ABSTRACT
In-vitro experiments performed in cat papillary muscles and strips of rat right ventricle suggest that the changes in myocardial contractility that follow acid-base disturbances are not a function of extracellular pH. Simultaneous changes in Pco2 and NaHCO3 concentration, with extracellular pH constant, decreased developed tension and maximal rate of rise of the tension (dT/dt) without significant changes in the time to peak tension when the muscle was exposed to the solution with higher Pco2 and NaHCO3 concentration. At an extracellular pH of 7.40, developed tension decreased 0.51 ± 0.13 g/mm² (P < 0.02) and dT/dt decreased 1.29 ± 0.50 g/sec (P < 0.05) with no significant change in time to peak tension (0.038 ± 0.022 sec). Changes in pH produced by increasing Pco2 at constant NaHCO3 concentration were followed by a significant decrease in contractility. A change of Pco2 from 20 to 90 mm Hg that produced a change in extracellular pH from 7.60 to 7.00 was accompanied by a decrease in developed tension of 0.67 ± 0.14 g/mm² (P < 0.01), in dT/dt of 2.63 ± 0.54 g/sec (P < 0.01) with no changes in time to peak tension (0.0017 ± 0.10 seconds). We were unable to show significant variations in contractility when extracellular pH was changed at a constant Pco2 of approximately 21 mm Hg (NaHCO3 7.5, 15, and 30 mm) or at a Pco2 of approximately 95 mm Hg (NaHCO3 15, 30, 60, 80 and 120 mm). Only when extracellular pH reached a value as high as 8.0 (Pco2 21 mm Hg, NaHCO3 80 mm) a small but significant increase in contractility was evidenced. Either Pco2 or intracellular pH could be the major determinants of the changes in myocardial contractility that follow acid-base alterations.

ADDITIONAL KEY WORDS cardiac performance developed tension time to peak tension intracellular pH extracellular pH Pco2 NaHCO3 cat rat
It is difficult to ascertain whether the changes in cardiac performance observed in isolated blood-perfused hearts, heart-lung, or more intact preparations are due to variation in blood pH, Pco₂, or both (3-10).

Recent experiments in our laboratory on heart-lung preparations or perfused dog hearts have demonstrated that heart performance is affected by changes in Pco₂ but not by changes in pH when Pco₂ is kept constant (11).

Considering the discrepancies between our results and those obtained with in-vitro preparations by other authors, experiments were undertaken on cat papillary muscles and strips of rat right ventricle to supply information about the effect of Pco₂, pH, and NaHCO₃ concentration on mammalian ventricular contractility. Evidence will be presented that in changing ventricular contractility during extracellular pH alterations, the way in which a change in extracellular pH was produced is more important than the change itself.

Methods

Cats and rats were anesthetized with sodium pentobarbital and the hearts immediately removed. Each heart was dissected while immersed in oxygenated Ringer's solution. Cat papillary muscles from the right ventricle and a few strips of rat right ventricle to supply information about the effect of Pco₂, pH, and NaHCO₃ concentration on mammalian ventricular contractility. Evidence will be presented that in changing ventricular contractility during extracellular pH alterations, the way in which a change in extracellular pH was produced is more important than the change itself.

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30°C. In some experiments temperature was maintained at 37°C, with no difference in results.

In the first series of experiments, the muscle was successively exposed to Ringer's solutions with different NaHCO₃ concentration and Pco₂, but the same pH. The experimental design was the following:

1. The muscle was first immersed in a solution containing 7.5 mM NaHCO₃ and equilibrated with a CO₂ in O₂ mixture with a Pco₂ of approximately 21 mm Hg. After a steady state was reached, the solution was switched to another containing 30 mM NaHCO₃ and Pco₂ 92 mm Hg. Both solutions had an extracellular pH of about 7.00.

2. A solution containing 35 mM NaHCO₃ and a Pco₂ of 45 mm Hg was changed by another in which NaHCO₃ concentration was 80 mM and Pco₂ 92 mm Hg. In both cases, extracellular pH was approximately 7.40.

3. From a Ringer solution with 30 mM NaHCO₃ and Pco₂ 21 mm Hg, the change was made to another containing 120 mM NaHCO₃ and Pco₂ 92 mm Hg; extracellular pH was maintained at a value of about 7.60.

