Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 9017–9021, December 1988
Biophysics

Ca^{2+} transient, Mg^{2+}, and pH measurements in the cardiac cycle by ^{19}F NMR

(^{19}F cation indicators/myoplasmic Ca^{2+} buffer/gated NMR spectra)

HEIDI L. KIRSCHENLOHR, JAMES C. METCALFE*, PETER G. MORRIS, GLENN C. RODRIGO, AND GERRY A. SMITH

Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QW, United Kingdom

Communicated by Hans L. Kornberg, June 14, 1988

ABSTRACT **^{19}F NMR indicators have been used to measure the free cytosolic cation concentrations ($[M^{n+}]_i$, where M is the atomic symbol and n is the value of the charge) of Ca^{2+}, H^+, and Mg^{2+} in perfused ferret hearts. The $[Ca^{2+}]_i$ transient, cytosolic pH (pH_i), and $[Mg^{2+}]_i$ have also been followed at 16 phases in the cardiac cycle in hearts paced at 1.25 Hz at 30°C. The initial $[Ca^{2+}]_i$ rose rapidly after a 50-ms delay, was maximal at >1.5 μM after 150 ms, and declined thereafter to the initial concentration. In contrast, no significant changes in pH_i (pH 7.03 ± 0.08) or $[Mg^{2+}]_i$ (1.2 ± 0.1 mM) were detected in the cycle. A decrease in developed pressure when the $[Ca^{2+}]_i$ indicator (but not the pH_i or $[Mg^{2+}]_i$ indicator) was loaded into hearts was substantially reversed by the addition of 50 μM $ZnCl_2$ to the perfusion medium. The Zn^{2+} was taken up into the myoplasm and displaced Ca^{2+} bound to the indicator, a symmetrically substituted difluoro derivative of 1,2-bis(*o*-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid (5FBAPTA), as evidenced by the appearance of the Zn–5FBAPTA resonance. The decrease in developed pressure caused by 5FBAPTA, therefore, may be due to its Ca^{2+} buffering effect on the myoplasm. By coloading hearts with the $[Ca^{2+}]_i$ and pH_i indicators, simultaneous measurement of several $[M^{n+}]_i$ was demonstrated, which should provide a useful addition to the methods available to monitor cardiac function and pharmacology.**

We have described a series of prototype indicators to measure the cytosolic free concentrations ($[M^{n+}]_i$, where M is the atomic symbol and n is the value of the charge) of Ca^{2+} (1, 2), H^+ (2), Mg^{2+},† and Na^+ (3) by ^{19}F NMR. The use of the indicators in functional cells was demonstrated by following increases in $[Ca^{2+}]_i$ in lymphocytes stimulated with mitogen (1) and in rat basophil leukemic (2H3) cells activated by antigen (2). The indicators were primarily intended, however, to extend the measurement of $[M^{n+}]_i$ to isolated tissues and organs and ultimately to *in vivo* animal studies.

The design of each indicator is based on the specific and reversible chelation of a single cation that titrates over its normal intracellular concentration range without interference from other ions. Direct readout of $[M^{n+}]_i$ is obtained from the change in the ^{19}F NMR spectrum of the indicator on binding its specific cation. The ^{19}F NMR Ca^{2+} indicator used here, 5FBAPTA (1), is the symmetrically substituted difluoro derivative of the tetracarboxylate Ca^{2+} chelator 1,2-bis(*o*-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid (4). 5FBAPTA titrates with Ca^{2+} over the physiological range of $[Ca^{2+}]_i$ from ≈ 0.1 μM to 10 μM and retains the selectivity of the parent structure for Ca^{2+} over Mg^{2+} and H^+. Under the conditions of the present studies, Ca^{2+} is in slow exchange with 5FBAPTA on the NMR time scale so that the two resonances observed correspond to the Ca–5FBAPTA complex and free 5FBAPTA. $[Ca^{2+}]_i$ is obtained from the relative proportions of the two components and the affinity of the Ca–5FBAPTA complex (1).

The ^{19}F NMR cytosolic pH (pH_i) indicator FQuene (2) is a derivative of the fluorescent pH_i indicator Quene 1 (5). FQuene has similar fluorescence properties to Quene 1 and, therefore, can be used to measure pH_i in cell suspensions by two independent spectroscopic assays. The ^{19}F NMR $[Mg^{2+}]_i$ indicator is the (+)-isomer of fluorocitrate [(+)-FCit]. Both (+)-FCit and FQuene operate in the fast-exchange ^{19}F NMR regime, and pH_i and $[Mg^{2+}]_i$ are determined directly from the chemical shifts of the respective ^{19}F NMR resonances.† All of the indicators are loaded into cells as their acetoxymethyl ester (AME) derivatives, which are nonpolar and permeate freely into cells where they are hydrolyzed by esterases to release the free indicators.

Preliminary studies with the $[Ca^{2+}]_i$ and pH_i indicators in perfused rat (2) and ferret hearts (†, 6) showed that it should be possible to follow changes in $[M^{n+}]_i$ during the cardiac cycle by using the gated acquisition techniques developed for studies of perfused hearts by ^{31}P NMR (7). We report the use of the indicators loaded into ferret hearts to measure $[Ca^{2+}]_i$, pH_i, and $[Mg^{2+}]_i$ during the cardiac cycle and to characterize the effect of 5FBAPTA on the developed pressure.

