The Effects of Substrates on Contractility of Isolated Rat Atria Depressed by Hydrochloric Acid[1] (35221)

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Energy-yielding substrates have served as useful tools for the study of the mechanism of cardiac-depressant agents on glycolytic sequences of the heart. In our previous demonstrations by the use of certain substrates, the inhalation anesthetic halothane was found to inhibit either glucose uptake or an early stage in its metabolism in the cardiac cells (1, 2). By relating cardiac metabolism to functional activity, depression of myocardial contractility by iodoacetate (3, 4); 2-deoxyglucose (4, 5); citrate (3, 6, 7); or bicarbonate-free medium (7–10) was shown to occur as a result of an inhibition of the glycolytic pathway.

The present experiment investigated another cardiac depressant by the use of exogenous substrates; in particular this study attempts to elucidate the role of glycolysis on the contractile activity of rat atria depressed by low pH.

Methods. Male, Sprague-Dawley rats, weighing 180 to 200 g which had *ad libitum* access to food and water, were employed. Atria were removed from decapitated rats and suspended in 50 ml of modified Krebs–Ringer bicarbonate glucose medium (1, 2) of the following composition (m*M*): NaCl, 120; KCl, 4.8; $CaCl_2$, 1.22; $MgSO_4 \cdot 7H_2O$, 1.33; KH_2PO_4, 1.2; $NaHCO_3$, 25.3; glucose, 5.55. The medium was aerated with 95% O_2 and 5% CO_2 to maintain a pH of 7.4 at 30°. A constant resting tension of 750 mg was maintained throughout the experiment. The developed tension was recorded with a Statham strain gauge, and the atria were electrically stimulated at a rate of 200 pulses/min. An equilibration period of 60 min was allowed before readings were taken. The experimental values of contractility (peak tension) were compared with control values obtained at zero time (following equilibration) and expressed as percentage change in developed tension.

In the experiments with low pH, hydrochloric acid (20 m*M*) was added to the bathing medium following the 1-hr equilibration period. A pH electrode was previously placed in the tissue bath, and the pH was measured throughout the experimental period after the addition of hydrochloric acid.

In the experiments with substrate-free medium, the medium was changed to the substrate-free medium (*i.e.*, free of glucose) following the 1-hr equilibration period.

Results. Effect of hydrochloric acid (20 mM) on atrial contractility and medium pH. Figure 1 shows the alterations in atrial developed tension and pH in the medium by the addition of 20 m*M* hydrochloric acid. It is evident that exposure of atria to the acid solution resulted in a marked transient depression of the contractility that returned toward the control value within 5 min. This was followed by a gradual decrease in the force of contraction, despite the fact that the medium contained 5.5 m*M* glucose. This is in contrast to the control data from the normal medium which showed only a small decrement in developed tension over the same period of time. As shown in Fig. 1, the depressed contractility was restored to the control level after the acidic medium was replaced with normal Krebs–Ringer bicarbonate glucose medium, indicating that the negative inotropic change was reversible. Figure 1 also shows that, by the addition of hydrochloric acid, the pH of the medium dropped rapidly to 5.6, and gradually returned to 6.7, within 5

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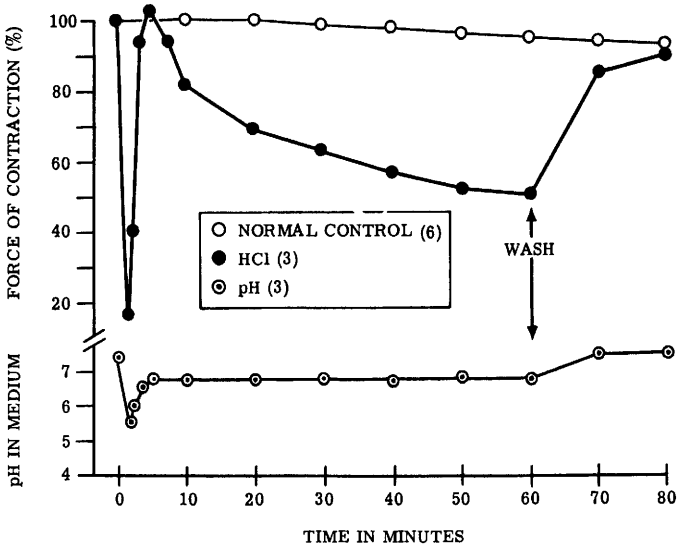

