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DETERMINATION OF INTRACELLULAR POTASSIUM ION CONCENTRATION IN ISOLATED RAT VENTRICULAR MYOCYTES

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Summary: The sarcoplasmic potassium concentration of a suspension of rat ventricular myocytes, prepared by collagenase-induced disruption of the myocardial mass, was determined by a null-point technique. Addition of digitonin resulted in a release of potassium from the cells which was interpreted as a flux from the sarcoplasm. The intracellular potassium concentration was estimated to be 113±6mM.

Considerable variation exists in the literature as to the value of the intracellular $[K^+]$ in cardiac muscle. Variation between 85 and 175mM has been reported (see (1)) despite the excellent selectivity of the K^+ exchanger valinomycin (2) that has been used to make these measurements - usually incorporated into an ion-selective micro-electrode. Whether these variations reflect true species differences or is a consequence of differences in experimental technique is unclear. Measurement of total cellular [K] by atomic absorption spectrophotometry has been used to measure total cell [K] in rat ventricular myocyte suspensions and yield values towards the lower end of the spectrum (3), but the technique does not allow one to differentiate between free and bound concentrations of the ion and does not distinguish between sarcoplasmic levels and those contained in intracellular organalles. The null-point determination of cytoplasmic ion levels circumvents the above difficulties (4). Digitonin is used to render selectively the surface membrane of a cell hyperpermeable to small ions and molecules whilst leaving intracellular organelles intact. When a suspension of cells is treated with digitonin the cytoplasmic pool will be added to the suspension medium; the $[K^+]$ of the suspension medium will then either be increased or reduced depending upon whether the cytoplasmic $[K^+]$ is more or less than that of the suspension medium.

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By altering the $[K^+]$ of the suspension medium the concentration at which no net change occurs will be equivalent to the cytoplasmic level of that ion. The use of such a method with liver cell suspensions (4) or red blood cell suspensions (5) has yielded values of cytoplasmic calcium ion concentrations which are similar to those obtained by other techniques.

Digitonin has been shown by us (6) to affect selectively only the sarcolemma of adult rat ventricular myocyte suspensions. We have therefore used this technique to estimate the sarcoplasmic $[K^+]$ of such cells, as an accurate estimate of this variable is required if a complete understanding of the role of the sarcolemma in regulating cell function is to be gained.

Materials and Methods: Suspensions of adult rat ventricular myocytes were prepared by perfusing the myocardial mass with collagenase (3) and were stored in a Krebs-Henseleit medium of the following composition: NaCl, 118mM; KCl, 2.6mM; $NaHCO_3$, 14.5mM; KH_2PO_4, 1.2mM; $CaCl_2$, 0.5mM; glucose 11.1mM; bovine serum albumin (fraction V), 1mg/ml. The medium was lightly gassed with 95% O_2/5% CO_2 to maintain a constant pH of 7.35.

Samples of the cell suspension were sedimented in an albumin-free Krebs-Henseleit medium. The pellet was resuspended in a medium of altered $[K^+]$, in which KCl iso-osmotically replaced NaCl, and the cells allowed to sediment again. This procedure was repeated in the medium of altered $[K^+]$. 200µl aliquots of the final suspension were fixed in one part of 5% (v/v) glutaraldehyde and two parts of Krebs-Henseleit medium for subsequent estimation of cell number. Cell counts were made in quadruplicate on an improved Neubauer haemocytometer stage.

The remainder of the cell suspension was introduced into a closed chamber. The roof of the chamber contained a ceramic dip-cast K^+ selective electrode with valinomycin and tetraphenylborate as the sensor (7). Reference electrodes were 4M NaCl bridges. Signals from the electrodes were displayed, via J-FET input stages, to a moving-paper chart recorder (6). The K^+ sensitive electrode was calibrated by adding samples of a 50mM KCl standard solution to the suspension medium. The original [K] of the medium was measured on a flame photometer (Instrumentation Laboratories, 543). Although the ion-selective electrode measures K^+ activity the results are expressed as concentrations, with respect to the KCL concentration standards utilised. All experiments were performed at 37°C.

