Journal of Physiology (1990) **424**, *pp.* 343–365
With 9 figures
Printed in Great Britain

COMPARISON OF INTRACELLULAR pH TRANSIENTS IN SINGLE VENTRICULAR MYOCYTES AND ISOLATED VENTRICULAR MUSCLE OF GUINEA-PIG

BY C. BOUNTRA*, T. POWELL AND R. D. VAUGHAN-JONES†
From the University Laboratory of Physiology, Parks Road, Oxford OX1 3PT

(*Received 14 August 1989*)

SUMMARY

1. Intracellular pH was recorded (double-barrelled pH-selective microelectrodes) in single ventricular myocytes and whole papillary muscles isolated from guinea-pig heart. Both preparations were acid-loaded by various manoeuvres (addition and removal of external NH_4Cl or CO_2) in order that a comparison could be made of the size and speed of intracellular pH changes and hence of the apparent intracellular buffering power (β).

2. For the same acid-loading procedure, the size of intracellular pH (pH_i) changes was about threefold larger in the isolated myocyte than in whole papillary muscle. The rate of initial acid loading as well as the subsequent rate of pH_i recovery (caused by acid extrusion from the cell) were also threefold faster in the myocyte. Estimates of apparent intrinsic (non-CO_2) buffering power, based upon the size of pH_i changes during acid loading, were 15–20 mmol l^{-1} for the myocyte and about 70 mmol l^{-1} for whole muscle. This latter value is similar to previous estimates of β in heart.

3. When acid extrusion was reduced by applying a high dose of amiloride (1 mmol l^{-1}), then the size of the pH_i change during acid loading increased greatly in papillary muscle but changed much less in the myocyte; β now appeared to be about 30 mmol l^{-1} in whole muscle but remained essentially unchanged in the myocyte.

4. We conclude that previous values for β in cardiac muscle have been greatly overestimated because of the presence of sarcolemmal acid extrusion. Paradoxically, this error in estimating β is far less evident in the isolated myocyte. We suggest that this is because a much more rapid acid loading is achievable in the myocyte so that acid loading will be blunted less by acid extrusion than in whole muscle. We present a simple mathematical model that demonstrates this phenomenon. We conclude that β in ventricular muscle is likely to resemble that measured in the isolated myocyte, i.e. 15–20 mmol l^{-1}.

5. Slow acid loading in whole ventricular muscle will also affect the kinetics of pH_i changes. The model indicates that the rate of pH_i recovery from an acid load in papillary muscle does not reflect the pH_i dependence of acid extrusion. Instead, it is heavily influenced by the slow rate of acid loading. This emphasises that great care

* Present address: Department of Molecular Science, Glaxochem Research, Greenford Road, Greenford, Middlesex.
† To whom correspondence should be addressed.

MS 7885

should be taken when interpreting the kinetics of pH_i changes in multicellular ventricular preparations.

INTRODUCTION

Much of our understanding of the mechanism regulating intracellular pH (pH_i) in cardiac muscle has been derived from measurements of pH_i in multicellular preparations (e.g. Ellis & Thomas, 1976*a*, *b*; Deitmer & Ellis, 1980; Piwnica-Worms, Jacob, Horres & Lieberman, 1985; Kaila & Vaughan-Jones, 1987). In the present work we have measured pH_i in single guinea-pig myocytes using a double-barrelled pH-selective microelectrode. We have compared the response of pH_i in the myocyte to various acid-loading procedures with the response of pH_i measured in a multicellular ventricular preparation. Our results clearly demonstrate that the magnitude and speed of pH_i changes in the isolated myocyte are much larger and faster than in the corresponding multicellular preparation. The larger pH_i changes in the myocyte could arise from a lower intracellular buffering power (β) in the isolated cell. We have investigated this possibility but conclude, instead, that previous estimates of β in multicellular ventricular tissue ($\sim$70 mmol l^{-1}; Thomas, 1976) are artificially high. We suggest that intracellular buffering power in mammalian ventricle is more akin to the value that we derive for the isolated myocyte (15–20 mmol l^{-1}). The selection of a much lower value for β has a major effect on the value of transmembrane acid fluxes calculated from measured changes in pH_i. Accurate determination of β is therefore an essential prerequisite for the accurate characterization of sarcolemmal acid transport.

A preliminary report of this work has been published (Bountra, Powell & Vaughan-Jones, 1987).

METHODS

The basic apparatus and methods were similar to those described previously (e.g. Powell, 1984; Bountra & Vaughan-Jones, 1989).

Multicellular ventricular preparation. Briefly, a guinea-pig heart was removed after the animal (albino, 300–600 g) had been killed by cervical dislocation and exsanguination. Fine papillary muscles (diameter 0·5–1·0 mm) were dissected from the right ventricle, sutured at their base with fine silk thread and pinned in the experimental chamber, lightly stretched over a small platform made from four stainless-steel pins (100 μm diameter) which were anchored, like 'goal-posts', to the base of the chamber. In a few experiments, thin strips of muscle (1·0–2·0 mm diameter) were dissected from the inner ventricular wall and pinned in the chamber with the undamaged surface exposed for electrode recording. In all cases, solution was continuously perfused through the chamber (volume 100 μl) at a rate of 1·5–2·0 ml min^{-1}. Temperature was maintained at 36 $\pm$ 0·5 °C.

Isolated ventricular myocytes. Albino guinea-pigs (300–600 g) were maintained on standard laboratory diets and tap water, available *ad libitum*. Suspensions of dissociated cardiac ventricular myocytes were prepared by retrograde perfusion of the heart, via the aorta, with crude collagenase as described in detail previously (Powell, Terrar & Twist, 1980; Powell, 1984; Mitchell, Powell, Terrar & Twist, 1987). After isolation, the cells were suspended in culture medium in sterile 20 ml tubes at room temperature. The culture medium used was Dulbecco's modified Eagle medium (MEM) in 25 mmol l^{-1} HEPES containing sodium pyruvate (5 mmol l^{-1}), glucose (5 mmol l^{-1}) and horse serum (5% v/v, mycoplasmic-screened). For electrical recording, aliquots of cells were

perfused with a solution identical to that used in the papillary muscle experiments, to which had been added 1 mg ml^{-1} fraction V bovine serum albumin. Myocytes used in this study were quiescent in the absence of stimulation, had clear cross-striatum and displayed all of the electrical activity reported previously for guinea-pig ventricular myocytes (Mitchell *et al.* 1987).

Solutions. The basic modified Tyrode solution contained (mmol l^{-1}): NaCl, 140; KCl, 4·5; $CaCl_2$, 2·5; $MgCl_2$, 1·0; glucose, 11; HEPES, 10 or 20; pH 7·4 at 37 °C, adjusted with 4 M-NaOH. In experiments with isolated myocytes 1·0 mg ml^{-1} albumen was added. Solutions were equilibrated with 100% O_2 (whole muscles, isolated myocytes) or air (isolated myocytes). For solutions equilibrated with CO_2, HEPES was replaced by 22 mmol l^{-1} $NaHCO_3$ equilibrated with nominally 5% CO_2 + 95% O_2 (British Oxygen), pH 7·40. Other modifications to these CO_2/HCO_3^- solutions are indicated in the text. NH_4Cl (5–10 mmol l^{-1}) was added to solutions shortly before use (from a 1 M stock solution) with no osmotic compensation. Amiloride, obtained from Sigma chemicals, was dissolved in Tyrode solution on the day of the experiment.

Double-barrelled pH-selective microelectrodes. These were constructed according to the method of Bountra & Vaughan-Jones (1989). One barrel (for recording membrane potential) was filled with 3 M-KCl and had an electrical resistance of 12–40 MΩ. The other barrel (pH-selective) was pre-silanized with dimethyl, trimethyl silylamine vapour and filled at its tip with a short column (Vaughan-Jones & Kaila, 1986) of H^+-selective sensor (Ammann, Lanter, Steiner, Schulthess, Shijo & Simon, 1981), the remainder of the barrel being filled with 500 mmol l^{-1} NaCl + 50 mmol l^{-1} HEPES adjusted to pH 7·2 with 4 M NaOH (20 °C). Electrodes were calibrated with standard Tyrode solution (10 mmol l^{-1} HEPES, pH 7·4) and Tyrode solution adjusted to pH 6·4 (HEPES replaced by 10 mmol l^{-1} PIPES). Acceptable electrodes displayed a sensitivity of 54–60 mV per pH unit.

Electrical arrangements. For experiments with whole muscle, arrangements were as described by Bountra & Vaughan-Jones (1989). For experiments with isolated myocytes they were as described by Mitchell *et al.* (1987). Voltage-clamping of myocytes, when required, was achieved with a switched (3 kHz) single-microelectrode clamp (Axoclamp; Axon Instruments) using the voltage barrel of the double-barrelled pH electrode. The signal from the pH barrel was monitored using the 0·001 preamplifier head (high impedance and ultra-low input bias current) of the Axoclamp system.

Estimation of intrinsic, intracellular buffering power (β). The apparent value of β (mmol l^{-1} per pH unit) was estimated from experiments where pH_i was varied by addition and removal of CO_2 or NH_4Cl (Roos & Boron, 1981).

