Intracellular pH and cell-to-cell transmission in sheep Purkinje fibers

Milton L. Pressler
Krannert Institute of Cardiology, Departments of Medicine and Physiology and Biophysics, Indiana University School of Medicine, Indianapolis, IN 46202; and Department of Physiology, University of Berne, Berne, Switzerland

ABSTRACT Intracellular pH (pH_i) is a significant modifier of cell-to-cell communication in some tissues but its role is uncertain in heart tissue. The present studies examined the effect of cytosolic protons on electrotonic spread and conduction velocity in cardiac Purkinje fibers. Cable analysis provided values for internal longitudinal resistance (r_i) and pH-selective microelectrodes monitored pH_i during CO_2 and HCO_3^- alterations. Resting fibers developed changes in r_i that were proportional to intracellular free proton concentration ($[H^+]_i$) during CO_2 changes at constant $[HCO_3^-]$. However, the effects on r_i were small between pH_i 6.9–7.8 and predicted only a 2.2% increase in r_i per 10 nM increase in $[H^+]_i$. Other findings suggested that titration of cytosolic protons may not directly produce the changes in r_i: (*a*) For an equal change in $[H^+]_i$, the effects on r_i were roughly three times greater (6.8% increase per 10 nM rise in $[H^+]_i$) if bicarbonate was lost during CO_2 changes. (*b*) pH-associated changes in r_i were preceded by a time delay (1–5 min) producing hysteresis in the $[H^+]_i$–r_i relation during successive perturbations. (*c*) The same CO_2 variations modified the direction and magnitude of r_i differently during pacing than at rest. The cumulative results suggest that the action of protons on r_i in the heart may be subordinate to another regulator or mediated by another pH-dependent substance or reaction.

INTRODUCTION

Cell-to-cell communication is affected by cytosolic protons and calcium ions (for reviews, see Loewenstein, 1981; Spray and Bennett, 1985) but the mechanism of action of these ions is still uncertain. It has been found that intercellular diffusion of charged molecules is impeded by fixed negative charges within junctional membranes (Flagg-Newton et al., 1979; Brink and Dewey, 1980). Consequently, modulation of intercellular transfer might occur by titration of these anionic groups by H^+ and/or Ca^{2+}. One means to evaulate the relative importance of H^+ and Ca^{2+} as regulators might be to directly compare the mass action of each ion on a functional property of intercellular transmission. With regard to protons, several investigators have reported effects on intercellular communication during changes in pH_i. Turin and Warner (1980) observed that CO_2-induced intracellular acidosis (pH_i 6.30) led to electrical uncoupling of *Xenopus* embryonic cells obtained from 32-cell up to early blastula stages. In cardiac Purkinje fibers, Reber and Weingart (1982) found that internal longitudinal resistance (r_i) increased during acidification and decreased during alkalinization. In cells from amphibian and teleost embryos, Spray et al. (1981) observed that junctional-membrane conductance (g_j) was directly proportional to pH_i. Subsequent findings by Spray et al (1982) implied that pH_i might be the predominant factor for physiological control of g_j (proton hypothesis) since g_j seemed much more sensitive to changes in pH_i than intracellular Ca^{2+}. However, in cardiac tissue, the relative role of H^+ and Ca^{2+} in regulating intercellular coupling has not been fully resolved. In paired cardiac cells, Noma and Tsuboi (1987) have found that g_j is more sensitive to changes in Ca^{2+} than H^+. Furthermore, there is recent evidence (Burt, 1987) that protons and Ca^{2+} may act synergistically to affect junctional permeability of cultured neonatal rat heart cells.

In this paper, the relationship between pH_i and r_i was studied in cardiac Purkinje fibers and found to vary with experimental conditions. Changes in r_i were investigated at rest and after pacing (i.e., during a pause between action potentials). In resting fibers, CO_2 alterations resulted in changes in r_i proportional to $[H^+]_i$. However, the effects on r_i were approximately threefold larger for the same changes in $[H^+]_i$ if bicarbonate also varied along with CO_2. Other experiments showed that repetitive activity modified the magnitude and even direction of the H^+-related changes in r_i. A time delay was observed between the onset of changes in pH_i and r_i. The results suggest that the effects of pH_i on r_i in the heart may be mediated indirectly or that protons are subordinate to another intracellular substance or enzymatic reaction. A preliminary report of these findings has been presented (Pressler, 1985).

Dr. Pressler's present address is Krannert Institute of Cardiology, Indiana University School of Medicine, 1001 West 10th Street, Indianapolis, IN 46202.

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METHODS

Purkinje fibers were dissected from sheep hearts obtained at a slaughterhouse. In some experiments, hearts of adult mongrel dogs were used after anesthesia with intravenous sodium secobarbital (30 mg/kg). Preparations were studied at room temperature (21–28°C) or at 36 ± 1°C. Composition of solutions and methods for pH–ion-selective microelectrode (pH-ISE) fabrication, calibration, and recording have been described (Pressler, 1988). Superfusates were adjusted to have equal Na^+, K^+, and Mg^{2+} concentrations and osmolality. A fraction of the calcium was inactive in HCO_3^--buffered Tyrode's solution: 17.9% at pH 8.85 (n = 3); 11.0% at pH 7.36 (n = 26); and 12.7% at pH 6.83 (n = 8). Consequently, a Ca-selective macroelectrode was used to equalize free extracellular calcium concentration at 2.0 ± 0.05 mM in Hepes-, Pipes-, and HCO_3^--buffered Tyrode's solutions.

Details of the cellular electrophysiological methods, theory and technique of unidimensional cable analysis, and the limitations of these techniques have been reported (Pressler et al., 1982; Pressler, 1984). Defined therein are the pertinent cable properties (e.g., space constant [λ], input resistance [R_{in}], membrane time constant [τ_m]) and the mathematical relations linking these properties to the intrinsic or unit constants (internal longitudinal resistance per unit length [r_i], membrane resistance times unit length [r_m], membrane capacitance per unit length [c_m]). Several factors seemed to enhance reliability and reduce spontaneous variation of r_i: (*a*) selecting unbranched strands without damaged or depolarized regions, (*b*) use of thin fibers (≤450 μm outer diameter) of long length (>3–4 λ), (*c*) maintenance of stable impalements, and (*d*) making repetitive measurements over a short interval. The reproducibility of r_i in normal Tyrode's was as follows: single measurements of r_i over 20–45 s varied ± 2.6% from the mean (n = 9); mean values of r_i measured 3–37 min apart varied ±2.7% (n = 7).

Cable analysis was done with three fixed microelectrodes (one current-injecting, two recording) rather than a "roving technique" so as to save time, preserve geometrical relationships, and minimize damage to the preparation from repeated impalements. Three-dimensional voltage decay was avoided by placing recording microelectrodes more than one fiber diameter from the current source. The validity of the method in resting and paced Purkinje fibers was tested previously (Pressler, 1984). Fig. 1 diagrams the electrode placements used to measure pH_i and either cable properties or interelectrode conduction velocity (θ). The method used to measure θ has been described (Pressler et al., 1982).

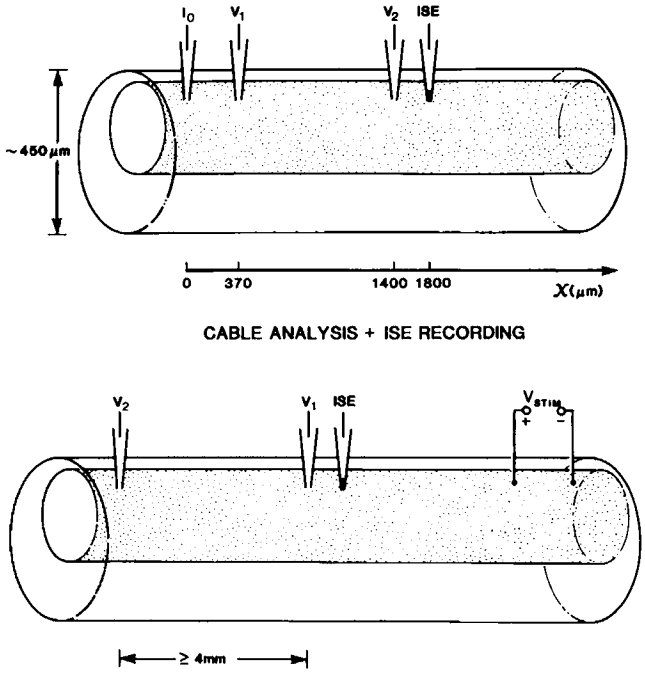

FIGURE 1 Diagram of microelectrode postions for cable analysis + ISE recording (*top*) or conduction velocity + ISE recording (*bottom*). Abbreviations: I_o, intracellular current-injecting microelectrode; V_1, V_2, voltage recording sites; ISE, ion-selective microelectrode recording site; x, distance from the point of current application (x = 0); V_{stim}, external stimulating electrode.

