Nature of Endogenous Substrates Used to Support Contractility of Isolated Rat Atria[1] (35696)

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Bicarbonate-free medium produces a complete, noncompetitive block in the glycolytic cycle based on the following information: (i) depression of atrial contractility by bicarbonate-free medium is overcome by acetate, lactate, and pyruvate but not by fructose or glucose (1); (ii) neither ^{14}C glucose nor ^{14}C fructose were converted to ^{14}C fructose diphosphate by isolated rat diaphragm in this medium (2, 3). These two experiments suggest a block in glycolysis at or above the phosphofructokinase step. The rate of contractile depression induced by bicarbonate-free medium in the presence of glucose is faster than that induced by glucose-free medium (4). Thus, the action of this inhibitor cannot be simply explained by a block in glucose uptake or phosphorylation of glucose to glucose-6-phosphate. If one of these sites were involved, the rate of depression would be similar to, but not greater than, that due to glucose-free medium. Thus, the site of blockade would appear to be at the glucosephosphate isomerase or phosphofructokinase (PFK) step. The former step cannot be the site since fructose, which is ineffective as a source of energy for the contractile process in bicarbonate-free medium, is not metabolized via this step (1). We are left with the PFK step as the most likely site inhibited by bicarbonate-free medium. This medium can then serve as a useful tool for determining the nature of endogenous substrates useful for the contractile process. Addition of this glycolytic inhibitor to atria bathed in substrate-free medium should have little or no effect if the major source of energy for contraction is derived from lipid or some other substrate below the PFK step. On the other hand, if glycogen is the major supplier of energy then bicarbonate-free medium should have profound effects. Addition of bicarbonate-free medium to atria incubated in substrate-free medium did, indeed, depress the force of contraction markedly, but not completely. This suggests that some material above the PFK step, probably glycogen, is a major, but not exclusive, source of fuel for the contractile process in the absence of exogenous substrates.

Citrate, another known inhibitor of PFK, was also tried. It likewise produced a profound depressant action on atria in substrate-free medium. Unlike bicarbonate-free medium, however, its effects were partially reversible by 20 m*M* glucose.

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 (1) 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; and 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).

1. *Bicarbonate-free experiments.* The procedures were conducted by means of techniques previously described by Ko *et al.* (5).

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The bicarbonate-free medium was prepared by replacing sodium bicarbonate from the Krebs–Ringer bicarbonate medium with an equivalent 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. 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 one free of glucose; then to the bicarbonate-free medium 15 min later.

2. *Citrate experiments.* After a 60-min equilibration period in the normal Krebs–Ringer bicarbonate glucose medium, and a further 15 min period in glucose-free medium, 1.5 m*M* sodium citrate was added to the bathing medium.

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

Results. Effects of citrate and bicarbonate-free medium on contractility of substrate-depleted atria. Following a 1-hr equilibration period in the normal Krebs–Ringer bicarbonate glucose medium (zero time in Fig. 1) the medium was changed to one free of glucose (substrate free). After 15 min incubation of atria in this substrate-free medium, 1.5 m*M* citrate was added to atria, or the substrate-free medium was again changed to bicarbonate-free medium. Figure 1 shows that the force of contraction of atria declined due to prolonged activity in substrate-free medium, in comparison with the normal control level. However, it is also evident that the substrate-free treated atria were markedly depressed by citrate, or bicarbonate-free medium.

Nature of inhibition of glycolysis in atria caused by citrate and bicarbonate-free medium. The previous experiment suggested that a substance above the PFK step, probably glycogen, is a major contributor to the force of contraction in the absence of exogenous