For the case of NH_4Cl, β was estimated following NH_4^+ removal using the equation:

$$\beta = \frac{[H^+]_i}{\Delta pH_i} = \frac{[NH_4^+]_o 10^{(pH_o - pH_i)}}{\Delta pH_i}, \quad (1)$$

where the numerator represents the increase in the intracellular concentration of acid equivalents ($[H^+]_i$; mmol l^{-1}) following the removal of external ammonium ($[NH_4^+]_o$; the increase in intracellular acid equivalents is assumed to equal the intracellular concentration of NH_4^+ at the moment of ammonium removal), and where the denominator (ΔpH_i) equals the fall of pH_i that this produces. $[NH_4^+]_o$ can be calculated from the total concentration of external ammonium chloride (C) and its pK, using a rearrangement of the Henderson–Hasselbalch equation:

$$[NH_4^+]_o = C/[10^{(pH_o - pK)} + 1].$$

For the case of CO_2, β was estimated following addition of CO_2 using a similar equation:

$$\beta = \frac{[HCO_3^-]_o 10^{(pH_i - pH_o)}}{\Delta pH_i}. \quad (2)$$

The numerator again represents the increase in intracellular concentration of acid equivalents (mmol l^{-1}; equal to the intracellular HCO_3^- concentration at peak acidosis) and the denominator equals the fall of pH_i that this has produced. Note that, for simplicity, $[HCO_3^-]_o$ is assumed here to equal the total concentration of $NaHCO_3$ added to the solution.

Equations (1) and (2) have been derived assuming that dissolved NH_3 and CO_2 readily equilibrate across the cell membrane and that their respective pK values and solubility coefficients

do not change in extracellular compared with intracellular fluid (see Roos & Boron, 1981, for further discussion).

RESULTS

Measurements of pH_i in the isolated myocyte and in multicellular tissue of guinea-pig ventricle

Figure 1 shows measurements of pH_i in the isolated myocyte using a double-barrelled pH-selective microelectrode. In Fig. 1*A*, the electrode recorded pH_i (upper trace) and membrane potential (lower trace). In Fig. 1*B*, a recording from a different cell, the membrane potential (middle trace) was voltage-clamped at its resting

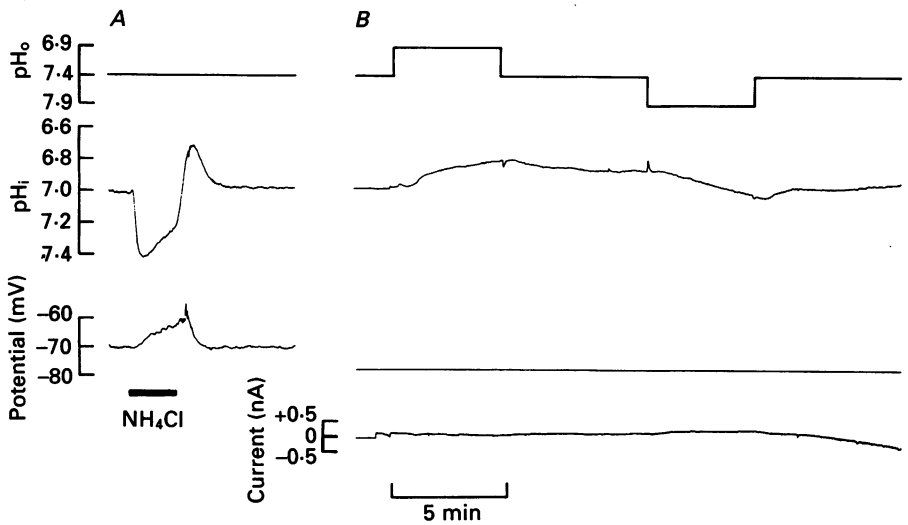

Fig. 1. Changes of intracellular pH in an isolated guinea-pig ventricular myoctye recorded using a double-barrelled pH-selective microelectrode. *A*, effect of adding and removing external NH_4Cl (5 mmol l^{-1}); addition indicated by bar. pH_o maintained constant (7·40). Traces show pH_i (upper) and membrane potential (lower). *B*, effect of changing pH_o (indicated at top of figure). Traces show pH_i (upper), voltage-clamped membrane potential (middle, −78 mV) and membrane current (lower). Single-microelectrode switched clamp (3 kHz).

potential of −78 mV using a switched-clamped technique (3 kHz, see Methods) in combination with the conventional voltage-recording barrel of the double-barrelled pH microelectrode. Membrane current is displayed in the lower trace. In both Fig. 1*A* and *B*, resting pH_i was close to 7·0 (HEPES-buffered Tyrode solution, pH_o 7·4 at 36 °C, 100% O_2). Average pH_i recorded under these conditions was $6{\cdot}99 \pm 0{\cdot}01$ (S.E.M., $n = 51$) at a mean membrane potential of $-77{\cdot}8 \pm 0{\cdot}5$ mV. When the same type of microelectrode was used to record pH_i in multicellular ventricular preparations from guinea-pig (papillary muscle or ventricular strips) then a comparable value was obtained: pH_i $6{\cdot}99 \pm 0{\cdot}02$ (S.E.M., $n = 12$) at a mean membrane potential of $86{\cdot}5 \pm 1{\cdot}7$ mV (again in HEPES-buffered Tyrode solution). Thus the enzymic dissociation of single ventricular myocytes does not seem to disturb the measured value of resting pH_i.

Intracellular pH transients in whole muscle and myocytes

Figure 1 shows manoeuvres that alter pH_i in the isolated myocyte. Superfusion of 5 mmol l^{-1} NH_4Cl at a constant bulk pH_o (Fig. 1*A*) produces a transient intracellular alkalosis (see e.g. Roos & Boron, 1981). Conversely, removal of the NH_4Cl induces an

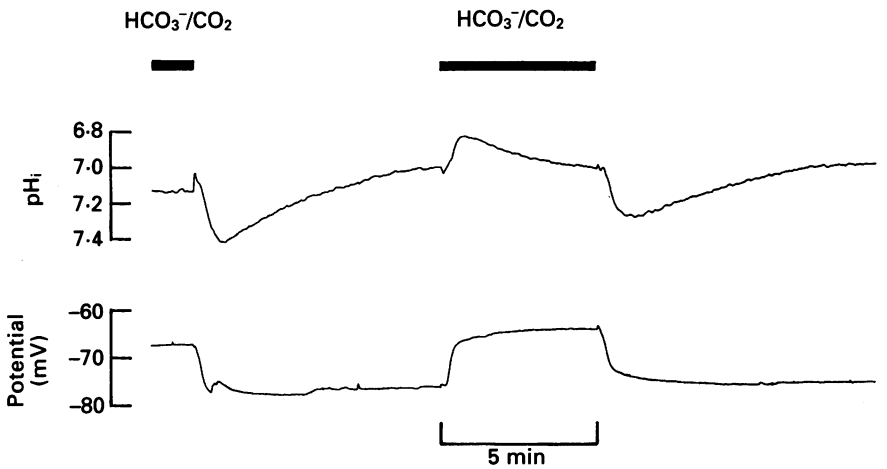

Fig. 2. Effect on pH_i of an isolated myocyte of adding (bar) and removing 5% CO_2/22 mmol l^{-1} HCO_3^-, (CO_2/HCO_3^- replaced by 10 mmol l^{-1} HEPES/100% O_2). pH_o constant throughout (7·40). Traces show pH_i (upper) and membrane potential (lower).

intracellular acid load which is followed by a prompt recovery of pH_i to control levels, this latter effect presumably occurring via the extrusion of acid equivalents from the cell (Deitmer & Ellis, 1980). Figure 1*B* shows that changes in extracellular pH produce slow changes in pH_i (Fig. 1*B*), which are 20–40% of the magnitude of change in pH_o, again similar to those observed previously in multicellular tissue (Ellis & Thomas, 1976*b*).

Figure 2 demonstrates that an intracellular acid load can be induced in a single myocyte by switching from a HEPES-buffered to a 5% CO_2/HCO_3^--buffered Tyrode solution at constant bulk pH_o (7·4). As with the NH_4Cl removal experiment, the acidosis is only transient. Similarly, removal of the CO_2/HCO_3^- buffer system induces a transient alkalosis. These results should be compared with those reported in the sheep Purkinje fibre (Ellis & Thomas, 1976*a*) where pH_i in the steady state is up to 0·2 units more acid in CO_2/HCO_3^--buffered Tyrode solution. Interestingly, a similar pH_i in the two solutions was also obtained in multicellular ventricular tissue (see e.g. the end of the experiment shown in Fig. 6*B*).

Figure 3 compares pH_i in a single myocyte (*A*) with that in a strip of ventricular muscle (*B*) during addition and removal of external NH_4Cl. It is immediately apparent that the changes of pH_i induced by this manoeuvre are larger and faster in the isolated myocyte. Thus, addition of 5 mmol l^{-1} NH_4Cl increased pH_i by 0·35 units in the myocyte, whereas addition of twice as much NH_4Cl (10 mmol l^{-1}) to the ventricular strip increased pH_i by only 0·2 units. Similarly, a much large acidosis was produced in the myocyte following NH_4Cl removal. Indeed, in preliminary experiments we found that superfusing an isolated myocyte with 10 mmol l^{-1} NH_4Cl

produced such large changes in pH_i that it was very difficult to maintain electrode impalement, especially during the acidosis following removal of the NH_4Cl. Figure 4 compares the rate of recovery from the acid load plotted as a function of pH_i for the isolated myocyte and for the multicellular preparation. In both cases the recovery

