CHARACTERIZATION AND FUNCTION OF ATP RECEPTORS ON HEPATOCYTES FROM THE LITTLE SKATE RAJA ERINACEA

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Introduction. Hormonal regulation of hepatocyte function, mediated via increases in cytosolic Ca^{2+} (Ca_i^{2+}), is well established in higher life forms but has not been investigated in elasmobranchs. We therefore examined Ca_i^{2+} signaling in hepatocytes from the little skate, Raja erinacea. Hepatocytes were isolated by collagenase perfusion, then Ca_i^{2+} was measured in populations of hepatocytes and in individual cells. For measurements of Ca_i^{2+} in populations of hepatocytes, cells were loaded with the Ca^{2+}-sensitive dye indo-1 and examined by ratio spectrofluorometry using a Perkin-Elmer LS-5B spectrometer. For measurements of Ca_i^{2+} in single hepatocytes, cells were loaded with the Ca^{2+}-sensitive dye rhod-2 and examined by confocal line scanning microscopy using a BioRad MRC-1000 Confocal Imaging System. Results. Baseline Ca_i^{2+} in hepatocyte populations was 315±153 nM (n=230), which is higher than values typically seen in mammalian cells. In each of 91 skate hepatocyte populations, ATP (100 μM) induced a rapid increase in Ca_i^{2+}, followed by a decrease to a level that was elevated above baseline, similar to the pattern seen in mammalian hepatocytes. A representative ATP-induced Ca_i^{2+} response is shown in Figure 1. Other hormones that act on mammalian hepatocytes, including vasopressin (60 nM), angiotensin (60 nM), and phenylephrine (80 μM), induced no such Ca_i^{2+} increase. In addition, no Ca_i^{2+} increase was induced by VIP (800 nM). The initial phase of the ATP-induced Ca_i^{2+} increase was seen even in Ca^{2+}-free medium, while the late, sustained (plateau) phase of the increase was not. Similar dose-response curves were seen upon stimulation with ATP, ADP, UTP, and 2-methylthio ATP. ATP-γS was likewise effective at increasing Ca_i^{2+}, but AMP, adenosine, β-γ-methyl ATP, CTP and GTP (each at concentrations of 100 μM) induced little or no Ca_i^{2+} increase (Figure 2). In single hepatocytes, ATP, ADP, UTP, and 2-methylthio ATP each predominantly induced a sustained increase in Ca_i^{2+} at high concentrations (10-100 μM), a single transient Ca_i^{2+} increase at a concentration of 1 μM, and repetitive transient Ca_i^{2+} increases (i.e., Ca_i^{2+} oscillations) at the lowest concentrations that elicited a response (10-100 nM). A maximal concentration of ATP (100 μM) also induced a marked, transient increase in bile flow in the isolated perfused skate liver, from a baseline rate of 3.8±1.7 μl/gm liver/hr to a peak flow rate of 11.1±2.5 μl/gm liver/hr (mean±SD, $p<0.005$). In contrast, 100 μM adenosine had no such effect on bile flow. Summary. These findings demonstrate that skate hepatocytes possess P_2 purinergic receptors which transduce signals that result in intracellular plus extracellular Ca^{2+} mobilization. One effect of these signals is to stimulate bile secretion. The broad specificity of the response to ATP and related compounds suggests that multiple types of P_2 receptors may be expressed on skate hepatocytes. Alternatively, these cells may possess a single, primitive purinergic receptor from which other P_2 subtypes subsequently evolved. Acknowledgements. We thank John N. Forrest, Richard Solomon and Patricio Silva for useful discussions and Robin M. Jones, Jurgen Kaljuvee and Anh Truong for technical assistance. This work was supported by a Young Investigator Award (to MHN) from the Center for Membrane Toxicity Studies (P30 ES03828), a Fiterman Award for Basic Research (to MHN) and a Student Research Award (to KM) from the American Gastroenterological Association, a Liver Scholar Award from the American Liver Foundation (to MHN), and the Hepatocyte Isolation, Liver Perfusion and Morphology Core Facilities of the Yale Liver Center (P30 DK34989).

