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Sodium/calcium exchange in ventricular muscle

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Summary. Ventricular cells possess two Ca extrusion mechanisms, a Na/Ca exchange system and a Ca pump. Reversing the exchanger by extracellular Na removal causes $[Na]_i$ to decrease, and the cells take up mmolar quantities of calcium. Since $[Ca]_i$ shows only a marginal increase the calcium load must be buffered. The capacity of the SR is limited so the mitochondria probably buffer a large part of this load. However, when Ca uptake into the mitochondria is blocked, the gain in Ca is still mmolar and the increase in $[Ca]_i$ still marginal, suggesting an additional buffering site.
Measurements of the Na/Ca stoichiometry on sarcolemmal vesicles gave a value of 3, but in ventricle values of around 2.5 or 3 are found. Reasons for this are discussed, as are the differences amongst the different methods of Ca measurement.
The interaction of the sarcolemmal Ca pump and the exchanger are considered and it is suggested they could interact via $[Na]_i$. At rest both systems could remove Ca from the cell but on a large perturbation the Na/Ca exchange would be the more important of the two.
Key words. Ferret heart; Na/Ca exchange; Ca buffering; Ca homeostasis.

Introduction

The inflowing calcium current is one of the sequence of events leading to contraction in heart muscle[45] and a rough calculation shows that unless some mechanism or mechanisms are present to expel Ca from the cell, the cytoplasmic Ca concentration ($[Ca]_i$) would, in a few minutes reach mmolar values. To remove Ca from the cytoplasm three mechanisms exist and these are shown diagrammatically in figure 1. There is a Na/Ca exchange system and a Ca pump, both of which can efflux Ca from the cell. Ca buffer systems also exist and while they can prevent a rise in cytoplasmic Ca concentration, to maintain Ca homeostasis this buffered Ca must eventually be actively expelled from the cell (reviewed in Carafoli[9]). In this article we will look at the evidence for a Na/Ca exchange system, describe possible sites for Ca buffering and discuss the role of the exchanger and the pump. We will not consider the role of the Na/Ca exchange system during the action potential for this is covered in the article by D. Noble.

Na/Ca exchange system

To investigate the Na/Ca exchange system a rapid perfusion chamber, shown diagrammatically in figure 2, was used. Ferret trabeculae, around 250 µm in diameter are fixed at one end to a small support and tied at the other end by a wire loop

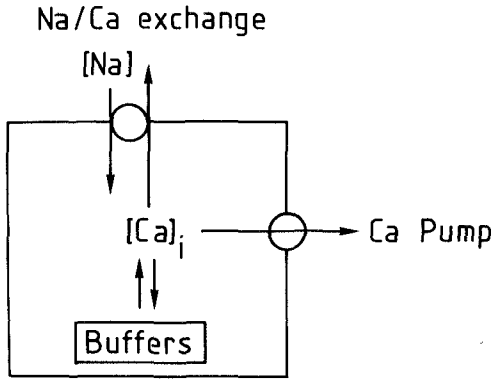


Figure 1. Diagram of the three systems capable of removing Ca from the cytoplasm.

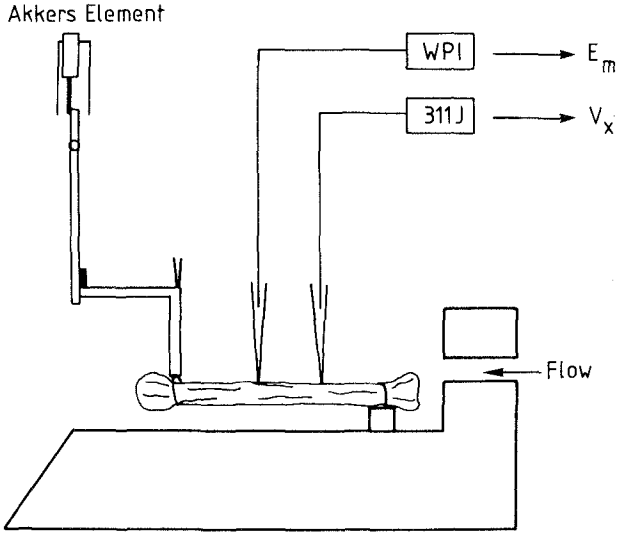


Figure 2. Diagram of recording system. Tension is measured by an Akkers element, the membrane potential (E_m) via conventional recording and the potential of the ion selective microelectrode (V_x) via the 311J Analogue Devices amplifier.

to a tension transducer. The method allows measurement not only of tension but also of the membrane potential and the potential of various cation selective microelectrodes[4, 10, 38].