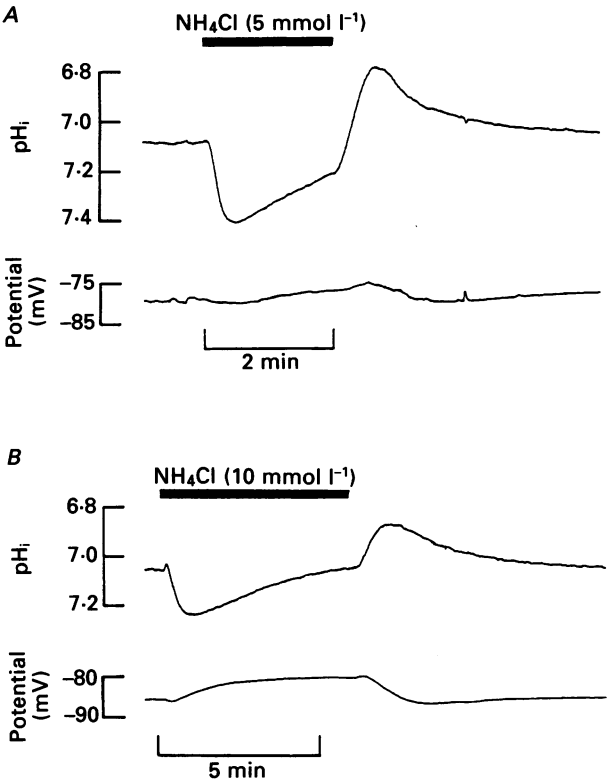

Fig. 3. pH_i transients are larger and faster in isolated ventricular myocytes than in whole ventricular muscle. *A*, isolated myocyte. The pH_i transients are induced by addition and removal of 5 mmol l^{-1} NH_4Cl (bar). *B*, ventricular muscle strip. Note the higher dose of NH_4Cl (10 mmol l^{-1}) than was used in *A* and note the slower time scale. HEPES-buffered Tyrode solution used in both *A* and *B*.

rate declines as pH_i increases from 6·8 to 7·2, but at all points the rate is considerably faster in the isolated myocyte. Table 1 pools data from many experiments for the kinetics of pH_i recovery from an intracellular acid load produced either by NH_4Cl removal or by CO_2 application. The half-time ($t_{0.5}$) for pH_i recovery is, on average, 2–3 times shorter in the isolated myocyte than in the multicellular tissue.

Assuming that pH_i recovery is caused by net acid extrusion, any comparison among recovery rates in the two preparations should take into account possible differences in the intrinsic intracellular buffering power (β). In the absence of a CO_2/HCO_3^- buffer system, β is a measure of the non-CO_2 buffering due, most likely, to intracellular proteins. Following an intracellular acid load, acid extrusion across the membrane can be estimated from the product of β and pH_i recovery rate,

$$J_H^e = \beta S \, dpH_i/dt, \quad (3)$$

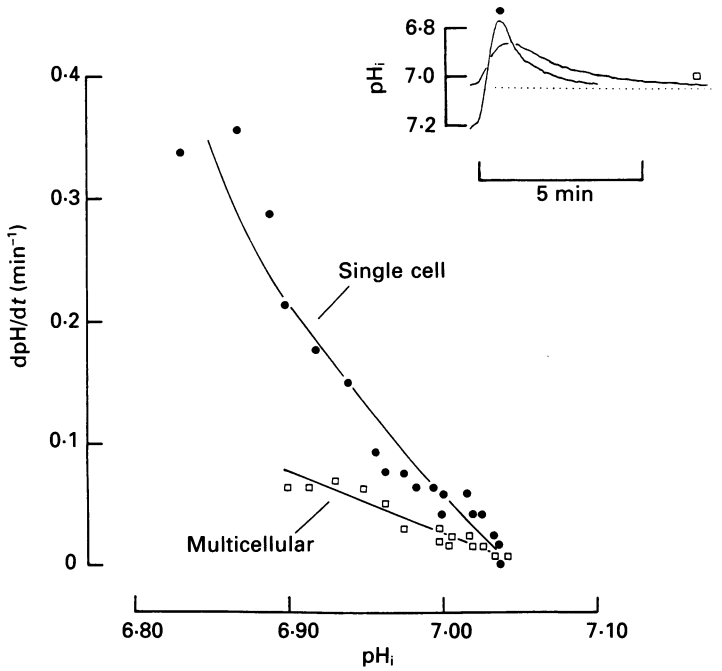

Fig.4. pH_i recovery is faster in isolated myoctyes. Plot of pH_i recovery rate (dpH_i/dt) following an intracellular acid load (induced by NH_4Cl removal) as a function of pH_i for an isolated myocyte (●) and ventricular muscle (□). Data derived from those illustrated in the inset (taken from Fig. 3).

TABLE 1. Data for kinetics of pH_i recovery from an intracellular acid load produced by NH_4Cl removal or CO_2 application

	$t_{0.5}$ for pH_i recovery (s)	
	Single myocyte	Multicellular tissue
NH_4Cl removal	58·9 ± 6·7 ($n = 31$)	142·8 ± 10·9 ($n = 10$)
+5% CO_2/HCO_3^-	97·3 ± 4·2 ($n = 9$)	192 ± 13·5 ($n = 6$)

where J_H^e is the net efflux of acid equivalents, dpH_i/dt the instantaneous rate of recovery of pH_i, S the surface area : volume ratio of the cell and β is the cell's intrinsic intracellular buffering power (when using CO_2/HCO_3^--buffered media, an additional component of buffering power due to the presence of intracellular HCO_3^- must be added to the intrinsic value of β, see Roos & Boron, 1981). In order to compare the kinetics of acid extrusion in myocyte and whole muscle, we therefore attempted to estimate the non-CO_2 buffering power in both preparations.

Intracellular buffering power

This is estimated by introducing a known amount of acid into the cell and measuring the resulting fall in pH_i. Intracellular (non-CO_2) buffering power (β) is then estimated using eqns (1) and (2) (see Methods). In the present work an acid load

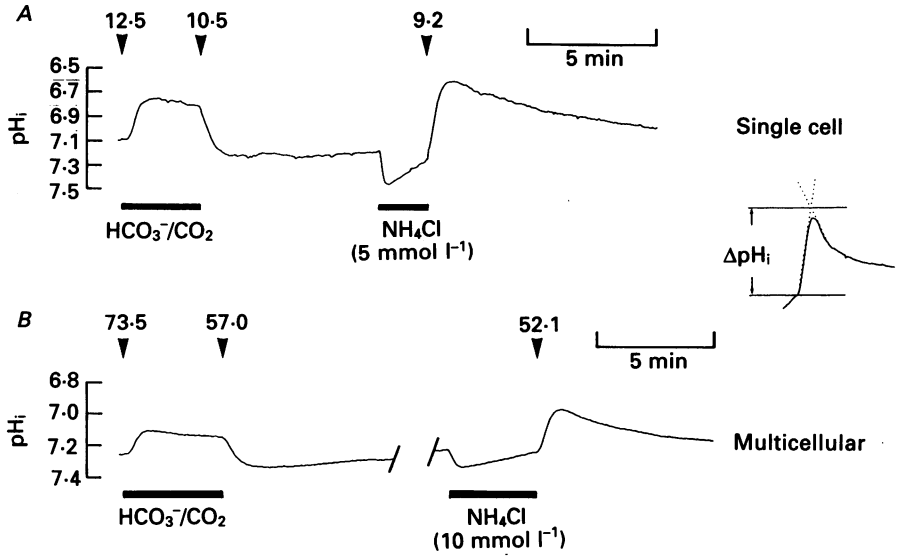

Fig. 5. Comparison of two methods to estimate intracellular intrinsic buffering power (β) in the isolated myocyte (*A*) and in papillary muscle (*B*). The two methods are (i) replacement of HEPES-buffered Tyrode solution with 5% CO_2/22 mmol l^{-1} HCO_3^--buffered Tyrode solution and (ii) addition/removal of external NH_4Cl while using HEPES-buffered Tyrode solution. pH_o 7·40 throughout both manoeuvres. The fall of pH_i during the acid-loading phase (ΔpH_i) was estimated using the 'back-extrapolation' method illustrated in the inset to the right of the figure. For further details, see text. Computed values of apparent β (mmol l^{-1}) are indicated at the arrows above the pH_i traces. Note that apparent β is uniformly higher in papillary muscle.

was induced by NH_4Cl removal (Fig. 3) or by addition of CO_2 (Fig. 2). Because acid extrusion mechanisms may attenuate the initial acidosis, it has been suggested that when measuring this initial fall of pH_i, one should back-extrapolate pH_i recovery to a point shortly after the beginning of the acid-loading procedure (Thomas, 1976; Aickin & Thomas, 1977; Aickin, 1984; see inset to Fig. 5 and section in small print below for further details). Figure 5 shows an experiment where apparent values of β were estimated in this way for an isolated myocyte (Fig. 5*A*) and for papillary muscle (Fig. 5*B*). The apparent value of β in the myocyte was uniformly lower than that estimated in the papillary muscle; in this particular case there was more than a fivefold discrepancy for β in the two preparations.

The back-extrapolation procedure for measuring buffering power is illustrated in the inset to Fig. 5. We have employed a simplified version of that used by Aickin & Thomas (1977) and Aickin (1984). The initial rate of acid loading and the maximum rate of pH_i recovery are both extrapolated linearly until they intersect at a point somewhat more acid than the peak level observed during the pH_i transient. The pH_i is then taken as the difference between the pH_i at this intersection point and that immediately before the acid load was applied. Thus some allowance is made for the

occurrence of acid extrusion during the loading period. The method differs from that of Aickin and Thomas in that those authors best-fitted an exponential curve to the pH_i recovery and then back-extrapolated this curve until it intersected the line defining the initial loading rate. We have not adopted this procedure because pH_i recovery is not truly exponential (cf. Fig. 4). Our own method of linear extrapolation is also simpler to apply (Fig. 5 inset) although it may result in a slightly smaller estimate of ΔpH_i and thus some overestimation of apparent β. The difference in β obtained by the two methods is, however, only small. For example, for the case shown in Fig. 3*B*, apparent β estimated using the Aickin and Thomas method is 96 mmol l^{-1}, and using our own method, 103 mmol l^{-1}. If no back-extrapolation is made at all, then apparent β estimated from the observed peak acidosis is 135 mmol l^{-1}. It is clear, therefore, that applying some form of correction can influence β significantly but that the difference obtained using the two types of correction is very small.