Data analysis

Measurements of r_i, r_m, and c_m were tabulated as a percentage of control values in HCO_3^--Tyrode's solution. For each point in the cable analysis experiments, three to eight electrotonic potentials were recorded at each of the two recording sites during a 20–40 s interval. The steady-state amplitude and time to reach 50% of this amplitude were measured from each electrotonic potential and the results averaged to determine a single point in the time course. Linear relations were fit by the method of least-squares. Statistical significance (defined as $P < 0.05$) between mean values was determined by applying Student's t test for paired or nonpaired data. For data with multiple "test" conditions, three-way analysis-of-variance (Snedecor and Cochran, 1967) was used to determine statistical significance and to analyze for interactions among two or three variables.

RESULTS

Activity-dependent effects of pH on cable properties

An initial set of nine experiments were performed in canine false tendons paced at 2 Hz (36°C; $[K^+]_o$ = 4 mM). Intracellular constant-current pulses were applied within diastole as previously described (Pressler, 1984). Addition or withdrawal of CO_2/HCO_3^- (by superfusion alternately with HCO_3^-- or Hepes-Tyrode's at a set pH) affected both the action potentials and electrotonic potentials of the cell bundle. Such effects were unrelated to the buffer substance (Hepes) per se. Fig. 2 shows an example of the effects during reintroduction of CO_2/HCO_3^- (which acidified the sarcoplasm). CO_2/HCO_3^- exposure shortened the action potential significantly. The transmembrane potential (V_m) and upstroke usually were little affected but depolarization of ~15–20 mV did occur in two cases during superfusion of Hepes-Tyrode's. Analysis of the amplitude and spatial decay of electrotonic potentials revealed a decrease in R_{in} and a consistent but variable increase in λ (no change occurred in τ_m) following exchange of Hepes-Tyrode's for CO_2/HCO_3^- Tyrode's (10% CO_2 [two experiments] 5% CO_2 [seven

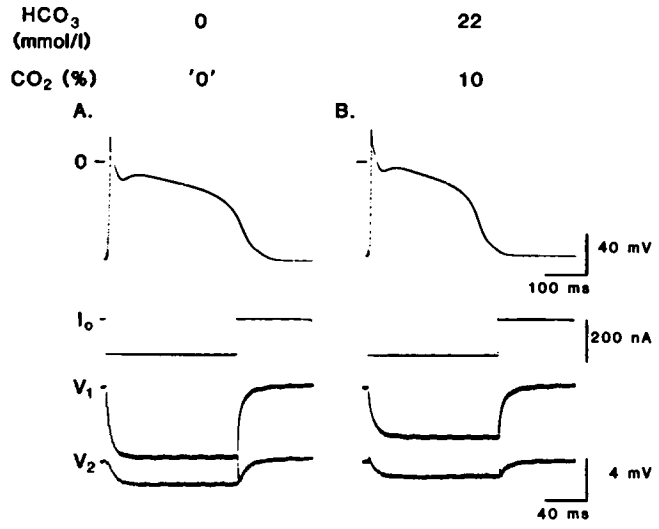

FIGURE 2. Effects of 10% CO_2 on electrical properties of a paced dog Purkinje fiber. Three microelectrode cable analysis in diastole. Each panel shows from top downwards: the action potential, current intensity (I_o), proximal electronic potential (V_1), and distal electrotonic potential (V_2). (*A*) Recordings in Hepes-Tyrode's solution gassed with 100% O_2; (*B*) recordings in HCO_3^- Tyrode's containing 90% O_2–10% CO_2. Action-potential duration shortened, input resistance decreased, and the space constant increased after 10% CO_2 exposure. The action-potential upstrokes were retouched.

experiments]). Table 1 summarizes the changes in passive electrical properties.

The effects on the cable constants were reversible and due to changes in r_i; there were no significant alterations of r_m or c_m (see Table 1). After 20–40 min superfusion with Hepes-Tyrode's, r_i had increased by a mean (±SD) of 52.8 ± 17.9% ($P < 0.001$) from control in 5% CO_2-Tyrode's. This was surprising from the standpoint of the proton hypothesis since loss of CO_2/HCO_3^- alkalinizes the cytoplasm and therefore might have been expected to decrease r_i. Consistent with the qualitative change in r_i was the finding that conduction velocity decreased after exposure to Hepes-Tyrode's (mean ± SD): 2.08 ± 0.24 m/s (5% CO_2-Tyrode's) vs. 1.95 ± 0.20 m/s (Hepes-Tyrode's; $n = 7$; $P < 0.01$ by paired t test). Intracellular pH was not measured during cable analysis in these preparations. Dog false tendons seemed poorly suited for such recordings since their contractility made it difficult to maintain multiple microelectrode impalements. The remainder of the experiments were performed in sheep Purkinje fibers. The robustness and weak contractility of sheep fibers allowed more precise and detailed studies.

The qualitative effects on cable properties described above in dog fibers were also observed in sheep: r_i increased during CO_2/HCO_3^- withdrawal in paced fibers. The aim of the experiments in sheep Purkinje fibers was to: (*a*) determine whether the alterations in r_i were primarily linked to changes in CO_2 or HCO_3^- and (*b*) assess whether the effects might be related to stimulation. Cable properties of the fibers were measured in 21.4 mM HCO_3^--Tyrode's and Hepes-Tyrode's at 36°C. Both solutions had the same extracellular pH (pH_o) but different CO_2 and HCO_3^- contents. It was too complicated to test multiple gradients of CO_2 and HCO_3^- under paced and resting conditions so only changes in the CO_2 gradients were explored. The results were grouped by the amount of difference in CO_2 (0%, 5%, 15%) between Hepes- and HCO_3^--Tyrode's. A total of 18 fibers were studied: 27 experiments were done during pacing at 2 Hz and 14 experiments at rest. In nine of the fibers, it was possible to maintain the impalements long enough to perform cable analysis both at rest and during pacing.

Control values of the cable constants in 5% CO_2-Tyrode's are shown in Table 2 and were similar to previous normal values (Weidmann, 1952; Pressler, 1984). An equilibration period (30–50 min) of rest or constant pacing preceded experimental interventions so that activity-related changes in r_m (Pressler, 1984) would reach steady state. Experiments were conducted without

TABLE 1 Electrical properties of dog false tendons in diastole

	HCO_3^--Tyrode's ($n = 7$)				Hepes-Tyrode's ($n = 7$)			
	V_m	λ	R_{in}	τ_m	V_m	λ	R_{in}	τ_m
	mV	*mm*	*kΩ*	*ms*	*mV*	*mm*	*kΩ*	*ms*
Mean	−85	2.08	153	19.3	−77*	1.68	201‡	17.9
SE	±1	±0.44	±23	±7.0	±4	±0.28	±33	±4.9
	r_i		r_m	c_m	r_i		r_m	c_m
	MΩ/cm		*kΩ · cm*	*nF/cm*	*MΩ/cm*		*kΩ · cm*	*nF/cm*
Mean	0.91		36.4	477	1.42‡		35.6	514
SE	±0.18		±11.3	±46	±0.29		±9.0	±87

HCO_3^--Tyrode's solution contained 22 mM HCO_3^- equilibrated with 95% O_2–5% CO_2; Hepes-Tyrode's contained 3 or 6 mM Hepes gassed with 100% O_2. Abbreviations: V_m, transmembrane potential; λ, space constant; R_{in}, input resistance; τ_m, membrane time constant; r_i, internal longitudinal resistance per unit length; r_m, membrane resistance times unit length; c_m, membrane capacitance per unit length; *$P < 0.05$; ‡$P < 0.01$.