At any given membrane potential the exchanger will cause the eliminiation or accumulation of calcium, depending on the respective gradients of Ca and Na. Removal of external Na will bring about an efflux of Na coupled with a gain of Ca.

Fig. 3A demonstrates an experiment where $[Na]_o$ was reduced from 155 mmol/l to 1.5 mmol/l and the $[Na]_i$ decreased from 15 mmol/l to around 1 mmol/l over a period of 150 s. Such decreases were not influenced by 50 µmol/l strophanthidin or by changing the pH_o from 7.4 to 9.5, suggesting that the major decrease was not due to either the Na/K pump or a Na/H exchange system. It could not be passive for in 1.5 mmol/l $[Na]_o$ the driving force for Na ions was still inward. If the decrease in $[Na]_i$ is due to Na/Ca exchange then changes in either $[Na]_o$ or $[Ca]_o$ should influence $[Na]_i$ and typical results of such an experiment are shown in figure 3B. Increasing $[Ca]_o$ or decreasing $[Na]_o$ were associated with concomitant small increases in tension, suggesting an increase in $[Ca]_i$ and supporting the idea of a coupled exchange[10].

In this series of experiments the average fall in $[Na]_i$ when $[Na]_o$ was reduced to 1.5 mmol/l was 13.4 mmol/l over a 6-min period (see fig. 6B in 10). On the basis of a 3 to 1 stoichiometry for the exchanger this would entail a Ca uptake of 4.5 mmol/l, more than enough to saturate the myofibrils.

However, tension measurements[10], microelectrode studies (Chapman[12], where earlier references are also given) and aequorin studies[1] show that while $[Ca]_i$ does increase slightly this increase is less than would occur during a twitch and could in no way account for mmolar quantities of Ca. An increase for instance in $[Ca]_i$ from 200 nmol/l to 1 µmol/l means only a Ca uptake of 8×10^{-4} mmol/l. Thus the Ca entering via the Na/Ca exchange must either be buffered and/or pumped out of the cell.

Ca buffering in heart cells

If the Ca is mainly buffered and not pumped out via the Ca pump, then in a Na-poor or Na-free solution heart cells should gain mmolar quantities of Ca. While we have not studied total Ca uptake ourselves, there are several reports in the literature of gains of Ca under Na-free or Na-poor conditions, and they are tabulated in table 1. The units expressing total Ca uptake used by the various authors have varied, and in an attempt to reduce them all to a common denominator, the Ca uptake has been expressed as the ratio of Ca present after zero or low Na perfusion, to calcium present under normal conditions, i.e. Ca-end/Ca-initial. The following calculation gives an idea of the expected ratio with mmolar uptakes of calcium. Assuming the calcium content/kg wet weight of tissue is around 2 mmol[21] and the uptake is 3 mmol/l then on the assumption of a wet/dry ratio of 4, an extracellular space of 20% of total water content and a $[Ca]_o$ of 2 mmol/l the expected ratio would be 2.2. However, this

Table 1. Ca uptake

Reference	$[Na]_o$ mmol/l	$[Ca]_o$ mmol/l	Time min	Ca-end/ Ca-initial
Mammalian ventricle				
Langer[24]	36	5	40	2.7
Wendt and Langer[47]	0	1.5	20	1.13
Bridge and Bassingthwaighte[6]	124 (Na loaded)	0→12	10	2.12
Mammalian atria				
Wollert[49]	0	1.8	30	2.05
Reuter and Seitz[37]	34.3	1.8	60	1.38
Cultured embryonic chick heart cells				
Murphy et al.[29]	0	2.7	15	1.88
Frog ventricle				
Nidergerke[31]	0	1.0	25	3.75