MATERIALS AND METHODS

Indicators and $[M^{n+}]_i$ Measurements. The apparent K_d for Ca^{2+} of 5FBAPTA was measured by UV spectroscopy of the indicator (0.5 μM) in a medium of 100 mM KCl and 25 mM Tris acetate (without EGTA), pH 7.1, titrated with $CaCl_2$ at 30°C. The residual Ca^{2+} in the medium before titration was 0.2 μM, measured by atomic absorption spectroscopy, and the UV spectrum of 5FBAPTA at 1 nM free Ca^{2+} was obtained by addition of 20 μM EGTA to a separate sample of the 0.5 μM 5FBAPTA solution. This titration gave the same value for the apparent K_d for Ca^{2+} of 0.54 μM at 30°C as UV titration in the citrate buffer used previously (1).

FQuene was synthesized from 4-fluoro-2-nitroaniline as described for Quene 1 (5). The ^{19}F NMR resonance of FQuene shifted by ≈4 ppm between pH 5 and 8 with an apparent pK value of 6.7.

The $[Mg^{2+}]_i$ indicator (+)-FCit was provided by E. Kun (University of California School of Medicine, San Francis-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: AME, acetoxymethyl ester; 5FBAPTA, symmetrically substituted difluoro derivative of 1,2-bis(*o*-aminophenoxy)-ethane-*N,N,N',N'*-tetraacetic acid; $[M^{n+}]_i$, cytosolic free concentration of a cation, where M is the atomic symbol and n is the value of the charge; pH_i, cytosolic pH; FCit, fluorocitrate.

*To whom reprint requests should be addressed.

†Morris, P. G., Smith, G. A., Metcalfe, J. C. & Rodrigo, G. C., Work in Progress 31, presented at the Sixth Annual Meeting of the Society of Magnetic Resonance in Medicine, August 17, 1987, New York.

co). The ^{19}F NMR frequency for the doublet (split by the methine proton) shifted by ≈3 ppm on raising the free Mg^{2+} concentration from 1 μM to 50 mM with an apparent K_d for Mg^{2+} of 4.2 mM, measured by direct ^{19}F NMR spectroscopy of 0.1 mM (+)-FCit in the above medium titrated with $MgCl_2$ at 30°C. A single peak, broadened by exchange of Mg^{2+} with (+)-FCit, was observed at free Mg^{2+} concentrations in the titration range for (+)-FCit. A reference chemical shift for (+)-FCit at the Mg^{2+} concentration of the perfusion medium (1 mM) was obtained by the addition of (+)-FCit to the perfusion medium at the end of the experiment (see legend to Fig. 5).

Indicator Loading. The AME derivatives of the indicators [50 mM stock solutions in $(CH_3)_2SO$] were injected into the polytetrafluoroethylene perfusion line at 200 μl/hr downstream of a bubble trap to prevent flocculation of the aqueous dispersion of the indicator. The esters, therefore, reached the heart at a calculated concentration in the perfusion medium, determined by the perfusion flow rate [4 ml per min per g (wet weight) of heart], of 5–10 μM for hearts of 8–4 g, respectively.

NMR Measurements. ^{19}F NMR spectra were recorded at 376 MHz by using 22° radio frequency (rf) pulses repeated at rates of either 5 Hz or 10 Hz. Control experiments were performed in which the rf pulse width and the repetition rate were varied. It was found that the $[Ca^{2+}]_i$ values measured from the relative areas of the bound and free 5FBAPTA resonances were unaffected. The effective ^{19}F T_1 relaxation times of the bound and free forms of the indicators, therefore, are equal under the conditions used in these experiments. This implies either that the intrinsic T_1 values of the two species are the same or that exchange between the two forms is fast compared with the intrinsic T_1 values. Data were accumulated in blocks of 1000, 2000, or 4000 free induction decays. To monitor changes in $[M^{n+}]_i$ during the cardiac cycle, the hearts were paced at 1.25 Hz and the gated spectra from 16 50-ms intervals in the cycle were accumulated over 100 min.

RESULTS AND DISCUSSION

Effects of 5FBAPTA on Cardiac Function. Perfusion of ferret hearts at 30°C with 5FBAPTA AME caused a rapid drop in the developed pressure in all experiments (Fig. 1*a*) well before the ^{19}F NMR spectrum of intracellular 5FBAPTA was detected between 5 and 10 min after commencement of loading. Studies with [^{3}H]5FBAPTA AME showed that, under the conditions described, ≈10% of the perfused indicator ester was taken up into the hearts, and the remainder was recovered unhydrolyzed in the expelled perfusate. Of the indicator ester retained in the heart, at least 95% was hydrolyzed to the free indicator, assayed after extraction of the radiolabeled material from the heart by separating the [^{3}H]5FBAPTA from unhydrolyzed ester by TLC (1). From these data and the 5FBAPTA AME concentrations of 5–10 μM passing through the heart, it can be calculated that the threshold of detection for intracellular 5FBAPTA in these experiments was 10–20 μM.

