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Alteration of cardiovascular and neuronal function in M_1 knockout mice

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Abstract

We used gene targeting to generate mice lacking the M_1 muscarinic acetylcholine receptor. These mice exhibit a decreased susceptibility to pilocarpine-induced seizures, loss of regulation of M-current potassium channel activity and of a specific calcium channel pathway in sympathetic neurons, a loss of the positive chronotropic and inotropic responses to the novel muscarinic agonist McN-A-343, and impaired learning in a hippocampal-dependent test of spatial memory.

Keywords: Seizure; Gene targeting; Learning; Electrocardiogram; Potassium channel; Sympathetic neurons; MAP kinase; Phospholipase C

Introduction

Muscarinic acetylcholine receptors (mAChR) are present in neurons in the central and peripheral nervous system, cardiac and smooth muscles, and a variety of exocrine glands. In mammals such as humans, rats, and mice, there are five subtypes of mAChR, each encoded by a distinct gene with no introns in its coding region. The five subtypes of mAChR, termed M_1–M_5, can be divided into two broad functional categories: the M_1, M_3, and M_5 receptors preferentially couple to the Gq family of G-proteins to mediate activation of phospholipase C, while the M_2 and M_4 receptors preferentially couple to the Gi family of G-proteins to mediate inhibition of adenylyl cyclase. This specificity is not absolute however, as the M_2 and M_4 receptors can also activate certain isozymes of phospholipase C via $\beta\gamma$ subunits released

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from the Gi proteins, and all five subtypes of mAChR can also couple to Gs to mediate stimulation of adenylyl cyclase activity [1].

As summarized recently elsewhere [1], there are many additional mechanisms for the regulation of intracellular cAMP levels by mAChR, including G-protein βγ-mediated and calcium/calmodulin-mediated stimulation of certain forms of adenylyl cyclase, and calcium/calmodulin- and cGMP-mediated activation of certain forms of phosphodiesterase. Activation of phospholipase C leads to increased intracellular calcium levels and production of diacylglycerol (which can activate protein kinase C), while changes in intracellular cAMP levels can lead to corresponding changes in the activity of cAMP-dependent protein kinase.

In addition to these "classical" second messenger pathways, muscarinic receptors can also activate tyrosine kinases, phosphatidylinositol-3–kinase, phospholipase D, and phospholipase A2. The mAChR can also activate mitogen-activated protein kinase (MAPK) pathways, such as the ERK 1/2, p38, and c-jun kinase pathways. Because of the cell specific expression of different signal transduction proteins and the crosstalk that can occur between different signaling pathways, there are a large number of potential signals that can be produced by a particular mAChR in different cell types.

There are similarly many potential mechanisms for the regulation of ion channel function by mAChR activation. Muscarinic receptor-mediated activation of inwardly rectifying potassium channels and inhibition of N and P/Q-type calcium channels results from direct interaction of Gβγ subunits (released from Gi-family proteins) with the respective channel proteins. In addition, mAChR can alter ion channel function by regulation of the phosphorylation of the ion channel proteins by kinases such as PKA, PKC, or tyrosine kinases.

In the central nervous system, mAChR have been implicated in a wide variety of processes, including memory and learning, control of movement, nociception, the generation of epileptic seizures, and the modulation of circadian rhythms. In the periphery, mAChR regulate the rate and force of contraction of the heart, contraction of many smooth muscles, and secretion from many exocrine glands [2]. Studies on the physiological roles of the various mAChR subtypes in vivo are complicated by the lack of a high degree of specificity for many subtype-selective drugs. In this chapter we describe studies designed to determine the functions mediated by the M_1 mAChR in mice.

Generation of M_1-deficient mice

In order to define the functions mediated by the M_1 mAChR, we used homologous recombination in embryonic stem cells to generate mice with a targeted deletion in the M_1 mAChR gene [3]. Homozygous M_1 mutant mice were born from crosses of heterozygote mice with the expected Mendelian ratio of 1:4, and did not exhibit significant differences in body weight, longevity, fertility, and overt behavior compared to wildtype mice. The total number of mAChR binding sites in the forebrains of knockout mice was reduced to approximately half of the level found in wildtype mice, while there was no significant difference in the number of mAChR sites in the cerebellum between wildtype and mutant mice. Immunoprecipitation analyses using subtype specific antibodies demonstrated that the expression of M_1 receptor in the brains of knockout mice was reduced to undetectable levels, while there were no changes in the levels of the M_2, M_3, and M_4 receptors. Immunocytochemical analyses dem-

onstrated that there were also no significant alterations in brain morphology or in the pattern or levels of expression of the M_2, M_3, and M_4 receptors.

The role of the M_1 receptor in mAChR-mediated signal transduction pathways was investigated in primary neuronal cultures prepared from wildtype and M_1 knockout mice (Hamilton, unpublished results). There were large decreases in mAChR mediated activation of phospholipase C ($\sim$ 60% decrease) and the MAPK kinases ERK1 and ERK 2 ($\sim$90% decrease) in cultures from mutant mice compared to controls. Thus, the M_1 receptor is the main mAChR subtype responsible for both PLC and MAPK activation in the forebrain.