Table 2. Stoichiometry of Na/Ca exchange

Reference	Method	n
Reeves and Hale[35]	Sarcolemmal vesicles	2.97 ± SE 0.03
Sheu and Fozzard[39]	$[Na]_i$; $[Na]_o$; E_m $[Ca]_i$; $[Ca]_o$	2.48
Lado et al.[22]	$[Na]_i$; $[Na]_o$; E_m $[Ca]_i$; $[Ca]_o$	2.55
Fry and Miller[17]	$[Na]_i$; $[Na]_o$; E_m $[Ca]_i$; $[Ca]_o$	2.51
Chapman[12]	$[Na]_i$; $[Na]_o$; E_m $[Ca]_i$; $[Ca]_o$	3.00 ± SE 0.07
Bridge and Bassingthwaighte[6]	changes in total Ca & Na	3

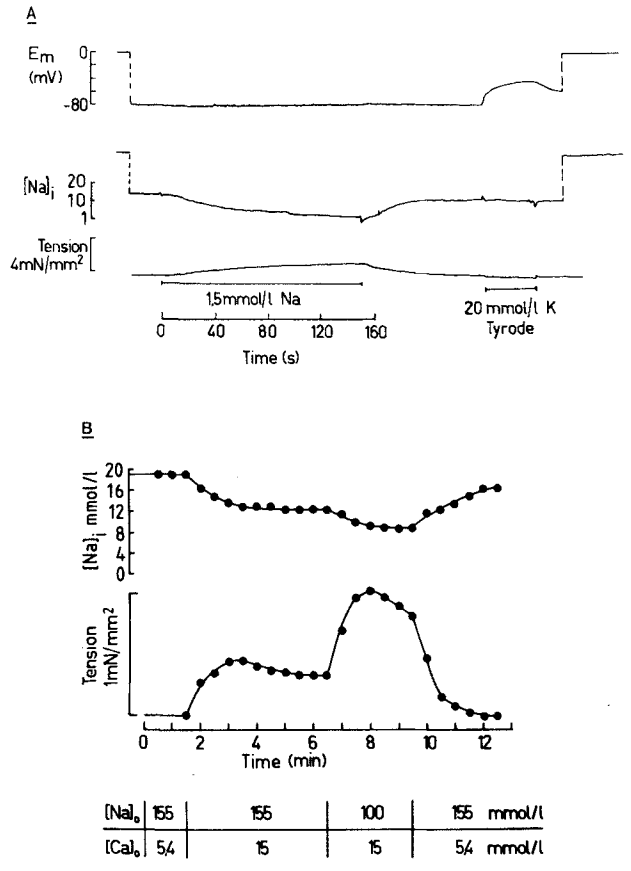


Figure 3. *A* Decrease in $[Na]_i$ during perfusion with $[Na]_o$ 1.5 mmol/l, Na being replaced by tetramethylammonium chloride. *B* Changes in $[Na]_i$ and tension on changing $[Na]_o$ and $[Ca]_o$ in the perfusion solution. Maximum tension increase was 5% of twitch tension.

ratio will vary somewhat depending on the value of the various parameters.

In table 1, with the exception of the results of Wendt and Langer[47], the values range from 1.38 to 3.75. Considering that some experiments were carried out in around 35 mmol/l $[Na]_o$ they all show considerable gains in total Ca. Why Wendt and Langer[47] found such a small increase is unclear. However their studies were carried out using ^{45}Ca and total Ca was not measured in these experiments.

From these measurements it would appear that the major part, if not all of the Ca that enters the cell due to the exchanger remains in the cell. Since this Ca is not in the cytoplasm it must have been taken up by intracellular organelles or bound to intracellular sites and in this sense buffered[11].

Mitochondria and the sarcoplasmic reticulum

The most likely stores for Ca in heart cells are the mitochondria and the sarcoplasmic reticulum[11]. In rat heart the mitochondria occupy 34% of the cell volume and the sarcoplasmic reticulum only 3.5%[32]. Morphometric studies on ferret heart have not yet been carried out, but an electronmicrograph from a ferret trabecula perfused in normal Tyrode solution shows not only excellent tissue preservation but also numerous mitochondria (fig. 4A).