TABLE 2 Electrical properties of sheep Purkinje fibers

	Resting ($n = 9$)				Paced 2 Hz ($n = 14$)			
	V_m	λ	R_{in}	τ_m	V_m	λ	R_{in}	τ_m
	mV	*mm*	*kΩ*	*ms*	*mV*	*mm*	*kΩ*	*ms*
Mean	−77	1.95	358	20.0	−82*	1.48	200‡	11.2‡
SE	±2	±0.22	±38	±2.7	±1	±0.13	±23	±1.3

Tyrode contained 21.4 mM HCO_3^- equilibrated with 95% O_2 – 5% CO_2; temperature 36°C. For abbreviations see Table 1.

directional bias but results were tabulated as percent changes from the values in HCO_3^--Tyrode's. During transition between HCO_3^-- and Hepes-Tyrode's, small changes in V_m (1–5 mV) were often observed but were not statistically significant. Most measurements were confined to two periods after solution exchange: 10–25 min (peak effects on r_i); and 40–60 min (quasi-steady state). Superfusion with Hepes-Tyrode's induced changes in λ, R_{in}, and τ_m that depended on (*a*) the amount of CO_2 loss, (*b*) time after solution exchange, and (*c*) the presence or absence of pacing during the experiment. Almost all of the effects of CO_2 on the cable constants could be explained by changes in r_i and were activity-dependent ($P < 0.001$, rest vs. pacing; see Fig. 3). There were no statistically significant effects of CO_2/HCO_3^- on r_m. Fig. 3, *A* and *B*, shows that the change in r_i varied with the amount of CO_2 (not HCO_3^-) lost during the solution transition. The difference in external HCO_3^- was constant between groups and yet the change in r_i grew progressively larger as the CO_2 gradient between HCO_3^- and Hepes-Tyrode's increased.

The direction of CO_2-induced changes in r_i was opposite in quiescent and active states. Fig. 3 *A* shows that r_i increased in paced fibers during loss of CO_2/HCO_3^- but decreased at rest (Fig. 3 *B*). In addition, the CO_2-dependent changes in r_i seemed to dissipate over time in resting fibers (Fig. 3, *B* vs *D*) but were maintained during pacing (Fig. 3, *A* and *C*). Two additional experiments supported the conclusion that changes in r_i depended upon activity. Cable properties were measured during NH_4Cl exposure in paced (2 Hz) then resting sheep Purkinje fibers. The effects of NH_4Cl on r_i were different during pacing than after an hour of quiescence. However, the activity-dependent changes in r_i seemed to be confined to the period of exposure to NH_4Cl (intracellular alkalinization). During washout of NH_4Cl (intracellular acidification), r_i increased in both resting and paced fibers.

Relation between changes in cable properties and pH_i

In retrospect, some loss of CO_2 occurred upon warming HCO_3^--Tyrode's in the perfusion system. This would not have altered the qualitative differences shown in Fig. 3 since the same solutions were used for both paced and resting fibers. However, the quantitative effects of CO_2 on r_i might have been damped. To reduce CO_2 losses, the rest of the experiments were done in an improved perfusion system with thick-walled Teflon tubing and faster flow rates. In addition, a pH-selective microelectrode was used

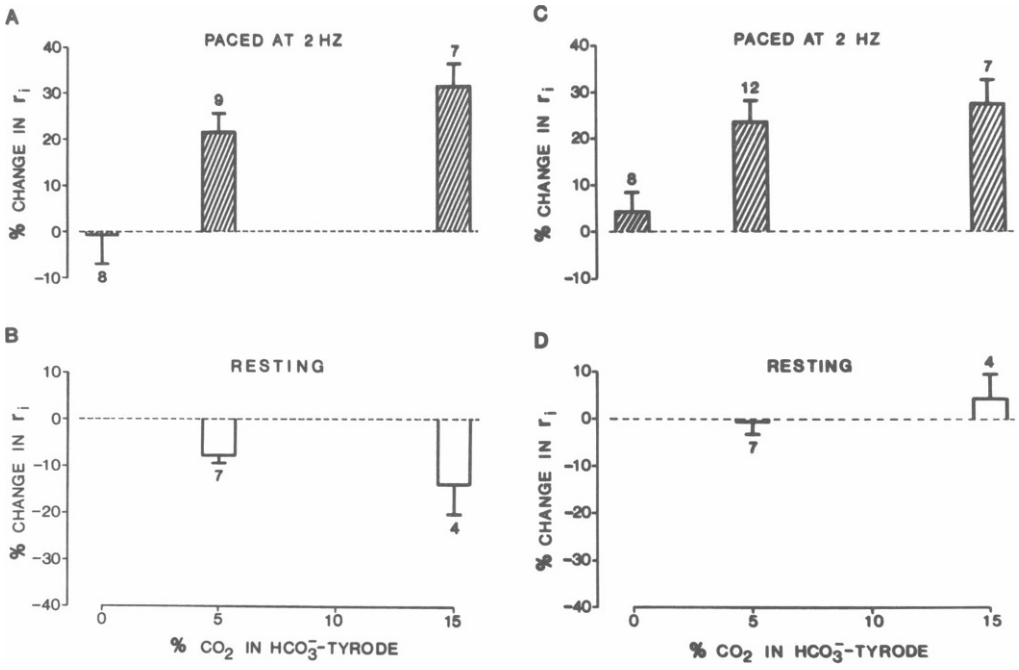

FIGURE 3 Effects of CO_2 removal on internal longitudinal resistance (r_i) in (*A*, *C*) paced and (*B*, *D*) resting sheep Purkinje fibers. Abscissa: percent CO_2 in 21.4 mM HCO_3^--Tyrode's; ordinate: r_i measured in Hepes-Tyrode's expressed as a percent change from HCO_3^--Tyrode's. (A and B) Peak effects 10–20 min after solution exchange; (*C* and *D*) quasi-steady-state changes at 40–60 min. The magnitude of effect on r_i depended upon the amount of CO_2 loss and on the presence of excitation. CO_2-induced changes in r_i during pacing were significantly different than at rest ($P < 0.001$). Bars depict means ± SE; the number of experiments is shown above each bar.

to monitor pH_i so that the time courses of changes in pH_i and r_i could be directly compared. Studies were performed in sheep Purkinje fibers at room temperature and confined to the period 10–20 min after solution exchange. The fibers were not voltage-clamped but variation in V_m throughout an experiment was small (-69 ± 1.8 mV standard deviation). Fig. 4 shows the effects of CO_2 alterations at constant $[HCO_3^-]$. Raising CO_2 from 5% to 15% produced a reversible 0.33 decrease in pH_i that reached a quasi-steady state after ~10 min; pH_i returned to control after 16–17 min in 5% CO_2-Tyrode's. As exemplified by Fig. 4, the fibers usually depolarized by a few millivolts, λ decreased and R_{in} increased during intracellular acidification. Similar findings were obtained in five other experiments. Alterations in CO_2 did not significantly alter τ_m.