Results and Discussion: Figure 1 shows the output from a K^+ sensitive electrode monitoring a suspension of rat ventricular myocytes. Two aliquots of a 50mM KCl standard solution were initially added to the chamber to provide a calibration for the electrode immediately prior to the experiment; the [K] was increased from 3.8 to 3.9 and 4.0mM. Subsequent addition of 20µl digitonin (final concentration 20µM) resulted in an initial step followed by a larger

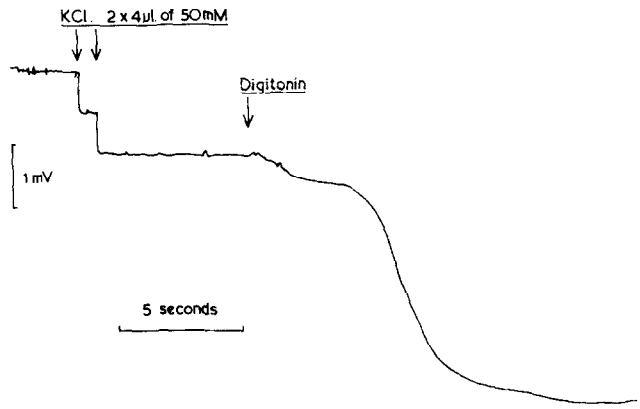
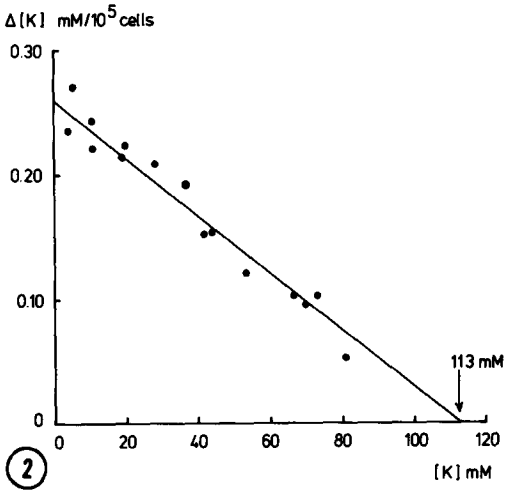


Figure 1. Output of a K^+ sensitive electrode monitoring a suspension of rat ventricular myocytes. The initial [K] was 3.8mM and two aliquots of a 50mM KCl solution were added to the chamber to achieve a final [K] of 4.0mM. Subsequent addition of digitonin (final concentration 20µM) resulted in an artefactual step and then a release of K^+ into the suspension medium. 6.5×10^4 cells/ml, 37°C.

deflexion. The initial response was an artefact due to digitonin addition, an equivalent response could be obtained in a cell-free suspension medium. The second response was due to the release of K^+ from the cytoplasmic pool into the suspension medium. The initial deflexion was not a result of K^+ contamination in the digitonin solution (shown by flame photometry) and was therefore ignored from the subsequent analysis. The total quantity of K released was estimated from the voltage deflexion of the second step and the electrode calibration curve. The amount was expressed in terms of the change in suspension $[K^+]$, $\Delta[K^+]$, per 10^5 viable cells.

A plot of $\Delta[K]$ versus suspension $[K^+]$ is shown in figure 2. A regression analysis of the data points yields a linear relationship ($p<0.001$) with an intercept on the abscissa at 113±6mM. This value is therefore equivalent to the sarcoplasmic K^+ level, as addition of the sarcoplasmic pool to the suspension medium results in no change in the measured $[K^+]$.

It is important to note that when viewed under a microscope the majority of cells are rod-shaped and have a well-defined sarcomere structure. However, a small proportion are rounded and non-viable. It has been shown that only the rod-shaped component participates in many cellular metabolic functions (6) but