Under conditions of Ca loading the sarcoplasmic reticulum will take up calcium but it has been previously argued that the capacity of the sarcoplasmic reticulum is limited to 0.4 mmol/l[11], i.e. too small to exert a large buffering effect. Based on this quantitative argument it was suggested that the mitochondria play a major role as a calcium buffering system[10–12]. Under normal conditions, because of the potential across the mitochondrial membrane, Ca ions pass down the electrochemical gradient into the mitochondria. However, due to the presence of a Na/Ca exchanger in heart mitochondria the Ca is extruded in exchange for Na. The process is referred to as Ca cycling[3] and with a $[Na]_i$ of 14.6 mmol/l[10] heart mitochondria contain little or no calcium, as has been substantiated in electron probe studies[20,48]. On Na removal the $[Na]_i$ falls to around 1 mmol/l, the Ca efflux pathway is blocked and there is a net uptake of Ca into the mitochondria[5,10,11]. Ca uptake by the mitochondria is a function of $[Mg]_i$[3,30] but estimations of $[Mg]_i$ in muscle tissue vary from 0.1 mmol/l to over 6 mmol/l[19] and to ensure that the Ca uptake mechanism could function in heart muscle we measured $[Mg]_i$ *in our* preparation using Mg sensitive microelectrodes[4]. These microelectrodes react not only to Mg but also to Na and K so calibration has to be carried out in a solution corresponding to the intracellular concentrations of Na and K. Using the mean measured values of $[Na]_i$[10] and $[K]_i$[38] the $[Mg]_i$ was found to be 0.4 mmol/l, a value well below that which would inhibit Ca uptake into the mitochondria[3,16].

Our value for $[Mg]_i$ of 0.4 mmol/l is much less than the value of around 3 mmol/l initially measured with these microelectrodes[19]. The main reasons for this are the use of measured values of $[Na]_i$ and $[K]_i$ in the calibrating solutions and the control of *each* impalement by K depolarizations[4,5]. The importance of impalement control is shown in figure 5 where seemingly stable impalements showed deviations on the difference signal of several mV. The deviation implies that one or both of the electrodes were not correctly in the cell, and such experiments had to be discarded. We would like to emphasize that failure to carry out such a test routinely can give values of $[Mg]_i$ that are too high.

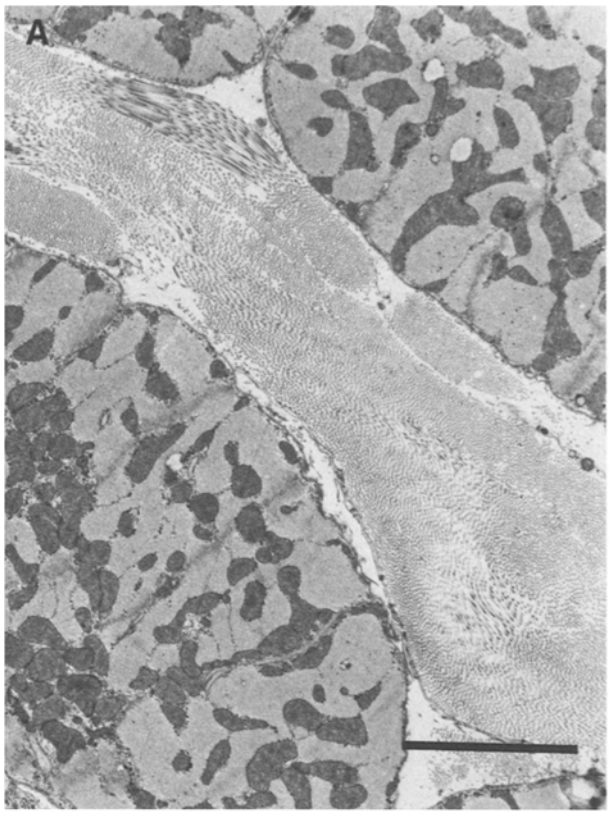
With the idea in mind that Ca could be deposited in the mitochondria in the form of granules, trabeculae were fixed after perfusion with 1.5 mmol/l $[Na]_o$. Electronmicrographs taken after such perfusion (fig. 4B) showed good tissue preservation but were indistinguishable from micrographs obtained during perfusion with normal Tyrode. This does not mean that Ca is not present in the mitochondria, but it is not in the form of granules.