The two resonances corresponding to Ca–5FBAPTA and free 5FBAPTA (Fig. 1*b*) demonstrated unequivocally that the observed spectrum was derived from the indicator rather than its ester, since the latter did not give a detectable high-resolution ^{19}F NMR spectrum. The ester has very low solubility in aqueous solutions and was present in the perfusion medium in suspension as micelles or very small droplets. The ^{19}F NMR spectrum of the ester was, therefore, very broad and was not detected above the baseline noise. It was also clear that the 5FBAPTA was contained within an intracellular compartment, since, in a large number of experiments, the averaged Ca^{2+} concentration determined from the relative areas of the bound and free components of the spectrum (1) varied between 0.40 μM and 0.55 μM, whereas

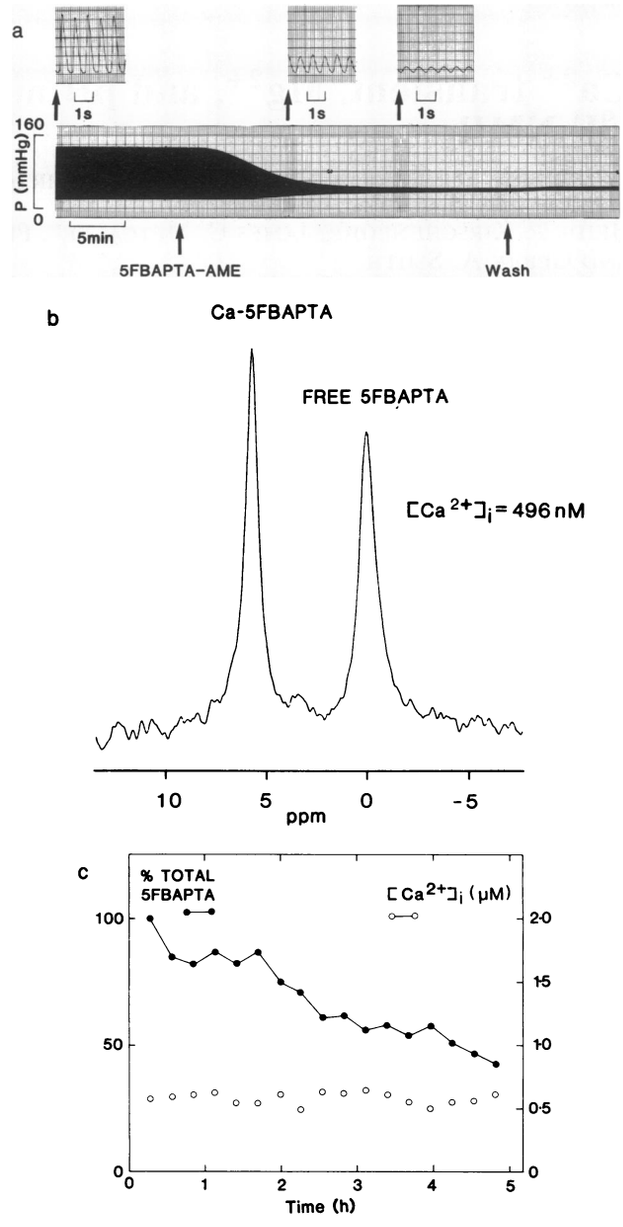

FIG. 1. (*a*) Developed pressure in a heart perfused with 5FBAPTA AME for 30 min. The final pressure was typically 10–20% of the initial pressure before perfusion with 5FBAPTA AME. The expanded traces show the slower rate of pressure increase and recovery as the heart was loaded with 5FBAPTA. (*b*) ^{19}F NMR spectrum of 5FBAPTA in a heart loaded as in *a* with spectral accumulation for 13 min. All 5FBAPTA spectra are referenced to free 5FBAPTA (0 ppm). The $[Ca^{2+}]_i$ is averaged over the cardiac cycle and includes the transient. (*c*) Time course of 5FBAPTA wash out from a heart loaded as in *a*. The half-time for 5FBAPTA efflux was ≈4 hr at 30°C. There was no significant change in the reported $[Ca^{2+}]_i$ during wash out and recovery of the developed pressure was <10% over 4 hr (data not shown).

the extracellular Ca^{2+} concentration in the perfusion medium was 1.8 mM. This Ca^{2+} concentration would saturate 5FBAPTA if it was in the extracellular compartment to give a single resonance at the Ca–5FBAPTA frequency.

The average $[Ca^{2+}]_i$ range was significantly higher than the values of 0.1–0.3 μM determined as the resting $[Ca^{2+}]_i$ during the cardiac cycle by fluorescent indicators (8), aquorin (9), or Ca^{2+}-sensitive microelectrodes (10). The high average $[Ca^{2+}]_i$ obtained from the 5FBAPTA spectra may be related to the decrease in developed pressure caused by loading with

5FBAPTA, although it was noted that the $[Ca^{2+}]_i$ indicated by 5FBAPTA did not vary significantly with the intracellular concentration of loaded indicator between 0.03 mM and 0.20 mM. It was also noted that after perfusion with the 5FBAPTA AME was completed, the intracellular 5FBAPTA was washed out of the heart with a half-time of ≈4 hr at 30° (Fig. 1*c*) and that during this process $[Ca^{2+}]_i$ remained approximately constant (Fig. 1*c*).

