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Invited review

Physiological roles of CNS muscarinic receptors gained from knockout mice

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ABSTRACT

Because the five muscarinic acetylcholine receptor subtypes have overlapping distributions in many CNS tissues, and because ligands with a high degree of selectivity for a given subtype long remained elusive, it has been difficult to determine the physiological functions of each receptor. Genetically engineered knockout mice, in which one or more muscarinic acetylcholine receptor subtype has been inactivated, have been instrumental in identifying muscarinic receptor functions in the CNS, at the neuronal, circuit, and behavioral level. These studies revealed important functions of muscarinic receptors modulating neuronal activity and neurotransmitter release in many brain regions, shaping neuronal plasticity, and affecting functions ranging from motor and sensory function to cognitive processes. As gene targeting technology evolves including the use of conditional, cell type specific strains, knockout mice are likely to continue to provide valuable insights into brain physiology and pathophysiology, and advance the development of new medications for a range of conditions such as Alzheimer's disease, Parkinson's disease, schizophrenia, and addictions, as well as non-opioid analgesics.

This article is part of the special issue entitled 'Muscarinic Receptors in the Central Nervous System'.

Contents

Abbreviations: CIN, cholinergic interneuron; CAMKIIα, Ca^{2+}/calmodulin-dependent protein kinase IIα; EPSP, excitatory postsynaptic potentials; HPA, hypothalamic-pituitary-adrenocortical; MAPK, mitogen-activated protein kinase; LTD, long-term depression; LTP, long-term potentiation; MSN, medium spiny neurons; mAChR, muscarinic acetylcholine receptor; PAM, positive allosteric modulator; PI, phosphatidyl inositol; PPI, prepulse inhibition; SNc, substantia nigra pars compacta; VTA, ventral tegmental area.

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1. Neuronal muscarinic receptor functions

1.1. Neuronal excitability, synaptic transmission, and neurotransmitter release

Unlike its classic neurotransmitter function in the periphery (e.g., at neuromuscular junctions), in the CNS, acetylcholine often functions as a modulator of neuronal activity. Acetylcholine, released from projection neurons and/or local interneurons, increases or decreases neuronal excitability and synaptic release of neurotransmitters, and modulates temporal patterns and coordination of activity between neurons (see Goldberg et al., 2012; Picciotto et al., 2012; Smythies, 2005). Given the complex and overlapping distribution of nicotinic and muscarinic acetylcholine receptor (mAChR) subtypes across the brain, combined with the difficulty in generating ligands with a high degree of selectivity at each muscarinic receptor subtype, transgenic mice lacking one or more functional mAChR subtypes ($M_1^{-/-}$ to $M_5^{-/-}$ mice) have been invaluable in dissecting these neuronal functions of acetylcholine (previously reviewed in Gautam et al., 2006; Matsui et al., 2004; Wess, 2012, 2004; Wess et al., 2007). Please see chapter 1 of the special issue, “Central muscarinic cholinergic system” for information about mAChR distribution and general characteristics.

1.1.1. Striatal functions

Acetylcholine in striatal tissues exemplifies cholinergic interneuron (CIN) function: representing only 1–2% of striatal neurons, the tonically active CINs are the major cholinergic input to the region and provide extensive innervation that potently modulate striatal functions (Gonzales and Smith, 2015). Striatal CINs express G_i-coupled M_2 and M_4 mAChRs (Bernard et al., 1992; Yan and Surmeier, 1996) and these are proposed to mediate suppression of dopamine transmission in the striatum (Bonsi et al., 2008; Shin et al., 2015; Zhang et al., 2002a). Initial investigation of dopamine release in slices from mAChR knockout mice suggested that multiple mAChR subtypes are involved in regulation of striatal dopamine, with some receptors (M_3 and M_4) mediating their effects indirectly via modulations of striatal GABA tone (Zhang et al., 2002b). While the effects of inactivation of different mAChR subtypes on dopamine release have been conflicting (Bendor et al., 2010; Forster et al., 2002; Foster et al., 2014b; Tzavara, 2004; Yamada et al., 2001; Zhang et al., 2002b), studies applying fast-scan cyclic voltammetry and amperometry have been valuable in the understanding of these, and conclude that a key action of mAChRs is to modulate acetylcholine tone from CINs (Shin et al., 2015; Threlfell et al., 2010). Depression of striatal dopamine transmission is proposed to arise from a disynaptic mechanism by which activation of M_2/M_4 autoreceptors on CINs creates an inhibitory outward current and decreased conductance, resulting in decreased cholinergic tone and subsequent nicotinic acetylcholine receptor-dependent dopamine transmission (Bonsi et al., 2008; Shin et al., 2015; Threlfell et al., 2010). This modulation is not simply inhibitory, but also makes dopamine release more sensitive to the frequency of neuronal firing, and seems to be controlled by M_2 and M_4 mAChRs in the dorsal striatum, but only by M_4 mAChRs in the ventral striatum/nucleus accumbens (Threlfell et al., 2010; Threlfell and Cragg, 2011). Consistent with this, whole-tissue striatal dopamine and metabolites were normal in $M_4^{-/-}$ mice (Dencker et al., 2012b), but $M_4^{-/-}$ mice displayed increased psychostimulant-induced extracellular dopamine efflux in the nucleus accumbens (Schmidt et al., 2011; Tzavara, 2004). No alteration of accumbal psychostimulant-induced dopamine efflux was detected in $M_2^{-/-}$ mice (Tzavara, 2004), in agreement with the notion that M_4, but not M_2 receptor activation exert inhibitory control on evoked dopamine release in ventral striatum.