Additional Ca buffers in heart muscle

We have presented indirect evidence that the mitochondria buffer Ca and the concept of mitochondrial buffering is supported by the recent work of Chapman[12] and by Fry et al.[18] who blocked mitochondrial uptake. However, there are indications that there may be other important buffer sources present.

When the calcium uptake into the mitochondria is blocked by cyanide[18] and/or carbonyl cyanide m-chlorophenyl hydrazone (CCCP)[12,18], Na removal still causes a decrease in $[Na]_i$. It is true that this fall in $[Na]_i$ is slowed and the rise in $[Ca]_i$ greater than in unpoisoned preparations, but there is still a mmolar decrease in $[Na]_i$ indicating a mmolar uptake of calcium. If this calcium is not pumped out and if the mitochondrial Ca uptake is blocked, then it must be buffered at some other site(s).

This situation of a significant buffer capacity still being present after cyaninde or CCCP is somewhat similar to that in squid axon where two buffer sources have been found, one sensitive to CCCP and/or cyanide and another insensitive to these agents[7]. Recently, Requena et al.[36] have shown that the CCCP insensitive buffer can be blocked by octanol, but this



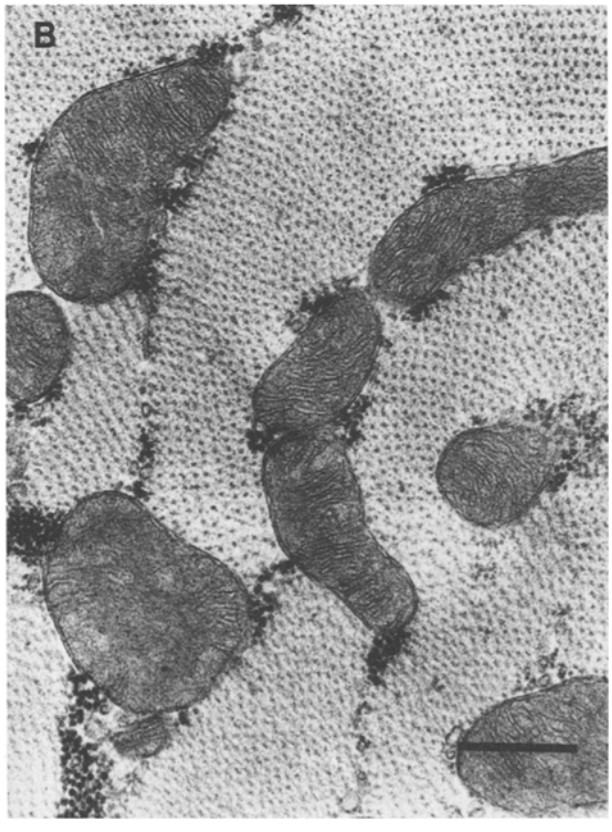


Figure 4. Electronmicrographs from ferret ventricular muscle preparations. Fixation by osmium tetroxide. *A* Normal tyrode solution (Bar 5 μm). *B* Preparation perfused in Na-poor tyrode solution (1.5 mmol/l) for 25 min (Bar 0.5 μm). (Courtesy of Dr P. S. Eggli)

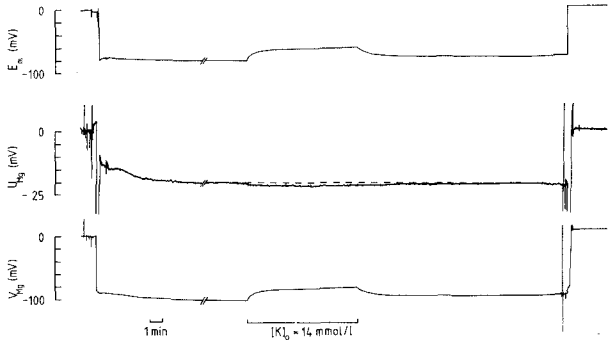


Figure 5. Experiment showing failure of the K depolarization impalement test. The traces from above downwards are membrane potential (E_m), Mg potential (U_{Mg}) and the potential of the Mg microelectrode (V_{Mg}), where $V_{Mg} = E_m + U_{Mg}$.
Depolarization of the preparation by increasing $[K]_o$ from 5 mmol/l to 14 mmol/l caused a deviation in the U_{Mg} signal of around 2 mV.

is not so in heart muscle (L. Blatter and J. McGuigan, unpublished observations).
Finally, it must not be forgotten that heart cells also possess a Ca pump. An efflux of 1.4 pmol/cm^2·s, for instance, could remove 4 mmol/l in a time interval of 10 min.