It was clear from experiments with a wide range of other chelators, including the pH_i and $[Mg^{2+}]_i$ indicators, that the effect on developed pressure was not attributable to the hydrolysis products of the AME groups. However, the experiments suggested that the decrease in developed pressure was correlated with the affinities of the chelators for Ca^{2+}, or possibly some other polyvalent cation. The simplest explanation for the effect of 5FBAPTA on the developed pressure is that it increases the buffering capacity for Ca^{2+} in the myoplasm. This is consistent with the slower time course of the contraction and the prolonged recovery phase in hearts loaded with 5FBAPTA (Fig. 1*a*), which may reflect the time taken for Ca^{2+}-ATPases to pump out myoplasmic Ca^{2+}. [^{3}H]5FBAPTA loaded under the same conditions as described for the unlabeled indicator gave estimated intracellular concentrations of 5FBAPTA between 0.06 mM and 0.11 mM. These values may be compared with estimates of the binding capacity for Ca^{2+} in the myoplasm of 70 μM for troponin and 50 μM for calmodulin (11). If titration of Ca^{2+} binding by these proteins provides a major part of the myoplasmic buffering capacity for Ca^{2+} over the $[Ca^{2+}]_i$ range from 0.1 to 10 μM, then the loaded 5FBAPTA would add substantially to this capacity.

Reversal of 5FBAPTA Effect on Pressure by Zn^{2+}. More direct evidence consistent with a buffering effect of 5FBAPTA on myoplasmic Ca^{2+} was obtained by perfusing hearts loaded with 5FBAPTA with medium to which 50 μM $ZnCl_2$ had been added. This resulted in a substantial restoration of the developed pressure (Fig. 2*a*). Zn^{2+} has a much higher affinity for 5FBAPTA ($K_a > 10^9\ M^{-1}$) than Ca^{2+}, and when taken up by the myoplasm, it displaced Ca^{2+} from 5FBAPTA. The kinetics of Zn^{2+} uptake were readily followed from the appearance of the resonance at the Zn–5FBAPTA frequency between the Ca–5FBAPTA and free 5FBAPTA peaks (1, 2) (Fig. 2*b*). In marked contrast to its effect on hearts loaded with 5FBAPTA, 50 μM Zn^{2+} caused a decrease in developed pressure in control hearts not loaded with 5FBAPTA (Fig. 2*c*). This effect of Zn^{2+} on control hearts was not reversed after removing Zn^{2+} from the perfusion medium for at least 100 min. It was concluded that the reversing effect of Zn^{2+} on the developed pressure in the 5FBAPTA-loaded hearts was correlated with the formation of the Zn–5FBAPTA complex that effectively reduces the Ca^{2+} buffering capacity of the 5FBAPTA in the myoplasm.

However, it remains to be demonstrated that all of the effect of 5FBAPTA on the developed pressure is attributable to its Ca^{2+}-buffering capacity. It was noted in the 5FBAPTA wash-out experiment (Fig. 1*c*) that as the intracellular 5FBAPTA concentration decreased, the effect on the developed pressure was essentially irreversible, although a small recovery was observed in some experiments. This would argue against the effect of 5FBAPTA on pressure being due solely to its buffering effect on changes in $[Ca^{2+}]_i$, although the time taken for the wash out of 5FBAPTA was lengthy and the possibility of a compensating decline in cardiac function during efflux cannot be eliminated. The data, therefore, leave open the possibility that Zn^{2+} or other minor intracellular cations that are effectively scavenged by 5FBAPTA may be involved in regulating contraction and we have noted that in some hearts loaded with 5FBAPTA there is a very small resonance at the Zn–5FBAPTA frequency.