We want to stress that Pco₂ and NaHCO₃ were not exactly the same in the different experiments. Before each experiment, different combinations of NaHCO₃ concentration and Pco₂ were tested to obtain the desired pH values, namely 7.00, 7.40, and 7.60. The more frequently used values of Pco₂ and NaHCO₃ were tested in Tables 1 and 2 and mentioned in the text; however, as can be seen in the figures in which individual experiments are shown, the actual values of Pco₂ and NaHCO₃ differed slightly from one experiment to another. The main objective of the experiment, however, was to expose the muscle to different Pco₂ and NaHCO₃ combinations with the same pH, and special care was taken to avoid a difference in pH of more than 0.03 units for a given pair of Pco₂ and NaHCO₃ values. The pH and Pco₂ of the solutions were measured anaerobically at 30°C with a Radiometer pHM 27 pHmeter and Gas Monitor PHA 27 and a Radiometer type E5036 Pco₂ electrode. In a few experiments, pH was measured, and Pco₂ calculated from the Henderson-Hasselbalch equation. pH was corrected for ionic strength (1) and for pH and temperature changes (16). The solubility coefficient for CO₂ employed was 0.035 mm/mM Hg. When the calculated Pco₂ values were compared with those actually measured, it was found that they differed by about 10%.

With this experimental design, changes in contractility at the same extracellular pH were analyzed after a steady state had been reached, usually approximately 15 minutes after the change in solution took place.

In a second series of experiments the effect of changes in extracellular pH produced by changes in Pco₂ at constant NaHCO₃ concentration were studied. The effects of changes in extracellular pH by changing NaHCO₃ concentration were reexamined at two different levels of Pco₂, 21 and 90 mm Hg.

In all the experiments in which NaHCO₃ concentration was varied, NaCl concentration was changed accordingly to keep osmolality constant.

In additional experiments, following a change in pH at constant Pco₂, contractility was assessed after periods longer than those used before. In these experiments, developed tension, maximal rate of rise of the tension and time to peak tension were recorded until after 60 minutes of the change in the solution.

The possibility that the lower O₂ concentration in the Ringer solution bubbled with high Pco₂ mixtures could account for changes in contractility was tested by exposing preparations to 100% O₂, and then after a stabilization period, the gas mixture was changed to 85% O₂ and 15% N₂. No effects on contractility were detected after this reduction in Pco₂. The data presented here belong to 26 different cat papillary muscles and 8 strips of rat right ventricle. The muscles were exposed to the solutions in a random sequence. Since no different responsiveness was found between cat papillary muscles and rat right ventricle, the results are presented together. Statistical analyses were carried out by paired sampling, and Student's t-test was used to evaluate difference. A P value smaller than 0.05 was considered significant.

Results

Experiments Maintaining Extracellular pH Constant.—The effect of simultaneously changing NaHCO₃ concentration and Pco₂ at constant extracellular pH on developed tension, rate of rise of the tension (dT/dt), and time to peak tension of a typical experiment is shown in Figure 1. The changes in developed tension and maximal dT/dt without changes in time to peak tension indicate that a decrease in contractility is produced when NaHCO₃ and Pco₂ are higher even though extracellular pH was 7.0 in both cases.

Table 1 summarizes the changes in developed tension, maximal dT/dt and time to peak tension of all the experiments performed at extracellular pH considered to be physiolog-
Effect of simultaneously changing $P_{CO_2}$ and $NaHCO_3$ concentration and maintaining extracellular pH at 7.0 on $dT/dt$ (rate of rise of tension) and developed tension of a cat papillary muscle.

FIGURE 1

Effect of simultaneously changing $P_{CO_2}$ and $NaHCO_3$ concentration and maintaining extracellular pH at 7.0 on $dT/dt$ (rate of rise of tension) and developed tension of a cat papillary muscle.

Changes in Extracellular pH by Changing $P_{CO_2}$ at Constant $NaHCO_3$ Concentration.—The results of all the experiments in which pH was changed by changing $P_{CO_2}$ from 20 to 90 mm Hg are summarized in Table 2. The increase in $P_{CO_2}$ was associated with a decrease in contractility, as can be seen from the changes in developed tension and maximal $dT/dt$ without significant change in time to peak tension.