Changes in r_i accounted for the significant changes in cable properties produced by CO_2. Fig. 5 depicts the analysis of the recordings illustrated in Fig. 4: r_i increased during intracellular acidification and decreased back to its initial value (within 2–4%) during pH_i recovery. However, the onset of changes in r_i was delayed compared to the rapid effects of CO_2 on pH_i (~10–15 s). Fig. 5 shows a particularly long latency of ~5 min prior to changes in r_i. In this experiment, almost 90% of the steady-state change in proton concentration had occurred before r_i was affected. A time lag of variable duration preceded the pH_i-related changes in r_i in each of six experiments during the first alteration of pH_i. During subsequent perturbations of pH_i, the latent period appeared to diminish. Fig. 6 demonstrates that CO_2 could both increase and decrease r_i. As pH_i increased from 7.33 to 7.68, r_i gradually decreased by 7.1%. When pH_i was lowered to 6.89, r_i increased to a value 20.4% greater than control. Hence, a 0.44-unit intracellular acidification (relative to control) resulted in a 2.9-fold greater change in r_i than a 0.35-unit alkalinization. In this fiber, the latency between changes in pH_i and r_i was shorter than before (1–2) min) but again seemed to decrease in duration as pH_i was repeatedly altered. There was no relation between the length of the delay and preparation diameter. In fact, several of the largest fibers (those in

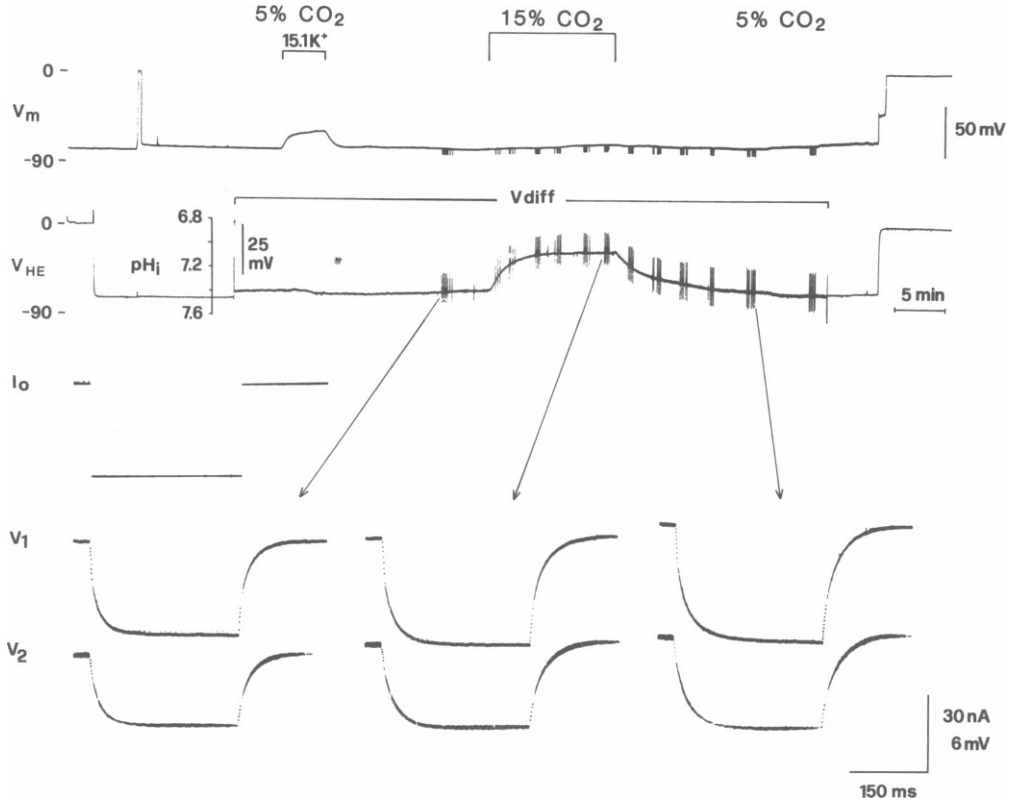

FIGURE 4 Cable analysis during pH_i changes in a resting sheep Purkinje fiber. Abbreviations: transmembrane potential (V_m); potential from a pH microelectrode (V_{HE}); for other abbreviations see Fig. 2. After obtaining stable recordings, V_m was subtracted electronically from V_{HE} ($-V_{diff}$) and the amplification was doubled. The brief transients locate the times of intracellular current injection. Intracellular pH decreased from 7.40 (5% CO_2) to 7.07 after 12 min in 15% CO_2-Tyrode's. Changes in V_1 and V_2 during intracellular acidification are analyzed in Fig. 5. Voltage and time scales as marked; I_o = 36 nA; 24.5°C.

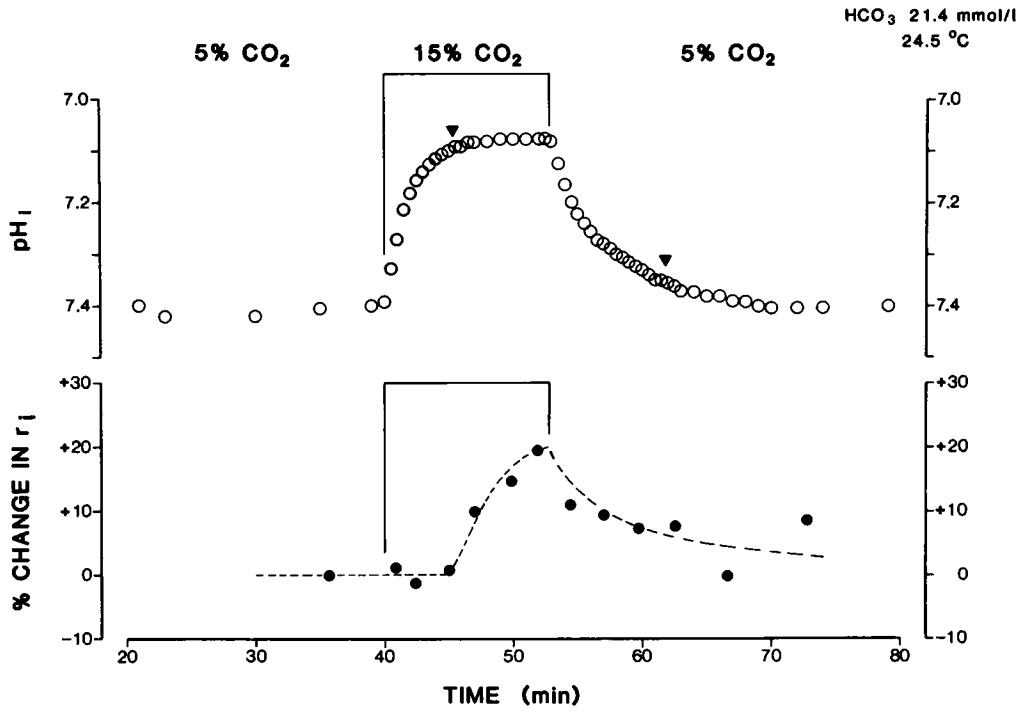

FIGURE 5 Time course of CO_2-induced changes in intracellular pH (pH_i) and internal longitudinal resistance (r_i). Analysis of the experiment shown in Fig. 4. After solution exchange, pH_i began to decrease within 10 s and reached 90% of the steady-state value of $[H^+]_i$ after ~7 min (*arrowheads*). A reversible increase in r_i occurred during intracellular acidification. However, a latent interval of ~5 min was observed before the initial change in r_i.