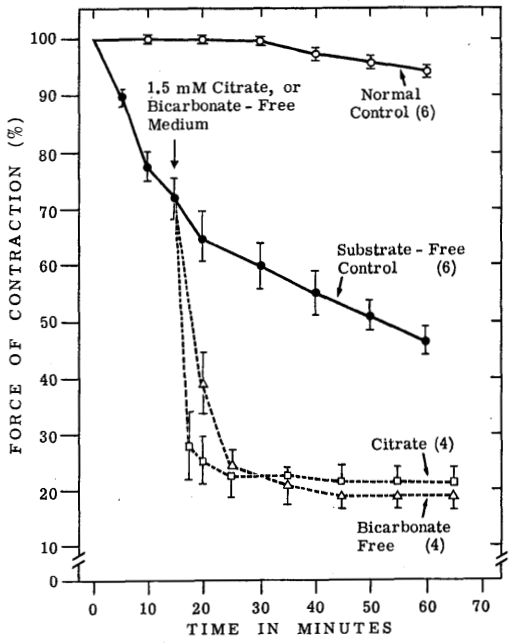

FIG. 1. Effect of citrate and bicarbonate-free medium on contractility of substrate-depleted atria: 0 time represents a 1-hr equilibration period in the normal Krebs–Ringer bicarbonate medium containing 5.56 m*M* glucose. At zero time, medium was changed to one free of glucose (substrate-free); 15 min later 1.5 m*M* citrate was added or medium was changed to bicarbonate- and substrate-free.

substrate since bicarbonate-free medium and citrate, known inhibitors of PFK, produced marked depressant effects in substrate-free medium. The experiments depicted in Fig. 2 were designed to demonstrate the nature of the inhibition of glycolysis produced by the above inhibitors. Glucose (20 m*M*), 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 in substrate-free medium. However, no effect was observed in atria depressed with bicarbonate and substrate-free medium.

Discussion. A number of experiments in isolated rat diaphragm and heart indicate that in the absence of bicarbonate, the phosphofructokinase (PFK) reaction is completely inhibited. Shaw and Stadie (2, 3) showed a failure of conversion of labeled glucose or

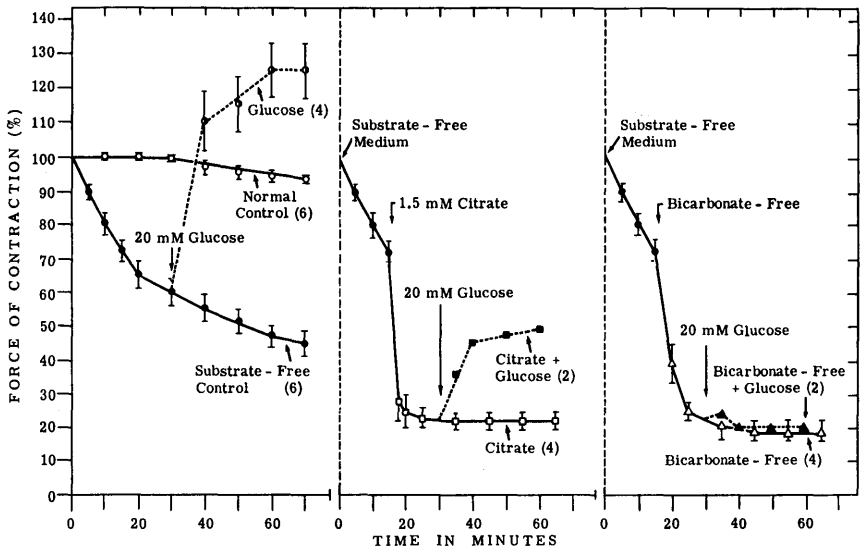

FIG. 2. Nature of the inhibition of glycolysis in atria caused by citrate and bicarbonate-free medium: At zero time, following 1-hr equilibration in normal Krebs–Ringer bicarbonate glucose medium, bath changed to one free of glucose (substrate-free); 15 min later, citrate (1.5 m*M*) was added (middle section) or medium changed to one free of bicarbonate and glucose (right section); glucose (20 m*M*) added at 30 min in all experiments.

