Rate of Depression of Atrial Contractility Inducted by Citrate, Bicarbonate-Free Medium, Hydrochloric Acid, and Halothane[1] (35462)

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This report is a continuation of studies dealing with the mechanism of action of the cardiac depressants citrate, bicarbonate-free medium, hydrochloric acid, and halothane in the isolated rat atrial preparation supplied with 5.5 m*M* glucose (1–5). All four of these depressants act by virtue of an inhibiting effect on glucose uptake or on glycolysis as evidenced by the following information. Contractile depression to approximately 50% of control levels by all of these agents can be overcome by pyruvate (5 m*M*). Additional glucose (20 m*M*) is ineffective in the presence of bicarbonate-free medium, HCl, or halothane but is partially effective in citrate-induced depression. Fructose (30 m*M*), which is equivalent to approximately 5 m*M* glucose as an energy source for contractility, is apparently metabolized via the phosphofructokinase step (6, 7). This sugar is effective against halothane-induced cardiac depression but not against that caused by citrate, bicarbonate-free medium or HCl (2, 4, 5).

On the basis of the above observations, the following conclusions are warranted: (i) bicarbonate-free medium, citrate, and HCl block either the uptake of glucose and fructose or some step in their metabolism prior to conversion of pyruvate to acetyl coenzyme A. (ii) Halothane blocks either the uptake of glucose or its metabolism to some step prior to the conversion of fructose-6-phosphate to fructose-1,6-diphosphate.

This study attempted to localize further the site of action of these inhibitors. By comparing the rate of development of depression induced by these cardiac depressants in the presence of 5.5 m*M* glucose with the rate of development of depression induced by glucose-free medium, one may determine whether the depressant is acting by interfering with glucose uptake or phosphorylation of glucose to glucose-6-phosphate by hexokinase. If one of these steps is the site of action, the depressant in appropriate concentration should affect the rate of depression like glucose-free medium, where there is no glucose to be taken up or phosphorylated. If the rate of depression is faster with the depressant this would imply another site of action.

This method should be helpful in elucidating the site of action of all cardiac depressants known to inhibit glycolysis.

In addition, data on the effects of 20 m*M* glucose on atrial contractility depressed by citrate, bicarbonate-free medium, halothane, hydrochloric acid, and glucose-free medium are included in an attempt to demonstrate the nature of the inhibition induced by the various inhibitors.

Methods. Male Sprague-Dawley rats, weighing 180–200 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 (8) 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 atria were electrically stimulated at 200/min in this medium and aerated with 95% O_2 and 5% CO_2 to maintain a pH of 7.4 at 30°. The developed tension of atria was determined as previously described by Ko and Paradise (1–5).

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1. Bicarbonate-free experiments. The procedures were conducted by means of techniques previously described by Ko *et al.* (9). The bicarbonate-free medium was prepared by replacing sodium bicarbonate from the Krebs–Ringer bicarbonate medium with an equilivalent concentration of sodium chloride and bubbling with 100% O_2. The pH of the bicarbonate-free medium was initially adjusted with dilute sodium hydroxide to 7.4 just prior to the experimental procedure. The pH electrodes, placed in the tissue bath to monitor the medium pH, demonstrated no significant change from 7.4 throughout the course of the bicarbonate-free experiments. After a 1-hr equilibration period in the normal Krebs–Ringer bicarbonate glucose medium, the medium was changed to the bicarbonate-free medium containing 5.5 m*M* glucose.

2. Halothane experiments. Halothane was administered to the medium by means of the anesthetistat previously described by Paradise and Griffith (10, 11). Halothane concentrations in the medium were determined at 10- to 30-min intervals with a gas chromatograph throughout the experimental period (10). Halothane administration was begun at zero time (following a 1-hr equilibration period). During the first 10 to 15 min the anesthetistat was adjusted to deliver enough halothane to achieve 50% depression of contractility. Following this period no further adjustments were made. Relatively stable concentrations of halothane were present in the bathing medium during the experimental period (approx 6 mg/100 ml of halothane in the medium).

3. Citrate experiments. Sodium citrate produced dose-dependent decreases in the force of contraction of atria (4). Thus, 1.5 m*M* sodium citrate was chosen for this experiment because it produced about the same degree of depression as that seen with the other cardiac depressants tested in this study. After a 60-min equilibration period in the normal Krebs–Ringer bicarbonate glucose medium, 1.5 m*M* sodium citrate was added to the bathing medium.

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

5. Substrate-free experiments. The normal medium was changed to substrate-free medium (*i.e.*, free of glucose) following the 1-hr equilibration period.

In Parts (1) and (5) above, following the equilibration period in normal medium, the bath was changed three times with either bicarbonate-free or substrate-free medium. This procedure required about 1 min. Citrate and hydrochloric acid were added to the medium from a needle and syringe. The needle was placed in the normal medium and the contents of the syringe delivered all at once. Halothane vapor was delivered to the normal medium via a vaporizer attached to a needle placed in the normal bathing medium. Delivery of the vapor was continued throughout the experiment at a constant rate such that the amount added equalled amount lost by evaporation.

