EVIDENCE FOR AN ATP-CONDUCTIVE PATHWAY IN RETINAL NEURONS OF THE SHARK SQUALUS ACANTHIAS

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ATP acts as a neurotransmitter in both the central (Edwards, et al., Nature 359: 144-7, 1992) and peripheral (Evans, et al., Nature 357: 503-5, 1992) nervous systems. Recent studies have also demonstrated that external ATP is associated with P_2 purinergic receptor activation in the retina (Kirischuk, et al., J. Physiol., 483: 41-57, 1995). It is possible, therefore, to suggest that brain tissues, and in particular the retina, may have specific transport mechanisms to elicit the release of ATP, which may then act as an autocoid regulator of neuronal function.

Recent studies have shown that the cystic fibrosis transmembrane conductance regulator (CFTR), an anion channel which is capable of conducting ATP as the charge carrier (Reisin et al., J. Biol. Chem., 269: 20584-20591, 1994), is expressed in mammalian brain tissues (Mulberg, et al., J. Neurochem., 64: 1662-8, 1995). The role of CFTR in the central nervous system, however, is as yet unknown. We have recently determined that cultured shark rectal gland (SRG) cells express a cAMP-activated electrodiffusional pathway that is permeable to both ATP and Cl^- (Cantiello et al, Bull. MDIBL 33: 47-48, 1994). Although the ATP-conductive pathway of SRG cells was activated by cAMP, a behavior expected for a CFTR-like molecule, this pathway showed rectifying properties in symmetrical ATP, and resistance to the CFTR-channel blocker diphenylamine carboxylic acid (DPC), and the Cl^- channel blockers DIDS, and anthracene-9- carboxylic acid (9AC), yet it was readily blocked by nifedipine (500 μM). In this report we used patch-clamp techniques to determine the presence of an ATP-conductive pathway in isolated shark retinal neurons.

Whole eyes were dissected from double pithed adult sharks. The cornea was dissected and the retina was removed with a spatula and transferred to a petri dish. The isolated retinas were minced and incubated for 30 min at room temperature in a papain solution (1mg/ml) in 280 mM NaCl buffer. 200 μl of the suspension was then placed into the patch clamp chamber and isolated neurons were allowed to attach for up to 30 min. The chamber was then perfused with saline to remove unattached neurons.

Whole-cell, cell-attached, and excised inside-out patch clamp techniques were applied to bipolar cells placed in a bathing solution containing 280 mM NaCl. The patch pipette was filled with 200 mM MgATP, pH 7.4. Under whole-cell conditions, asymmetrical ATP/Cl^- currents increased 1,440% with the addition of 1 mM cyclophenylthio-cAMP (cpt-cAMP) (22.8 ± 0.35 nS/cell, n=3 vs. 1.48 ± 0.10, n=3, $p<0.001$, Fig. 1). Whole cell

currents were highly linear (r=0.9991), thus indicating that the cAMP-stimulated pathway was permeable to both Cl^- (positive currents) and ATP (negative currents).