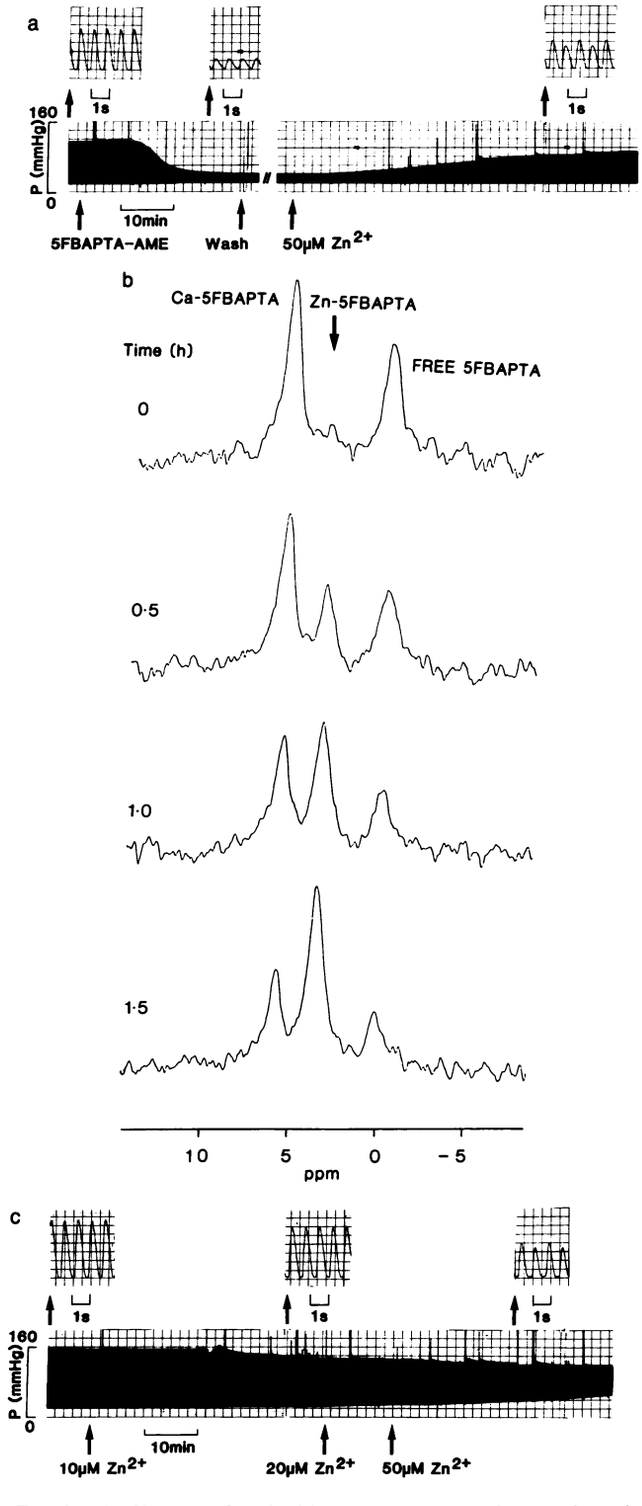

FIG. 2. (*a*) Heart perfused with 5FBAPTA AME for 30 min and perfused with medium for a further 30 min before the addition of 50 μM $ZnCl_2$. In the experiment shown the developed pressure eventually recovered to 73% of the value before perfusion with 5FBAPTA AME. (*b*) ^{19}F NMR spectra from the experiment shown in *a* accumulated for 13 min after the times indicated. The Zn–5FBAPTA resonance is identified from its chemical shift (+3.4 ppm from free 5FBAPTA; see ref. 1). There was no significant change in $[Ca^{2+}]_i$ during the uptake of Zn^{2+}. (*c*) Perfusion of a control untreated heart with medium containing $ZnCl_2$. (Spontaneous arrhythmias occurred more frequently in hearts perfused with medium containing Zn^{2+}.)

The $[Ca^{2+}]_i$ Transient. In experiments to monitor changes in $[Ca^{2+}]_i$ during the cardiac cycle, the hearts were paced at

1.25 Hz and gated spectra were accumulated over a period of 100 min in 16 bins, each of 50-ms duration (Fig. 3*a*). The $[Ca^{2+}]_i$ values calculated for each 50-ms interval are shown in Fig. 3*b* and spectra from the cycle are shown in Fig. 3*c*. $[Ca^{2+}]_i$ rose rapidly after a delay of 50 ms from an initial value of 0.5 μM to a peak of 1.5–2 μM 150 ms after stimulation. Thereafter, $[Ca^{2+}]_i$ returned more slowly toward the initial level. The form of the transient, with a delayed, rapid rise and a slower fall, is similar to, but more protracted than, that observed with aquorin injected into single myocytes (9). However, the $[Ca^{2+}]_i$ measured by both techniques was maximal before the maximal increase in developed tension. It, therefore, is clear that although the hearts are perturbed by the presence of 5FBAPTA, the $[Ca^{2+}]_i$ transient can nevertheless be readily detected.

Simultaneous $[Ca^{2+}]_i$ and pH_i Measurements. When hearts were perfused with the AME derivative of the pH_i indicator FQuene and loaded with similar intracellular concentrations of FQuene as for $[Ca^{2+}]_i$ measurements with 5FBAPTA, there was no significant effect on developed pressure (consistent with the low affinity of FQuene for Ca^{2+} and other polyvalent cations) and the average pH_i value was 7.02 ± 0.07 (± SEM, n = 6). The use of the ^{19}F NMR indicators to measure $[M^{n+}]_i$ for two ions simultaneously is illustrated in Fig. 4*a*. Loading 5FBAPTA into hearts after FQuene caused the normal decrease in pressure but did not affect the pH_i indicated by FQuene and gave $[Ca^{2+}]_i$ values within the range (0.40–0.55 μM) obtained for hearts loaded with 5FBAPTA alone. Gated acquisition experiments showed no variation in pH_i over the cycle (Fig. 4*b*) and this invariance is consistent with previous observations with gated ^{31}P NMR methods (7).