The dense and extensive axonal branching of CINs results in a widespread release of acetylcholine, which acts locally on cholinergic receptors on striatal output medium spiny neurons (MSN). Postsynaptically, excitability of MSN in response to excitatory and inhibitory inputs is modulated by M_1 receptor activation, via KCNQ potassium channel regulation and endocannabinoid-mediated signaling (Narushima et al., 2007; Shen, 2005). Several lines of evidence including knockout studies indicate that M_1 receptor stimulation enhances the dendritic excitability and spiking of MSN, making them more “responsive” to corticostriatal input (Ding et al., 2010). This modulation happens preferentially in the D_2-expressing indirect pathway MSN, thought to provide the inhibitory, “no-go”, side of striatal output (Ding et al., 2010). Measured at the level of striatal tissue rather than at the cell level, $M_1^{-/-}$ mice had significantly elevated extracellular dopamine levels despite normal whole-tissue levels (i.e., indicating increased release), and dopamine efflux in response to amphetamine was exacerbated (Gerber et al., 2001). M_4 mAChRs are densely co-expressed with dopamine D_1 receptors on MSNs (Ince et al., 1997; Yan et al., 2001), and were suggested to act as a functional antagonist of D_1 receptor-mediated cyclic AMP-dependent signaling pathways (Jeon et al., 2010; Onali and Olianas, 2002; see also section 3). M_4 receptors on MSN were also shown to mediate prolonged suppression of dopamine release, through a cannabinoid CB_2 receptor dependent mechanism (Foster et al., 2016). Corticostriatal glutamatergic transmission is depressed by stimulation of presynaptic M_4 mAChRs (Higley et al., 2009; Pakhotin and Bracci, 2007; Pancani et al., 2014). Finally, a specific Ca^{2+}/calmodulin-dependent protein kinase IIα (CAMKIIα) has been found to bind directly and selectively to the M_4 receptor upon calcium influx and to mediate potentiation of M_4 receptor signaling (Guo et al., 2010a, 2010b). Thus, overall, activation of striatal M_1 and M_4 receptors modulates dopaminergic signaling towards inhibition.

Striatal tissues receive dopaminergic projections from the ventral tegmental area (VTA) and the substantia nigra pars compacta (SNc), in which only the M_5 mAChR subtype has been detected. Stimulation of those M_5 receptors increases neuronal activity and dopamine release, and is necessary for sustained striatal dopamine release (Forster et al., 2002; Foster et al., 2014b). Dopaminergic VTA and SNc neurons in turn receive input from cholinergic neurons in the laterodorsal pontine tegmental nuclei, which are modulated by inhibitory M_2 and M_4 receptors (Kohlmeier et al., 2012). In $M_5^{-/-}$ mice, extracellular nucleus accumbens dopamine levels were comparable to wild-type controls at baseline, after depolarization (K^+)-induced release, and after electrical stimulation of the medial forebrain bundle (Basile et al., 2002; Schmidt et al., 2010). However, $M_5^{-/-}$ mice showed dramatically reduced dopamine efflux after stimulation of the laterodorsal or pedunculopontine tegmental nuclei, and electrically

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