Stoichiometry (n) of the Na/Ca exchanger

Reeves and Hale[35] by measuring Ca fluxes in cardiac sarcolemmal vesicles found a stoichiometry of 2.97±SE 0.03. These experiments provide strong evidence that the stoichiometry is 3 and the exchanger is electrogenic.
In heart muscle it is possible to calculate an apparent stoichiometry and if the exchanger is at or near equilibrium at rest the apparent stoichiometry should approach the vesicular stoichiometry, which on the basis of the experiments of Reeves and Hale[35] would be 3. If it is less than three it means that the exchanger, at rest, is producing an efflux of calcium from the cell. One major problem in the calculation of the apparent stoichiometry from the measured calcium and sodium gradients and the membrane potential is the measurement of $[Ca]_i$. This will be considered first before discussing the measurements of the stoichiometry of the Na/Ca exchange system.

$[Ca]_i$ in ventricular muscle

In Ca measurements it is usual to use EGTA as a calcium buffer in the calibrating solutions and since not all authors have used the same binding constants for EGTA the $[Ca]_i$ measurements have been recalculated using the published constants of Smith and Miller[42,43]. In these calculations no attempt has been made to judge the purity of the EGTA or to estimate the errors involved in pH measurement; both of these factors can introduce substantial errors[27,34,42]. The influence of Mg has been neglected. In the aequorin measurements only the results of Cobbold and Bourne[13] have been recalculated since Snowdowne et al.[41] measured the binding constant under the appropriate conditions and the method used by Allen et al.[2] requires no a priori knowledge of the constant.
Recalculation using the method of Smith and Miller gives larger values for the $[Ca]_i$ than those published by the majority of authors. However, the calculations are *not* to be regarded as giving *absolute* values for $[Ca]_i$ but rather as an

attempt to compare and contrast the three different methods of Ca measurements under standardized conditions.

The results are shown in figure 6 in the form of a histogram for microelectrodes[12, 14, 22, 25, 26, 39, 46], Quin 2[23, 28, 29, 33, 40, 44] and aequorin[2, 13, 41].

The microelectrode results, with the exception of the recent measurements of Chapman[12], give values which are on average larger than those of Quin 2. The Quin 2 was initially calibrated in 1 mmol/l Mg and lower values of Mg would shift the values to the left. The calibration of the aequorin signal also depends on the Mg concentration and on the basis of our $[Mg]_i$ measurements the values estimated for $[Ca]_i$ by aequorin would all be too high. This means that the Quin 2 and aequorin measurements are giving values of $[Ca]_i$ less than the earlier Ca microelectrode studies and raises the question of these earlier results overestimates the $[Ca]_i$. If this were so it would have repercussions on the stoichiometry calculated from the measured Na and Ca gradients and the membrane potential.

Apparent stoichiometry

Two methods have been used to estimate an apparent stoichiometry (n). Measurement of the Na and Ca gradients coupled with measurement of the membrance potential[12, 17, 22, 39] and measurememt of the total loss of Na and gain of Ca[6].

The various measurements, including the results of Reeves and Hale[35] are given in table 2. The values for n are those calculated by the authors since recalculation of $[Ca]_i$ in these experiments did not essentially alter the coupling ratio. The value of 3 found by Chapman[12] suggests that the exchanger is at equilibrium and under these conditions presumably the Ca leak is balanced by the pump. Three values of n are around 2.5 indicating that the leak is balanced both by the pump and the exchanger. The reason for the difference is unclear for the measured $[Ca]_i$ in these cases is similar and it would be expected that the Ca leak would also be similar. One possibility, as pointed out above, would be an overestimation of the $[Ca]_i$ in the experiments where n was around 2.5 since in these experiments the $[Ca]_i$ calculated for equilibrium conditions (n = 3) would be around 14 nmol/l. However, such a large error in $[Ca]_i$ seems unlikely.

