Determining the Location of GABA Receptor mRNA Transcripts in *Danio rerio*

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Introduction

γ-aminobutyric acid (GABA) is a neurotransmitter that has been shown to be present in the inner ear. GABA binds to two classes of GABA receptors (GABARs): $GABA_A$Rs are pentameric ion channels (isoforms include: α 1-6, β 1-3, γ 1-3, ρ 1-3, δ, ζ, and π); $GABA_B$Rs are G-protein coupled receptors (isoforms include: 1a, 1b, and 2). Studies have shown that $GABA_A$R and $GABA_B$R transcripts are located in the inner ear but their function is not yet fully understood. This is in part due to access to the inner ear being very limited. *Our experiments are designed to further our understanding of the role of GABA in the inner ear.*

To get around the limited access to the inner ear, we use zebrafish lateral line sensory tissue as a model because the sensory hair cells of the lateral line are closely related to the hair cells of the mammalian inner ear. Neuromasts containing hair cells and are distributed over the head and the trunk of zebrafish. Hair cells communicate with the central nervous system through connections with afferent and efferent neurons.

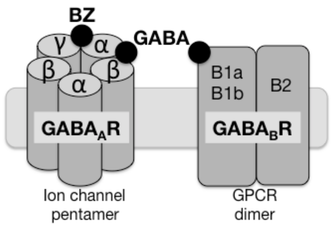

Figure 1: Schematic representation of the different GABARs. The image on the left represents the GABA(A)R which forms a pentameric ion channel formed by α, β, and γ isoforms. The image of the right represents the GABA(B)R which is a G-protein coupled receptor that is made up of B1a or B1b and B2 isoforms.

Hypothesis

Because hair cells of the mammalian inner ear and the zebrafish lateral line are closely related, *we hypothesize that lateral line cells express GABA receptors* and will be a useful model that can be utilized to study the function of GABA in the inner ear.

Experimental Methods

We first performed reverse transcription followed by polymerase chain reaction (RT-PCR) to determine which GABARs would be good candidates to look for in the lateral line. We have now begun to determine if candidate GABAR mRNA transcripts are located in the lateral line by performing *in situ* hybridization.

Results

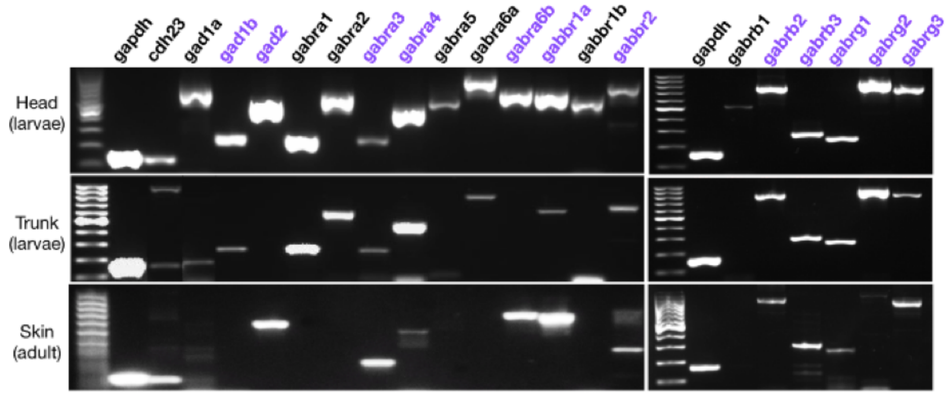

Figure 2: Expression of GABA related genes in zebrafish tissues. RNAs were extracted from tissues shown at left (RNeasy Mini Kit Qiagen) and RT-PCR was performed with primers designed to amplify GABAR genes (Superscript IV First-Strand Synthesis System (Invitrogen), GoTaq Hot Start PCR Master Mix (Promega)). PCR products were run on 1% agarose gels; the specific target genes are indicated above the gel. DNA ladders show 100-1000 bp standards. Control genes *cdh23* and *gapdh* were amplified along with test genes. Genes highlighted in purple are candidate lateral line genes based on their presence in the skin and/or trunk of zebrafish.

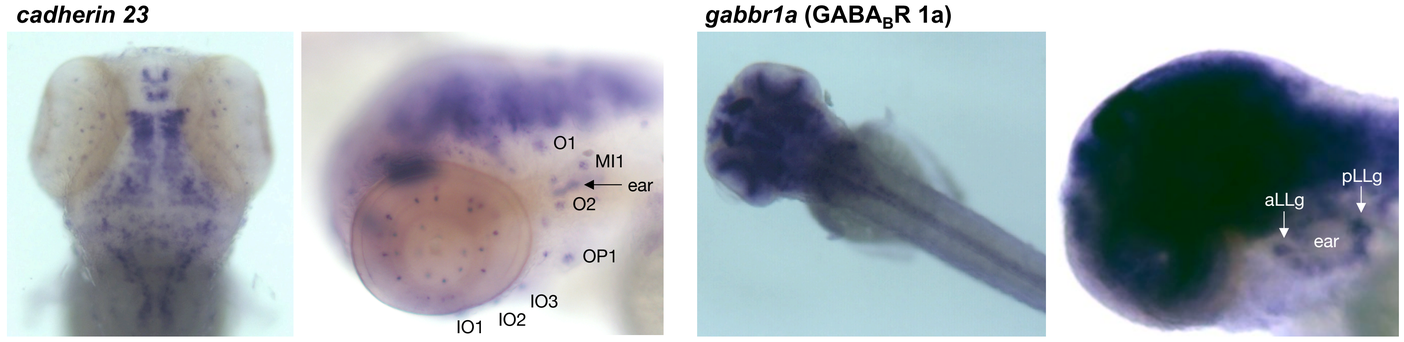

Figure 3: *In situ* hybridization images of 3dpf zebrafish larvae. *In situ* hybridization was used to locate the mRNA transcripts of the target genes indicated above. DNA templates were amplified (with primers designed using NCBI Primer BLAST) from cDNAs reverse-transcribed from whole-larvae mRNAs. Each antisense RNA probe was synthesized by performing *in vitro* transcription with DIG-RNA labeling mix (Roche) that incorporated DIG into the RNA probe. The probe was then integrated into the larvae and recognized by an anti-DIG antibody which reacted with labeling solution to create a purple precipitate. *Cdh23* was used as a control because it is known to be expressed at low levels in hair cells, the other targeted GABAR mRNAs were chosen because they were identified as lateral line candidates.

Conclusions

Our preliminary data support our hypothesis that lateral line cells express GABARs. Of the candidate GABAR genes tested so far, *gabrr1a* is the only one to exhibit any lateral line staining. *gabbr1a* is expressed in anterior and posterior lateral line ganglia (aLLg, pLLg), which is relevant because these ganglia contain the cell bodies of afferent neurons that innervate the lateral line *gabbr1a* encodes the $GABA_B$R subunit B1a, and our data suggest that there may be postsynaptic $GABA_B$Rs expressed in the lateral line.

Future directions

Our preliminary RT-PCR data suggested that there are several GABAR candidates that could be localized in the lateral line (Fig. 2). In the future, the rest of these GABAR subunits need to be located by performing *in situ* hybridization. Once the locations of these GABAR mRNA transcripts are discovered, then we can determine if the lateral line is a sufficient model to study GABA signaling in the inner ear.

Acknowledgments

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