silencing siRNA (Control). Cells were cultured for 48 h, and then seeded in 48-well plates at 120,000 cells/well. Twenty-four hours later the medium was aspirated, the cells were washed with HBSS/HEPES and then stimulated with increasing doses of bTSH (0 - 300 mU/ml/5.4 μM) in HBSS/HEPES with IBMX for 1 hour at 37 ºC. Subsequently, cAMP was determined in the cell lysates. The knockdown efficiency was 73±3.6% and 63.0±3.7% for ARRB1 and ARRB2, respectively. The data represent the mean±SEM of duplicate measurements in 2 experiments.

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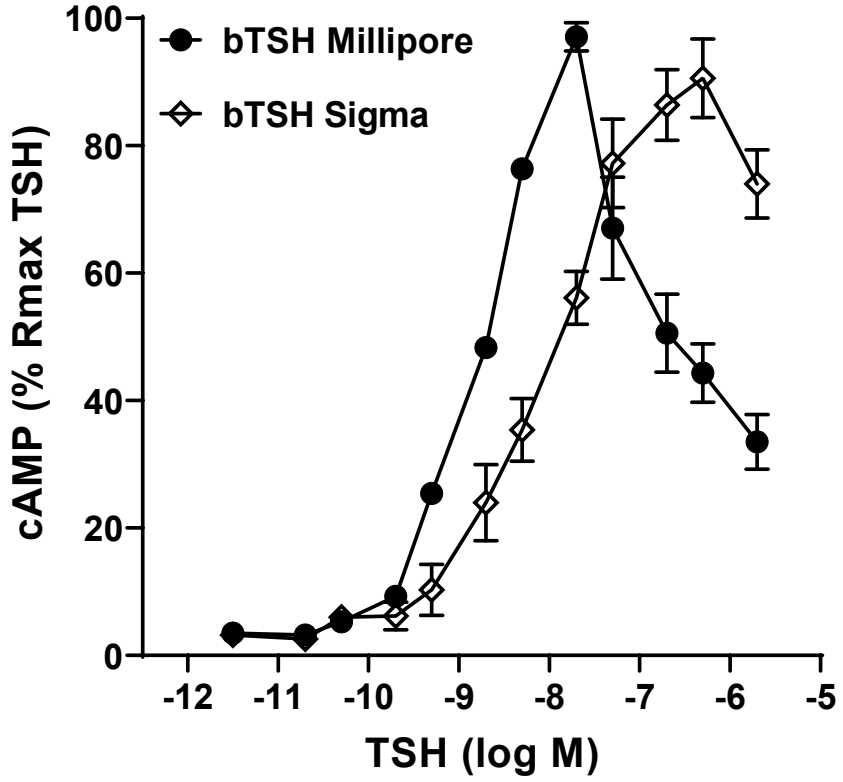

Supplemental Figure 3

Comparison of potency and efficacy of bovine TSH (bTSH) from different sources. bTSH was purchased from SIGMA (Catalog Number T8391) and Millipore (Catalog Number 609385). HEK-TSHR cells were incubated with increasing doses of bTSH from both sources (0 to 300 mU/ml (5.4 μM)) in HBSS/HEPES with IBMX at 37 ºC for 60 min. Thereafter, HEK-TSHR cells were lysed and cAMP was measured in the cell lysates. Despite no difference in efficacy, bTSH Millipore is more potent than bTSH SIGMA (1 nM versus 8 nM) and induces a biphasic dose response for cAMP production. The symbols represent the mean±SEM of duplicate measurements in 6 and 3 experiments for bTSH SIGMA and bTSH Millipore, respectively. Rmax = maximum response.