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Novel ryanodine-binding properties in mammalian retina

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Abstract

The ryanodine receptor (RyR)/Ca^{2+} release channel mobilizes Ca^{2+} from internal calcium stores to support a variety of neuronal functions. To investigate the presence of such a protein in mammalian retina, we applied ryanodine binding, PCR and antibodies against known RyRs. Surprisingly, ryanodine-binding properties of retinal endoplasmic reticulum-enriched membrane fraction were vastly different from those of skeletal and cardiac muscles ryanodine-binding proteins. In common with the skeletal and cardiac muscle, ryanodine bound with high-affinity to two or more types of binding site (K_{d_1} = 20.6 and K_{d_2} = 114 nM); binding was strongly stimulated by high concentrations of NaCl; it was inhibited by tetracaine and the protein appeared to possess an ATP-binding site. Unlike cardiac and skeletal muscle, RyRs in retina binding was Ca^{2+}-independent; inhibited by caffeine and dantrolene; less sensitive to ruthenium red; and unaffected by La^{3+}. Also, in retina, ryanodine rapidly associated to and dissociated from its binding sites. Furthermore, although the protein bound the ATP analog BzATP, retinal ryanodine binding was not stimulated by nucleotides. Immunostaining of bovine retinal sections with anti-RyR2 showed a strong staining of amacrine, horizontal and ganglion cells. Finally, using RT-PCR, the three known RyR isoforms were identified in retina. However, consistent with the novel binding properties, the peptide maps yielded by trypsin treatment and Western blotting demonstrate different patterns. Together, the results suggest that retina expresses a novel ryanodine-binding protein, likely to be a ryanodine receptor. Its presence in retina suggests that this protein might play a role in controlling intracellular Ca^{2+} concentration.

Keywords: Caffeine; Dantrolene; Retina; Ryanodine receptor; Ryanodine

Abbreviations: DAB, 3,3′-diaminobenzidine; EDTA, ethylene-diaminetetraacetate; EGTA, ethylene glycol bis (aminoethylether) tetraacetate; Mops, 3-(*N*-morpholino) propanesulfonic acid; PMSF, phenylmethylsulfonyl fluoride; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SR, sarcoplasmic reticulum; RyR, ryanodine receptor; Tricine, *N*-(2-hydroxy-1,1-bis (hydroxy-methyl)-ethyl)-glycine

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1. Introduction

Intracellular free Ca^{2+} ($[Ca^{2+}]_i$) plays an important role in regulating cell function in both contractile and non-contractile cells (Berridge, Lipp, & Bootman, 2000; Clapham, 1995). Intracellular $[Ca^{2+}]$ is regulated partially by mobilizing Ca^{2+} into and out of the endoplasmic reticulum (ER). In the ER, Ca^{2+} is taken up by Ca^{2+} pumps, and is released by two well-characterized Ca^{2+}-release channels: the inositol 1,4,5-triphosphate receptor ($InsP_3R$) (Berridge, 1993; Berridge et al., 2000) and the ryanodine receptor (RyR) (Coronado, Morrissette, Sukhareva, & Vaughan, 1994; Meissner, 1994; Shoshan-Barmatz & Ashley, 1998; Sutko & Airey, 1996). RyR exhibits ryanodine-sensitive Ca^{2+} release channel activity that is activated by Ca^{2+} binding to a high-affinity site and inactivated by Ca^{2+} binding to a low-affinity site. In addition to Ca^{2+}, the RyR is also activated by adenine nucleotides and caffeine, and is inhibited by Mg^{2+} and ruthenium red (for reviews see Coronado et al., 1994; Meissner, 1994; Shoshan-Barmatz & Ashley, 1998; Sutko & Airey, 1996). There are at least three RyR isoforms (RyR1–3), encoded by three distinct genes, which are widely distributed in different mammalian tissues, e.g., cardiac (Lai et al., 1992; Meissner, 2004; Otsu et al., 1990), skeletal muscle (Coronado et al., 1994; Meissner, 1994; Shoshan-Barmatz & Ashley, 1998; Sutko & Airey, 1996), brain (Ashley, 1989; Lai et al., 1992; McPherson & Campbell, 1990), and smooth muscle (Herrmann-Frank, Darling, & Meissner, 1991). In addition, the liver expresses a ryanodine-binding protein that differs from the three known RyR types in its binding properties, but its molecular nature has not yet been resolved (Shoshan-Barmatz, Pressley, Higham, & Kraus-Friedmann, 1991).

As in all neurons, intracellular Ca^{2+} in retinal neurons plays a critical role in transmitter release and in regulating a variety of signal transduction processes. Intracellular $[Ca^{2+}]$ is regulated by several mechanisms including Na/Ca exchanger (Schnetkamp, 2004), adenosine receptor (Hartwick, Lalonde, Barnes, & Baldridge, 2004; Stella, Bryson, Cadetti, & Thoreson, 2003) $InsP_3$ receptor (Wang, Sterling, & Vardi, 1999), and RyR. In invertebrate eyes, RyR contributes to light adaptation (Akopian & Witkovsky, 2002; Akopian, Gabriel, & Witkovsky, 1998; Arnon et al., 1997; Baumann, 2000; Walz, Baumann, Zimmermann, & von Ciriacy-Wantrup, 1995). In lower vertebrates, RyR and $InspP_3R$ were immunolocalized to several cell types (Akopian et al., 1998; Krizaj, Lai, & Copenhagen, 2003; Krizaj, Liu, & Copenhagen, 2004), and physiological recordings from ganglion cells showed that the activity of these receptors modulates GABAergic responses (Akopian et al., 1998). However, none of these studies pertain to mammalian retina in which anatomical and physiological connections are intensively studied. To start to understand RyR function in mammalian retina, we applied several approaches including, immunostaining, RT-PCR and characterization of ryanodine binding to retinal fractions enriched with endoplasmic reticulum. Surprisingly, we found that retina expresses novel ryanodine-binding properties distinguished from those of known RyRs, including Ca^{2+}-independence, inhibition by caffeine, and different kinetic parameters.

2. Materials and methods

2.1. Materials

ATP, BSA, EDTA, EGTA, $LaCl_3$, Mops, trypsin and Tris were obtained from Sigma (St. Louis, MO). Benzoyl–benzoyl–ATP (BzATP), and [α-^{32}P]BzATP were synthesized and purified as described previously (Zarka & Shoshan-Barmatz, 1993) with some modifications to scale down the amount of BzATP synthesized. [α-^{32}P]ATP was obtained from Amersham and [^{3}H]ryanodine (60 Ci/mmol) was purchased from NEN® Life Science Products Inc. (Boston, USA). Unlabeled ryanodine was obtained from Calbiochem. Ruthenium red (98% pure) was from Fluka. Monoclonal anti-RyR 34C antibodies were obtained from the Developmental Studies Hybridoma Bank (University of Iowa). A polyclonal, affinity purified antibody against a cardiac RyR was kindly provided by S. Fleischer (Vanderbilt University), and a monoclonal anti-cardiac antibody (MA3-916) was from Affinity Bioreagents (ABR) (Golden, CO, USA). Human retina and skeletal muscle Marathon-ready cDNA were obtained from BD Biosciences Clontech (CA, USA).

2.2. Membrane preparations

Retinal membrane fractions were prepared from about 10 freshly isolated or frozen bovine or rabbit