Results. Rate of depression of atrial contractility induced by cardiac depressants. The rate of contractile depression induced by substrate-free medium was compared with that induced by the cardiac depressants, citrate, halothane, hydrochloric acid, and bicarbonate-free medium (Fig. 1). Figure 1 shows that the depression rate of atria in substrate-free medium is slower than that caused by the depressants citrate, halothane, and bicarbonate-free medium. Hydrochloric acid produced an initial, marked, transient fall in contractility along with a pH change to 5.6 (5). This was followed by a slow rate of contractile depression more like that caused by substrate-free medium than by the other depressants. The pH remained constant at 6.7 from the 5-min period to the end of the experiment.

Effect of glucose on contractility of atria depressed by cardiac depressants. Glucose, added to atria exposed to substrate-free medium for 30 min, produced a marked increase in force of contraction (Fig. 2). A much smaller, but still definite, effect was achieved by glucose added to citrate-depressed atria. Still less effect was seen with atria depressed by HCl, and no effect whatsoever was ob-

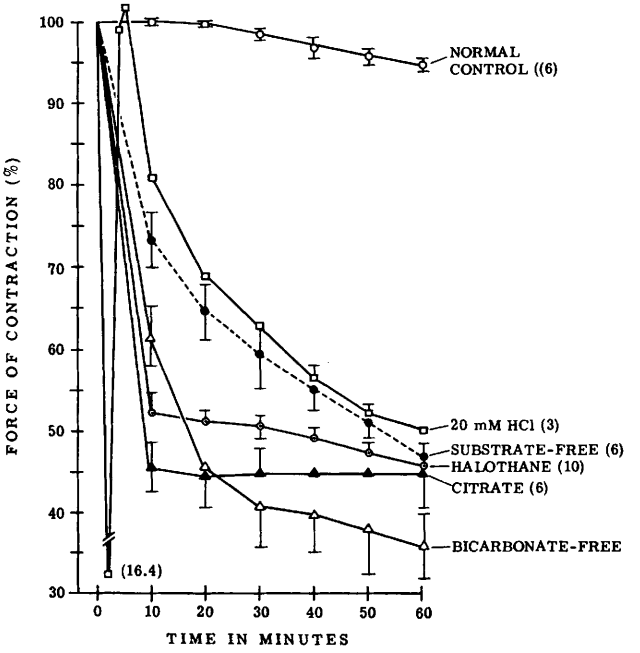


FIG. 1. Rate of depression of rat atrial contractility by various cardiac depressants: zero time represents a 1-hr equilibration period in the normal Krebs–Ringer bicarbonate glucose solution. Citrate (1.5 m*M*), halothane (6 mg/100 ml) or HCl (20 m*M*) added at zero time. Bathing medium changed to substrate-free, i.e., free of glucose or bicarbonate-free also at zero time. Vertical bars represent ± 1 standard error of the mean. Values in parentheses represent number of experiments.