Changes in Extracellular pH by Changing $NaHCO_3$ Concentration at Constant $P_{CO_2}$.—In this series of experiments, the statistical analysis shows that contractility remained unchanged in the experiments in which pH was changed by changing $P_{CO_2}$ from 20 to 90 mm Hg are summarized in Table 2. The variations in $NaHCO_3$ concentration during metabolic acidosis, normal, or alkalosis. Developed tension and rising rate of tension were reduced at the highest $P_{CO_2}$ and $NaHCO_3$ values.

TABLE 1

<table>
<thead>
<tr>
<th>pH</th>
<th>$P_{CO_2}$ (mm Hg)</th>
<th>$NaHCO_3$ (mEq/l)</th>
<th>T (g/mm²)</th>
<th>$T_T-T_T$ (g/mm²)</th>
<th>$dT/dt$ (g/sec)</th>
<th>$(dT/dt)<em>{B}- (dT/dt)</em>{A}$ (g/sec)</th>
<th>$TT_P$ (sec)</th>
<th>$TT_P (sec)$</th>
<th>$TT_{A}$ (sec)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7.00</td>
<td>21</td>
<td>7.5</td>
<td>2.70 ± 0.34</td>
<td>1.11 ± 0.28</td>
<td>8.67 ± 0.94</td>
<td>3.85 ± 0.94</td>
<td>0.53 ± 0.05</td>
<td>0.028 ± 0.015</td>
<td>9</td>
</tr>
<tr>
<td>B</td>
<td>7.00</td>
<td>92</td>
<td>30</td>
<td>1.50 ± 0.20</td>
<td>0.51 ± 0.13</td>
<td>5.02 ± 0.02</td>
<td>1.95 ± 0.50</td>
<td>0.49 ± 0.045</td>
<td>0.038 ± 0.022</td>
<td>6</td>
</tr>
<tr>
<td>A</td>
<td>7.30</td>
<td>45</td>
<td>35</td>
<td>2.49 ± 0.34</td>
<td>1.09 ± 0.13</td>
<td>5.30 ± 0.08</td>
<td>1.95 ± 0.50</td>
<td>0.49 ± 0.045</td>
<td>0.038 ± 0.022</td>
<td>6</td>
</tr>
<tr>
<td>B</td>
<td>7.30</td>
<td>92</td>
<td>80</td>
<td>1.39 ± 0.20</td>
<td>0.51 ± 0.13</td>
<td>5.02 ± 0.02</td>
<td>1.95 ± 0.50</td>
<td>0.49 ± 0.045</td>
<td>0.038 ± 0.022</td>
<td>6</td>
</tr>
<tr>
<td>A</td>
<td>7.62</td>
<td>21</td>
<td>30</td>
<td>2.56 ± 0.34</td>
<td>1.09 ± 0.13</td>
<td>5.30 ± 0.08</td>
<td>1.95 ± 0.50</td>
<td>0.49 ± 0.045</td>
<td>0.038 ± 0.022</td>
<td>6</td>
</tr>
<tr>
<td>B</td>
<td>7.62</td>
<td>92</td>
<td>120</td>
<td>2.11 ± 0.37</td>
<td>0.45 ± 0.09</td>
<td>5.26 ± 0.10</td>
<td>1.95 ± 0.50</td>
<td>0.49 ± 0.045</td>
<td>0.038 ± 0.022</td>
<td>6</td>
</tr>
</tbody>
</table>

$T$ = developed tension; $T_T-T_T$ = mean difference between A and B in developed tension obtained by paired sampling; $dT/dt$ = maximal rate of rise of the tension; $(dT/dt)_{B}-(dT/dt)_{A}$ = mean difference between A and B in the maximal rate of rise of tension obtained by paired sampling; $TT_P$ = time to peak tension; $TT_{A}$ = $TT_{B}$ = mean difference between A and B in the time to peak tension obtained by paired sampling; $N$ = number of experiments; Values are means ± 1 SE.
hypocapnia were not followed by significant changes in contractility.