FIG. 1. Effect of hydrochloric acid (20 m*M*) on contractility of atria and on medium pH: In this and subsequent experiments zero time represents a 1-hr equilibration period in normal Krebs–Ringer bicarbonate medium containing 5.5 m*M* glucose. Hydrochloric acid added at zero time. Medium changed to normal at 60 min. Values in parentheses are numbers of experiments.

min, then was maintained at this level throughout the experimental period. With enlargement of the time-course in the first 5 min, the relationship between hydrogen ion in the medium and contractility of the atria is demonstrated in Fig. 2. Figure 2 shows that the transitory depression of the atria is well correlated with the hydrogen ion concentration in the medium.

Effect of substrates on contractility of atria depressed by hydrochloric acid. Substrates were added to the bathing medium 30 min after the atria were depressed in the acid solution (Fig. 3). Pyruvate partially restored the contractility of atria depressed with hydrochloric acid, but additional glucose and fructose were without appreciable effect.

Effect of substrates on contractility of normal atria, or atria depressed with substrate-free medium. Developed tension of the atria progressively decreased in the substrate-free medium after the 1-hr equilibration period in

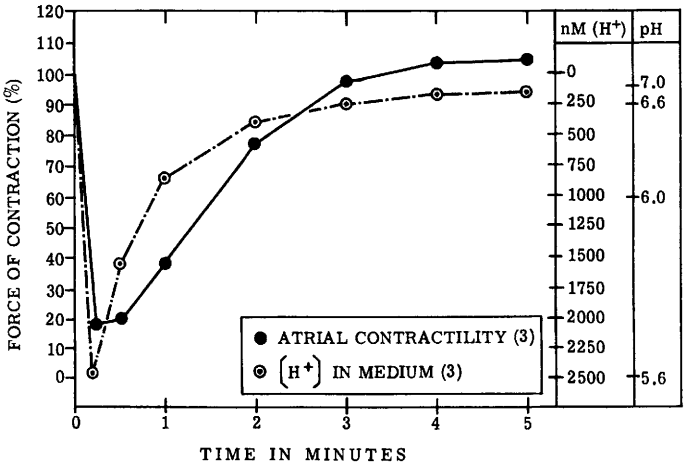

FIG. 2. Relationship between hydrogen ion concentration in medium, pH, and contractility: hydrochloric acid (20 m*M*) added at zero time.

