PRIMITIVE ORGANIZATION OF CYTOSOLIC Ca^{2+} SIGNALS IN HEPATOCYTES FROM THE LITTLE SKATE *RAJA ERINACEA*

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Cytosolic Ca^{2+} (Ca_i^{2+}) signals begin as inositol 1,4,5-trisphosphate (InsP3)-mediated, polarized Ca_i^{2+} waves in mammalian epithelia, and this signaling pattern directs fluid and electrolyte secretion and other cell functions (*Gastroenterol* 106:1349-1364, 1994). Ca_i^{2+} signals also occur in hepatocytes isolated from the elasmobranch *Raja erinacea* (little skate), and Ca_i^{2+} agonists induce secretion in skate liver as well (*Am J Physiol* 270:R561-R570, 1996), but the molecular basis for Ca_i^{2+} signals in this tissue is unknown. To investigate the organization of Ca_i^{2+} signals in elasmobranch epithelia, we examined Ca_i^{2+} signaling patterns and InsP3 receptor expression in polarized preparations of isolated skate hepatocytes (*J Exp Zool* 271:273-284, 1995).

Ca_i^{2+} signaling was examined by confocal line scanning microscopy using the fluorescent Ca^{2+}-sensitive dye rhod2, InsP3 receptor expression was examined by immunoblot, and the subcellular distribution of InsP3 receptors was determined by immunochemistry. ATP induced a rapid increase in Ca_i^{2+} in skate hepatocytes, as we have described previously (*Am J Physiol* 270:R561-R570, 1996; *Cell Calcium* 18:429-439, 1995), and as it does in mammalian hepatocytes. Unlike mammalian hepatocytes, the Ca_i^{2+} increase in skate hepatocytes began in the apical, basal or middle region of cells with similar frequency. To determine the role of InsP3 in ATP-induced Ca_i^{2+} signaling, cells were loaded using a transient permeabilization technique (*Hepatol* 15:107-116, 1992) with the InsP3 receptor antagonist heparin, or with de-*N*-sulfated heparin, which does not interact with the InsP3 receptor.

ATP-induced Ca_i^{2+} signals were blocked in cells loaded with heparin but not with de-*N*-sulfated heparin, suggesting that the increases in Ca_i^{2+} were mediated by InsP3. Immunoblot analysis using isoform-specific antibodies (*Nature* 396:81-84, 1998; *J Biol Chem* 270:11678-11683, 1995) showed that the type I but not the type II or III InsP3 receptor was expressed in skate liver. Confocal fluorescence immunochemistry revealed that the type I InsP3 receptor was distributed throughout the hepatocyte (Figure 1), rather than concentrated apically as in mammalian epithelia (*J Biol Chem* 269:4693-4696, 1994; *Am J Physiol* 267:G338-G349, 1994). These findings demonstrate that ATP-induced Ca_i^{2+} signals are mediated by InsP3 in skate hepatocytes, as they are in mammalian hepatocytes. However, in skate hepatocytes Ca_i^{2+} signals begin at loci throughout the cell rather than as an organized apical-to-basal Ca_i^{2+} wave; this non-polarized signaling pattern likely is because the InsP3 receptor is distributed throughout these cells, rather than being concentrated apically as in mammalian epithelia. This primitive organization of Ca_i^{2+} signaling may in part explain the observation that Ca^{2+}-mediated events such as secretion occur much less efficiently in elasmobranchs.

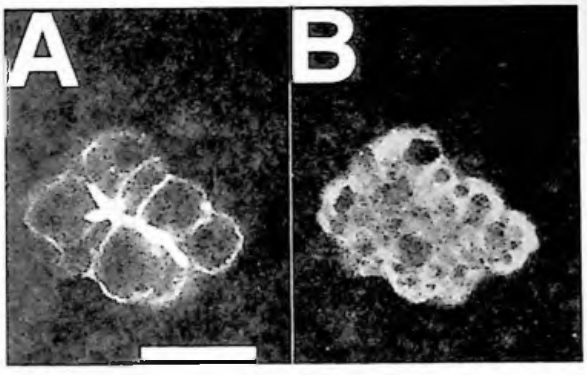

Figure 1. Subcellular distribution of the type I InsP3 receptor in skate hepatocytes, as determined by confocal immunofluorescence microscopy. (A) Rhodamine-phalloidin labeling of an isolated cluster of skate hepatocytes identifies the plasma membranes of the cells. Labeling is most intense along the apical membranes (*J Exp Zool* 271:273-284, 1995). Scale bar. 50 μm. (B) Distribution of the type I InsP3R, labeled with an isoform-specific antibody (*Nature 342*:192-195, 1989). This image demonstrates the nonpolarized distribution of the type I InsP3R in skate hepatocytes, since receptor labeling is diffusely distributed rather than concentrated in an apical trigger zone. No labeling was seen in hepatocytes stained with primary antibody directed against the type II or type III InsP3R, or in cells stained with secondary antibody alone.

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