$[Mg^{2+}]_i$ Measurements with (+)-FCit. Racemic FCit is toxic: it binds to aconitase and inhibits the tricarboxylic acid cycle (12, 13). However, the interaction is highly stereospecific and the pure (+)-FCit isomer (14) is not toxic in several types of cells that have been loaded with (+)-FCit to cytosolic concentrations of at least 1 mM (unpublished data). When loaded into ferret hearts, (+)-FCit gave a single resonance that was detectable 10–15 min after commencing perfusion with (+)-FCit AME (Fig. 5*a*). In most experiments loading with (+)-FCit caused a small increase in developed pressure. To check on the calibration of the $[Mg^{2+}]_i$ measurement at the end of the experiment, 0.5 mM sodium (+)-fluorocitrate was added to the perfusion medium in which the Mg^{2+} and Ca^{2+} concentrations were adjusted to 5 mM and 0.1 mM, respectively. The extracellular Ca^{2+} concentration was adjusted to the lowest value (0.1 mM) consistent with maintenance of cardiac function for calibration of $[Mg^{2+}]_i$, since Ca^{2+} also binds to (+)-FCit and shifts the ^{19}F NMR resonance. No contracture of the hearts was observed in the low Ca^{2+} medium and when perfusion with the normal medium [containing 1 mM $MgCl_2$ and 1.8 mM $CaCl_2$, without sodium (+)-fluorocitrate] was resumed, the functional status of the heart was restored. The calibration procedure gave a (+)-FCit resonance corresponding to the 5 mM Mg^{2+} concentration in the perfusion medium and a separate resonance from the intracellular (+)-FCit that was not shifted by the change in perfusion medium.

The average $[Mg^{2+}]_i$ obtained with (+)-FCit was 1.2 ± 0.22 mM (± SEM, n = 5), similar to the value of 1.4 mM reported for rat myocardium with ^{31}P NMR measurements of the upfield shift of the β resonance of the Mg-ATP^{2-} complex (15). Changes in extracellular Mg^{2+} concentration in the perfusion medium over the range from 10 μM to 10 mM had no effect on $[Mg^{2+}]_i$. This was consistent with data for pig lymphocytes with (+)-FCit and for mouse thymocytes with the fluorescent $[Mg^{2+}]_i$ indicator quin-5, which both showed negligible changes in the indicated $[Mg^{2+}]_i$ over the same extracellular Mg^{2+} concentration range (unpublished observations). When gated acquisition experiments were performed on hearts loaded with (+)-FCit, no variations in $[Mg^{2+}]_i$ (1.2 ± 0.1 mM) were detected (Fig. 5*b*). These data suggest that there is close regulation of $[Mg^{2+}]_i$ to 1.2 mM and that the $[Ca^{2+}]_i$ transient is imposed on a constant $[Mg^{2+}]_i$ background.