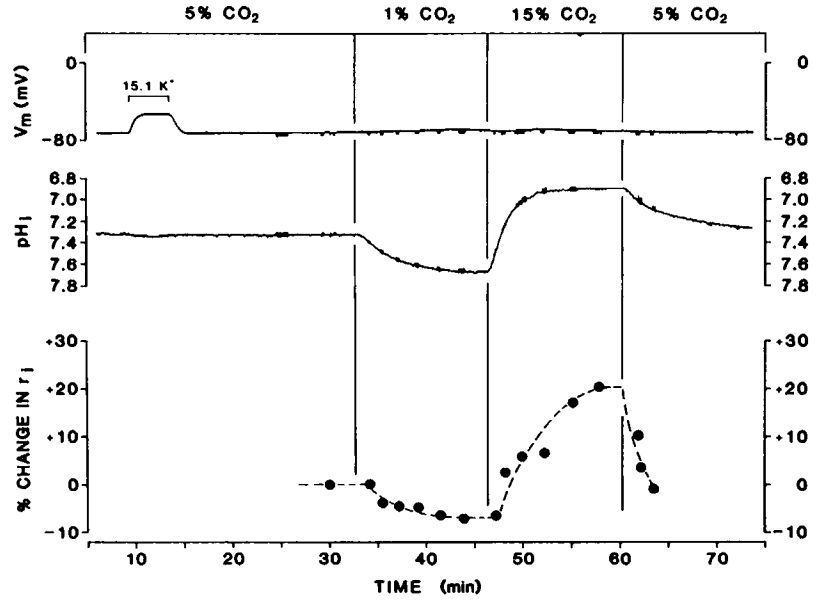

FIGURE 6 Effects of CO_2 on pH_i and internal longitudinal resistance in a resting sheep Purkinje fiber. Larger alterations in r_i developed during acidification than alkalinization. A time delay was noted from the onset of change in pH_i to the initial change in r_i (1.5 and 0.9 min for the first two solution exchanges, respectively). Each value of r_i represents the mean of several measurements. For abbreviations see Figs. 4 and 5.

which diffusion of CO_2 might have required the most time) had the shortest latency.

The delay between changes in pH_i and r_i could not be quantified precisely since r_i was not measured continuously. The precision to which latency could be measured was ±0.5–1 min or roughly the time required to record a set of three to five electrotonic potentials. However, the borders of the delay could be delineated by the points before (lower boundary) and after (upper boundary) r_i changed >2% from its prior value. In six experiments, the lower bound to the latency averaged 2.5 min initially and decreased to 0.6 min during the second alteration of r_i. The corresponding upper limits to the delays during successive perturbations were 4.7 and 2.2 min on average, respectively. During the first latent period, $[H^+]_i$ changed by a mean of 28–37 nM (lower and upper bounds) before a significant effect on r_i was detectable. This result meant that roughly 56–75% of the proton gain or loss occurred before r_i initially began to change.

From six experiments in five resting sheep Purkinje fibers, the mean (±SD) pH_i values 10–20 min after CO_2 alteration were: 7.72 ± 0.05 (n = 2), 1% CO_2; 7.39 ± 0.03 (n = 6), 5% CO_2 (control); 7.42 ± 0.05 (n = 5), 5% CO_2 (recovery); 7.02 ± 0.10 (n = 6), 15% CO_2. Respective values of $[H^+]_i$ from the same measurements were: 24 ± 3 nM, 1% CO_2; 49 ± 5 nM, 5% CO_2 (control + recovery); 121 ± 28 nM, 15% CO_2. r_i (mean ± SD) decreased to 92.5 ± 0.6% ($P < 0.05$) of its value in 5% CO_2-Tyrode's during alkalinization in 1% CO_2-Tyrode's. Conversely, r_i increased to 117.1 ± 4.1% of control after transition to 15% CO_2-Tyrode's ($P < 0.001$). There was no significant difference between initial and final values of r_i during control and recovery periods (100% vs. 98.8 ± 5.3%). At a constant $[HCO_3^-]$, the maximum variation in r_i over the pH_i interval 6.9–7.8 was ~25–30%. The effects on r_i became larger as pH_i decreased below 7.0–7.1. The relation between r_i and pH_i seemed best described by a shallow curve which in turn could be explained by the fact that pH_i is logarithmic. As shown below in Fig. 8, the relation between r_i and $[H^+]_i$ was linear (r = 0.926; $P < 0.001$).

pH_i-related changes in r_i at constant pH_o

Extracellular pH varied during the above experiments and it seemed important to test whether the same r_i–pH_i relation was obtained when pH_o was held constant. Fig. 7 *A* shows one experiment of this type in which pH_i and r_i were measured simultaneously during CO_2/HCO_3^- alterations (resting fibers). The changes in r_i were monotonic and symmetrical to the alterations in pH_i. Once again a delay was noted between the onset of changes in pH_i and r_i. The lag between changes in these two parameters was more obvious when r_i was plotted as a function of $[H^+]_i$ (Fig. 7 *B*). During transitional periods, significant hysteresis in the time course developed because r_i was constant during changes in $[H^+]_i$. In general, the extent of hysteresis shown in Fig. 7 *B* ($[HCO_3^-]$ loss) was comparable to other results in which $[HCO_3^-]$ was constant (e.g., Fig. 5). The major difference between the two cases was that the decrease in r_i during intracellular alkalinization in Pipes-Tyrode's was much larger than expected from similar pH_i changes when $[HCO_3^-]$ was constant. In two experiments, the mean ± SD decrease in $[H^+]_i$ during exposure to Pipes-Tyrode's was 69 ± 6 nM (mean pH_i increased from 7.13 to 7.76). There was an average decrease in r_i of 43.0 ± 3.4% during this drop in proton concentration. In contrast, the data reported in the previous section ($[HCO_3^-]$ constant) would have predicted only a 15% decrease in r_i, a 2.9-fold smaller effect.

Fig. 8 summarizes the r_i–$[H^+]_i$ data obtained in resting fibers. The values of r_i were those measured at the steady pH_i reached 10–20 min after a change in CO_2. The slope of the line relating r_i and $[H^+]_i$ at constant $[HCO_3^-]$ predicted a 2.2% increase in r_i for every 10 nM increase in $[H^+]_i$. Although based on fewer results, Fig. 8 shows that the r_i–$[H^+]_i$ relation was much steeper during $[HCO_3^-]$ loss (6.8% increase in r_i for every 10 nM increase in $[H^+]_i$).

Relation between pH_i and changes in conduction velocity

Speed of action-potential propagation was measured during short-term changes in CO_2/HCO_3^- to add perspective to the pH_i-associated changes in r_i. Admittedly, other electrical parameters affect conduction velocity, but several of these factors, e.g., membrane capacitance, action-potential amplitude, and upstroke velocity, were affected little by the above alterations in CO_2 content. Therefore, it seemed appropriate to evaluate whether CO_2-related effects on r_i alone could predict part or all of the changes in θ.

Experiments were conducted in sheep Purkinje fibers at 22.9 ± 0.9°C constantly paced (1–2 Hz) except for 4–5-s pauses to measure pH_i. The same changes in CO_2/HCO_3^- used to determine the r_i–pH_i relation were now used to study the relation between θ and pH_i. As shown in Fig. 9, there were several consistent findings: (*a*) CO_2-induced changes in θ (at constant extracellular $[HCO_3^-]$) were monophasic, reached a steady value within 10–20 min and were predictable. Intracellular acidification resulted in a decrease in θ, and intracellular alkalinization, an increase in θ. Larger changes in θ were observed upon acidification than alkalinization. Pooling