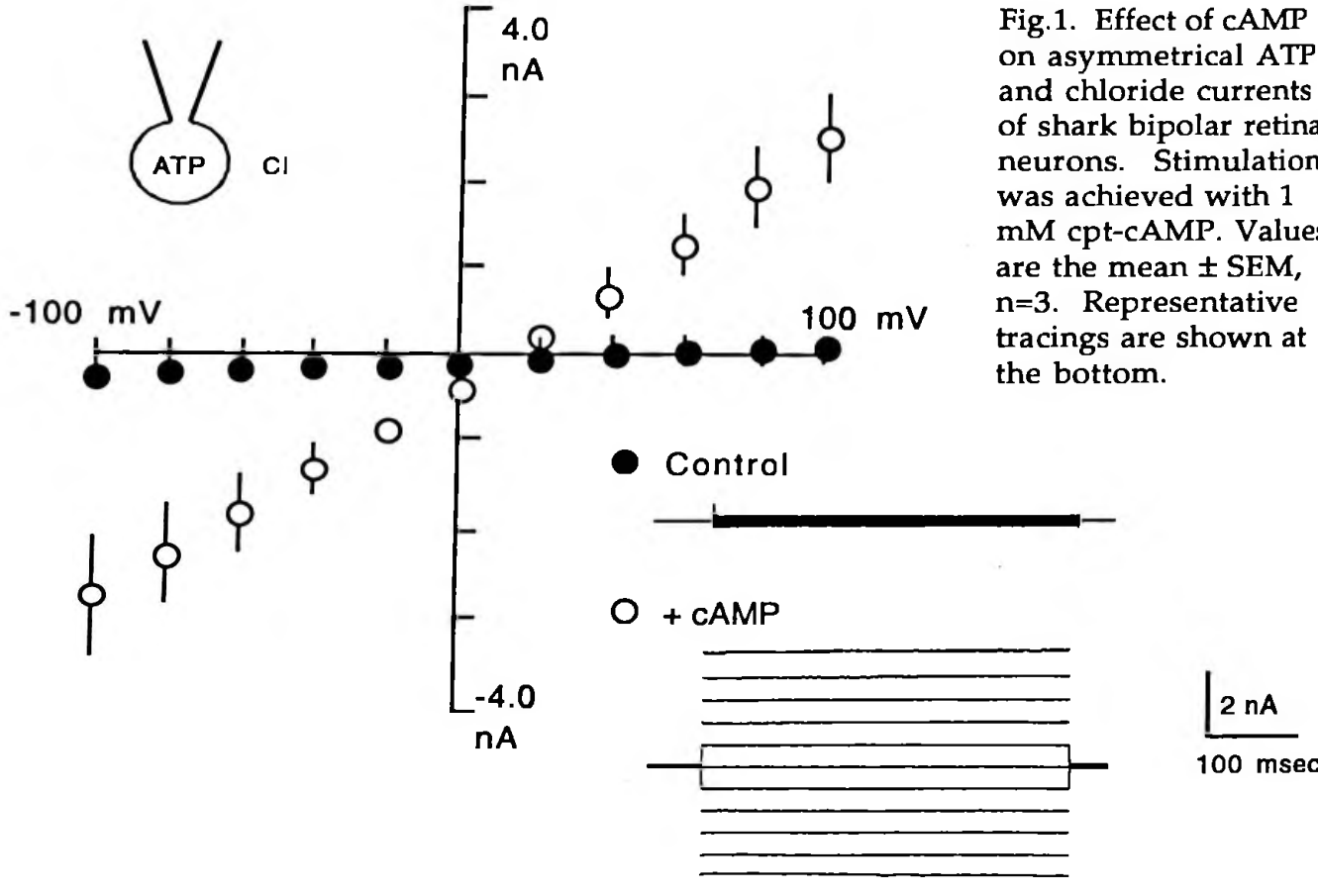

Fig.1. Effect of cAMP on asymmetrical ATP and chloride currents of shark bipolar retinal neurons. Stimulation was achieved with 1 mM cpt-cAMP. Values are the mean ± SEM, n=3. Representative tracings are shown at the bottom.

Under cell-attached and excised, inside out conditions, spontaneous ATP channels were observed in all cases (9 and 5 experiments, respectively, Fig. 2). Channel activity was insensitive to the anion channel blockers diphenylamine carboxilic acid (DPC), glibenclamide, and also resistant to nifedipine.

The data indicate that shark retinal neurons express an ATP-permeable channel sharing some functional similarities with a similar pathway in the rectal gland, namely ATP permeability and activation by cAMP. Immunocytochemistry studies with anti-CFTR antibodies (#13-1 or #24-1, Genzyme Corp. Framingham MA, data not shown) showed a strong labeling of amacrine cells and the inner plexiform layer, a region of synaptic junctions between bipolar cells and the ganglion cell layer. This finding suggests the presence of a protein with structural homology to a known ATP transporter, CFTR (Reisin, et al., op. cit.). However, the retinal ATP pathway was insensitive to anion channel blockers known to inhibit mammalian CFTR. Interestingly, the horizontal cell layer, which facilitates synaptic junctions between

