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Update article

Protein kinase C bound with A-kinase anchoring protein is involved in muscarinic receptor-activated modulation of M-type KCNQ potassium channels

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This article is dedicated to the memory of the late Professor Emeritus Yasutomi Nishizuka.

Abstract

The second messenger for closure of M/KCNQ potassium channels in post-ganglionic neurons and central neurons had remained as a 'mystery in the neuroscience field' for over 25 years. However, recently the details of the pathway leading from muscarinic acetylcholine receptor (mAChR)-stimulation to suppression of the M/KCNQ-current were discovered. A key molecule is A-kinase anchoring protein (AKAP; AKAP79 in human, or its rat homolog, AKAP150) which forms a trimeric complex with protein kinase C (PKC) and KCNQ channels. AKAP79 or 150 serves as an adapter that brings the anchored C-kinase to the substrate KCNQ channel to permit the rapid and 'definitive' phosphorylation of serine residues, resulting in avoidance of signal dispersion. Thus, these findings suggest that mAChR-induced short-term modulation (or memory) does occur within the already well-integrated molecular complex, without accompanying Hebbian synapse plasticity. However, before this identity is confirmed, many other modulators which affect M-currents remain to be addressed as intriguing issues.

Keywords: Excitable membrane; Synaptic transmission; KCNQ/M K^+ channels; A-kinase anchoring protein; Protein kinase C; Muscarinic acetylcholine receptor; Postsynaptic modulation

1. Introduction

One of the recent topics is a signaloplex of phospholipase C (PLC) which conveys signals from receptors to ion channels, including M-type (KCNQ2/3) potassium channels (Brown and Adams, 1980; Brown, 1988; Singh et al., 1998; Wang et al., 1998; Shapiro et al., 2000; Selyanko et al., 2000; Jentsch, 2000; Robbins, 2001), as reviewed by Delmas et al. (2004) and Shapiro (2004). Molecular organization of PLC signaling in microdomains is without doubt important in the suppression of M-currents, as was originally reported by Higashida, Brown and others in NG108-15 cells expressing native bradykinin receptors and cloned muscarinic acetylcholine receptors (mAChRs) (Yano et al., 1984; Higashida and Brown, 1986; Fukuda et al., 1988; Neher et al., 1988; Robbins et al., 1991). However, the details of the pathway leading from receptor-stimulation to suppression of M-current had remained as a 'mystery in the neuroscience field' for over 20 years (see *Slow Synaptic Responses and Modulation* edited by Kuba et al. (2000) and Ikeda and Kammermeier (2002)), from the early works on synaptic events in sympathetic ganglia (Kuba and Koketsu, 1978), the first report on M-current (Brown and Adams, 1980) and the

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Berridge's note on second messengers in 1986 (Berridge, 1986).

2. Protein kinase C associated with A-kinase anchoring protein

Hoshi et al. published a paper in 2003 which stresses that the signaling pathway from mAChRs to KCNQ/M K^+ channels is mediated by A-kinase anchoring protein (AKAP; AKAP79 in human, or its rat homolog, AKAP150) (Carnegie and Scott, 2003). AKAP forms a trimeric complex with protein kinase C, found by the late Nishizuka and his fellow researchers (PKC; Nishizuka, 1986; Shearman et al., 1989), and KCNQ channels (Cooper et al., 2000; Fig. 1). AKAP150 serves as an adapter that brings the kinase to the substrate KCNQ channel to permit the rapid and 'definitive' phosphorylation of serine residues, resulting in avoidance of signal dispersion. Results by Hoshi et al. (2003) are thus the first demonstration of anchoring-dependent PKC modulation of ion channels. mAChR-induced short-term modulation (or memory) for periods of time ranging from a few seconds to some minutes does occur within the already well integrated molecular complex, without accompanying Hebbian synapse plasticity (Brown and Milner, 2003).

For the role of kinases, it has been reported that Src kinase possesses a powerful inhibitory effect on KCNQ channels, though Src is not an initiator of M-current inhibition following mAChR stimulation (Gamper et al., 2003).