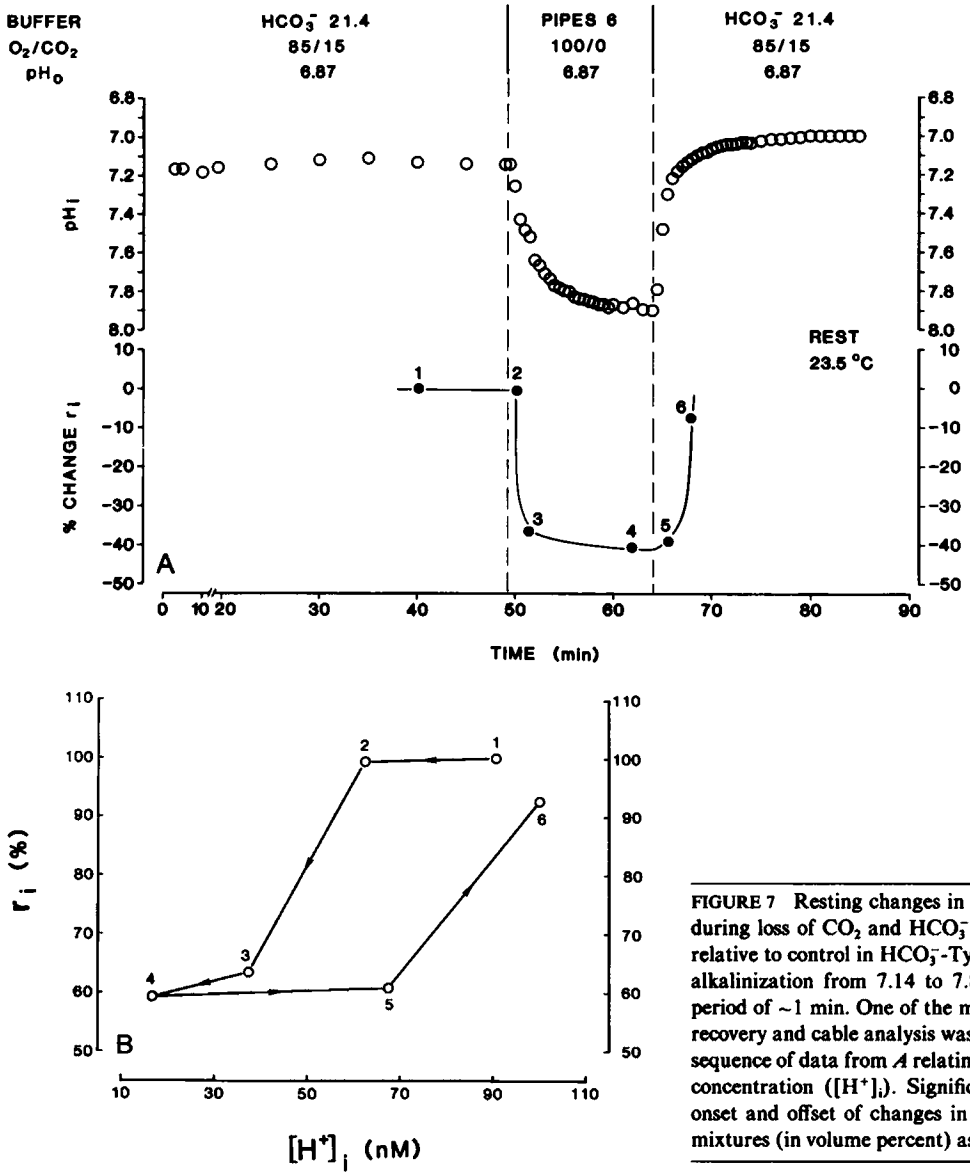

FIGURE 7 Resting changes in pH_i and internal longitudinal resistance during loss of CO_2 and HCO_3^- (constant pH_o). Values of r_i are shown relative to control in HCO_3^--Tyrode's (100%). (*A*) During intracellular alkalinization from 7.14 to 7.88, r_i decreased by 40% after a latent period of ~1 min. One of the microelectrodes became dislodged during recovery and cable analysis was terminated after point 6. (*B*) Temporal sequence of data from *A* relating effects on r_i to intracellular free proton concentration ($[H^+]_i$). Significant hysteresis was evident during the onset and offset of changes in r_i. Buffers (in millimolar) and gaseous mixtures (in volume percent) as marked.

measurements from other fibers, θ was 13.4 ± 6.3% smaller in 15% CO_2-Tyrode's than 5% CO_2-Tyrode's (n = 5); conversely, θ in 1% CO_2-Tyrode's was 2.6 ± 0.4% greater than control (n = 2). (*b*) Assuming constancy of c_m and τ_{ap}, the measured values of θ followed the direction and approximate magnitude of the behavior predicted by the equation $\theta^2 = [c_m \cdot r_i \cdot \tau_{ap}]^{-1}$, where τ_{ap} denotes the time constant of potential rise of the action-potential foot (Tasaki and Hagiwara, 1957). The broken line in Fig. 9 depicts the theoretical effects on θ computed from the steady-state changes in r_i obtained during alterations of pH_i (solid line relation in Fig. 8). The correspondence between observed and predicted values of θ was reasonably close but did not take into account any latency for pH_i-related changes in r_i.

The interrelationship between pH_i, r_i, and θ was even more complex when pH_i was altered by a different means. Under conditions of constant pH_o but changing CO_2 and $[HCO_3^-]$, θ continued to vary with time even as pH_i approached a steady value (see Fig. 10). The hysteresis in the pH_i–θ relation contrasted with the parallel time courses of pH_i and θ observed when $[HCO_3^-]$ was constant (e.g., Fig. 9). Disparity in the pH_i–θ relationship developed because θ differed when pH_i was increasing vs when pH_i was decreasing. For example (Fig. 10), at pH_i 7.5 on withdrawal of CO_2/HCO_3^-, θ was ~1.6 m/s; at pH_i 7.5 on reexposure to CO_2/HCO_3^-, θ was ~1.4 m/s. The time course of resting changes in r_i (Fig. 7) did not explain the hysteresis of θ shown in Fig. 10. Unfortunately, no simultaneous measurements of r_i and pH_i could be performed during pacing to examine whether a different succession of events occurred during activity.

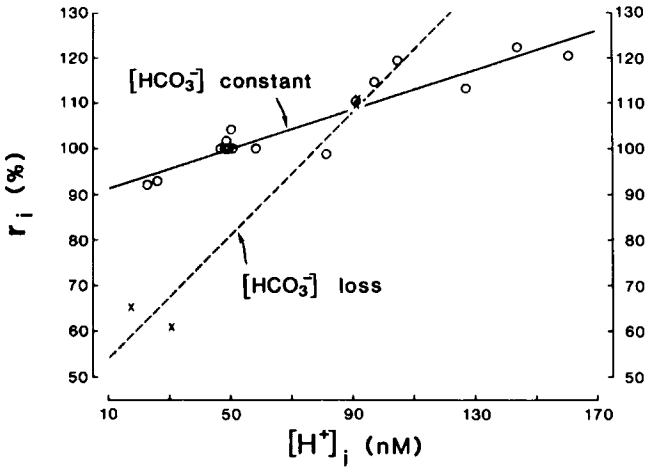

FIGURE 8 Summary of relationship between internal longitudinal resistance and $[H^+]_i$ during CO_2 changes in resting Purkinje fibers. Values of r_i observed when $[H^+]_i$ had reached a steady state are shown as a percent of control in 5% CO_2-Tyrode's (=100%). Symbols: (O) constant extracellular $[HCO_3^-]$; (X) loss of HCO_3^- during transition into Pipes Tyrode's solution. A linear relation was observed between r_i and $[H^+]_i$ but the steepness of the relation varied with buffer conditions. Linear regression was used to draw best-fit lines.

DISCUSSION

Relation between protons and internal longitudinal resistance in heart

In embryonic cells of amphibians, protons appear to be a principal regulator of junctional membrane conductance (Spray et al., 1981). It is less certain how applicable the "proton hypothesis" is in cardiac cells. The present work was undertaken to further examine the relation between H^+ and regulation of intercellular communication in Purkinje fibers. Internal longitudinal resistance was measured during changes in pH_i induced by transient alteration of CO_2 and HCO_3^-. The results confirmed the findings of Reber and Weingart (1982) that changes in pH_i are associated with small and reversible changes in r_i. Resting Purkinje fibers subjected to changes in CO_2 developed steady-state changes in r_i that were proportional to $[H^+]_i$ (see Fig. 8). However, other findings suggested that protons may compete with other regulatory processes to modify r_i in cardiac tissue. To date, the

