The Effects of Substrates on Contractility of Isolated Human Atria[1] (34797)

KYE-CHANG KO AND RAYMOND R. PARADISE

Department of Pharmacology, Indiana University School of Medicine, Indianapolis, Indiana 46202

The relative abilities of various substrates to maintain the functional activity of the myocardium have been extensively investigated (1–10). Although numerous studies on cardiac metabolism involving the use of cardiac tissue slices (11–15), isolated hearts from animals (16–24), and hearts contracting *in situ* (25-28) have attempted to relate the effects of substrates on the functional activity of the myocardium, no direct demonstration confirming the utilization of individual substrates for the contractile process of isolated human heart in the *in vitro* system has been made.

To demonstrate direct evidence of substate utilization by human heart, we used isolated human atria contracting in an *in vitro* system. In order to investigate the relationship between metabolizable substrates and contractile behavior of human atria, one can introduce the substrates after the force of contraction has declined due to prolonged activity in substrate-free medium, and determine the effect of individual substrates on the mechanical performance. We have carried out such experiments with glucose, pyruvate, lactate, acetate, and fructose.

Methods. Isolated right atrial appendages were obtained at the time of catheter replacement for cardiac surgery in patients with atrial septal defect, pulmonary stenosis, or aortic stenosis. Atria were obtained from both male and female patients 7–10 years old. Immediately upon removal from the patients, the auricular appendages were placed in modified Krebs–Ringer bicarbonate glucose medium of the following composition (m*M*): NaCl 120; KCl 4.8; $CaCl_2$ 2.44; KH_2PO_4 1.20; $MgSO_4 \cdot 7H_2O$ 1.2; $NaHCO_3$ 25.3; glucose 20 at pH 7.4. The medium was previously aerated with 95% O_2 and 5% CO_2 and placed in an ice bath. The atria were transported in this iced medium from the operating room to the laboratory (about 5 min). The auricular appendages were trimmed of fatty tissue, cut into 10- to 15-mm lengths and then rapidly transferred into a tissue bath containing the modified Krebs–Ringer bicarbonate glucose medium (as above) at 37°.

The experimental apparatus and procedures for recording the developed tension of the atria were the same as previously reported for rat atria (10, 24). A constant resting tension of 750 mg was maintained throughout the experimental period. The developed tension was recorded with a Statham strain gage, and the atria were stimulated electrically at a rate of 70 pulses per 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 (after equilibration) and expressed as percentage of change in developed tension.

Results. Effect of glucose on contractility of isolated human atria. Human atria were initially incubated in the Krebs-Ringer bicarbonate medium containing 5.5 m*M* glucose at 37°. The force of contraction declined rapidly, however, in this medium. Increasing the concentration of glucose to 20 m*M* resulted in a steady force of contraction for 6 hr. In order to find the optimum concentration of glucose, a series of atria were initially equilibrated in 20 m*M* glucose for 1 hr. The medium was then changed to one containing either 30, 40, or 60 m*M* glucose. The force of contraction was followed for an additional

[1] This investigation was supported by Grants HE-07718, H-6308, and RR-00162 from the U.S. Public Health Service.

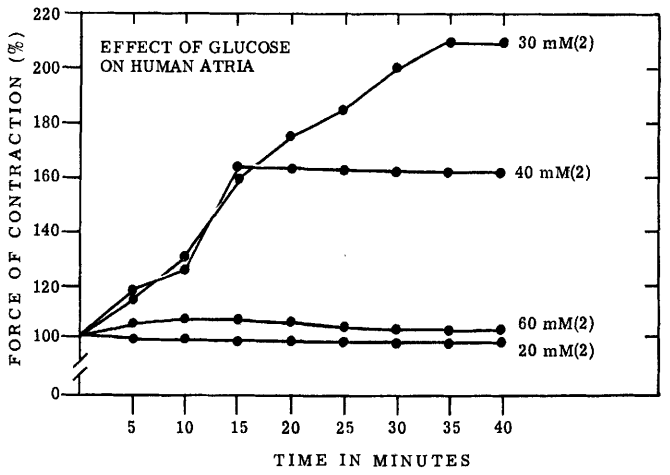

FIG. 1. Effect of glucose on human atria. Atria were equilibrated for 1 hr with 20 m*M* glucose prior to start of experiment. At zero time the medium was either changed to one containing 30, 40, or 60 m*M* or left unchanged (20 m*M*). Figures in parentheses represent number of experiments. All points represent mean values.

40 min and compared to control atria in 20 m*M glucose*. Figure 1 demonstrates the maximally effective concentration of glucose to be 30 m*M*. At higher concentrations osmotic effects may be operating.