3. Interaction of possible second messengers with A-kinase anchoring protein

Delmas et al. (2004) depict the downstream signal from PLC to PKC as only diacylglycerol (DAG) in their scheme and claimed that Ca^{2+} is crucial mainly in the microdomain around bradykinin receptors. However, since Ca^{2+} and calmodulin (CaM) can release PKC from AKAP79 in vitro (Faux and Scott, 1997), they could be messengers for releasing PKC from the KCNQ/AKAP/PKC complex in vivo. Indeed AKAP79 has been reported to harbor Ca^{2+}-activated molecules such as calcineurin (CaN) and CaM (Faux and Scott, 1997). If this addition to the signaloplex is important, then what is the role of CaN bound directly to the KCNQ channels (Marrion, 1996)?

Indeed it is reported that CaM binds directly to the C-terminal region of KCNQ channels and acts as a mediator in the Ca^{2+}-dependent modulation of KCNQ channels (Wen and Levitan, 2002; Yus-Najera et al., 2002; Gamper and Shapiro, 2003; Shapiro, 2004). One simple possibility is that CaM bound to AKAP could serve as a pool for the KCNQ bound CaM. The main effect of CaM then should be reconsidered from the view point of its binding to AKAP.

Furthermore the targeting of AKAP79 to the cell surface membrane is also mediated through phosphatidyl-4,5-bisphosphate (PIP_2) (Dell'Acqua et al., 1998). Thus, PIP_2 may play a role in regulation of KCNQ channels both directly and indirectly through AKAP (Suh and Hille, 2002; Zhang et al., 2003; Ford et al., 2003). The recovery process of M-current may also require an AKAP–PIP_2 interaction.

