SOLUTIONS WITH LOW pH IMPROVE SURVIVAL OF VENTRICULAR CARDIOMYOCYTES ENZYMATICALLY ISOLATED FROM *SQUALUS ACANTHIAS*

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It is well known that the blood and plasma of the dogfish shark, *Squalus acanthias*, are generally at alkaline levels (pH ≅ 7.8). It is perhaps less known that the pericardial fluid is quite acidic (pH 5.3; Forster, R. P., and Hannafin, J. A., *Comp. Biochem. Physiol.* 65A:445-451, 1980). In this report we present results showing that low pH may have cardioprotective effect on enzymatically isolated shark ventricular myocytes.

Ventricular myocytes were isolated in 5 steps using different solutions. First, the heart was removed from the dogfish shark (*Squalus acanthias*), and placed into a beaker containing a Ca^{2+}-free elasmobranch physiological solution (solution #1: 270 mM NaCl, 4 mM KCl, 10 mM $MgCl_2$, 0.5 mM KH_2PO_4, 0.5 mM Na_2SO_4, 350 mM urea, 10 mM glucose and 10 mM HEPES titrated to a pH of 5.4, 7.2 or 7.4). Second, the heart was mounted on a modified Langendorff apparatus, the two major coronary arteries were canulated, and the heart was perfused for 20 minutes with Ca^{2+} free elasmobranch physiological solution bubbled with 100% oxygen (solution #1). Third, the heart was perfused with 25 ml of the Ca^{2+}-free solution containing 20 mg collagenase (type B, Boehringer Manheim) and 7 mg protease (type XIV, Sigma; solution #2) for 20 min. Fourth, the enzymes containing solution was then washed out using solution #1 containing 0.2 mM Ca^{2+} (solution #3). The heart was then removed from Langerdorff apparatus and placed into petri dishes containing the solution #3, and it was gently shaken to release the myocytes. Different batches of isolated myocytes were transferred to different storage solutions (#4), modified as described below, for later use in experiments and measurements on numbers of viable cells. Damaged cells floated and were completely rounded or partially contracted with twisted ends. Viable cells attached to glass surfaces, were long (150-200 μm) and slender (≅ 5 μm) with straight finely tapered ends, and had clear striation.

It was a striking and consistent observation that the yield of viable freshly dissociated cells was very high (≅ 80%) when both experimental and storage solutions (#1-4) were acidic with a pH of 5.4. This finding contrasted sharply with the lower yield of ≅ 20% typically obtained using solutions titrated to pH of 7.2 (Näbauer, M., and Morad, M., *J. Physiol.* 457:627-637, 1992).

To gain more insight into the cardioprotective effects of low pH, the following experiments were carried out. Cells isolated at pH 5.4 using the procedure described above were separated into two batches. One batch was transferred to storage solutions (#4) with different pHs and observed for 8 hours. Each intervention was examined in 3-4 hearts. Myocytes maintained at pH 5.4 had a 2-3 fold higher survival rate than cells stored at pH 7.4. This finding suggests that the continued survival of myocytes depends on high $[H^+]o$ ruling out the possibility that high (70-80%) yield may have resulted from the modulating effect of low pH on the activity of digestive enzymes.

Variation of both pH and extracellular Ca^{2+} concentration during storage demonstrated that cells' survival depended both on low pH and $[Ca^{2+}]_o$, such that cells stored in Ca^{2+}-free solution could tolerate solutions with pH 7.2. Thus, enzymatically isolated myocytes show low Ca^{2+}-tolerence, indicating that poor survival may be linked to Ca^{2+} overload.

In the next series of experiments we explored strategies to prevent Ca^{2+} overload. In one group we dispersed the cells in solution #3 where $[K^+]_o$ was increased to 20 mM, as in cardioplegic solutions. This attempt, however, failed to increase cell survival at pH 5.4 or 7.2 even when the cells were subsequently transferred to solution with normal K^+. This finding suggests that partial depolarization was not sufficient to prevent Ca^{2+} overload. Isolation of myocytes at low pH in K^+-free solutions (#1-3) also produced low cell yield and poor survival. This may indicate that low pH does not stimulate Na^+-H^+ exchange sufficiently to alleviate the build up of intracellular Na^+ thought to mediate many of the deleterious effects caused by depriving the Na^+-K^+-ATPase of extracellular K^+. Finally we found that elevated Na^+ during isolation (#2-4), diminished rather than improved cell viability independent of pH.

Our experiments show that low pH improves the yield and viability of enzymatically dispersed dogfish cardiomyocytes during long periods of storage by improving Ca^{2+} tolerance. The involvement of the Na^+-H^+ exchanger in this effect remains a possibility, but requires verification with the use of specific blockers.

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