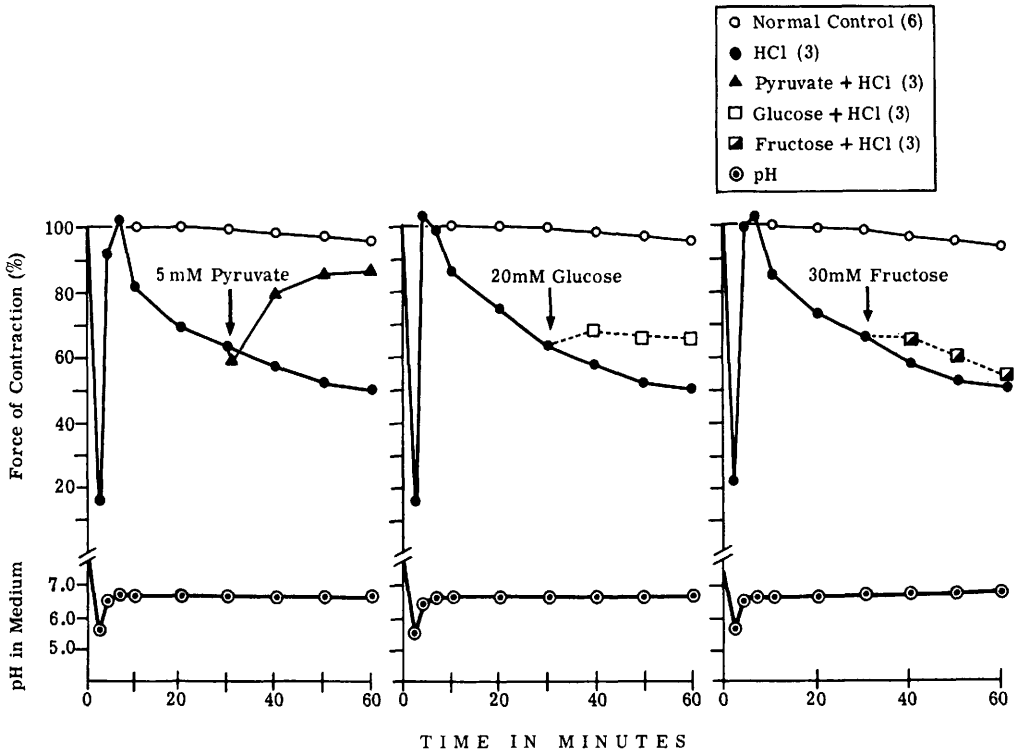

FIG. 3. Effect of substrates on contractility of atria depressed by hydrochloric acid (20 m*M*). HCl added at zero time; substrates added at 30 min.

Krebs-Ringer bicarbonate glucose medium (Fig. 4). Substrates were added after 30-min incubation in the substrate-free or normal medium. Pyruvate produced only partial restoration of the depressed contractility in the substrate-free medium, a result similar to that seen in the presence of HCl, but was without effect on the normal atria. Fructose (30 m*M*) produced almost complete recovery of the force of contraction in the substrate-free experiment but produced no increase in the developed tension of the normal atria. Glucose (20 m*M*) produced a marked increase, higher than the control levels, in the contractile activity of either substrate-depleted, or normal atria. The marked effects of glucose and fructose in atria depressed by substrate-free medium is in sharp contrast to their effects in HCl media and suggests a block in the utilization of these substrates by HCl. The similarity of effectiveness of pyruvate in both HCl- and substrate-free treated atria suggests that the utilization of this substrate is not interfered with by HCl. These studies thus indicate a defect in the utilization of glucose and fructose prior to their conversion to pyruvate.

Discussion. Interest in the effect on the heart of changes in external pH, well outside the physiological range, goes back to Gaskell in 1880 (11) and Mines (12). In the present investigation, addition of 20 m*M* hydrochloric acid resulted in a biphasic action on both contractility and pH. Initially, there occurred a rapid marked fall in contractility to 18% of the control value and in pH to 5.6. This was followed, within 5 min, by a return of contractility to 102% of control and pH to 6.7. After these initial changes the contractility fell slowly over the next 55 min to 50% of the control value despite the fact that the pH remained constant at 6.7.

The rapid and marked initial fall in contractility is reasonably well correlated with the change in hydrogen ion concentration and probably reflects the influence of hydrogen ions on some readily accessible cellular compartment, involved intimately with the con-