Figure 6 illustrates a third method used to estimate β in a myocyte and a papillary muscle. In this case, the preparations were perfused with Tyrode solution buffered using 5% CO_2/20 mmol l^{-1} HCO_3^- (pH_o 7·35). The P_{CO_2} was then elevated to 10% (constant HCO_3^-; pH_o 7·06). This induced an internal acidosis which was partly transient; pH_i initially decreased but then slowly recovered to a more alkaline value, but this secondary pH_i recovery was incomplete. Although pH_i behaved qualitatively in a similar way in the multicellular preparation, the acidoses were again smaller, the secondary pH_i recoveries much slower and the apparent value of β 4–5 times larger than in the myocyte (Fig. 6*A* and *B*).

Table 2 summarizes apparent values of β determined by the above three methods and averaged for the two preparations. It is notable that, in a given preparation, the apparent value of β is reasonably constant and does not vary greatly with the method of determination. Buffering power appears, however, to be consistently lower in the isolated myocyte.

In Fig. 7 net acid extrusion during pH_i recovery from an acid load is estimated for the two preparations. Acid efflux has been calculated as $\beta\, dpH_i/dt$, assuming a constant surface area: volume ratio. The pH_i recovery rates shown in Fig. 4 have been multiplied by an appropriate value for β similar to that listed in Table 2 (multicellular tissue, 70 mmol l^{-1}; isolated myocyte, 15 mmol l^{-1}). Surprisingly, acid extrusion computed at any pH_i is remarkably similar in the two preparations, despite the great differences in pH_i recovery rate and apparent intracellular buffering power. We shall reconsider this similarity in the Discussion.

Influence of Na^+–H^+ exchange inhibitors upon the apparent value of β

An alternative method to the back-extrapolation procedure for measuring β is to inhibit directly all acid extrusion mechanisms before inducing an intracellular acid load. The experiments shown in Fig. 8*A* (isolated myocyte) and *B* (papillary muscle) illustrate the effect of the Na^+–H^+ exchange inhibitor, amiloride, upon pH_i recovery and upon the estimated value of β. It can be seen that, at 1 mmol l^{-1}, amiloride greatly slowed recovery of pH_i from an acid load (NH_4Cl removal) confirming that, as in other cardiac cells, pH_i recovery is at least partly mediated by acid efflux via Na^+–H^+ exchange. In papillary muscle, the initial acid load produced by NH_4Cl removal was greatly increased in the presence of amiloride, whereas it was increased much less in the isolated myocyte. This effect of amiloride to increase the initial acid load also influences estimates of β. In Fig. 8*B*, β is reduced from an apparent value of 61 mmol l^{-1} (control) to a value of 29 mmol l^{-1} (in amiloride). By contrast, the

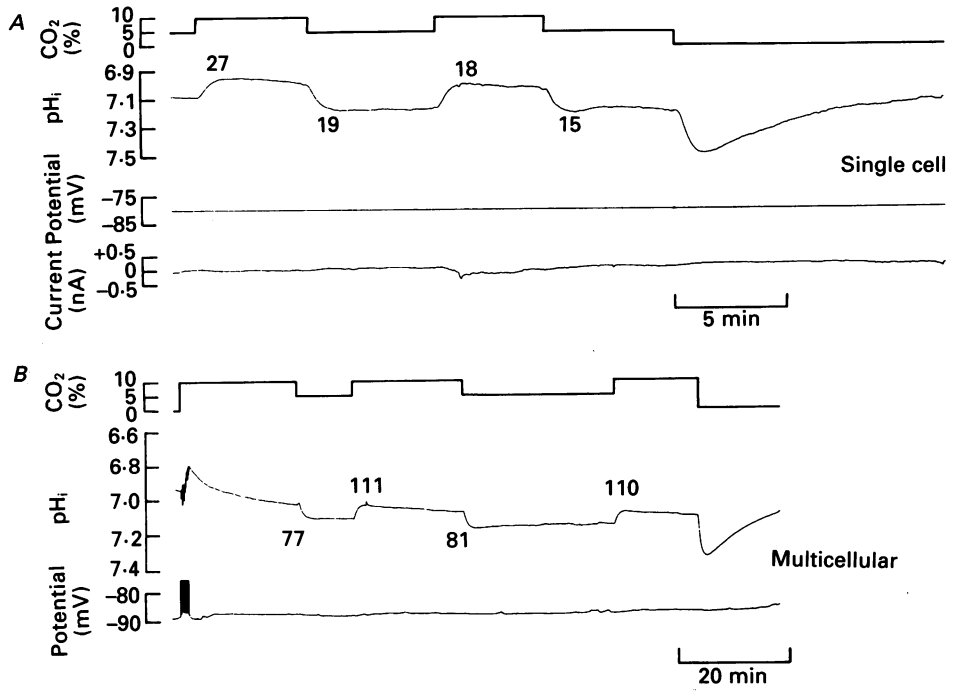

Fig. 6. A third method of estimating apparent β in the isolated myocyte (*A*) and papillary muscle (*B*). The P_{CO_2} of Tyrode solution was altered (5–10% CO_2) while HCO_3^- concentration was held constant at 20 mmol l^{-1} (P_{CO_2} indicated at top of *A* and *B*). At the end of each experiment, the solution was finally switched to HEPES–Tyrode solution. The pH of the various solutions was 7·06 (10% CO_2), 7·35 (5% CO_2), and 7·4 (0% CO_2, 10 mmol l^{-1} HEPES + 100% O_2). Estimates of apparent β (mmol l^{-1}) are shown close to each pH_i trace. Note that when P_{CO_2} is raised at constant $[HCO_3^-]_o$, there is an internal acidosis followed by a slow and incomplete recovery of pH_i. Despite the lack of a rapid pH_i recovery, apparent β is still higher in papillary muscle than in the myocyte. *A*, myocyte; traces show pH_i (upper), voltage-clamped membrane potential (middle) and membrane current (lower). *B*, papillary muscle; traces show pH_i (upper) and membrane potential (lower). Note the slower time scale in *B*.

TABLE 2. Apparent values of β determined by three different methods

	β (mmol l^{-1})	
	Single myocyte	Multicellular tissue
$-NH_4Cl$ (HEPES)	19·91 ± 1·36 (n = 34)	71·1 ± 4·1 (n = 12)
+ 5% CO_2/22 mmol l^{-1} HCO_3^-	20·58 ± 2·5 (n = 12)	70·0 ± 17·4 (n = 5)
5–10% CO_2, and 10–5% CO_2 (20 mmol l^{-1} HCO_3^-)	19·8 ± 2·7 (n = 4)	70·6 ± 24 (n = 2)

value of β estimated in the isolated myocyte was virtually unaffected by amiloride (14 mmol l^{-1} in control and 14·9 mmol l^{-1} in amiloride).

Table 3 pools data obtained from nine preparations (five papillary muscles, four myocytes). In all cases, amiloride (in some cases ethylisopropyl amiloride, EIPA, was

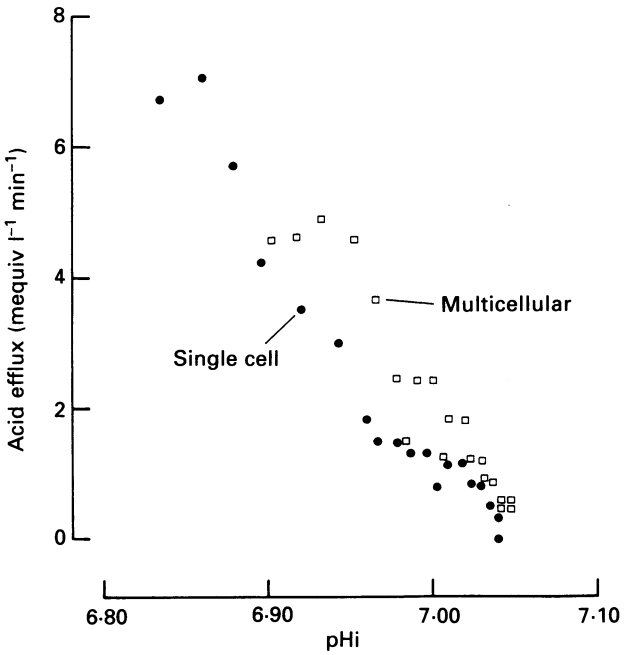

Fig. 7. Estimate of net acid efflux as a function of pH_i for the myocyte (●) and ventricular muscle (□). Data from Fig. 4. Acid efflux estimated as $\beta\, dpH_i/dt$, where β was chosen to resemble the apparent value listed in Table 2 (myocyte, $\beta = 15$ mmol l^{-1}; ventricular muscle, $\beta = 70$ mmol l^{-1}). Note that net acid afflux appears similar in the two preparations.

used, 100 μmol l^{-1}) increased the acid loading produced by NH_4Cl removal, with by far the larger effect occurring in whole muscle (cf. Fig. 8). The effect of this on the mean apparent value of buffering power (Table 3) was to reduce it from 74 to 33 mmol l^{-1} in whole muscle and from 18 to 14 mmol l^{-1} in the myocyte although, in this latter case, the decrease was not statistically significant ($P < 0·05$). These data clearly indicate that in multicellular tissue, acid extrusion when it is functional will blunt the initial intracellular acid load thus resulting in an overestimate of intracellular buffering power.