Effect of substrates on contractility of isolated human atria. After an equilibration period in the Krebs–Ringer bicarbonate medium containing 30 m*M* glucose, the atria were incubated in a similar medium but without glucose for 30 min (Fig. 2). This resulted in a rapid drop in contractility. Addition of 30 m*M* glucose or pyruvate promptly restored the force of contraction to the control level. Fructose, lactate, or acetate, all at 30 m*M*, partially restored the contractility.

Discussion. Using coronary sinus catherization in men *in vivo* Bing and his co-workers (29) and also Goodale and Hackel (26) have found that the human heart utilizes glucose, pyruvate, and lactate. Bernsmeier and Rudolph have reported that the human heart utilizes about 11 g of glucose and 10 g of lactic acid per day (30). However, the functional importance of substrates directly on the isolated human heart has not been demonstrated.

The present study shows that glucose or pyruvate were more effective than acetate, lactate, or fructose as energy sources for the contractile process in the isolated human atria. The marked positive inotropic action of pyruvate (30 m*M*) on the substrate-depleted human atria is in marked contrast to its effects on rat atria. In the rat the maximally effective concentration is about 2.5 m*M* and it is only partially effective in restoring the contractility of substrate-depleted or 2-deoxy-glucose-treated atria (21). Higher concentrations are less effective and even depressant if glucose is in the medium (9). The apparent preference of the rat heart for glucose over pyruvate has been attributed to an extra effect of glucose on the contractile process due to early steps in its metabolism in addition to its ability to generate ATP (9, 21). Under the conditions of the present human atrial experiment it appears that early steps in the metabolism of glucose may not be important for a fraction of the contactile activity since pyruvate is as effective as glucose.

Besides the species difference, however, it should be pointed out that the rat atria were at 30° in a medium containing 1.22 m*M* calcium and stimulated at a rate of 200 pulses per min. The human atria were at 37° with 2.44 m*M* calcium with stimulus rate of 70 pulses per min. Temperature, *per se,* may affect the utilization of glucose as evidenced

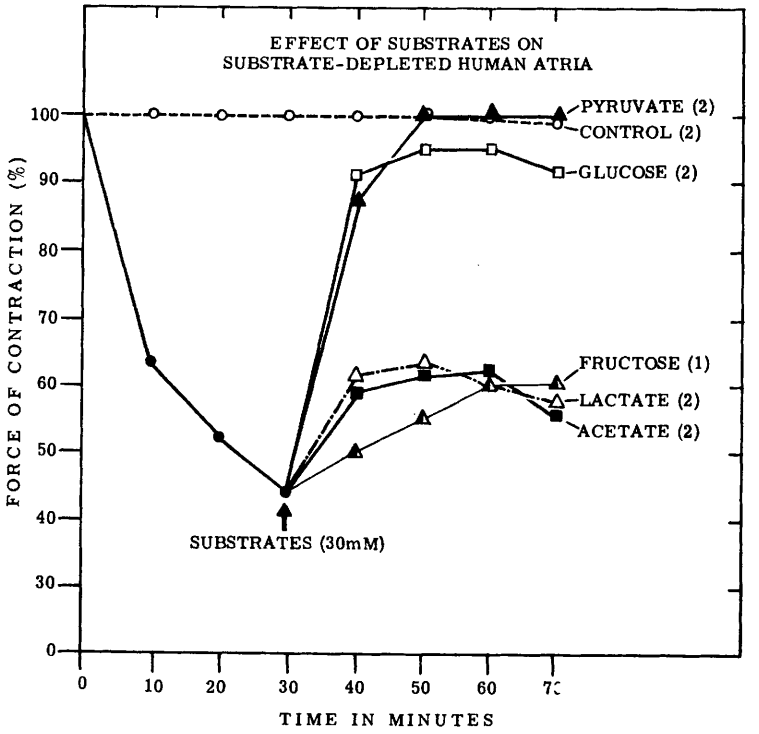

FIG. 2. Effect of substrates on substrate-depleted human atria. After equilibration in 30 m*M* glucose the medium was changed at zero time to one free of glucose. After 30 min in this medium various substrates (30 m*M*) were added. Control represents atria in 30 m*M* glucose throughout the experimental period. Figures in parentheses represent number of experiments. All points represent mean values.

by the greater positive inotropic action of glucose at 27° in the isolated cat papillary muscle preparation than at 37° (7).

Fructose has been shown to serve as excellent substrate for the maintenance of contractility by the isolated rat atria when used at a concentration of 30 m*M* (24). Opie *et al.* (22) found fructose (5 m*M*) to be taken up and metabolized to CO_2 less than 1/5 as rapidly as glucose (5 m*M*). Gimeno *et al.* (9) demonstrated that fructose, at concentrations of 5.5 or 11 m*M*, is utilized for contractility but not nearly so efficiently as the corresponding concentrations of glucose. Thus the uptake of fructose or its conversion to fructose-6-phosphate may be rate-limiting, higher concentrations of fructose than glucose being necessary for similar effects. A similar situation may exist in the human atria since fructose is less effective than glucose at similar concentrations.