Since Bridge and Bassingthwaighte[6] measured total changes in Na and Ca it cannot be decided from these experiments if the exchanger is at equilibrium or not.

Relationship between Na/Ca exchange and the Ca pump

Heart cells possess two calcium extrusion mechanisms (see fig. 1) and as Prof. H. Schatzmann, Berne, has pointed out to us, both extrusion mechanisms must influence each other through the internal sodium concentration. If for the sake of argument the Ca pump reduced the level of $[Ca]_i$ to below that of the equilibrium point of the Na/Ca exchange the exchanger would react by exchanging Na for Ca and thus reduce $[Na]_i$. As this Ca is pumped out both systems will come to a new steady state set by the reduced $[Na]_i$. In this way the Ca pump could modulate a much more powerful Na/Ca exchange system. There is evidence for the exchanger controlling $[Na]_i$ for if the Na/K pump is blocked by cardiac glycosides, $[Na]_i$ plateaus and does not reach an equilibrium state[15]. Recently Murphy et al.[29] have shown that after pump inhibition, blocking the ATP production produces a further increase in $[Na]_i$ presumably due to ATP depletion influencing the extrusion of Ca through the Ca pump.

Both the Ca pump and the Na/Ca exchanger can remove Ca from the cell and the fraction that each system can efflux at

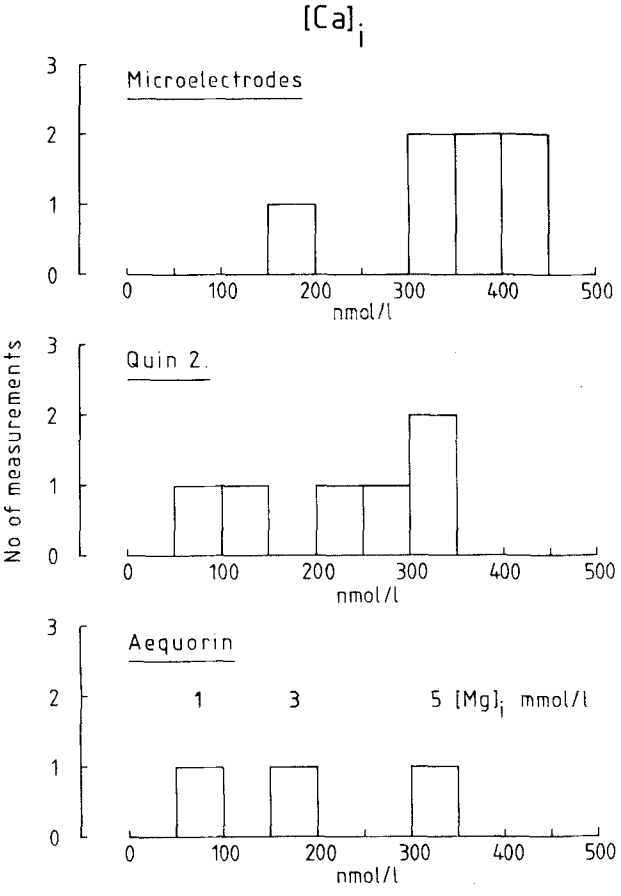


Figure 6. Histogram of the $[Ca]_i$ measured by microelectrodes, Quin 2 or aequorin. For further details see text.

rest depends not only on the maximal efflux of each system (J_{max}) but also on the appropriate affinities for Na and Ca (see fig. 10 in Blatter et al.[5]). Thus it is possible that at rest active efflux via the pump could be more important than the efflux through the exchanger[8]. However, since J_{max} of the exchanger is some 60 times greater than the J_{max} of the pump[9], during a perturbation of the system the exchanger will be the more important of the two.

Conclusions

It is now apparent that the Na/Ca exchanger system can move mmolar quantities of calcium into and out of heart cells. The cells can buffer significant amounts of calcium in mitochondria and in the sarcoplasmic reticulum but the site of the additional Ca buffering systems is not precisely known. Both the pump and the exchanger can extrude calcium but the relationship between the two systems has not yet been worked out in detail.

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