ABSTRACT

Muscarinic acetylcholine receptors (mAChR) belong to the family of G-protein coupled receptors. There are five subtypes of mAChR denoted M_1 to M_5 that are widely and differentially distributed in both the central nervous system and periphery and play an important role in many specific physiological functions. Impairment of muscarinic neurotransmission occurs in serious disorders such as Alzheimer's disease, schizophrenia or Parkinson's disease that are accompanied by cognitive decline mainly due to the disruption of M_1 receptor signaling in the brain. Unfortunately, the high degree homology of the orthosteric binding site among muscarinic receptor subtypes makes it very difficult to obtain subtype-selective agonists. One of the few known selective agonists is xanomeline that preferentially activates the M_1 and M_4 subtypes. Xanomeline exerts unique interactions with muscarinic receptors comprising reversible binding to the orthosteric domain, and wash-resistant allosteric interaction with a secondary binding site.

The basis of xanomeline functional selectivity remains largely unknown. In an attempt to probe into such mechanisms we investigated the immediate and long-term effects of xanomeline on activation of muscarinic receptors, using intact Chinese hamster ovary (CHO) cells expressing individual subtypes of mAChR. CHO cells expressing M_2 and M_4 receptors were transiently transfected with the promiscuous $G\alpha_{16}$ G-protein subunit to enable measuring changes in intracellular calcium level as an indicator of receptor activation at all subtypes of mAChR. In saturation binding experiments we assayed changes in receptor density and binding characteristics following xanomeline treatment.

We found that short (20s) stimulation with 0,1–10 μM xanomeline resulted in a fast increase in intracellular calcium level at all mAChR subtypes that was most pronounced at M_1 and M_4 receptors. At these two subtypes increased calcium level did not return to its basal level value. After longer (1, 3, or 10min) stimulation with 10 μM xanomeline calcium level did not return to its resting level even after 1h washing at M_1, M_3 and M_4 receptors. Increased calcium level at M_1 and M_4 receptors showed oscillations and subsequent stimulation with the classical agonist carbachol after 1h washing did not induce any additional response at the M_1 subtype. Wash-resistantly bound xanomeline behaved as a long-lasting antagonist at M_5 receptors. Unlike classical orthosteric agonists, 10 min xanomeline treatment did not induce receptor internalization at any receptor subtype. The classical antagonist N-methylscopolamine (NMS) inhibited the effects of wash-resistantly bound xanomeline at M_1–M_4 receptors. Withdrawal of NMS resulted in reappearance of calcium response at M_1 and M_4 receptors. Wash-resistantly bound xanomeline decreased the potency of NMS binding to a different extent at all receptor subtypes both after 10 and 60 min washing, which indicates competition for the orthosteric binding site.

In summary, xanomeline behaves in a similar manner at all mAChR subtypes regarding the characteristics of its reversible and wash-resistant binding and immediate effects. Nevertheless, our results indicate that distinct activation mechanisms exist at M_1 and M_4 receptors. This conclusion is foremost based on the higher efficacy of xanomeline to induce the primary fast calcium response at M_1 and M_4 than at other subtypes. The ability of xanomeline to induce fully developed oscillating calcium response in a long-term scale only at M_1 and M_4 receptors also supports this notion.