fructose to fructosediphosphate in bicarbonate-free medium along with other biochemical data indicating the importance of bicarbonate for progression of the PFK reaction in the diaphragm. In bicarbonate-free medium, glucose is relatively ineffective in maintaining the contractile activity of rat ventricle strips (6–8). Rice and Berman (9, 10) demonstrated that the oxidation of glucose by heart strips incubated in bicarbonate-free medium is lower than the oxidation of pyruvate or acetate. In contrast, they have observed that in medium containing bicarbonate, glucose maintains contractile activity (7), and Hood and Saunders (11) have reported that glucose is rapidly oxidized in this medium. In the presence of glucose, the contractile activity of rat atria was markedly depressed by bicarbonate-free medium. This depression was overcome by pyruvate, lactate, and acetate, but not by fructose or additional glucose (1). Furthermore, glucose uptake or phosphorylation were recently ruled out as the site of blockade produced by bicarbonate-free medium since the rate of contractile depression induced by this medium in the presence of glucose was much faster than that induced by merely omitting glucose from the medium (4). Taken together, these experiments suggest that bicarbonate is an essential component of the bathing medium for the PFK reaction to proceed in isolated rat heart experiments.

The fact that bicarbonate-free medium has a marked depressant action, not reversed by glucose, in the absence of exogenous substrate, indicates that some endogenous material, probably glycogen, can serve as a source of fuel for the contractile process in the absence of exogenous substrate. This functional study thus confirms biochemical studies suggesting that glycogen is used by the heart *in vitro* in the absence of exogenous substrate (12). The marked depression seen with citrate, a well-known inhibitor of PFK (1, 13–15), serves to further verify the importance of glycogen for the contractile process in the absence of exogenous substrate.

That bicarbonate-free medium does not completely depress contractile activity indicates that some endogenous substrates below the PFK step are used for the contractile process. The most likely substrates would be lipid and perhaps lactate.

Summary. In the absence of exogenous substrate, bicarbonate-free medium produced a marked depressant effect on the force of contraction of isolated rat atria which was not reversed by 20 m*M* glucose. Since the negative inotropic effects of bicarbonate-free medium are due to an inhibition of phosphofructokinase (PFK) this indicates that endogenous substrate above the PFK step, probably glycogen, is very important for the contractile process, at least in the absence of exogenously supplied substrate. Citrate, another inhibitor of PFK, also produced a marked depressant action on the force of contraction in the absence of exogenous substrate. The nature of its inhibitory effect was different from that produced by bicarbonate-free medium since depression by citrate was partially overcome with 20 m*M* glucose. Since bicarbonate-free medium did not completely suppress the contractility of the atria, endogenous substrates below the PFK step, probably lipid and/or lactate, besides glycogen, can serve as a source of fuel for the contractile process in substrate-free medium.

1. Ko, K.-C., and Paradise, R. R., Proc. Soc. Exp. Biol. Mel. **134**, 469 (1970).

2. Shaw, W. N., and Stadie, W. C., J. Biol. Chem. **227**, 115 (1957).

3. Shaw, W. N., and Stadie, W. C., J. Biol. Chem. **234**, 2491 (1959).

4. Ko, K.-C, and Paradise, R. R., Proc. Soc. Exp. Biol. Med. **136**, 1222 (1971).

5. Ko, K.-C., Gimeno, A. L., and Berman, D. A., Amer. J. Physiol. **216**, 853 (1969).

6. Covin, J. M., and Berman, D. A., J. Pharmacol. Exp. Ther. **125**, 137 (1959).

7. Berman, D. A., and Saunders, P. R., Circ. Res. 3, 559 (1955).

8. Covin, J. M., and Berman, D. A., J. Pharmacol. Exp. Ther. **117**, 443 (1956).

9. Rice, L. I., and Berman, D. A., J. Pharmacol. Exp. Ther. **127**, 11 (1959).

10. Rice, L. I., and Berman, D. A., Amer. J. Physiol. **200**, 727 (1961).

11. Hood, J. E., and Saunders, P. R., Amer. J. Physiol. **190**, 525 (1957).

12. Neely, J. R., Whitfield, C. F., and Morgan, H. E., Amer. J. Physiol. **219**, 1083 (1970).

13. Passonneau, J. V., and Lowry, O. H., Biochem. Biophys. Res. Commun. **13**, 372 (1963).

14. Parmeggiani, A., and Bowman, R. H., Biochem. Biophys. Res. Commun. **12**, 268 (1963).

15. Garland, P. B., Randle, P. J., and Newsholme, E. A., Nature (London) **200**, 169 (1963).

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