Ryanodine receptor isoforms in the neonatal rat cochlea

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Intracellular Ca^{2+} plays an essential role in many aspects of cochlear function. The ryanodine receptor (RyR) intracellular Ca^{2+} release channel has been implicated in the regulation of both auditory neurotransmission and sound transduction (Bobbin, 2002; Kennedy and Meech, 2002; Sridhar *et al.*, 1997). Nevertheless, there is a lack of data on the localisation of RyR mRNA and protein in the cochlea.

RyR isoform (RyR1, RyR2, RyR3) mRNAs were amplified from postnatal day 10 rat spiral ganglion neuron (SGN), organ of Corti, and whole cochlea cDNA using the reverse transcription-polymerase chain reaction (RT-PCR) with RyR isoform-specific primers. The identity of the cDNA PCR products was confirmed by sequencing. Ca^{2+} imaging of RyR-mediated Ca^{2+} store release was imaged using 300μm neonatal rat cochlear slices loaded with 10μM fluo 4-AM.

All three RyR isoform mRNA transcripts were detected in the whole rat cochlea and SGN cDNA. However, only RyR1 and RyR3 mRNA transcripts were detected in the organ of Corti. As previously reported, a primary antibody recognising both RyR1 and RyR2 revealed protein expression in the SGN cell bodies and organ of Corti in adult rat cochlea (Whitehead *et al.*, 2002). However, a RyR2-specific antibody showed staining only in the SGN cell bodies, thus the organ of Corti labelling probably reflects RyR1 expression. Functional expression of RyR in the neonatal rat cochlea was confirmed by increases in intracellular Ca^{2+} in the SGN cell bodies and the organ of Corti with bath superfusion of the RyR agonist caffeine (5mM).

This study confirms the expression of multiple RyR isoform mRNA transcripts in the neonatal rat cochlea, and the expression of functional RyR protein in the SGN and organ of Corti. Co-expression of all three RyR isoform mRNA transcripts in the SGN cell bodies suggests a complexity of RyR-mediated Ca^{2+} signalling associated with auditory neurotransmission. The detection of RyR1 and RyR3 mRNA and the immunolabelling for RyR1 in the organ of Corti suggests that both isoforms contribute to regulation of sound transduction.

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