Electrophysiological analyses of M_1-deficient mice

Muscarinic receptors regulate the activity of a variety of ion channels in sympathetic neurons. The M-current is a voltage-gated potassium current which exhibits slow (but sustained) activation with a steep voltage dependence near the threshhold for action potential firing. Because it is the main potassium channel which is active near threshold, it is believed to play a major role in regulating neuronal excitability. Muscarinic receptor activation closes the channel and thus leads to membrane depolarization, making the cell more susceptible to firing. Treatment of dissociated sympathetic neurons from wildtype mice with the muscarinic agonist oxotremorine-M significantly decreased the magnitude of the M-current, while the M-current in sympathetic neurons from knockout mice was insensitive to oxotremorine-M [3]. This was not due to a defect in the M-current channel itself, as treatment with angiotensin II resulted in suppression of the M-current in sympathetic neurons from both wildtype and mutant mice. These results demonstrate that the M_1 receptor is the only subtype of mAChR which mediates suppression of the M-current potassium channel mouse in sympathetic neurons.

There are two pathways for the reduction of voltage-sensitive calcium channel activity by mAChR in sympathetic neurons: a “fast” membrane-delimited pathway, mediated by $\beta\gamma$ subunits released from pertussis toxin sensitive G-proteins, and a “slow” pathway mediated by pertussis toxin-insensitive G-proteins and a diffusible second messenger [4]. Consistent with previous pharmacological studies carried out in rat, mice lacking the M_1 receptor exhibit a loss of the slow pathway but retain the fast pathway. In contrast to previous results observed in rat sympathetic neurons, where the M_4 receptor mediates the fast pathway, analyses of M_2 and M_4-deficient mice clearly demonstrate that the M_2 receptor mediates modulation of the fast calcium channel pathway.

The role of the M_1 receptor in the regulation of potassium channel activity in hippocampal neurons was also examined by electrophysiological analysis of pyramidal cells in the CA1 region of hippocampal slice preparations [5]. In contrast to the loss of M-current regulation seen in sympathetic neurons, mAChR suppression of the M-current was not altered in CA1 hippocampal neurons from M_1 knockout mice. Muscarinic suppression of the afterhyperpolarization current (a calcium activated potassium current) and the leak potassium current were also not altered in the M_1 knockout mice. Consistent with these genetic results, mAChR inhibition of all three current was not blocked by M_1-toxin. Thus, the M_1 receptor is not required for the regulation of the activity of these three potassium channels in mouse CA1 pyramidal cells.

Role of the M_1 receptor in seizure initiation

Systemic administration of the partial muscarinic agonist pilocarpine to mice and rats results in tonic-clonic seizures which are similar to those exhibited by patients with temporal lobe epilepsy. Pharmacological studies have demonstrated that pilocarpine-induced seizures can be prevented by administration of pirenzepine, suggesting the M_1 and/or M_4 receptor may mediate seizure initiation. Mice homozygous for the mutant M_1 receptor do not exhibit seizures following the administration of doses of pilocarpine which cause multiple tonic-clonic seizures in wildtype mice [3]. Heterozygous mice with one wildtype allele are almost as resistant as homozygous mutant mice to pilocarpine-induced seizures. Mutant and wildtype mice exhibit similar seizure sensitivity to administration of kainic acid. Thus, the M_1 receptor is required for the initiation of seizures in the pilocarpine model of epilepsy.

Role of the M_1 receptor in memory and learning

Muscarinic receptors have been implicated in a variety of paradigms involving memory and learning, particularly hippocampal based learning paradigms [6,7], and in the induction of long-term potentiation (LTP) in hippocampal CA1 pyramidal cells [8,9]. LTP is widely used to study neuronal plasticity at the molecular and cellular level. When tested in a hippocampal-based spatial learning task, M_1 mutant mice show a significant impairment when compared to wildtype mice (Anagnostaras, unpublished results). The M_1 mutant mice also exhibit a defect in establishment of LTP in hippocampal CA1 neurons after minimal stimulation of the Schaffer collaterals. The M_1 mutant mice exhibit a severe impairment in the consolidation of hippocampal-based context conditioning but normal cue conditioning, which is based on the amygdala. [10]. These results indicate that the M1 receptor plays an important role in hippocampal-based memory and learning and neuronal plasticity.

Role of the M_1 receptor in cardiac function

There is conflicting data in the literature on the possible expression and function of M_1 receptors in the heart [11,12]. We found that there was no mRNA encoding the M_1 receptor detectable in mouse atria or ventricles by reverse transcriptase/polymerase chain reaction analysis, indicating negligible expression of the M_1 receptor in mouse heart (Hardouin, unpublished results). Injection of the novel muscarinic agonist McN-A-343 into wildtype mice caused an increase in the rate and force of cardiac contraction which was blocked by the beta-adrenergic antagonist propranolol. This is consistent with previous suggestions that McN-A-343 activates mAChR in the sympathetic ganglia to stimulate the heart . The positive chronotropic and inotropic effects of McN-A-343 were completely absent in the M_1 knockout mice [13]. Thus, McN-A-343 activates M_1 receptors in the sympathetic ganglia to produce the increased rate and force of contraction of the heart.

Conclusions

The work described here has provided new insights into the role of the M_1 mAChR in the functioning of the central and peripheral nervous systems and the cardiovascular system. The

ability to produce mice not just with targeted deletions in genes but also with tissue-specific or inducible gene deletions and with genes containing point mutations which can alter the function of the resulting proteins will likely provide even greater insights in the future on the functions of the mAChR in vivo.

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