Because changes of NaHCO₃ at constant Pco₂ did not seem to influence myocardial contractility in the time used in these experiments, it seemed interesting to study the same phenomena during longer periods of exposure to a given NaHCO₃ concentration. This was done in experiments in which, after a given change in pH took place, contractility was assessed for 1 hour. The values of developed tension and maximal dT/dt of five papillary muscles exposed for 1 hour at each different solution are shown in Figure 2. It should be mentioned that the changes from one solution to another were done in a random sequence, and not in the progressive way shown in the figure. When NaHCO₃ concentration was changed at a constant Pco₂ of 97 mm Hg, there were no significant changes in developed tension or maximal dT/dt even though pH was altered over a relatively large range (6.70 to 7.40).

The same lack of response was also observed when pH was changed from 7.00 to 7.60 at Pco₂ of 24 mm Hg in six additional experiments (Fig. 3). However, in this set of experiments, when NaHCO₃ concentration was increased and extracellular pH 8.0 was reached, a small but statistically significant increase in developed tension and dT/dt was observed.

Discussion

The results obtained in the present experiments show that, in producing a change in contractility during extracellular pH alterations, the way in which the change in pH is produced is more important than the change in pH itself. This can be better illustrated in Figure 4 in which the changes in developed tension of strips of rat right ventricle are plotted as a function of changes in pH originated by changes in Pco₂. Panel A shows the effect of changing Pco₂ at two different HCO₃⁻ concentrations (30 and 60 mmol), and B, the effect when using HCO₃⁻ concentrations of 15 and 30 mmol. It can be seen that, for a given extracellular pH value, developed tension is higher when HCO₃⁻ and Pco₂ are
Summary of the changes in developed tension and maximal dT/dt as a function of time in five experiments in which NaHCO₃ concentration was changed from 15 mM up to 80 mM at constant Pco₂. After a given change in NaHCO₃ concentration, the muscle remained exposed to that particular solution for 1 hour. The change from one solution to another was made in a random sequence and not in the progressive way shown in the figure. Vertical bars represent 1 SE on either side of the mean.

lower. This is consonant with the results obtained by exposing the muscles to different Pco₂-HCO₃⁻ combinations with the same external pH. Up to this point, the decrease in contractility observed when changing from a low Pco₂-low HCO₃⁻ to a high Pco₂-high HCO₃⁻ solution, could be explained in two ways: contractility was decreased because of the increase in Pco₂ or as a result of the increase in HCO₃⁻ concentration. The last possibility was discarded when it was demonstrated that, when Pco₂ was maintained constant, changes in HCO₃⁻ concentration that produced a variation in extracellular pH from 6.70 to 7.60 were not followed by changes in myocardial contractility. In addition, the experiments in which developed tension and dT/dt were recorded at intervals of 15 minutes for 1 hour after the change in pH of the medium show that the lack of change in contractility is not due to a failure to reach a steady state, as could be suggested by the results of Pannier and Leusen (17).

Whether the discrepancy between our results and those of V. Williams (1, 2) is a matter of atrium vs. ventricle or a different response of the rabbit is not apparent to us. Experiments are in progress to compare the behavior of both types of muscle. However, the complicated geometry of the atrial preparation together with the method of stimulation used could account at least partially for the differences. In effect, it is not difficult to conceive that changes in conduction velocity like those described in their paper can bring about changes in the synchronicity with which the fibers are contracting.

Several investigations have proposed intracellular pH changes as a determinant of the changes in myocardial contractility that follow acid-base alterations (3, 6, 9, 18). There is
Considerable evidence regarding the fact that CO₂ influences intracellular pH to a greater extent than HCO₃⁻ does (19); whereas CO₂ equilibrates rapidly across the cell membrane, HCO₃⁻ does not seem to enter the cell, or, at best, diffuses at a slower rate (20). Even though it is possible to assume that in our experiments a change in Pco₂ was followed by a change in intracellular pH, the present experiments give no aid in deciding whether intracellular pH or Pco₂ changes are responsible for the variations in contractility observed.