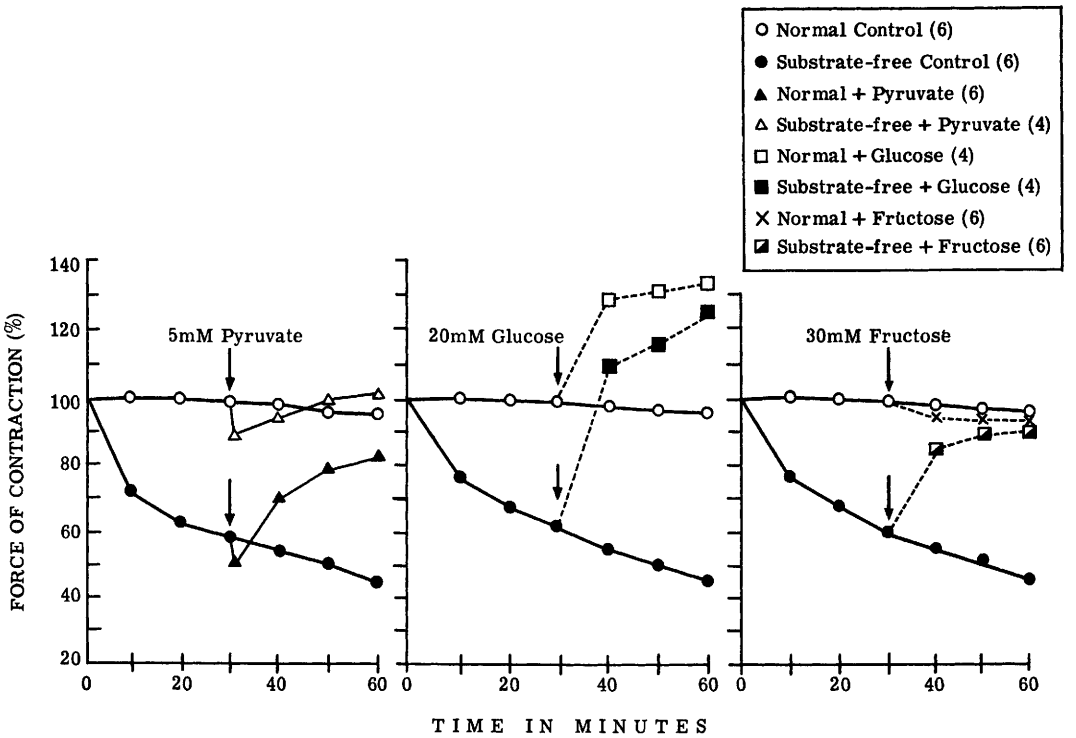

FIG. 4. Effect of substrates on contractility of normal atria and atria depressed by substrate-free medium: medium changed to substrate free at zero time; substrates added at 30 min.

tractile process. The possibility of an interaction of hydrogen ion with the cell surface resulting in marked changes in electrical activity which could affect the contractile process was considered and rejected on the basis of the following observations. Lorkovic (13) has demonstrated with frog ventricle that by changing the external pH from 9.0 to 5.0 the membrane potential and the amplitude of the action potential were only little changed whereas the mechanical activity decreased and almost disappeared. Ko *et al.* (14) reported similar results with rat atria in that the magnitude of the action potential duration showed only little alterations over the range of pH from 4.0 to 10.

The secondary, gradual decrease in contractility to 50% of control values in 1 hr is clearly not a function of extracellular hydrogen ion concentration as the pH remained constant at 6.7 during this period. The mechanism of this depression is involved with an inhibition of glycolysis since pyruvate is effective in overcoming the contractile depression whereas glucose and fructose are not. Our results implicating a block in glycolysis by low pH with the functional consequence of decreased contractility is consistent with previous biochemical data indicating that glucose utilization by red cells (15), leukocytes (15, 16), skeletal (17) and cardiac (18) muscle is decreased in acidosis *in vitro*. The mechanism of this inhibition has been attributed to a block in phosphofructokinase activity by low intracellular pH (19). This enzyme is known to have a pH optimum of about 8 (20). Reduction of extracellular pH below 6.85 results in intracellular acidosis (21). Another possible contributory factor to the decreased contractility may be related to a decrease in intracellular potassium and increase in hydrogen and sodium ions by low external pH (22). Indeed glucose utilization for contractile activity is blocked in rat ventricle strips placed in potassium-free media (23). Potassium is lost from the cells under such conditions (24). However, pyruvate and succinate are also not effective as energy

sources for the contractile process in potassium-free media (23). This is in contrast to the marked effectiveness of pyruvate in the low pH media reported here. It is possible that the intracellular potassium changes in low pH medium are less severe than corresponding changes in potassium-free medium and the effects of these changes are confined to the glycolytic cycle in the low pH studies, although there is no evidence on this point. Further speculation is unwarranted.

Summary. Addition of 20 m*M* hydrochloric acid to isolated rat atria results in a biphasic action on both contractility and pH. Initially contractility fell to 18% of the control value and pH fell from 7.4 to 5.6. This was followed, within 5 min, by a return of contractility to 102% of control and pH to 6.7. Following these initial changes the contractility fell slowly over the next 55 min to 50% of the control value despite the fact that the pH remained constant at 6.7. The initial rapid fall in contraction was correlated reasonably well with the changes in external hydrogen ion concentration and probably reflects the action of hydrogen ions on some readily accessible cellular compartment involved in the contractile process. The secondary, gradual decrease in contractility to 50% of control values in 1 hr is clearly not a function of extracellular hydrogen ion concentration as the pH remained constant at 6.7 during this period. The mechanism of this depression is involved with an inhibition of glycolysis since pyruvate is effective in overcoming the contractile depression whereas glucose and fructose are not.

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