The rate of initial acid loading

It is apparent that not only is pH_i recovery rate slower in multicellular tissue, but also the rate of initial acid *loading* is slower. Figure 8 shows that the time to peak acidosis following NH_4Cl removal (measured under conditions where acid extrusion

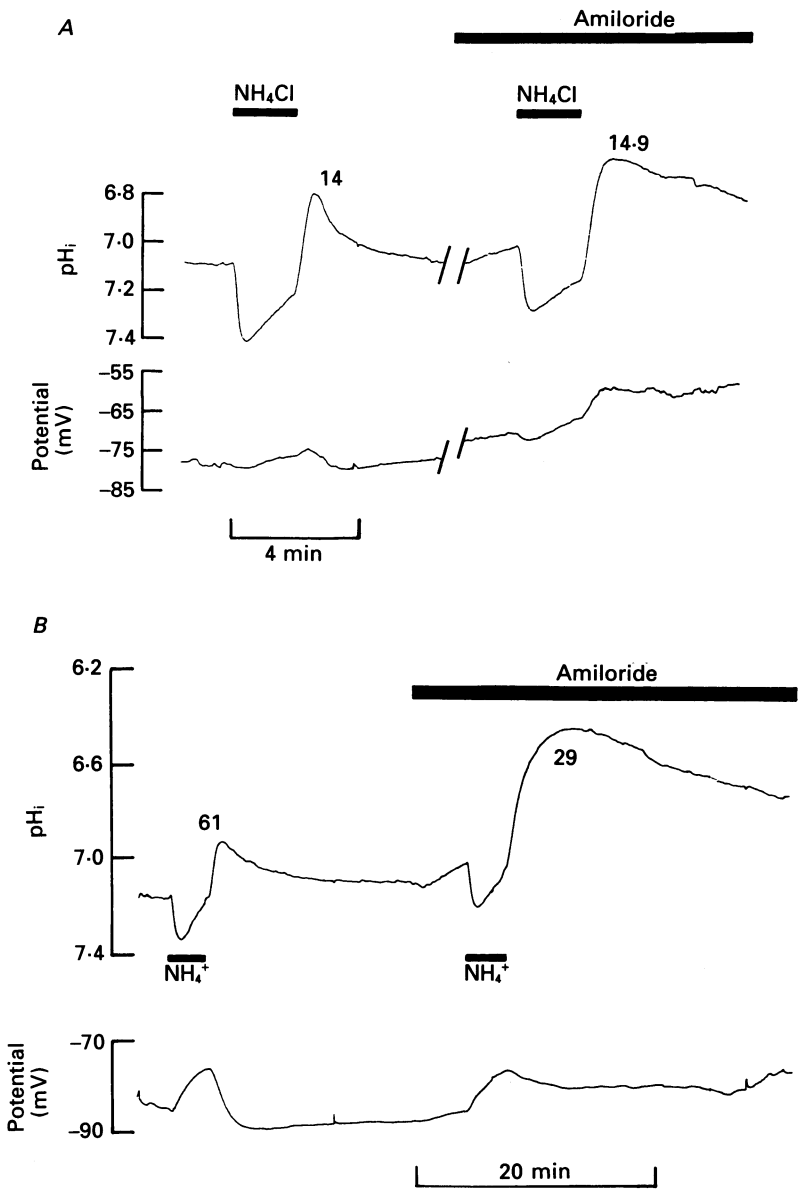

Fig. 8. Effect on pH_i recovery and upon apparent β of reducing acid efflux by adding amiloride. *A*, myocyte; 5 mmol l^{-1} NH_4Cl added as indicated by bars above pH_i trace. Break in traces indicates a period of 2·5 min. *B*, papillary muscle; 10 mmol l^{-1} NH_4Cl added. Computed values of apparent β (mmol l^{-1}) are indicated by the pH_i traces. Note that amiloride influences apparent β in papillary muscle but not, in this case, in the isolated myocyte.

has been slowed greatly by amiloride) is considerably longer in the papillary muscle compared with the isolated myocyte. In three experiments, the half-time ($t_{0.5}$) to peak acidosis (in amiloride) was 1·95 ± 0·5 min (S.E.M.) for papillary muscle compared with 0·23 ± 0·03 min for the isolated myocyte. Even in the absence of amiloride, when

TABLE 3. Apparent values of β measured by inhibiting acid extrusion with amiloride

	β (mmol l^{-1})	
	Single myocyte	Multicellular tissue
Control	18·2 ± 3·4	73·9 ± 10·4
Amiloride	13·6 ± 0·6*	32·8 ± 3·7†
	(n = 4)	(n = 5)

* Difference not statistically significant ($P > 0.05$). † Difference statistically significant ($P < 0.02$).

acid extrusion is functional, it is clear that the initial acid loading rate (NH_4Cl removal or P_{CO_2} elevation) is slower in multicellular tissue (cf. Figs 3, 5, 6 and 8). When acid extrusion rate is reduced by amiloride, as in Fig. 8, the slow fall of pH_i following NH_4Cl removal will reflect more closely the true acid loading rate. As outlined in the Discussion, the significantly slower loading rate in multicellular tissue may be expected to distort severely the subsequent pH_i recovery. The phenomenon of slow acid loading may therefore seriously affect measurements of the kinetic properties of acid extrusion in multicellular tissue.

DISCUSSION

The present work reports the first measurements of pH_i in ventricular myocytes using ion-selective microelectrodes. The resting pH_i of about 7·00 is similar to that which we record in ventricular muscle isolated from the same species and the value is within the range of previous electrode measurements of pH_i in cardiac tissue (e.g. Ellis & Thomas, 1976*a*,*b*; Vanheel, de Hemptinne & Leusen, 1985; Kaila & Vaughan-Jones, 1987). Estimates of pH_i using fluorescent indicators in cultured avian myocytes and in isolated mammalian ventricular myocytes are also in the range 7·0–7·4 (Piwnica-Worms *et al.* 1985; Eisner, Nichols, O'Neil, Smith & Valdeolmillos, 1989; Weissberg, Little, Cragoe & Bobik, 1989). The important question of whether electrode and fluorescent probe techniques furnish identical readings of pH_i must, however, remain unanswered until simultaneous recordings using the two methods are performed in the same cardiac cell.

The behaviour of pH_i in an isolated myocyte resembles qualitatively that observed in multicellular tissue, but there are striking quantitative differences: the intracellular acidoses induced in isolated myocytes are 2–3 times larger than in whole-muscle preparations, and the subsequent rates of pH_i recovery are 2–3 times faster. Possible reasons for these differences are considered below.

Intracellular buffering power of cardiac muscle

Our initial estimate of β in whole ventricular muscle obtained in the absence of amiloride was about 70 mmol l^{-1} which is similar to that reported previously for isolated cardiac muscle (Ellis & Thomas, 1976*a*). Values of 71 mmol l^{-1} (Clancy & Brown, 1966) and 45 mmol l^{-1} (Elliott, 1987) have also been quoted for perfused dog and ferret hearts. Until now it has been assumed that β in cardiac muscle is inherently rather high when compared with other cells such as invertebrate neurones, skeletal muscle, smooth muscle and epithelial cells, where values for β of $<$ 35 mmol l^{-1} are more commonly quoted (cf. Roos & Boron, 1981). It could be argued that a high H^+ buffering power in cardiac muscle is required to match the fact that the tissue is extremely active metabolically. This idea, however, is inconsistent with our observation that isolated myocytes appear to have values of β over three times *lower* than multicellular tissue (15–20 mmol l^{-1}; Table 3). Our results suggest that the estimate for β in ventricular muscle is artificially high because Na^+–H^+ exchange extrudes much of the imposed acid load before loading is complete. As discussed later, this occurs to a much lesser extent in the isolated myocyte. Substantial acid extrusion attenuates the initial acidosis thus giving the appearance of high internal buffering. The problem has been recognized previously in other tissues (Aickin & Thomas, 1977; Boron, 1977; Aickin, 1984; Szatkowski & Thomas, 1989). Although, in many cases, a back-extrapolation of the pH_i recovery (when measuring initial acidosis) is believed to remove most of the overestimate of β (Thomas, 1976; Aickin & Thomas, 1977; Vanheel, de Hemptinne & Leusen, 1986) it is now clear that, in cardiac muscle, this technique is inadequate (Aickin, 1984, also concludes that it is inadequate in multicellular smooth muscle preparations). In ventricular muscle, inhibition of acid extrusion with amiloride reveals a much lower value of β (about 30 mmol l^{-1}), which is closer to that measured in the isolated myocyte. We conclude, therefore, that the non-CO_2 buffering power of cardiac muscle is not unusually high, but rather that β approaches values quoted for many other cell types (Aickin, 1984; Szatkowski & Thomas, 1989) i.e. 15–30 mmol l^{-1}. A value of β of $\sim$ 25 mmol l^{-1} has also recently been reported by Eisner *et al.* (1989) for isolated ventricular myocytes and a value of $\sim$ 20 mmol l^{-1} (at pH_i 7·2) quoted for the sheep Purkinje fibre (Vaughan-Jones & Wu, 1989).

The present determinations of β do not take account of possible variation of β with pH_i. Recent work in the sheep Purkinje fibre (Vaughan-Jones & Wu, 1989) indicates that β is not constant over the physiological range but increases roughly linearly as pH_i falls, a doubling of β occurring as pH_i decreases from 7·4 to 6·4. To date there is no information concerning any pH_i dependence of β in the isolated ventricular myocyte or in ventricular muscle although a rise of β at $pH_i < 6{\cdot}4$ has been reported recently for the ischaemic myocardium (Wolfe, Gilbert, Brindle & Radda, 1988). The technique used for estimating β in the present work involved inducing relatively large changes of pH_i (up to 0·4 pH units). Thus any variation of β within this range would be averaged out in a single measurement. In both the myocyte and in ventricular muscle, we found that resting pH_i was very similar ($\sim$ 7·00) A shallow dependence of β upon pH_i as found in the Purkinje fibre would therefore exert a negligible influence upon apparent differences in β estimates in the two preparations.