In experiments performed in this laboratory and reported elsewhere (21), an indirect attempt to dissociate the effect of intracellular pH from that of CO₂ was made. Measurements in dogs indicate that a sharp rise in intracellular pH follows the administration of THAM, whereas similar amounts of NaHCO₃ induce only minor changes (22). Therefore, it is not unreasonable to assume that changing from a solution containing NaHCO₃ with a given Pco₂ and pH to another with the same pH and Pco₂ in which NaHCO₃ has been replaced by THAM, an increase in intracellular pH or Pco₂ would take place. This hypothesis was tested in papillary muscles, and the replacement of NaHCO₃ by THAM was not followed by variations in developed tension or maximal dT/dt when the effect of hyponatremia was avoided, a factor that was not considered in previous experiments (23, 24). This finding, however, gives no definitive answer to the problem, and three possible explanations could be offered:

1. THAM did not produce a significant increase in intracellular pH, or the increase produced was not enough to elicit a change in contractility.

2. A negative inotropic effect of THAM is
Changes in developed tension as a function of changes in pH originated by variations in Pco₂ at constant NaHCO₃ concentration (two strips of rat right ventricle). Figures in parentheses are Pco₂ values in mm Hg.

masking the effect mediated through changes in intracellular pH.

3. Despite intracellular pH changes, contractility did not change because Pco₂ was maintained constant.

When HCO₃⁻ concentration was increased at constant Pco₂, an increase in contractility was found only when pH reached a value as high as 8.0. This could mean either that extracellular pH has some additional effect evident only when such a degree of alkalinity is produced, or that, in this circumstance an uptake of HCO₃⁻ or release of H⁺ by the cell is produced.

Considering the data collected by other authors and by ourselves, it seems that the extent to which the dissociation between the effect of intracellular pH and Pco₂ on myocardial contractility will be successful will depend on the availability and appropriateness of the information about heart cell pH variations during acid-base derangements.

Present information about the subject is inadequate.

The changes in developed tension reported here are due entirely to changes in the rate of development of the force. There were no significant changes in the time to peak tension. In the absence of changes in the stiffness of the series elastic element, one may conclude that the degree of activation of the contractile element is increased without major changes in the duration of the active state. This is further assessed by the force-velocity curves obtained.

**FIGURE 5**

Force-velocity curves during single isometric contractions obtained in three different experiments. CEV = contractile element velocity; P = load. The upper curve belongs to an experiment in which Pco₂ and HCO₃⁻ concentration were simultaneously changed without changing the extracellular pH. In the middle panel, the effect of increasing Pco₂ at constant HCO₃⁻ concentration upon the force-velocity curve is shown. The lower curve belongs to an experiment in which pH was changed at constant Pco₂.
during single isometric contractions, shown in Figure 5. When pH was kept constant, in spite of different PCO₂ and HCO₃⁻ values, either force or contractile element velocity were lower when PCO₂ was higher. If the velocity of the contractile element is compared at the lowest common tension levels, a change in the estimated Vma becomes apparent. A similar fact is observed in the middle panel, in which pH was changed by changes in Pco₂. When pH was changed at constant PCO₂, no difference could be observed in the force-velocity curves.

The mechanism by which an increase in Pco₂ can affect myocardial contractility cannot be explained by this experiment. Several theoretical possibilities besides a direct action of H⁺ or CO₂ upon the contractile machinery can be contemplated. The first hypothesis devolves from the fact that interventions that increase myocardial contractility are frequently associated with changes in myocardial potassium balance (25, 26), a release of potassium from the cell occurring during an increase in performance. This hypothesis is related to the findings of Brown and Good (27), who demonstrated that a decrease of the ratio of intracellular to extracellular H⁺ activity promotes a net loss of potassium from the skeletal muscle cell; on the other hand, the demonstration that hypercapnia is associated with an uptake of potassium by the myocardium gives support to this theory (28). The second theoretical possibility to consider is the influence of Pco₂ or intracellular pH on the process of Ca release and sequestering. In this respect it is interesting to remark that in a paper by Carvalho and Leo (29), changes in the binding properties of sarcoplasmic reticulum membranes of skeletal muscle with changes in pH are shown. This finding could perhaps give a clue into the possibility that pH acts at the excitation-contraction coupling level.

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