The marked effect of pyruvate compared to lactate may warrant clinical trials of pyruvate instead of lactate in Ringer's lactate solution for use in surgical patients with minimum blood loss (31).

Summary. Isolated strips cut from the right atrial appendage of 7- to 10-year-old patients undergoing cardiac surgery were placed in Krebs–Ringer bicarbonate medium at 37° and stimulated at 70 pulses per min. If the medium contained 5.5 m*M* glucose as substrate, the force of contraction declined rapidly. Contractility could be maintained at a constant level for 6 hr with 20 m*M* glucose. The optimal concentration of glucose, however, was 30 m*M*. Equilibration of atria in 30 m*M* glucose was followed by incubation in glucose-free medium for 30 min in another series and resulted in a marked fall in contractility. Introduction of 30 m*M* glucose or pyruvate resulted in a prompt and complete

restoration of contractile force to control levels. Fructose, lactate, or acetate, also at 30 m*M*, restored the force only partially.

We thank Dr. Harold King for supplying us with the atrial preparations.

1. Nakamura, K., Saunders, P. R., Webb, J. L., Lawson, H. C., and Thienes, C. H., Amer. J. Physiol. **158**, 269 (1949).

2. Masuoka, D. T., Berman, D. A., and Saunders, P. R., Amer. J. Physiol. **170**, 301 (1952).

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

4. Webb, J. L., Brit. J. Pharmacol. **5**, 87 (1950).

5. Webb, J. L., and Hollander, P. B., Circ. Res. **4**, 618 (1956).

6. Garb, S., Penna, M., and Scriabine, A., Amer. J. Physiol. **180**, 103 (1955).

7. Garb, S., and Scriabine, A., Amer. J. Physiol. **180**, 101 (1955).

8. Gimeno, A. L., Gimeno, M. F., Savino, E. A., and Bedners, A. S., Proc. Soc. Exp. Biol. Med. **123**, 875 (1966).

9. Gimeno, A. L., Lacuara, J. L., Gimeno, M. F., and Savino, E. A., Proc. Soc. Exp. Biol. Med. **130**, 1033 (1969).

10. Ko, K-C., and Paradise, R. R., Anesthesiology **31**, 532 (1969).

11. Webb, J. L., Saunders, P. R., and Thienes, C. H., Arch. Biochem. **22**, 451 (1949).

12. Pearson, O. H., Hastings, A. B., and Bunting, H., Amer. J. Physiol. **158**, 251 (1949).

13. Miller, O. N., and Olson, R. E., J. Biol. Chem. **199**, 457 (1952).

14. Brin, M., and Olson, E. E., J. Biol. Chem. **199**, 475 (1952).

15. Brin, M., Olson, R. E., and Stare, F. J., J. Biol. Chem **199**, 467 (1952).

16. Cavert, H. M., and Johnson, J. A., Amer. J. Physiol. **184**, 575 (1956).

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

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

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

20. Williamson, J. R., J. Biol. Chem. **240**, 2308 (1965).

21. Gimeno, A. L., Lacuara, J. L., Gimeno, M. F., Ceretti, E., and Webb, J. L., Mol. Pharmacol. **2**, 77 (1966).

22. Opie, L. H., Shipp, J. C., Evans, J. R., and Leboeuf, B., Amer. J. Physiol. **203**, 839 (1962).

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

24. Paradise, R. R., and Ko., K.-C., Anesthesiology, **32**, 124 (1970).

25. Bing, R. J., Circulation **12**, 635 (1955).

26. Goodale, W. T., and Hackel, D. B., Circ. Res. **1**, 509 (1953).

27. Alella, A., Williams, F. L., Bolene-Williams, C., and Katz, L. N., Amer. J. Physiol. **185**, 487 (1956).

28. Harris, P., Howel-Jones, J., Bateman, M., Chlouverakis, C., and Gloster, J., Clin. Sci. **26**, 145 (1964).

29. Bing, R. J., Siegel, A., Vitale, A., Balboni, F., and Sparks, E., J. Clin. Invest. **32**, 556 (1953).

30. Bernsmeier, A., and Rudolph, W., *in* "Verhandlungen der deutschen Gesellschaft fuer Kreislaufforschung," 27th meeting, |R. Thauer, ed.), p. 59. Dietrich Steinkopf Verlag, Darmstadt (1961).

31. Miller, J. R., Stoelting, V. K., and Paradise, R. R., Anesth., Analg. **42**, 640 (1963).

Received Feb. 2, 1970. P.S.E.B.M., 1970, Vol. 134.