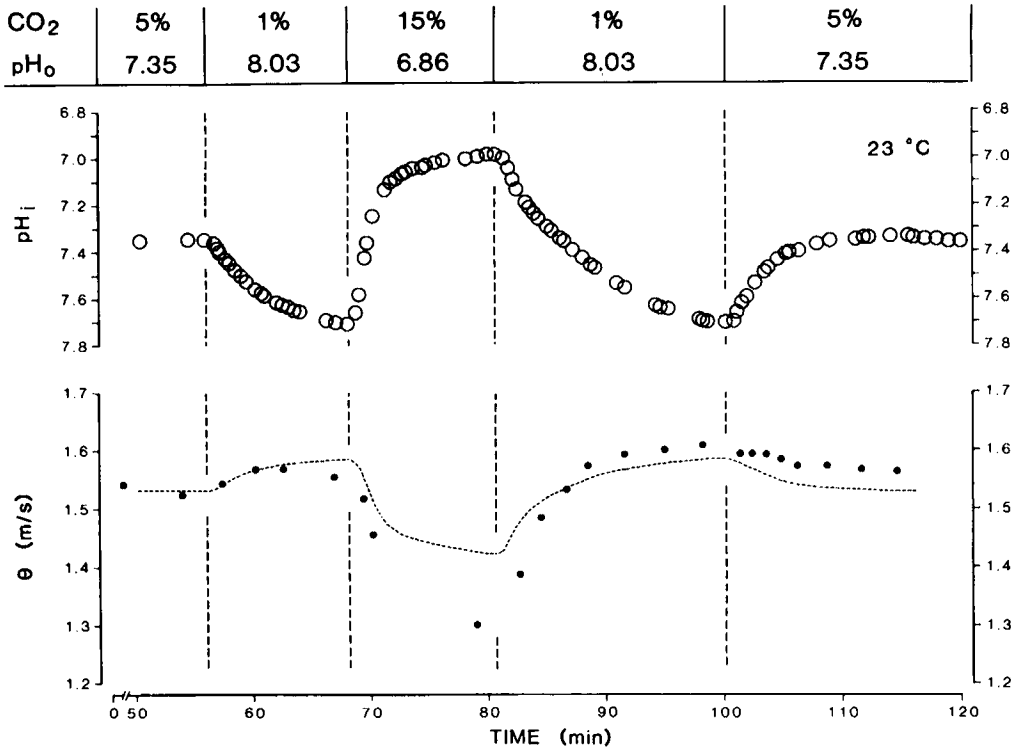

FIGURE 9 Relation between intracellular pH and interelectrode conduction velocity (θ) during CO_2 alterations in a sheep Purkinje fiber. Extracellular $[HCO_3^-]$ was constant at 21.4 mM. CO_2-induced changes in θ were monophasic and followed the changes in pH_i. The broken line shows the calculated effects on θ expected from resting changes in r_i alone. The expected changes in r_i were obtained from the solid line, $[H^+]_i$–r_i relationship shown in Fig. 8. pH_i was measured during 4–5-s pauses in the pacing train.

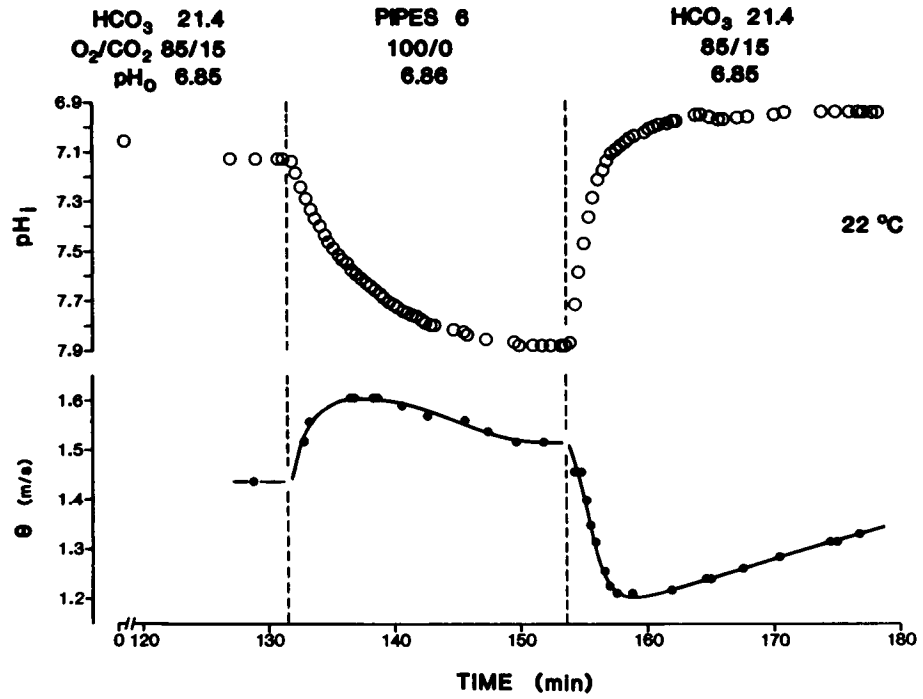

FIGURE 10 Changes in intracellular pH and interelectrode conduction velocity during combined CO_2/HCO_3^- withdrawal. pH_i was measured during intermittent 4–5-s pauses in pacing. Conduction velocity initially increased during intracellular alkalinization but the peak effects were not sustained. Hysteresis in the pH_i-θ relationship occurred upon return to control conditions. The time course of changes in θ would not be predicted from the pH_i-related effects on r_i shown in Fig. 7. For abbreviations, see Fig. 9.

exact site and mechanism of action of H^+ have not been determined.

It is widely believed that protons affect intercellular transmission by altering some intracellular constituent rather than an external component of the surface membrane (Spray et al., 1981). The present results support this conclusion since changes in r_i occurred even under conditions where variations in extracellular pH were small or negligible (Fig. 7). Furthermore, pH-related swelling or shrinking of extracellular clefts probably did not produce the effects on r_i. Clefts do not modify the calculation of r_i from cable properties in a model that accounts for the complex structure of Purkinje fibers (Levin and Fozzard, 1981). However, it is open to interpretation which intracellular component(s) of r_i was most sensitive to changes in pH_i. Intracellular pH may alter both myoplasmic resistance (r_{myo}) and junctional membrane resistance (r_j) and the role of each cannot be separated by cable analysis. Given this caveat, there is reason to suspect that pH-related changes in r_{myo} were subordinate to those of r_j. Myoplasmic resistivity in barnacle muscle fibers increases only 1.2% with pH changes from 7.2 to 6.1 (Caillé, 1975). In addition, alterations in cell volume affecting r_{myo} were probably minor over the range of pH_i reported in this paper. Data from skeletal muscle fibers exposed to 15% CO_2 (Huguenin et al., 1980) would predict only a 5% gain in cell water from the measured fall in intracellular K^+. Recent observations support the crucial role of cell junctions in the regulation of intercellular transmission. Warner et al. (1984) and Hertzberg et al. (1985) have shown that microinjection of antibodies to gap-junctional protein interrupts electrical transmission and intercellular dye transfer. Consequently, it seems logical to conclude that most of the pH_i-related changes in r_i reflected the pH-dependence of r_j.

The above statements concerning pH-dependence should not be misconstrued as suggesting that protons alone directly regulate r_i in the heart. Several findings in the present study seem to conflict with a simple proton hypothesis: (*a*) Changes in r_i were activity-dependent (Fig. 3) even though the imposed changes in pH_i were virtually the same in resting and paced fibers (Pressler, unpublished observations). (*b*) Hysteresis in the time course of changes in $[H^+]_i$ and r_i (see Fig. 7) resulted in a highly variable relation between the two parameters. (*c*) The magnitude of pH_i-associated changes in r_i depended on the method used to change pH_i (Fig. 8). Alterations in

r_i were approximately threefold larger for the same pH_i range when HCO_3^- also was lost from the medium than when $[HCO_3^-]$ was constant. These findings, albeit indirect, cumulatively suggest that the effects of H^+ on r_i are subsidiary to another regulator or are mediated indirectly via some interposed substance, reaction, or combination thereof.