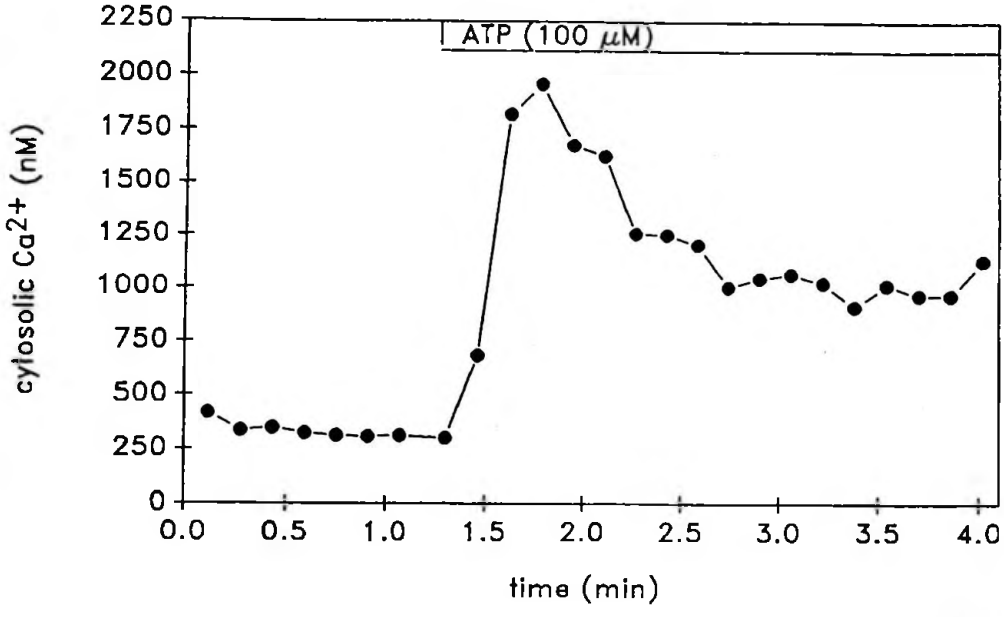

Figure 1. Effect of 100 μM ATP on Ca_i^{2+} in isolated skate hepatocytes. ATP induces a Ca_i^{2+} increase that rapidly peaks, then decreases to a plateau that is elevated above baseline. Tracing is representative of the pattern seen in n=91 separate experiments.

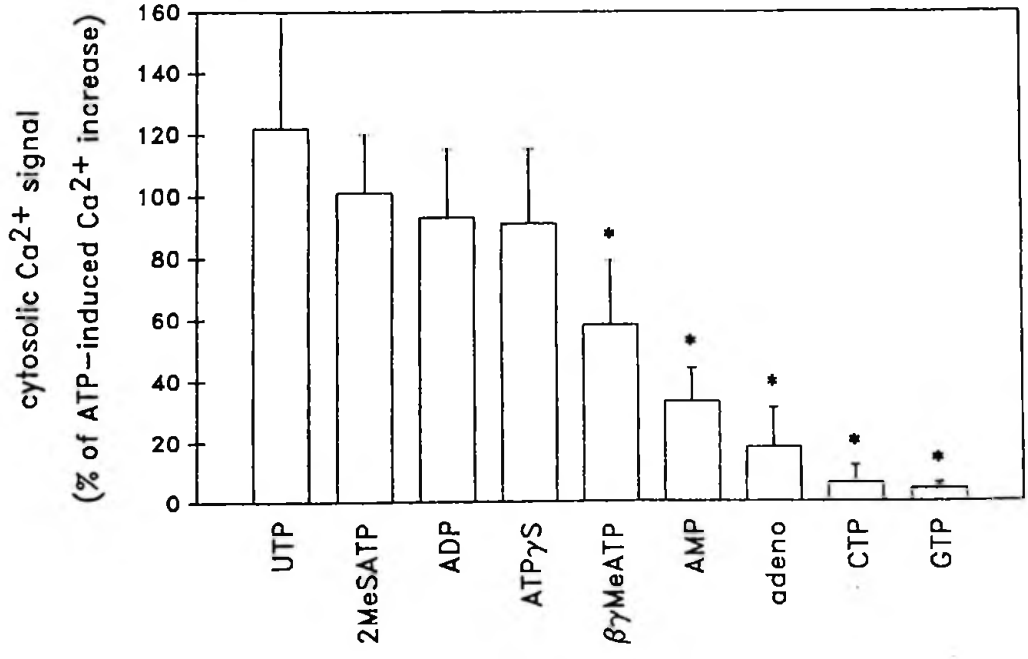

Figure 2. Ability of different agonists to increase Ca_i^{2+} in skate hepatocytes. All agonists were used at a concentration of 100 μM, and the resulting Ca_i^{2+} increase was compared to the increase induced by 100 μM ATP in pair-matched hepatocytes (range of n, 3-7 pairs). To normalize these data, ATP-induced Ca_i^{2+} increases were taken as 100%. Values are mean±SD (*$p<0.005$).