When β is determined in the presence of amiloride, its apparent value still remains somewhat higher in ventricular muscle (33 mmol l^{-1}) than in the ventricular myocyte

(14 mmol l^{-1}; see Table 3). Part of this difference may be due to the incomplete inhibition of acid extrusion by amiloride (see Fig. 8*A* and *B*). Thus, because of the particularly slow rate of acid loading into whole muscle (see next section), residual acid extrusion would still lead to some overestimate of β in this tissue. Alternatively, one should ask if other factors conspire to produce genuine differences of β in the two preparations. For example, β, in the myocyte may be affected by the enzymic method of cell separation. A *reduction* of β would occur if cell dispersion resulted in a significant increase of cellular volume. Intrinsic buffers would then become diluted and β would fall. However, a doubling of cell volume would be required to reduce β from 33 to 14 mmol l^{-1} (Table 3) and it seems hard to argue that cell disaggregation should produce such a dramatic effect. Alternatively enzymic digestion might conceivably remove some intracellular buffers if the enzymes themselves were to penetrate into the cytoplasm. There is no evidence, however, to suggest transmembrane penetration of collagenase (see Powell, 1984). Indeed such an event would most likely prove catastrophic for the cell. It seems unlikely, therefore, that cell isolation results in a significant charge of β. It is far more likely that β in myocytes and whole muscle is the same and that its value equals the low value measured consistently in myocytes (i.e. 15–20 mmol l^{-1}).

Although the present work places an upper limit on the magnitude of β, the experimental results cannot identify the chemical nature of the buffers. Intracellular acidosis does not significantly alter the intracellular levels of phosphate compounds (Allen, Elliott & Orchard, 1987) so that changes in energy metabolism during pH_i changes are unlikely to contribute to estimates of β. Similarly, under resting conditions, passive H^+ buffering by phosphate compounds will not contribute more than a few mmol l^{-1} (Wolfe *et al.* 1988). While H^+ uptake at various organelles cannot be excluded, the most likely explanation for fast, initial H^+ buffering is the titration of ionizable groups on cytoplasmic proteins. These groups remain unidentified. A recent report by House, Miller & O'Dowd (1989) has highlighted the presence of up to 10 mmol l^{-1} imidazoles in rat ventricular tissue. In the erythrocyte, imidazoles form the bulk of the buffering by haemoglobin so that it is tempting to speculate that they may also make the largest contribution to intrinsic buffering by proteins in cardiac tissue.

The problem of slow acid loading

The overestimation of β caused by acid extrusion appears to be large in whole muscle (by at least 40 mmol l^{-1}) but small or even negligible in the myocyte (by about 4 mmol l^{-1}; Table 3). At first sight this result is puzzling since one might expect the fast pH_i recovery in the myocyte to produce the greater reduction of total acid loading and hence the larger overestimate of β. A likely explanation is afforded by the observation that initial acid *loading* is much slower in multicellular tissue. When slow loading occurs (i) it will permit acid extrusion to reduce peak acidosis and (ii) it will slow the subsequent rate of pH_i recovery. This latter effect will occur since acid extrusion will be offset by late acid loading. We have modelled mathematically the effect on the pH_i transient of combining an acid loading phase with an acid extrusion mechanism whose dependence on pH_i resembles that illustrated in Fig. 7. This is illustrated in Fig. 9*A*: results of the model have been plotted for the cases

where initial acid loading is slow (i.e. ventricular muscle), fast (i.e. isolated myocyte) and instantaneous. Further details are given in the Appendix. The model assumes identical cell surface area: volume ratios and an identical pH_i dependence of acid extrusion in all three cases. In addition, the 'true' intracellular buffering power is, in all three cases, assumed to be identical and of low value (15 mmol l^{-1}). The model simulations indicate that when acid loading is slow and acid extrusion proceeds at a comparable rate (the 'multicellular' curve in Fig. 9*A*), then the change of pH_i will be smaller, slower and delayed in time when compared with that expected if initial acid loading were instantaneous (the 'instant load' curve in Fig. 9*A*). In the case computed for the isolated myocyte, the rate of initial acid loading was assumed to be tenfold faster than in whole muscle (i.e. similar to the difference observed experimentally, see p. 355). As a result, the time course of pH_i recovery becomes very similar to that computed for instant acid loading, although the recovery is still displaced a little along the time axis. Peak acid loading is also predicted to be much larger in the myocyte compared to whole muscle. A most interesting result is that when the pH_i curves computed for both myocyte and whole muscle are used to estimate the *apparent* value of β, employing the back-extrapolation technique, then β appears to be 21 mmol l^{-1} in the myocyte and 68 mmol l^{-1} in whole muscle! In both cases the real value of β has been set in the model to equal 15 mmol l^{-1}. This example thus emphasizes that (i) slow acid loading results in an overestimate of β and (ii) the value of β estimated experimentally from a pH_i transient is closer to its real value in the myocyte.

Figure 9*B* shows further results of the model. Here the rate of pH_i recovery has been plotted as a function of pH_i. Assuming a constant value for β, the relationship computed following instant acid loading provides an accurate kinetic description of the pH_i dependence of the acid extrusion mechanism. In the case modelled for the isolated myocyte this is also true over most of the range of pH_i computed (Fig. 9*B*), although there is an increasing distortion at the lower values of pH_i. This occurs where pH_i recovery rate has been computed just following peak acidosis, a time when significant acid loading is still occurring. On the other hand, the recovery rate modelled for multicellular tissue is much slower than that expected from acid extrusion alone. Simultaneous slow acid loading results in a spuriously low estimate of acid extrusion over the *whole period* of pH_i recovery. Thus, in this latter case, pH_i recovery from an acid load cannot be used to quantify the kinetic properties of acid extrusion.

From the above analysis one must conclude that, unless a more rapid initial acid loading can be made, the quantification of pH_i regulation in whole ventricular muscle will be fraught with error. It is most surprising, therefore, that the pH_i dependence of acid extrusion determined in the present work for both ventricular muscle and the isolated myocyte appears to be virtually identical (Fig. 7). This result, however, may be fortuitous: owing to slow acid loading, estimates of β in ventricle will, in the absence of amiloride, be artificially *high* by a factor of 3 or more and, as discussed above, estimates of pH_i recovery rate are likely to be spuriously *low*, again by a factor of 2–3. Consequently when computing sarcolemmal acid extrusion (the product of the above two terms; see eqn (3)) these two errors will

tend to cancel each other. The derived pH_i dependence of acid extrusion in multicellular tissue will then resemble that measured in the myocyte. Of course, such a convenient cancellation of errors cannot always be anticipated, so that we would counsel caution when analysing kinetic changes of pH_i in whole ventricular muscle.

Are other cardiac tissues or even myocytes free of the above problems? Figure 9*B* indicates that, even where acid loading is rapid, the use of indirect acid loading techniques to examine pH_i regulation can deliver errors when pH_i recovery rate is measured shortly after peak acid loading. Nevertheless, the experimentally measured value of β in myocytes is not affected greatly by the addition of amiloride indicating that, in general, errors will be much less than in whole muscle.

Many previous analyses of pH_i regulation in heart have utilized the multicellular, sheep Purkinje fibre. In our experience this tissue is much more amenable to quantitative analysis than whole ventricular muscle. Firstly, estimates of β in this tissue, obtained without taking the precaution of inhibiting acid extrusion, are moderately low (~ 35 mmol l^{-1}, Ellis & Thomas, 1976*a*) suggesting that any overestimate of β caused by acid extrusion will be less than in ventricular muscle. Nevertheless, estimates of β in the Purkinje fibre obtained in the absence of acid extrusion (Vaughan-Jones & Wu, 1989) are lower still (~ 20 mmol l^{-1}), so that some overestimate will have occurred. Secondly, recent work (M. L. Wu & R. D. Vaughan-Jones, unpublished observations) indicates that the acid loading phase following NH_4Cl removal (measured in Na^+-free solution in order to inhibit Na^+–H^+ exchange) is up to 10 times faster than the time for pH_i recovery from an acid load (measured in Na^+-containing solution). This is similar to the situation observed in the isolated myocyte. Much of the pH_i recovery phase in the Purkinje fibre should therefore be free of contamination by late acid loading.

Possible causes of slow acid loading.

A number of factors may contribute to the slower acid loading in whole ventricular muscle compared to the isolated myocyte. If the ratio of cell surface area: volume were greater for the myocyte, this would assist a more rapid equilibration of CO_2 or NH_3. There would have to be, specifically, a large increase in surface area in the myocyte since, as argued above, major changes of volume during cell isolation seem unlikely. Diffusion delays in the complex extracellular space of multicellular tissue will also result in a slow ionic equilibration and hence a slow loading (ionic diffusion delays should be minimal in the myocyte). In addition, when combined with differing diffusion rates and dissimilar surface area: volume ratios, significant *transmembrane* movements of HCO_3^- or NH_4^+ ions could lead to disparate acid loading rates in the two preparations. The possibility that surface pH effects in single cells and whole tissue (Vanheel *et al.* 1986) are different has also not been rigorously investigated to date. Whatever the mechanisms underlying slow acid loading, the problem is most certainly far less serious in single myocytes where reasonably prompt loading can be seen. It is even possible that the rate-limiting step here is the rate of bath exchange upon switching to a new solution. This was measured to be $t_{0.5} \sim 0.15$ min by using a pH electrode in the bath to record solution changes, a value which is similar to the measured $t_{0.5}$ for acid loading into myocytes listed on p. 355.