One regulatory process that could confer pH dependence is phosphorylation of junctional proteins by protein kinases. cAMP-dependent protein kinase is one possible candidate. Junctional permeability and degree of cell-to-cell coupling have been correlated with intracellular levels of cAMP in cultured mammalian cells (Flagg-Newton et al., 1981) and heart tissue (De Mello, 1984). Recently, Saez et al. (1986) have found that membrane-permeant cAMP derivatives increased junctional-membrane conductance of voltage-clamped pairs of rat hepatocytes. In the same paper, Saez et al. (1986) demonstrated incorporation of ^{32}P into the 27-kD gap-junctional polypeptide when small groups of hepatocytes were incubated with ^{32}P. Whether a similar mechanism might pertain to cardiac cells is currently a focus of our investigation. Preliminary results (Pressler and Hathaway, 1987) suggest that cAMP-dependent protein kinase phosphorylated a 47-kD protein of dog heart gap junctions. Ca^{2+}/calmodulin (CaM)-dependent protein kinase also has been proposed as a regulator of r_j. Some evidence is available that inhibitors of CaM modify cell-to-cell coupling (Peracchia, 1984). However, it seems less likely that CaM mediates pH-dependent changes in either r_i or r_j since the Ca^{2+}-affinity of CaM is virtually independent of pH between 5.5–7.5 (Haiech et al., 1981). One reason to favor the involvement of an enzymatic process in the regulation of r_i is because enzymes require time for activation. This might explain the hysteresis and delay between changes in pH_i and changes in r_i. In fact, the decreasing latency accompanying successive perturbations of pH_i would be more consistent with the activation of a cellular enzyme than a diffusional process. The failure of previous workers (Reber and Weingart, 1982) to identify a time lag may have been a consequence of higher temperatures at which the measurements were performed (35°C). If such an enzymatic event were temperature-dependent, the present experimental conditions (ca. 24°C) may have facilitated the detection of a latent period.

Activity-dependence of r_i

An interesting characteristic of the mechanism modifying r_i in cardiac Purkinje fibers was its activity dependence. Under some experimental conditions (CO_2 and HCO_3^- loss, NH_4Cl exposure), pH-related changes in r_i were different at rest than during pacing. This result has been discussed above as evidence favoring the indirect action of H^+ to modify conductance of the junctional membrane. The exact mechanism for the effects of activity on r_i remains uncertain. A previous paper (Pressler, 1984) reported no significant changes in r_i consequent to pacing per se (0–2 Hz) and yet Fig. 3 shows that activity clearly modified the effects of CO_2 on r_i. Other studies (Pressler, 1988) reveal activity-dependent changes in $[H^+]_i$ that per se would be too small to significantly alter r_i. It bears mentioning that the activity-dependent effects on r_i were observed during marked alkalinization of the cytosol. Consequently, changes in intracellular Na^+ (Cohen et al., 1982) and free calcium concentration ($[Ca^{2+}]_i$) (Lado et al., 1982) that occur during stimulation might have affected r_i when $[H^+]_i$ was small. In particular, it is tempting to speculate that the activity-dependent effects on r_i were directly or indirectly linked to changes in Ca^{2+}. Intracellular alkalinization is known to increase Ca^{2+} influx through Ca channels (Kurachi, 1982) and also enhances Ca^{2+} release from the sarcoplasmic reticulum (Fabiato, 1985). An increase in $[Ca^{2+}]_i$ would be in the right direction to explain the increase in r_i observed in paced fibers during CO_2 loss (see Fig. 3).

The findings in this paper suggest that the mass action of H^+ alone cannot explain the behavior of r_i during cardiac activity. Since there is an interdependence between cytosolic H^+ and Ca^{2+} (Hess and Weingart, 1980; Vaughan-Jones et al., 1983), it seems logical to query the role of Ca^{2+}. In cardiac fibers, elevation of $[Ca^{2+}]_i$ to micromolar levels increases r_i (Dahl and Isenberg, 1980), and Ca^{2+} alters the ultrastructural appearance of gap junctional particles (Dahl and Isenberg, 1980; Shibata and Page, 1981). Noma and Tsuboi (1987) and Veenstra and DeHaan (1987) have observed significant Ca^{2+} dependence of cardiac r_j at physiological $[Ca^{2+}]_i$ and pH_i. However, other evidence suggests a lesser role for Ca^{2+} in regulation of gap-junctional resistance. Spray et al. (1982) observed that r_j was insensitive to $[Ca^{2+}]_i$ with half-maximal changes in r_j around 0.1 mM. Dye coupling between cultured neonatal rat myocardial cells also was found to be insensitive to elevation of $[Ca^{2+}]_i$ alone (Burt, 1987). Furthermore, Maurer and Weingart (1987) have reported that $[Ca^{2+}]_i$ had to be elevated to the point of a sustained contracture in order to substantially alter r_j of pairs of rat or guinea pig myocytes. Part of the controversy stems from trying to independently test the effects of H^+ and Ca^{2+}; those agents which alter pH_i also affect $[Ca^{2+}]_i$ and vice versa. Till proven otherwise, it seems justified to continue to consider that Ca ions or a Ca^{2+}-dependent enzyme may play a role in regulating junctional transmission in the heart. If a Ca^{2+}-dependent enzyme was a component of the regulatory process, it

might also account for the findings of latency and activity dependence noted in the present work.

Significance of r_i to conduction

Acidosis is known to slow the conduction of action potentials in cardiac tissue (Marrannes et al., 1981; Kagiyama et al., 1982). Alterations in conduction velocity may develop by one of several mechanisms: (*a*) changes in density of excitatory current generated during the action potential, (*b*) alterations in excitation threshold, (*c*) variation of the volume or geometry of the conductor, and/or (*d*) changes in the passive elements of the biological circuit (c_m, r_i, r_o, where r_o = external longitudinal resistance per unit length). Of these factors, the present study has focused on the pH-related changes in the passive-circuit elements, especially r_i. One question that arises is "What is the functional significance of the changes in r_i to conduction?" Cable theory would predict (Tasaki and Hagiwara, 1957; Walton and Fozzard, 1983) that alterations in θ vary inversely with $\sqrt{r_i}$ if all other passive elements remain constant. As shown in Fig. 9, the observed changes in θ produced by alterations in CO_2 content ($[HCO_3^-]$ constant) correlated reasonably well with changes in θ predicted to occur from alterations in r_i alone. There was good agreement over the pH_i range 7.2–7.8 and all directional changes in θ were anticipated. However, slowing of conduction was greater than expected during marked intracellular acidification and no latency was apparent between changes in pH_i and θ. Furthermore, worse concordance between measured and theoretical changes in θ was found under other experimental conditions. Hysteresis was observed between pH_i and θ when pH_i was changed by transient exposure to Pipes-Tyrode's solution (Fig. 10). This hysteresis could not be accounted for by the time course of pH_i-related changes in r_i in resting fibers (Fig. 7). Other experiments (Pressler, unpublished observations) have shown no substantive difference in the amount of change in pH_i when resting and paced fibers were challenged with an acid load. Hence, the question is unanswered as to what factor(s) account for the varying relation between pH_i and θ. It is possible that some of the changes in θ resulted from transient changes in surface pH (Marrannes et al., 1981) that affected excitatory current intensity or threshold. However, it is also conceivable that part of the discrepancies between changes in pH_i, θ and r_i (Figs. 8 and 10) might have stemmed from analyzing an active property like θ based on measurements of r_i in quiescent fibers. In paced fibers, it seems plausible that the time course of r_i during pH_i changes might be very different from that observed at rest. Whatever the explanation for the hysteresis, the activity dependence of r_i and the puzzling changes in θ both suggest that regulatory processes characterized at rest may not pertain to behavior under active conditions.

In conclusion, the results of this investigation have shown that the pH_i-associated changes in r_i are preceded by a time delay and vary with activity. Furthermore, the relation between pH_i and r_i was affected by experimental conditions despite equivalent changes in pH_i. Activity dependence, methodological dependence, and the existence of a time delay between changes in pH_i and r_i are observations which seem inconsistent with the hypothesis that the regulation of r_i in the heart occurs solely through the mass action of protons. Instead, these results seem to infer that direct effects of H^+ on r_i are subordinate to another mechanism or that pH_i-associated changes in r_i are mediated by some interposed substance or reaction. Possible candidates for such regulatory mechanisms include $[Ca^{2+}]_i$, pH-dependent Ca^{2+}-binding proteins, phosphorylation of junctional membrane proteins and/or some combination thereof.

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