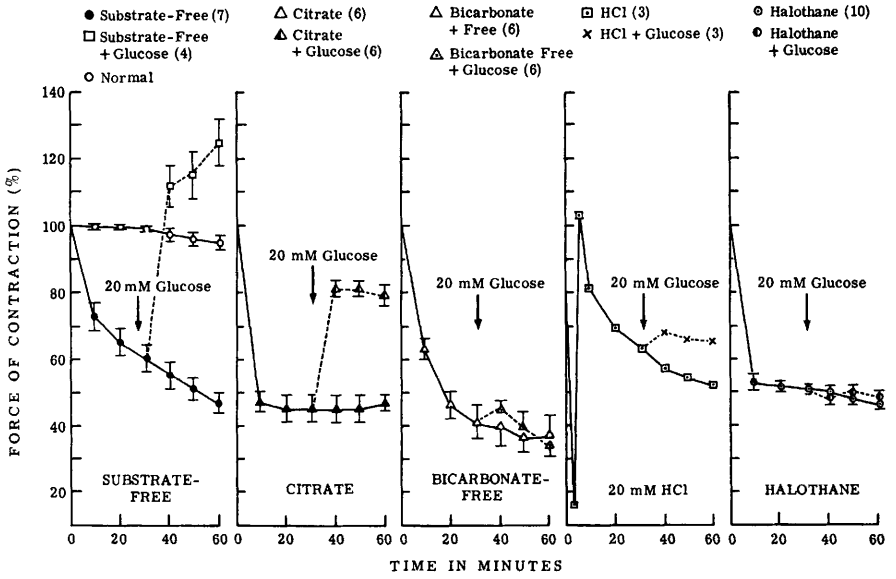


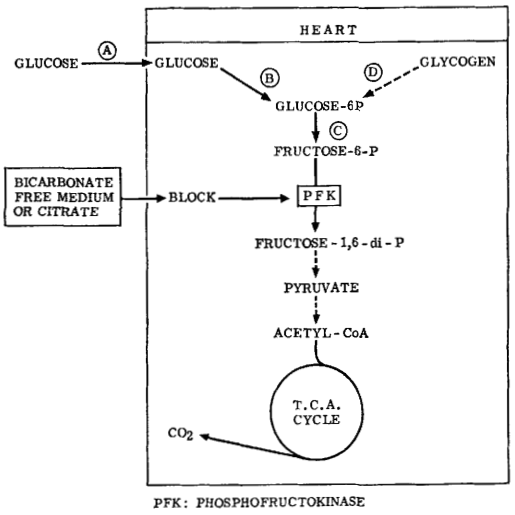
FIG. 2. Effect of 20 m*M* glucose on contractility of rat atria depressed by substrate-free medium and by various cardiac depressants: citrate (1.5 m*M*), halothane (6 mg/100 ml) or HCl (20 m*M*) added at zero time. Bathing medium changed to substrate-free or bicarbonate-free at zero time. Glucose (20 m*M*) added at 30 min.

served in atria depressed by halothane or bicarbonate-free medium.

Discussion. The very rapid decline in contractility induced by citrate, bicarbonate-free medium, and halothane indicate that these cardiac depressants act by a mechanism which is different from that operating in the glucose-free experiments in which the rate of decline was much slower. In the glucose-free experiments there is no exogenous glucose to be taken up or to be metabolized, so atria exposed to this medium must rely on endogenous substrate stores, probably glycogen, to provide energy for contraction. If citrate, bicarbonate-free medium, or halothane were depressant by virtue of an inhibitory action on glucose uptake or phosphorylation to glucose-6-phosphate the rate of depression would have been similar to, or certainly no greater than, that produced by glucose-free medium. Thus, uptake or phosphorylation of glucose is ruled out as the site of action of citrate, bicarbonate-free medium, or halothane. Experiments previously reported for citrate and bicarbonate-free medium, in which pyruvate was effective while fructose was not effective in counteracting the depressant action of these agents pointed to the phosphofructokinase (PFK) step (Fig. 3), or some earlier step in glycolysis as the key site (4). This study rules out sites A and B leaving C, the glucose phosphate isomerase step, or the PFK step. Site C is probably not involved since fructose is apparently not metabolized via this step (12). This leaves the PFK step as the most likely site for the depressant action of citrate and bicarbonate-free medium. These studies thus confirm biochemical evidence which implicates PFK as a site inhibited by these inhibitors (6, 7, 13–15).

This study also shows that the primary site of action of halothane is not step A or B by the same reasoning. Coupled with previous experiments which showed that pyruvate and fructose, but not glucose, could overcome the contractile depression induced by halothane, these results lead to site C, the glucose phosphate isomerase step, as the most likely site for halothane depressant action.

The situation with hydrochloric acid is a bit more complex. Following an initial rapid transient depressant effect the rate of decline



PFK: PHOSPHOFRUCTOKINASE

FIG. 3. Schematic representation of glycolytic cycle.

of contractility was very slow with this agent, similar to that seen with glucose-free medium. This might indicate that HCl was acting by inhibiting glucose uptake or phosphorylation to glucose-6-phosphate. Another interpretation, however, is that HCl acts at the PFK step by a slow process involving a gradual increase in hydrogen ion concentration. This study cannot differentiate between these two possibilities.

Some information concerning the nature of the inhibition caused by the various depressants can be obtained from Fig. 2. The fact that 20 m*M* glucose was partially effective in overcoming citrate depression may indicate that citrate is a competitive inhibitor of PFK. Additional fructose-6-phosphate, coming from glucose, being sufficient to partially overcome citrate block. Halothane and bicarbonate-free medium, although depressing the heart to the same degree as citrate may be noncompetitive inhibitors since their actions were not affected at all by additional glucose. Hydrochloric acid may also be acting as a noncompetitive inhibitor since glucose produced little, if any, effect in its presence.

Summary. Citrate, bicarbonate-free medium, and halothane all produced rapid depressions of atrial contractility, depression occurring much more quickly than that seen by merely omitting glucose from the bathing

medium. This suggests a site of action for these agents which is different from the glucose uptake step or the hexokinase step involving phosphorylation of glucose to glucose-6-phosphate. The results are consistent with previous reports suggesting the phosphofructokinase step as the site of action of citrate and bicarbonate-free medium and also suggests the glucose phosphate isomerase step as the site of halothane action. Hydrochloric acid produced a slow decline in contractility, similar to that seen with glucose-free medium. This can be interpreted as either a rapid effect on glucose uptake or phosphorylation or a slowly developing effect at the phosphofructokinase step. Addition of 20 m*M* glucose partially overcame the depression induced by citrate but had little or no effect on that produced by bicarbonate-free medium, halothane, or hydrochloric acid. This suggests that citrate depression is surmountable and probably competitive in nature while the other depressants may be of the noncompetitive type.

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