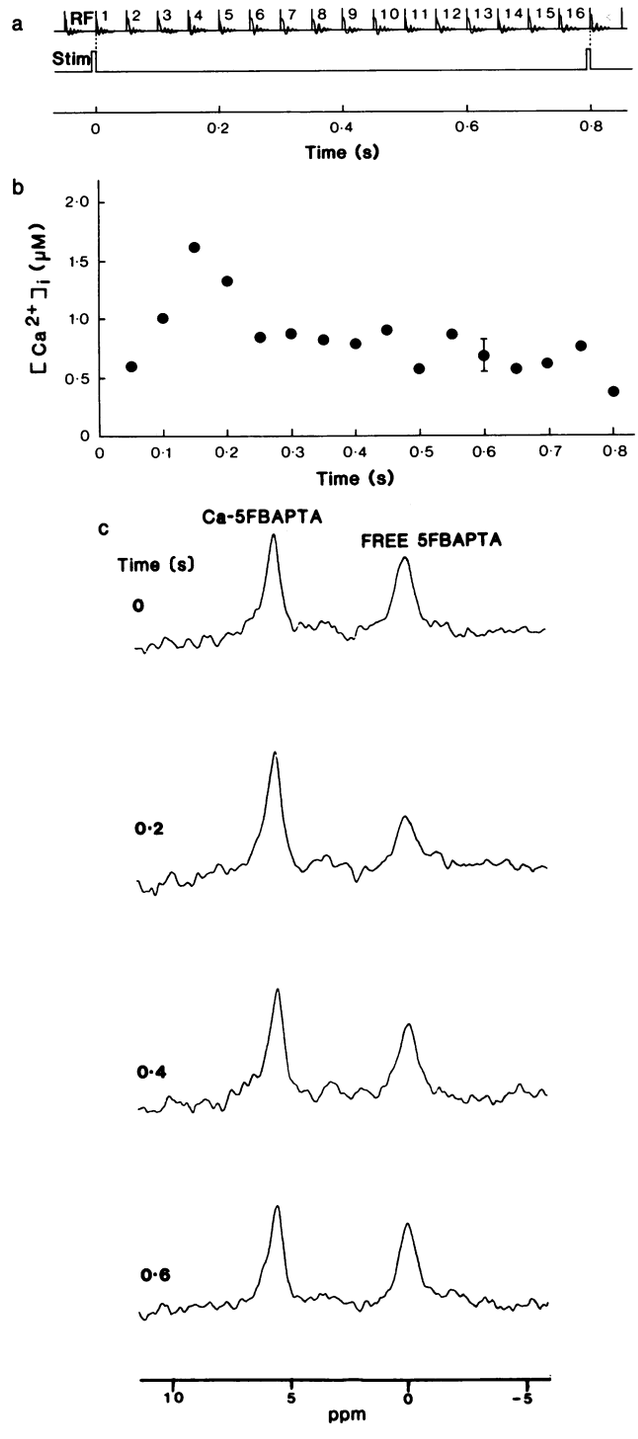

FIG. 3. $[Ca^{2+}]_i$ transient measurement in the cardiac cycle at 30°C. (*a*) Sequence of 22° rf pulses at 50-ms intervals in the cycle (stimulation at 1.25 Hz). (*b*) $[Ca^{2+}]_i$ values calculated from spectra accumulated for 100 min. (*c*) ^{19}F NMR spectra at 0.2-s intervals within the cardiac cycle from the experiment shown in *b*.

CONCLUSIONS

The pH_i and $[Mg^{2+}]_i$ ^{19}F NMR indicators should prove to be useful adjuncts to the corresponding ^{31}P NMR measure-

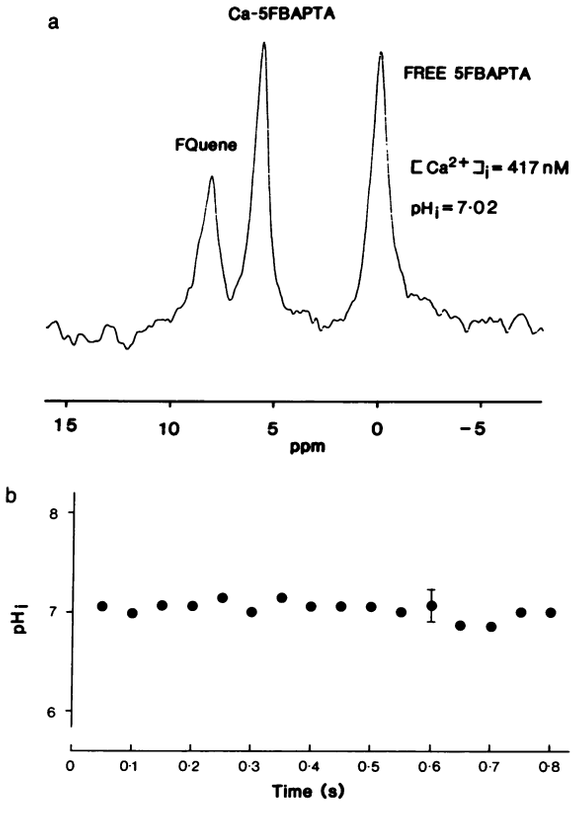

FIG. 4. (*a*) ^{19}F NMR spectrum of FQuene accumulated for 13 min after perfusing with FQuene AME for 30 min and then with 5FBAPTA AME for 30 min. The pH_i indicated by FQuene was unaffected by loading the heart with 5FBAPTA. (*b*) pH_i measurement in the cardiac cycle by gated acquisition of FQuene spectra as described for 5FBAPTA in Fig. 3. The average pH_i value was 7.03 ± 0.08 (± SEM).