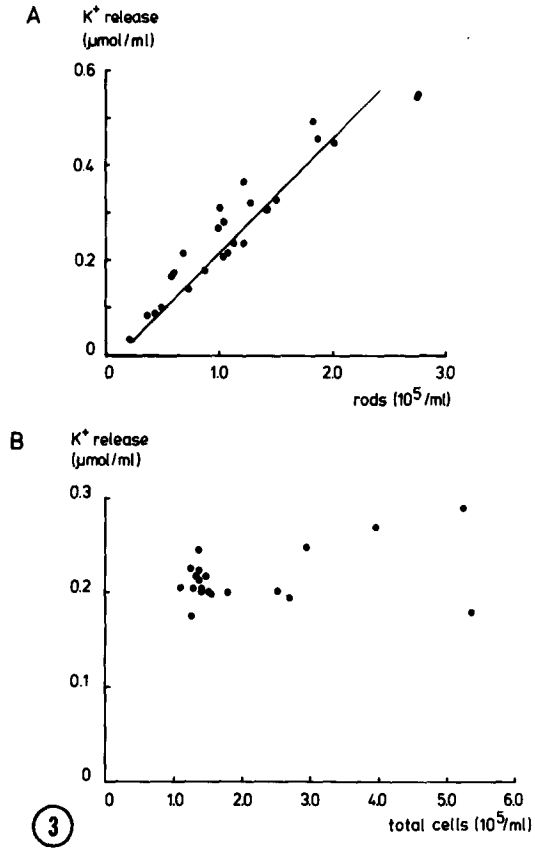


Figure 2. A plot of the change of suspension [K], Δ[K]mM/10^5 cells, as a function of the suspension [K]. The line was obtained by linear regression.

Figure 3. Part A shows the relationship between the quantity of K^+ released from a suspension of myocytes after digitonin addition and the number of rod-shaped cells present in the suspension. The line was obtained by linear regression. In part B the K^+ release is plotted as a function of the total cell number when the number of rod-shaped cells was kept constant at 1x10^5/ml, 37°C.

it is important to specifically exclude the rounded cells from contributing to the observed release of potassium upon digitonin addition, because the release of K^+ is measured in terms of the number of rod-shaped cells in the suspension (i.e. figure 2). The results in figure 3 indicate that this assumption is true. In part A the absolute quantity of K^+ released is plotted as a function of the number of rod-shaped cells regardless of the rounded cell number present (the proportion of rod-shaped cells was about 85% of the total cell number in these experiments). It can be seen that a close correlation exists between the amount of K^+ released and the number of rod-shaped cells, with the intercept of the line not significantly different from zero. In part B K^+ release is plotted as

a function of total cell number in the suspension (rod-shaped and rounded cells) but when the rod-shaped cell number was kept constant at 10^5/ml - i.e. cell suspension were contaminated with varying numbers of rounded cells that had been kept from the preparative stages of the myocyte suspension. The lack of correlation ($r=0.22$, $n=20$) implies that the release of K^+ is not a function of the total cell number but only the number of rod-shaped cells, as the latter was constant the quantity of K^+ released was also invariant.

The mean value of 113mM for the sarcoplasmic $[K^+]$ is equivalent to an intracellular activity of 86mM, assuming an activity coefficient of 0.76. These values are similar to the total cell [K] of 120mM obtained in this preparation (3) and that reported in rabbit ventricle (8). If the membrane potential of the myocyte was solely determined by the transmembrane K^+ gradient a value of -89mV can be calculated, which is close to the recorded value in this preparation (3).

Higher values of $[K^+]_i$ concentration (assuming a similar K^+ activity coefficient) have been recorded by other workers in the guinea-pig ventricle (e.g. 137mM (10); 157mM (11)); ferret ventricle (142mM; J.A.S. MgGuigan, personal communication) and somewhat intermediate values measured in the cat ventricle (121mM (12)). It seems therefore that true species differences may occur and that the rat ventricle may have values for $[K^+]_i$ towards the lower end of the spectrum.

One possible source of error in these experiments is the assumption that $[K^+]_i$ remains constant when extracellular levels are raised. If $[K^+]_i$ increased as the extracellular concentration was raised an overestimation of the true value would result. Lee and Fozzard (13) have shown that raising extracellular $[K^+]$ up to 50mM did not alter intracellular K activity in rabbit myocardium and more recently it has been demonstrated in ferret ventricle that increasing extracellular $[K^+]$ up to 80mM is also without effect (J.A.S. McGuigan, personal communication).

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