STUDIES EXAMINING THE NEUROTRANSMITTER PROPERTIES OF HORIZONTAL CELL POPULATIONS IN THE GOLDFISH RETINA.

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Purpose: Gamma-aminobutyric acid (GABA) is the only viable transmitter candidate of retinal horizontal cells (HC). In the goldfish retina, GABAergic markers have been exclusively associated with H1 HCs. We re-examined the neurotransmitter properties of goldfish HC populations to determine whether HC types other than H1 HCs express GABA immunoreactivity, and examine the ability of goldfish HCs to release various amino acid neurotransmitter candidates upon activation of HCs with the glutamatergic agonist, kainate.

Methods: Techniques were derived from Marc and Liu, 1984; Marc et al., 1995; Marc, 1999a,b. GABA immunocytochemistry was performed on Golgi-impregnated retinas (Kalloniatis and Marc, 1990). Partial removal of Golgi reaction product from impregnated cells was required to enhance immunogenicity while reducing non-specific IgG adherence to the original Golgi reaction product. Neuronal transmitter efflux was activated in vivo in the 125 µM kainate and quantitative changes in asp, ala, gly, GSH, gln, glu and GABA signals immunochemically measured (Marc et al., 1995). Verification of endogenous and kainate activation of HCs was measured with AGB permeation (Marc, 1999a,b).

Fig. 1. Golgi-impregnation and GABA immunocytochemistry. Panel A: Golgi-impregnated horizontal cells, H1, H2 and H3 horizontal cells. Note the dense array of AGB+ H1 HCs. The scale bar equals 50 µm. Panel B: Shows the Golgi-impregnated H3 HC in the whole-mount retina prior to sectioning. Panel C: Demonstrates characteristic rod contacts of the rod HC shown in C3. The large pale ring outlines its dendritic arbor, while the pale arrows point to a few contacts with rod spherules. The adjacent, smaller pale ring outlines a pattern of cone contacts characteristic of H1 HCs. The scale bar equals 20 µm.

Fig. 2. Only H1 HCs are GABA immunoreactive. Sets of HC images (A1-A2, A3, B1-B2-B3, and C1-C2-C3) processed for Golgi impregnation and GABA immunofluorescence. The fluorescent images have been inverted and GABA-labeled HCs added as a colored layer in panels A1, A2, B1, C1. In panel A2, the H1 HC is GABA+, while the H2 HC is GABA-. Likewise, the H3 HC in panel B2 and the rod HC (RH) in panel C2 are GABA-. The dots denote the positions of representative cells, while the pale rings roughly outline their dendritic arbor. Panel A: Demonstrates characteristic cone contacts of H1 and H2 HCs shown in panel A3. The small blue circles outline examples of such contacts. Panel B: Shows the Golgi-impregnated H3 HC in the whole-mount retina prior to sectioning. Panel C: Demonstrates characteristic rod contacts of the rod HC shown in C3. The large pale ring outlines its dendritic arbor, while the pale arrows point to a few contacts with rod spherules. The adjacent, smaller pale ring outlines a pattern of cone contacts characteristic of H1 HCs. The scale bar equals 20 µm.

Fig. 3. Only H1 HCs contain GABA. Serial sections of normal goldfish retina were probed for GABA signals at different amplification times. At 4 min, standard GABA signals can be calibrated (Marc et al., 1995). H1 HC signals contain ~1 mM GABA and most ACs contain ~10 mM GABA. By extending the amplification time, estimates of GABA content in other structures can be made. At 4 min + 80 sec, H1 HC signals were amplified 10-fold, and H2, H3 and rod HC signals remained far below the 1 mM level normally found in H1 HCs. In fact, extending the H2, H3 and rod HC signal line to intersect the 1 mM line required a 316-fold amplification. Thus H2, H3 and rod HCs could contain no more than 3 µM GABA. The true value is likely zero. Even so, 3 µM GABA cannot support either conventional synaptic transmission or reverse transport.

Conclusions: A total of 173 Golgi-impregnated horizontal cells were identified in retinas processed for GABA immunocytochemistry: 133 were designated as H1 HCs and all were found to be GABA+. The remainder were identified as either H2 HCs (5), H3 HCs (19), or rod HCs (16) and were not found to label for GABA. An examination of normal goldfish retina probed for GABA signals at different amplification times revealed that the concentration of GABA in H2s, H3s and rod HCs must be no more than 3 µM, over 300 times less than the GABA levels in H1 HCs. As this concentration cannot support synaptic vesicular release or reverse transport, this further establishes their non-GABAergic nature. "AGB response" studies reveal that all HC types are activated by kainate and endogenous glutamate. However, GABA is the only endogenous amino acid transmitter candidate tested that was released by HCs (H1s) in response to kainate activation. Thus neither aspartate, alanine, glycine, GSH, glutamine, nor glutamate can subserve HC feedback/feedforward operations via reverse transport. However, vesicular release cannot deplete cytosolic pools of neurotransmitters, so non-H1 HCs could use vesicular glutamate release and a sign-inverting glutamate receptor for feedback. In this respect, all HCs contain sufficient glutamate to support vesicular release (Marc et al., 1990, 1995). H1 HCs do form conventional synapses onto glycinergic interplexiform cells, and this could be their major GABAergic output. If all HCs use the same molecular mechanism for cone feedback, then the signal cannot be GABA. If GABA is the bona fide feedback signal from H1 HCs, then H2/H3 cells must use a different mechanism that mimics the same types of biophysical properties. In any event, the feedback process remains an enigma.