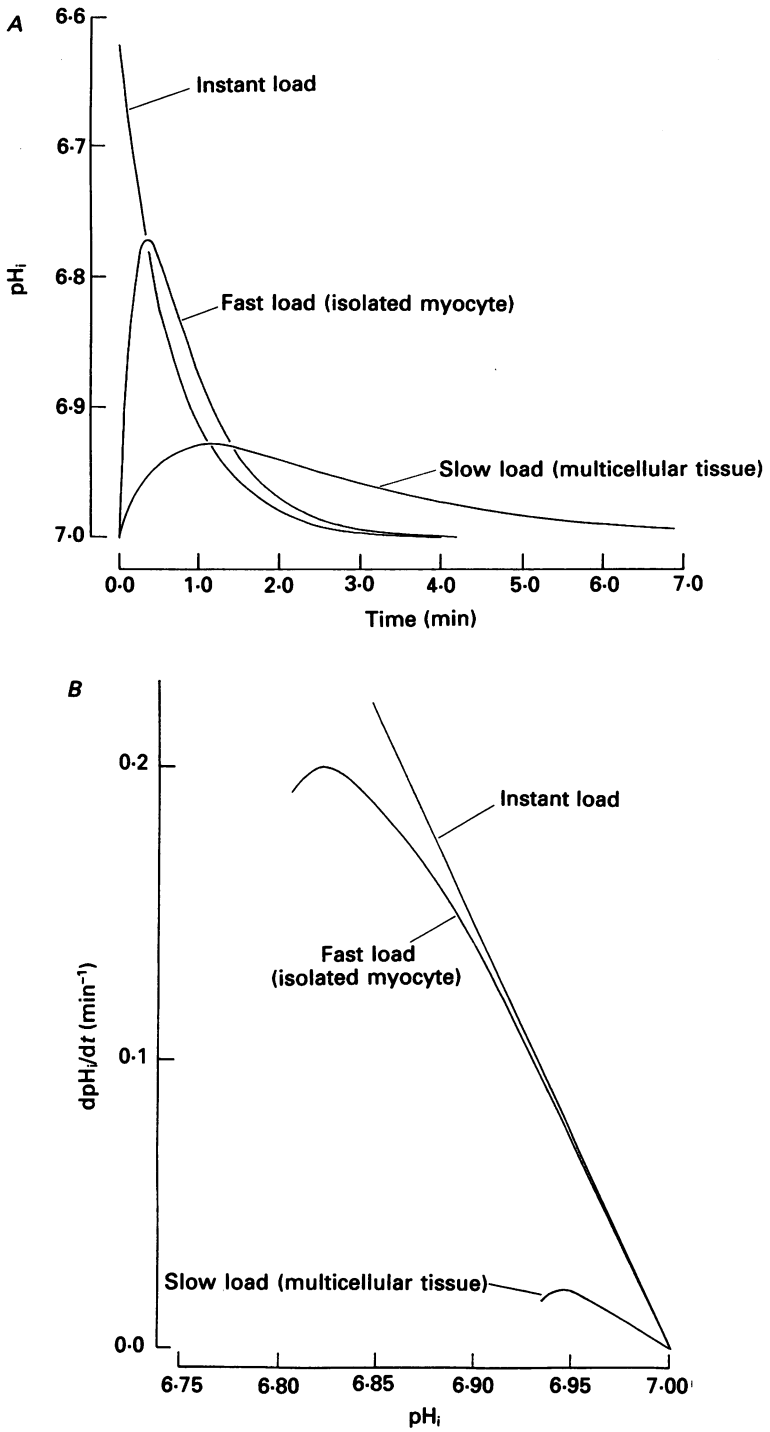

Fig. 9. For legend see facing page.

Regulation of pH_i in ventricular tissue

Some features observed in the present work relate to the regulation of pH_i in mammalian ventricular tissue.

Following an intracellular acid load either in the presence of a CO_2/HCO_3^- buffer system or in an O_2/HEPES system, pH_i usually returns fully to control levels of pH_i (Figs 2 and 3) providing bulk pH_o remains constant at 7·4. A similar result has been observed in rabbit and cat ventricular muscle (Vanheel *et al.* 1985). In the sheep Purkinje fibre, however, steady-state pH_i is more acidic in CO_2/HCO_3^--buffered solutions than in HEPES solutions even though pH_o remains at 7·40 (e.g. Ellis & Thomas, 1976*a*). Vanheel, de Hemptinne & Leusen (1984) suggest that the lower pH_i is due to significant HCO_3^- conductance in the Purkinje fibre membrane resulting in the passive outward leak of HCO_3^- ion and thus a sustained acid loading whenever CO_2/HCO_3^--buffered solutions are employed. Similarly the operation of HCO_3^- efflux/Cl^- influx via sarcolemmal Cl^-–HCO_3^- exchange in cardiac muscle could also result in a decrease of steady-state pH_i (Vaughan-Jones, 1979). The lack of a sustained acid loading in ventricular fibres might therefore suggest either that resting HCO_3^- conductance is considerably lower in these cells and/or that anion exchange in ventricle is inactive at resting levels of pH_i and pH_o.

A further observation is that, in myocyte and whole muscle, after raising P_{CO_2} from 5 to 10% at a constant extracellular [HCO_3^-] (thus simultaneously reducing pH_o), there is a partial, secondary recovery of pH_i from the internal acid load. A similar phenomenon is evident in smooth muscle (Aickin, 1984). In the sheep Purkinje fibre and in skeletal muscle, however, there is no recovery of pH_i (Ellis & Thomas, 1976*b*; Aickin & Thomas, 1977). Once again the reason for the recovery in ventricular tissue is not known but its occurrence may contribute to the observation that elevation of P_{CO_2} produces a fall followed by a slow secondary rise of developed tension in mammalian ventricular muscle (Allen & Orchard, 1983). Since intracellular acidosis reduces myofibrillar Ca^{2+} sensitivity, the slow secondary recovery of pH_i would promote an increase in Ca^{2+} binding and thus a slow secondary recovery of tension. A similar conclusion has been reached in a recent abstract by Allen *et al.* (1987) reporting upon phosphorus NMR measurements of pH_i in perfused ferret heart.

Fig. 9. *A*, model illustrating the effect upon peak intracellular acidosis and the subsequent rate of pH_i recovery of varying the rate of initial acid loading. As acid loading rate is reduced, peak acidosis is also reduced and pH_i recovery is slowed. See text plus Appendix for further details. Fast initial acid loading, $k = 4{\cdot}95\ min^{-1}$; slow $k = 0{\cdot}48\ min^{-1}$. *B*, further results of model. pH_i recovery rate is plotted as a function of pH_i. Graph labelled 'Instant load' displays the true properties of the acid extrusion mechanism adopted in the model. The other two traces show the apparent pH_i dependence of the acid extrusion mechanism derived using the pH_i recoveries for whole muscle and isolated myocyte, computed in *A*, i.e. for the conditions of fast and slow initial acid loading. Note that pH_i recovery rate computed following a slow load is greatly reduced compared with that observed following either an instantaneous or a fast acid load. As discussed in the text, this implies that the time course of pH_i recovery in whole ventricular muscle does not accurately reflect the pH_i dependence of the acid extrusion mechanism.

Finally, the activation of acid efflux as a function of pH_i shown in Fig. 7 resembles that described in other cardiac cells in that acid efflux increases steeply with a fall of pH_i in the range 7·1–6·8. The activation is attributable to Na^+–H^+ exchange since it is amiloride sensitive. We have recently recorded a transient rise of intracellular Na^+ activity in the isolated myocyte (double-barrelled Na^+-selective microelectrode) following internal acid loading (NH_4Cl removal; C. Bountra, T. Powell & R. D. Vaughan-Jones, unpublished observations) which would again be consistent with activation of the exchanger.

Conclusions

A comparison of pH_i transients in ventricular myocytes and in ventricular muscle leads to the conclusion that the transients are smaller and slower in whole muscle because the architecture of this preparation introduces significant time delays in the initial acid-loading procedure. This can lead, in turn, to a serious overestimate of β in isolated ventricular muscle and to substantial distortion of the time course of pH_i recovery. Both problems will affect analyses of acid-equivalent transport in this tissue if these are based upon pH_i measurements. We conclude that single cells are far less affected by these problems and are therefore more suitable for quantitative studies of ventricular pH_i regulation. Intracellular intrinsic buffering power in these isolated cells is in the region of 15–20 mmol l^{-1}.

APPENDIX

Model of acid loading and acid extrusion in cardiac cells

Acid loading reduces pH_i and stimulates the extrusion of acid equivalents. We assume that, during this manoeuvre, pH_i is governed only by the balance between these two fluxes. The net flux of acid across the membrane is therefore given by:

$$\beta \, dpH_i/dt = J_o^H - J_i^H, \tag{1}$$

where β is the intracellular buffering power (non-CO_2; this model assumes the absence of CO_2/HCO_3^- buffers), J_o^H is the unidirectional acid efflux (e.g. via Na^+–H^+ exchange) and J_i^H the unidirectional acid influx (via the acid-loading procedure).

The kinetics of acid extrusion have been approximated to a linear equation of the form:

$$J_o^H = -SpH_i + w, \tag{2}$$

where S and w are constants. This is similar to that shown in Fig. 7 in that, for positive values of J_o^H, net acid extrusion is roughly linearly dependent upon $-pH_i$ in the range ~6·8–7·1 (although at values of $pH_i > 7·2$ the system is virtually inactive).