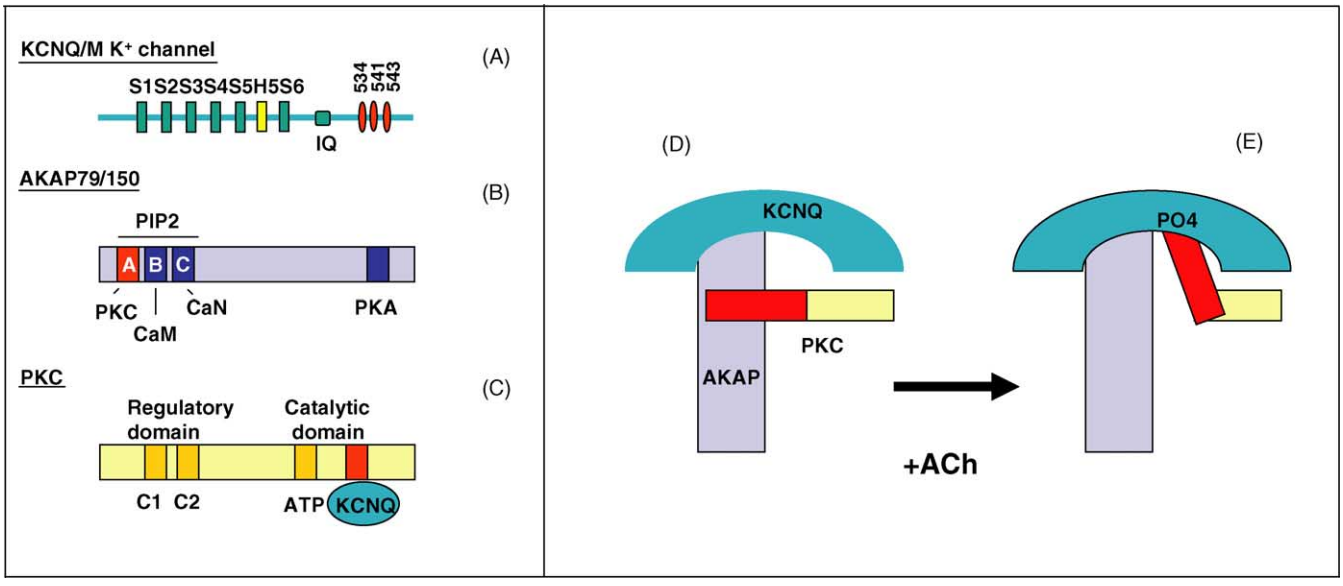

Fig. 1. Structures of the KCNQ channel, AKAP97/150 and PKC: (A) the KCNQ channel indicates the transmembrane domains (S1–S6), pore region (H5), and the potential phosphorylation sites of serine residues in the C-terminal tail focused in this study; (B) four domains in AKAP represents binding sites for PKC, CaM, CaN, and PKA. At the N-terminal portion of AKAP can bind to PIP_2 in the membrane. (C) C1 and C2 in the regulatory domain show the binding sites for phorbol ester or diacylglycerol and calcium, respectively. ATP and phosphoryl-transfer sites where the substrate (KCNQ) receives phosphates are shown. (D, E) Schematic diagram of the trimeric complex before and after mAChR stimulation. PKC binds to AKAP97/150 at its catalytic domain and masked before stimulation. PKC is released from AKAP and activated, then phosphorylates nearby serine residues of the KCNQ channel bound to AKAP.

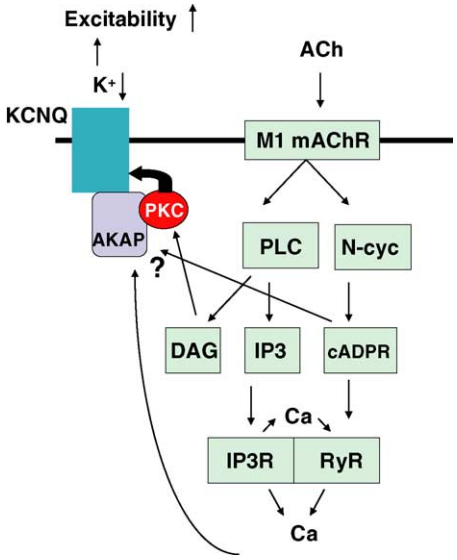

Fig. 2. Models for M(KCNQ)-current inhibition by activation of muscarinic acetylcholine receptors. Stimulation of mAChRs by ACh leads to activation of phospholipase C (PLC) and ADP-ribosyl cyclase (N-cyc), resulting in production of diacylglycerol (DAG), inositol-1,4,5-trisphosphate (IP3), and cyclic ADP-ribose (cADPR) in NG108-15 cells. Cytosolic Ca^{2+} (Ca) is elevated by Ca^{2+} release both from IP_3 receptor (IP3R) activation or ryanodine receptor (RyR) with a concerted action of Ca^{2+} and cADPR. DAG activates PKC bound to AKAP at its catalytic domain, and subsequently, Ca^{2+} may release PKC. Then, the PKC phosphorylates KCNQ channels, which resulted in closure of M-channels, producing an increase in membrane excitability. Unidentified signal from cADPR to the KCNQ–AKAP complex may also contribute to M-channel inhibition.

One messenger not considered by Delmas et al. (2004) and Shapiro (2004) is cyclic ADP-ribose (cADPR) (Lee, 2001; Higashida et al., 2001). To our knowledge the almost complete block of M-current inhibition by its antagonist, 8-amino-cADPR, is the only example of a cytosolically applied substance to produce such a dramatic effect, all be it in NG108-15 cells (Higashida et al., 1995, 1997, 2000; Bowden et al., 1999). Though not completely understood, the possible role of cADPR in KCNQ channel closure in downstream of mAChRs is schematized in Fig. 2.

Upstream from the above the role of G_q versus G_{11} also needs considering. Although these two G-protein subtypes tend to be considered as super imposable, there is evidence that this PLC signaling domain may diverge at the level of the G-protein. For example, mAChR-mediated inhibition of M-current in rat superior cervical ganglion neurons was attenuated by G_q antisense but not G_{11} (Haley et al., 1989). In G_q deficient mice muscarinic and bradykinin-mediated inhibition remained and was only removed by the further addition of a $G_{q/11}$ antibody (Haley et al., 2000). Therefore, both G proteins seem to be necessary for different receptors.

4. Conclusion

In conclusion, after 18 years the PKC-hypothesis in M-current inhibition originally proposed by Higashida and Brown (1986) is re-evaluated from the fact of involvement of PKC/AKAP bound to KCNQ/M channels reported by Hoshi et al. (2003). However, still there reported many other modulators which affect on M-currents. So what is the ‘‘take home message’’ (pun intended)? Is there a requirement for PIP_2, calcium, CaM, CaN, cADPR, PKC, and Src all to act in concert (AND) to close M-channels? Will a mixture of a few of them work (EITHER) or will any one signal do the job (OR)?

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