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Diacylglycerol levels modulate the cellular distribution of the nicotinic acetylcholine receptor

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ABSTRACT

Diacylglycerol (DAG), a second messenger involved in different cell signaling cascades, activates protein kinase C (PKC) and D (PKD), among other kinases. The present work analyzes the effects resulting from the alteration of DAG levels on neuronal and muscle nicotinic acetylcholine receptor (AChR) distribution. We employ CHO-K1/A5 cells, expressing adult muscle-type AChR in a stable manner, and hippocampal neurons, which endogenously express various subtypes of neuronal AChR. CHO-K1/A5 cells treated with dioctanoylglycerol (DOG) for different periods showed augmented AChR cell surface levels at short incubation times (30 min–4 h) whereas at longer times (18 h) the AChR was shifted to intracellular compartments. Similarly, in cultured hippocampal neurons surface AChR levels increased as a result of DOG incubation for 4 h. Inhibition of endogenous DAG catabolism produced changes in AChR distribution similar to those induced by DOG treatment. Specific enzyme inhibitors and Western blot assays revealed that DAGs exert their effect on AChR distribution through the modulation of the activity of classical PKC (cPKC), novel PKC (nPKC) and PKD activity.

1. Introduction

Diacylglycerol (DAG) is involved as a second messenger in different cell signaling cascades and cellular events (Carrasco and Merida, 2007; Merida et al., 2010). It has also been demonstrated that proteins responding to DAG, such as PKCs, PKDs and other kinases, have at least one copy of the conserved domain 1 (C1) in their amino acid sequence. In addition, DAGs' conical shape and small polar head group make them especially apt to move in slow flip-flop oscillations in the membrane, favoring vesicle budding, fusion and fission (Huttner and Zimmerberg, 2001; Lev, 2006). DAG therefore has a dual role in mammalian cells as both a modulator of membrane dynamics and a second messenger, tightly regulating DAG levels. The interrelationship between DAG metabolism and signaling functions is crucial to ensure correct maintenance of homeostasis during cell growth and development (Joshi et al., 2013; Liu et al., 2014; Meguro et al., 2006). Modification of membrane DAG levels and other non-membrane DAG pools, in combination with certain intracellular responses, is crucial for specifying the nature, intensity and duration of a given signal in the modulation of cellular processes.

The Golgi complex is the only organelle with relatively stable DAG levels in the absence of any stimuli. In contrast, DAGs located in the plasma membrane are generated and quickly degraded in response to extracellular signals through e.g. PKC/phospholipase C (PLC) and sphingomyelin synthase 2 (SMS2) pathways (Gallegos et al., 2006; Huitema et al., 2004; Lev, 2006).

Intracellular DAG accumulation in muscle triggers the activation of novel PKCs with subsequent impairment of insulin signaling (Schmitz-Peiffer, 2000, 2013). Although lipid accumulation has been thoroughly studied in relation to the insulin pathway, little is known about the consequences of this condition on the muscle-type nicotinic acetylcholine receptor (AChR), a key molecule in neuromuscular transmission.

The AChR is an integral membrane protein that belongs to the ligand-gated ion channel superfamily. It is composed of five homologous subunits organized pseudo-symmetrically around a central

Abbreviations: 4α-PMA, 4α-phorbol 12-myristate 13-acetate; AChR, muscle-type nicotinic acetylcholine receptor; aPKC, atypical protein kinase C; BTX, α-bungarotoxin; cPKC, classical protein kinase C; CPT, choline phosphotransferase; DAG, diacylglycerol; DAGK, diacylglycerol kinase; DOG, dioctanoylglycerol; GGOH, geranylgeraniol; nPKC, novel protein kinase C; PC, phosphatidylcholine; PDBu, phorbol 12-13-di butyrate; PKD, protein kinase D; PVDF, polyvinylidene fluoride; ROT, rottlerin; TTBS, Tween–Tris buffer solution.

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pore (Karlin, 2002). The binding of the natural neurotransmitter acetylcholine (ACh) to the AChR produces a conformational change, which opens the channel, thus allowing positively charged ions to enter the cell (Karlin, 2002). Previous research from our laboratory has shown that AChR is extremely sensitive to its lipid environment (reviewed in Barrantes (2004, 2010)). Furthermore, we have demonstrated that cholesterol and sphingomyelin are particularly important for correct AChR distribution and trafficking (Baier and Barrantes, 2007; Borroni et al., 2007; Borroni and Barrantes, 2011; Gallegos et al., 2008; Pediconi et al., 2004; Roccamo et al., 1999). Additional work from our laboratory also demonstrated that ceramides differentially modulate AChR trafficking to the plasma membrane in a concentration-dependent manner (Gallegos et al., 2008). A precise lipid balance is therefore required for AChR to reach the plasma membrane in a functional way (Baier and Barrantes, 2007; Gallegos et al., 2008; Roccamo et al., 1999).

The purpose of the present work was to study the modulation of muscle-type AChR distribution and function by DAGs in CHO-K1/A5 cells, a cell line model system that heterologously expresses adult-type muscle AChR (Roccamo et al., 1999). To this end, different experimental strategies were implemented to set cellular DAG levels. We demonstrate that AChR distribution in the cell line expressing adult muscle-type receptor is modified in a time-dependent manner by DAG oversupply acting through conventional and novel PKC (cPKC and nPKC) and PKD. These results are further confirmed using hippocampal neurons in primary culture.