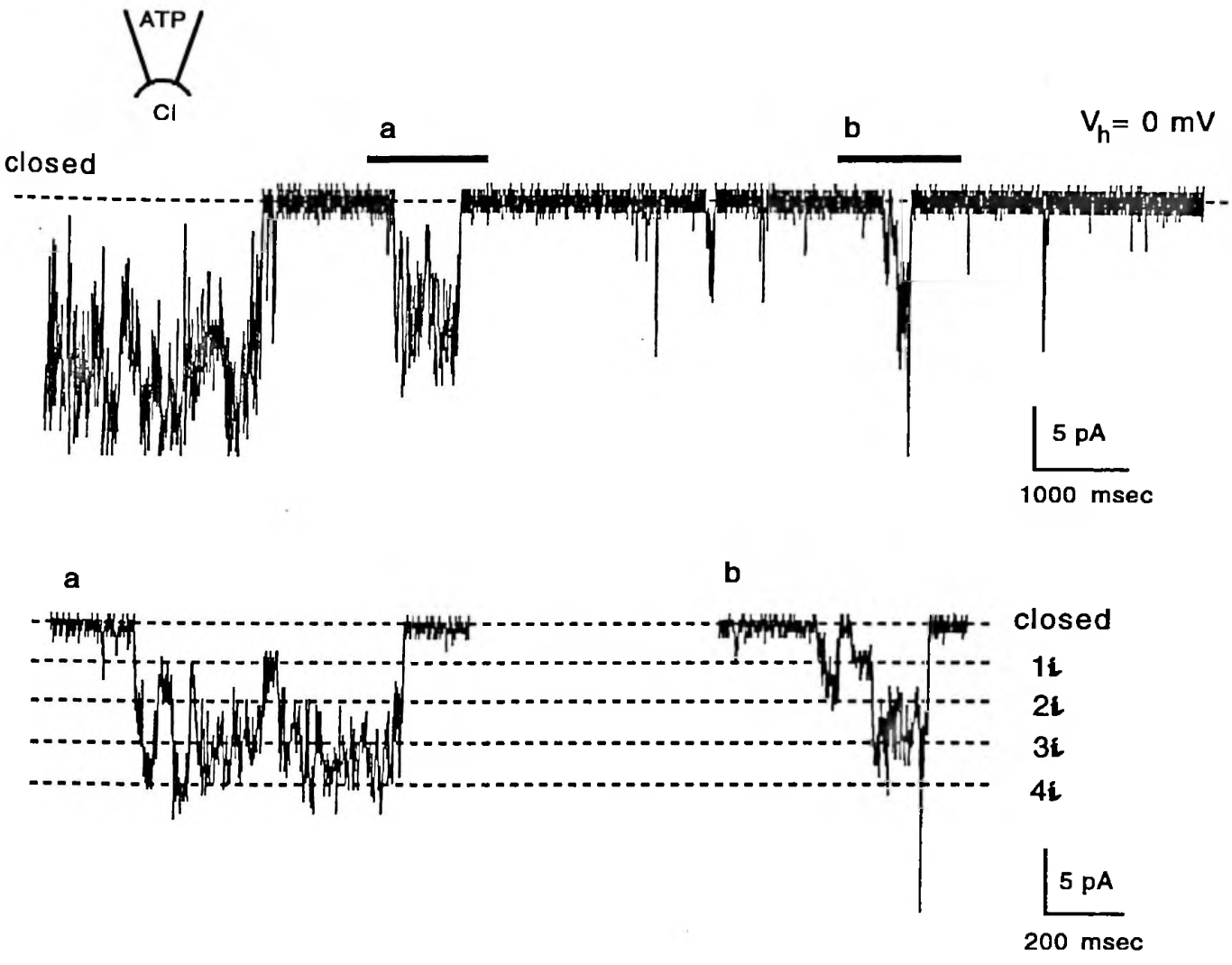

Fig. 2. Single channel ATP currents of shark bipolar neurons. ATP currents were spontaneously activated after patch excision. Pipette solution was 200 mM MgATP, and bathing solution contained 280 mM NaCl. Expanded tracings on bottom represent portions of top tracing underlined by the solid lines indicated (a & b). Tracings are representative of 5 experiments.

individual photoreceptors, stained strongly with an anti-P-glycoprotein antibody (Ab-1, Oncogene Science, Uniondale, NJ), suggesting the presence of another ATP transporter (Abraham, et al., op. cit.) structurally distinct from that in the lower retina. The focus of this study, however, was on bipolar cells, which form junctions between the inner and outer plexiform layers.

The ATP pathway in these cells is most consistent with the description of an ATP transport mechanism similar to that of mammalian CFTR. Clearly this needs to be further explored since it is possible that more than one ATP pathway may exist in the retina. The data in this report however, indicate the presence of an as yet unknown ATP pathway whose function and physiological relevance may be associated with the delivery of cellular ATP in the retina.

The studies were funded by grants from the Cystic Fibrosis Foundation and the Center for Membrane Toxicity Studies.