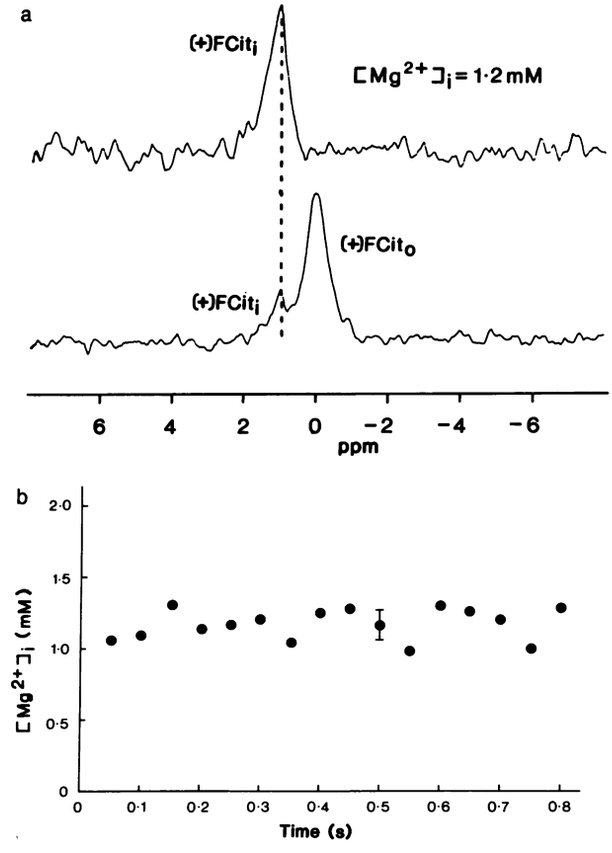

FIG. 5. (*a*) ^{19}F NMR spectra of (+)-FCit accumulated for 13 min after perfusing with (+)-FCit AME for 30 min (upper trace). (+)-FCit (0.5 mM) was then added to the perfusion medium and the Mg^{2+} and Ca^{2+} concentrations were adjusted to 5 mM and 0.1 mM, respectively (lower trace). The extracellular (+)-FCit [(+)$FCit_o$] resonance from the (+)-FCit in the medium provides a reference (set at 0 ppm) for the chemical shift of (+)-FCit in 5 mM Mg^{2+}. The chemical shift of the intracellular (+)-FCit [(+)$FCit_i$] resonance was unaffected by the change in the perfusion medium (dashed line). (*b*) $[Mg^{2+}]_i$ measurement in the cardiac cycle by gated acquisition of (+)-FCit spectra as described for 5FBAPTA in Fig. 3. The average $[Mg^{2+}]_i$ was 1.2 ± 0.1 mM (mean ± SEM).

ments. In some tissues the latter are limited by the low resting levels of inorganic phosphate (<1 mM) so that precise pH_i measurements are difficult, and the sensitivity of the [β-^{31}P]ATP measurements is restricted by the small shift on titrating over the physiological $[Mg^{2+}]_i$ range. Direct comparisons of pH_i and $[Mg^{2+}]_i$ by both ^{31}P and ^{19}F NMR assays are feasible for the same heart preparation by using a $^{31}P/^{19}F$ switchable NMR probe. This will also facilitate monitoring of metabolic regulation by ^{31}P NMR in hearts and other tissues loaded with the ^{19}F NMR indicators.

The advantages of $[Ca^{2+}]_i$ measurements with 5FBAPTA include direct identification of the Ca–5FBAPTA resonance (and other M^{n+}-5FBAPTA species), internal calibration of $[Ca^{2+}]_i$ from the spectra, the absence of background ^{19}F NMR signals from unhydrolyzed 5FBAPTA or any endogenous cellular components, and freedom from motion artifacts due to contraction. The limitations of this indicator are the time taken to measure $[Ca^{2+}]_i$ transients and the effects on developed pressure at the required intracellular concentrations of 5FBAPTA (although the perturbations assumed to be due to Ca^{2+} or M^{n+} buffering by 5FBAPTA are of interest). Indicators incorporating more ^{19}F nuclei than 5FBAPTA to increase ^{19}F NMR sensitivity should greatly enhance the range of $[Ca^{2+}]_i$ measurements that can be made and also permit the use of chemical-shift imaging techniques to provide localized measurements of $[Ca^{2+}]_i$, which would be of interest in defining pathological effects on cardiac function.

We are very grateful to Dr. E. Kun for the gift of the (+)-FCit. We thank Dr. Deborah Harding for her help with part of this study. H.L.K. is the recipient of a Royal Society European Fellowship, and the work is supported by a grant from the British Heart Foundation (to J.C.M. and P.G.M.).

1. Smith, G. A., Hesketh, T. R., Metcalfe, J. C., Feeney, J. & Morris, P. G. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 7178–7182.
2. Metcalfe, J. C., Hesketh, T. R. & Smith, G. A. (1985) *Cell Calcium* **6**, 183–195.
3. Smith, G. A., Morris, P. G., Hesketh, T. R. & Metcalfe, J. C. (1986) *Biochim. Biophys. Acta* **889**, 72–83.
4. Tsien, R. Y. (1980) *Biochemistry* **19**, 2396–2404.
5. Rogers, J., Hesketh, T. R., Smith, G. A. & Metcalfe, J. C. (1983) *J. Biol. Chem.* **258**, 5994–5997.
6. Marban, E., Kitakaze, M., Kusuoka, H., Porterfield, J. K., Yue, D. T. & Chacko, V. P. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 6005–6009.
7. Morris, P. G., Allen, D. G. & Orchard, C. H. (1985) *Adv. Myocardiol.* **5**, 27–38.
8. Lattanzio, F. A. & Pressman, B. C. (1986) *Biochem. Biophys. Res. Commun.* **139**, 816–821.
9. Cobbold, P. H. & Bourne, P. K. (1984) *Nature (London)* **312**, 444–446.
10. Marban, E., Rink, T. J., Tsien, R. W. & Tsien, R. Y. (1986) *Nature (London)* **286**, 845–850.
11. Wier, W. G. & Yue, D. T. (1986) *J. Physiol. (London)* **376**, 507–530.
12. Peters, R. (1954) *Endeavor* **13**, 147–154.
13. Kirsten, E., Sharma, M. L. & Kun, E. (1978) *Mol. Pharmacol.* **14**, 172–184.
14. Dummel, R. J. & Kun, E. (1969) *J. Biol. Chem.* **244**, 2966–2969.
15. Kushmerick, M. J., Dillon, P. F., Meyer, R. A., Brown, T. R., Krisander, J. M. & Sweeney, H. L. (1986) *J. Biol. Chem.* **261**, 14420–14429.