2. Materials and methods

2.1. Materials

All chemicals were from Sigma Chemical Company (St. Louis, MO, USA) unless otherwise specified. [^{125}I]iodine was from Perkin Elmer (Wellesley, MA, USA). 1-Palmitoyl-2-oleoyl-sn-glycerol (POG) was from Avanti Polar Lipids (Alabaster, AL, USA). Nile red and Alexa Fluor^{488}-conjugated BTX (Alexa Fluor^{488}-BTX) were from Molecular Probes (Eugene, OR, USA).

Rabbit polyclonal antibodies anti-PKD, anti-pPKD (Ser^{916}), anti-pPKCα/βII (Thr$^{638/641}$) and anti-pPKCδ/θ (Ser$^{643/676}$) were from Cell Signaling (Beverly, MA, USA). Mouse monoclonal antibodies anti-PKCβ, anti-PKCη and anti-PKCδ were from BD Biosciences (San Jose, CA, USA). Rabbit polyclonal antibody anti-pPKCη (Thr^{655}) was from Life Technologies (Grand Island, NY, USA). HRP-conjugated goat anti-rabbit and goat anti-mouse antibodies were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA).

2.2. Cell culture and protein content

CHO-K1/A5 cells were grown in Ham's F12 medium supplemented with 12% fetal bovine serum as previously described (Roccamo et al., 1999). Dissociated neuronal cultures were prepared from hippocampi of embryonic day 19 rats, as previously described (Brocco et al., 2003). When required, lipids and enzyme

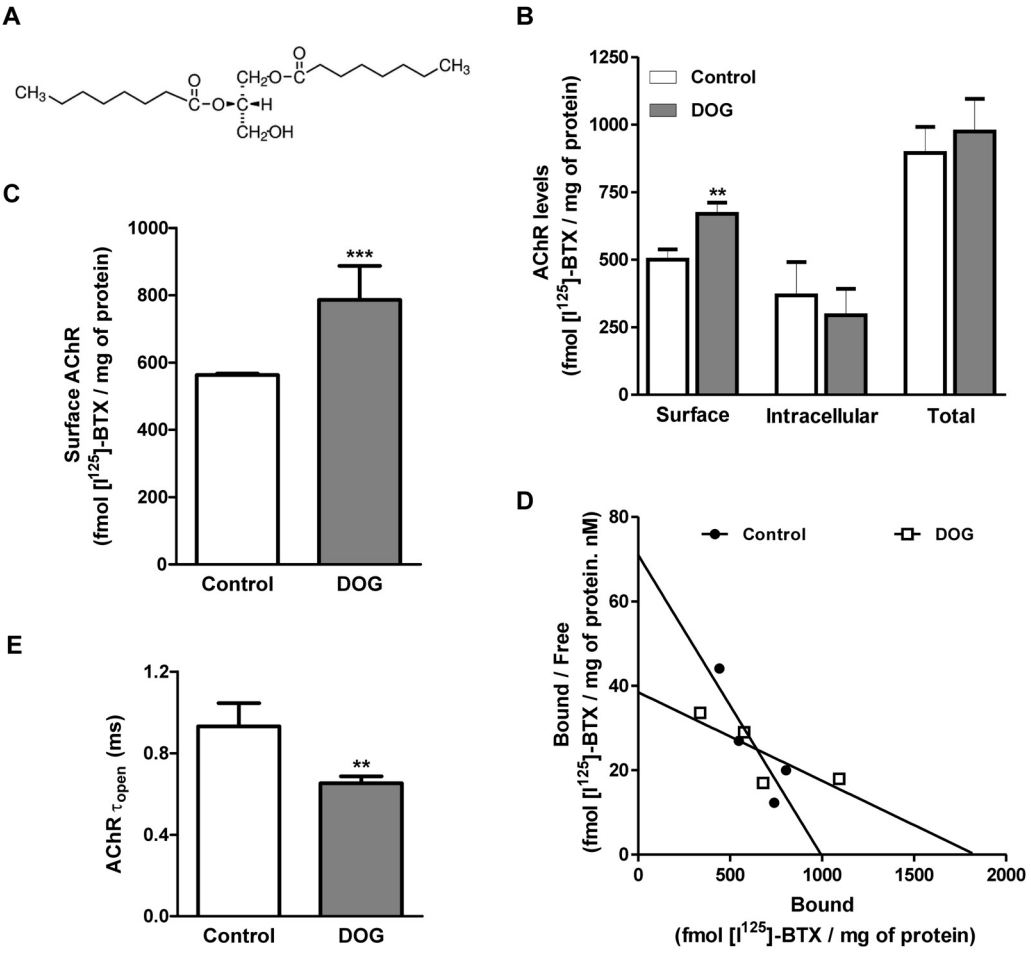

Fig. 1. Exposure to DOG from 30 min to 4 h increases surface AChR levels and alters ligand binding affinity and channel properties. (A) Schematic representation of DOG chemical structure. (B) CHO-K1/A5 cells were exposed to 300 μM DOG for 4 h at 37 °C and at the end of the incubation period cells were probed for surface, intracellular and total [^{125}I]-BTX binding. (C) CHO-K1/A5 cells were incubated with 300 μM DOG for 30 min at 37 °C and at the end of the incubation period surface [^{125}I]-BTX binding was quantified. (D) Scatchart-plots obtained from control and treated cells exposed to DOG for 30 min. (E) Histogram showing the mean open time of AChR activated with ACh after incubation of CHO-K1/A5 cells for 30 min at 37 °C with 300 μM DOG. Data represent average ± SD from $n = 4$. $^{**}p < 0.01$, $^{***}p < 0.001$.