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β_1-adrenergic receptors exhibit voltage-dependence of agonist efficacy

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In recent years it has been shown for certain Gi- and Gq-coupled GPCRs that changes in membrane potential (V_M) alter ligand binding-induced receptor activation. In the present study we aim to investigate whether activation of Gs-coupled β_1-ARs by the synthetic and endogenous ligands isoprenaline (Iso) and adrenaline (Adr), respectively, also depends on the membrane potential.

In order to directly monitor the influence of V_M on changes in receptor conformation induced by ligand binding we used a FRET-based biosensor of β_1-AR [1] combined with voltage-clamp electrophysiology. Transmission of voltage-dependence to downstream signalling was measured with a FRET assay of YFP-tagged β_1-AR and Turquoise-tagged arrestin 3 (Tur-arr3).

Depolarization reduced the Iso-induced activation of β_1-AR indicating that this receptor is voltage-sensitive. Voltage-induced attenuation of receptor activity was about equal when nonsaturating (1 µM) or saturating (100 µM) concentrations of Iso were applied (23±3.6% vs 18±2.4%, mean ± sem). The offrate of receptor inactivation induced by depolarization was over 100-fold faster than the offrate induced by agonist withdrawal (k: 3.07±1.2 s^{-1} vs 0.02±0.002 s^{-1}, 1 µM Iso, F test p=0.0011) identifying drug efficacy as the target of voltage-dependence. Voltage-dependence is also transmitted to downstream signalling. The interaction of arrestin and receptor is reduced by depolarization in both, Iso and Adr stimulated cells but, here, an increase in agonist concentration significantly decreased the relative inhibitory effect of depolarization (Iso: 28±3.2%/16±2.3%, 100 nM/10 µM, $p<0.05$; Adr: 29±1.1%/10±1.7%, 1 µM/100 µM, $p<0.001$). As speed of voltage- and washout-induced dissociation also differed significantly (τ: Iso: 3.2±0.2 s/44.5±12.9 s, 100 nM, $p < 0.05$; Adr: 1.3±0.1 s/6.8±0.7 s, 1 µM, $p<0.001$) we compared offkinetics under constant potentials (-90 mV or +45 mV) to elucidate whether voltage-dependent inactivation is due to changes in efficacy or affinity. Washout of Iso was moderately accelerated under constant depolarization (τ: 30.8±2.7 s/20.0±1.5 s, 100 nM, $p<0.001$) whereas washout of Adr showed no significant difference between holding potential and depolarization (τ: 3.7±0.3 s/ 3.6±0.3 s, 1 µM, $p>0.05$), indicating at most minor changes in affinity. We also determined the amount of charge moved across the membrane and the half maximal potential of activation from a V_M-FRET response relation curve fitted to a Boltzmann equation. The half maximal potentials (V_{50}) for both, Iso and Adr stimulated receptors were in the physiological range (V_{50} Iso:-28.0 mV, V_{50} Adr:-26.6 mV) and calculated z-values of less than one charge (Iso: 0.35, Adr: 0.48) are in line with values from the literature for M_2-muscarinic and α_{2A}-adrenergic receptors [2,3,4].

Taken together these data suggest that the β_1-AR is voltage-dependent with highest sensitivity in the physiological range. A major contribution to this phenomenon comes

from a fast alteration in agonist efficacy by voltage, suggesting that voltage regulates these receptors on a faster time scale than classical agonist binding would allow.

References:

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