For convenience, the rate of acid loading (NH_4Cl removal) is assumed to be exponential, being maximal initially (J_{max}^{in}) and then declining with time (t) to zero.

$$J_i^H = J_{max}^{in} \exp^{-kt}, \tag{3}$$

where k is a constant. Combining eqns (1), (2) and (3) gives

$$\beta \, dpH_i/dt = w - SpH_i - J^{in}_{max} \exp^{-kt},$$

which, upon integration, has the solution:

$$pH_i = pH_i^o + C[\exp^{(-S/\beta)t} - \exp^{-kt}], \quad (4)$$

where $C = [J^{in}_{max}/(S+\beta k)]$ and pH_i^o is the value of pH_i before acid loading.

Equation (4) predicts a fall and then a recovery of pH_i with time. This is illustrated in Fig. 9*A*. The values of the constants used to model the pH_i transients in this figure are listed below. The same value of constant is used for both the isolated myocyte and for multicellular tissue *except* for the rate constant of acid loading (k) which is about tenfold faster in the myocyte.

The values of the constants are: pH_i^o, 7·0; w, 155·4 mmol l^{-1}; S, 22·2 mmol l^{-1} min^{-1} per pH_i unit; β, 15 mmol l^{-1}; k, (myocyte) 4·95 min^{-1} (multicellular) 0·48 min^{-1}. The value of β was chosen to approximate that calculated by experiment for the isolated myocyte (Tables 2 and 3). The constants k were chosen to resemble the experimentally measured half-time of acid loading ($t_{0.5}$) following NH_4Cl removal in the presence of amiloride (see p. 355 Results; $k = 0{\cdot}693/t_{0.5}$). The total amount of acid loaded into each cell type was chosen arbitrarily to be 5·67 mmol l^{-1}. Thus J^{in}_{max} for each preparation can be calculated as 28 (myocyte) and 2·72 (multicellular) mmol l^{-1} min^{-1}.

Note that, in Fig. 9, reducing the rate of acid loading (i) reduces peak intracellular acidosis and (ii) reduces the rate of the secondary recovery of pH_i. These two features of the model are considered in the Discussion.

Instantaneous acid loading

In this case all acid loading occurs at $t = 0$ so that eqn (3) can be ignored. The initial pH_i following acid loading (pH_i^A) will be given by:

$$pH_i^A = pH_i^o - A/\beta, \quad (5)$$

where A is the quantity of acid loaded into the cell (5·67 mmol l^{-1}) and pH_i^o is the value of pH_i before acid loading (which will also equal the final steady-state value after all acid extrusion). From eqns (2) and (5) it can be shown that:

$$pH_i = pH_i^o - (A/\beta)\exp^{(-S/\beta)t}. \quad (6)$$

Results obtained from eqn (6) are illustrated in Fig. 9*A*.

We wish to thank Victor Twist for valuable assistance in the preparation of the myocytes used in this work and Dr Keith Buckler who kindly measured for us the effect of EIPA on pH_i recovery in the isolated myocyte. This work was supported by grants from the British Heart Foundation to T.P. and R.D.V-J.

REFERENCES

AICKIN, C. C. (1984). Direct measurement of intracellular pH and buffering power in smooth muscle cells of guinea-pig vas deferens. *Journal of Physiology* **349**, 571–585.

AICKIN, C. C. & THOMAS, R. C. (1977). Micro-electrode measurement of the intracellular pH and buffering power of mouse soleus muscle fibres. *Journal of Physiology* **267**, 791–810.

ALLEN, D. G., ELLIOTT, A. C. & ORCHARD, C. H. (1987). The metabolic effects of acidosis in isolated ferret hears. *Journal of Physiology* **394**, 40*P*.

ALLEN, D. G., MORRIS, P. G., ORCHARD, C. H. & PIROLO, J. S. (1987). A nuclear magnetic resonance study of metabolism in the ferret heart during hypoxia and inhibition of glycolysis. *Journal of Physiology* **361**, 185–204.

ALLEN, D. G. & ORCHARD, C. H. (1983). The effects of changes of pH on intracellular calcium transients in mammalian cardiac muscle. *Journal of Physiology* **335**, 555–567.

AMMANN, D., LANTER, F., STEINER, R. A., SCHULTHESS, P., SHIJO, Y. & SIMON, W. (1981). Neutral carrier based hydrogen ion selective microelectrode for extra- and intracellular studies. *Analytical Chemistry* **53**, 2267-2269.

BORON, W. F. (1977). Intracellular pH transients in giant barnacle muscle fibers. *American Journal of Physiology* **233**, C61–73.

BOUNTRA, C., POWELL, T. & VAUGHAN-JONES, R. D. (1987). Comparison of micro-electrode measurement of intracellular pH in cardiac ventricular tissue and isolated ventricular cells of guinea-pig. *Journal of Physiology* **390**, 58*P*.

BOUNTRA, C. & VAUGHAN-JONES, R. D. (1989). Effect of intracellular and extracellular pH on contraction in isolated, mammalian cardiac muscle. *Journal of Physiology* **418**, 163–187.

CLANCY, R. L. & BROWN, E. B. (1966). In vivo CO_2 buffer curves of skeletal and cardiac muscle. *American Journal of Physiology* **211**, 1309–1312.

DEITMER, J. W. & ELLIS, D. (1980). Interactions between the regulation of the intracellular pH and sodium activity of sheep cardiac Purkinje fibres. *Journal of Physiology* **304**, 471–488.

EISNER, D. A., NICHOLS, C. G., O'NEILL, S. C., SMITH, G. L. & VALDEOLMILLOS, M. (1989). The effects of metabolic inhibition on intracellular calcium and pH in isolated cat ventricular cells. *Journal of Physiology* **411**, 393–418.

ELLIOTT, A. C. (1987). Phosphorus nuclear magnetic resonance studies of metabolite levels and intracellular pH in muscle. Ph.D. Thesis, University of London.

ELLIS, D. & THOMAS, R. C. (1976*a*). Direct measurement of the intracellular pH of mammalian cardiac muscle. *Journal of Physiology* **262**, 755–771.

ELLIS, D. & THOMAS, R. C. (1976*b*). Microelectrode measurement of the intracellular pH of mammalian heart cells. *Nature* **262**, 224–225.

HOUSE, J. P., MILLER, D. J. & O'DOWD, J. J. (1989). Differences in the distribution of the imidazoles of rat heart between atria and ventricles. *Journal of Physiology* 162*P*.

KAILA, K. & VAUGHAN-JONES, R. D. (1987). Influence of sodium–hydrogen exchange on intracellular pH, sodium and tension in sheep cardiac Purkinje fibres. *Journal of Physiology* **390**, 93–118.

MITCHELL, M. R., POWELL,T., TERRAR, D. A. & TWIST, V. W. (1987). Electrical activity and contraction in cells isolated from rat and guinea-pig ventricular muscle: a comparative study. *Journal of Physiology* **391**, 527–544.

PIWNICA-WORMS, D., JACOB, R., HORRES, R. C. & LIEBERMAN, M. (1985) Na/H exchange in cultured chick heart cells. pH_i regulation. *Journal of General Physiology* **85**, 43–64.

POWELL, T. (1984). Methods for the preparation and characterization of cardiac myocytes. In *Methods in Studying Cardiac Membranes*, ed. DHALLA, N. S., pp. 46–62. CRC Press, Boca Raton, FL.

POWELL, T., TERRAR, D. A. & TWIST, V. W. (1980). Electrical properties of individual cells isolated from adult rat ventricular myocardium. *Journal of Physiology* **302**, 131–153.

ROOS, A. & BORON, W. J. (1981). Intracellular pH. *Physiological Reviews* **61**, 296–434.

SZATKOWSKI, M. S. & THOMAS, R. C. (1989). The intrinsic intracellular H^+ buffering power of snail-neurones. *Journal of Physiology* **409**, 89–101.

THOMAS, R. C. (1976). The effect of carbon dioxide on the intracellular pH and buffering power of snail neurones. *Journal of Physiology* **255**, 715–735.

VANHEEL, B., DE HEMPTINNE, A. & LEUSEN, I. (1984). Analysis of $Cl^- - HCO_3^-$ exchange during

recovery from intracellular acidosis in cardiac Purkinje strands. *American Journal of Physiology* **246**, C391–400.

VANHEEL, B., DE HEMPTINNE, A. & LEUSEN, I. (1985). Intracellular pH and contraction of isolated rabbit and cat papillary muscle: effect of superfusate buffering. *Journal of Molecular and Cellular Cardiology* **17**, 23–29.

VANHEEL, B., DE HEMPTINNE, A. & LEUSEN, I. (1986). Influence of surface pH on intracellular pH regulation in cardiac and skeletal muscle. *American Journal of Physiology* **250**, C748–760.

VAUGHAN-JONES, R. D. (1979). Regulation of chloride in quiescent sheep-heart Purkinje fibres studied using intracellular chloride and pH-sensitive microelectrodes. *Journal of Physiology* **295**, 111–137.

VAUGHAN-JONES, R. D. & KAILA, K. (1986). The sensitivity of liquid sensor, ion-selective microelectrodes to changes in temperature and solution level. *Pflügers Archiv* **406**, 641–644.

VAUGHAN-JONES, R. D. & WU, M.-L. (1989). Intracellular intrinsic buffering power varies with pH_i in the sheep cardiac Purkinje fibre. *Journal of Physiology*. **418**, 165*P*.

WEISSBURG, P. L., LITTLE P. J., CRAGOE, E. & BOBIK, A. (1989). The pH of spontaneously beating cultured rat heart cells is regulated by an ATP–calmodulin-dependent Na^+/H^+ antiport. *Circulation Research* **64**, 676–685.

WOLFE, C. L., GILBERT, H. F., BRINDLE, K. M. & RADDA, G. (1988). Determination of buffering capacity of rat myocardium during ischaemia. *Biochimica et biophysica acta* **971**, 9–20.