The Effect of Fructose on Halothane-depressed Rat Atria

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Fructose produced dose-dependent increases in the force of contraction of isolated rat atria depressed by substrate-free Krebs-Ringer bicarbonate medium. The maximally effective concentration of fructose was 30 mM. The nonmetabolized sugar sucrose, also administered at 30 mM, was without effect when administered under the same conditions. Neither fructose (30 mM) nor glucose (20 mM) was effective in restoring force of contraction of atria depressed by Krebs-Ringer medium containing 5.5 mM glucose without bicarbonate; pyruvate (5 mM), however, produced a marked positive inotropic effect in this medium. Since bicarbonate is necessary for phospho-fructokinase activity, these results are taken as evidence that fructose is metabolized via this step to serve as an energy-yielding fuel for atrial contractility. In another experiment atria suspended in Krebs-Ringer bicarbonate glucose medium were depressed 50 per cent by approximately 6 mg/100 ml of halothane. Addition of 30 mM fructose to these depressed atria resulted in a marked increase in contractile force. The results are consistent with a previous report suggesting blockade by halothane of the uptake or utilization of glucose in the glycolytic pathway, and further pinpoint the blockade as an early step in the glycolytic sequence prior to the phospho-fructokinase step. (Key words: Halothane; Heart; Fructose; Contractility; Bicarbonate.)

THIS INVESTIGATION is a continuation of studies from our laboratory dealing with the mechanism of the depressant action of inhalation anesthetics on cardiac contractility.[1-5] We found, earlier, that anoxia and halothane produce similar decreases in contractility and potassium content in the perfused rat heart. That anoxia produced an increase in coronary flow rate not seen with halothane and produced irreversible damage to the contractile mechanism, again not seen with halothane, suggested that different biochemical changes were occurring with the two variables.[2]

Recently, we found that pyruvate partially restored the contractility of rat atria depressed 50 per cent with approximately 6 mg/100 ml halothane, and that lactate and acetate also partially restored the halothane-depressed atria, despite the fact that additional glucose had no significant effect on the depressed contractility.[3-5] From these findings we concluded that at least part of the negative inotropic action of halothane is the result of inhibition of glucose uptake or utilization in the glycolytic pathway of the heart. The site of blockade by halothane must precede the conversion of pyruvate to acetyl CoA.

The present studies represent an attempt to localize further the site of halothane action in the glycolytic sequence by using the metabolizable substrate, fructose. We found a few references with respect to the utilization of fructose by the heart, and those indicated that fructose was a poorly-utilized substrate compared with glucose.[6, 7] Therefore, we first determined dose–response curves in the substrate-depleted heart to determine if, and at what concentrations, fructose could serve as a source of fuel for the contractile process. Next, we attempted to determine whether fructose was metabolized via the phospho-fructokinase step and, finally, we observed the effect of fructose on the halothane-depressed atria. From the results we conclude: 1) fructose can serve as a source of fuel for the contraction of isolated rat atria; 2) metabolism of fructose occurs via the phospho-fructokinase step; 3) fructose partially restores the con-

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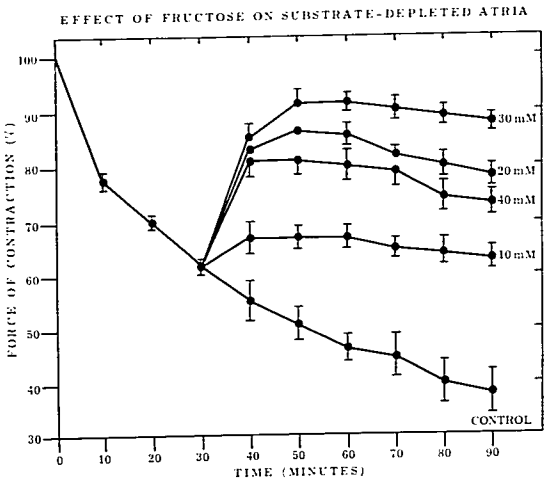

FIG. 1. Effect of fructose on substrate-depleted atria. In this and subsequent figures zero time is that time following a 60-minute equilibration of the atria in the normal Krebs-Ringer glucose medium. By substrate-depleted atria is meant exposure to substrate-free, but otherwise normal, medium. Fructose was added 30 minutes after exposure to substrate-free medium. Vertical bars indicate ± one standard error of the mean. Each curve represents six experiments.

tractility of atria depressed by halothane. Thus, the site of halothane blockade must be either the uptake of glucose or its utilization prior to the phospho-fructokinase step.

Methods

Male Sprague-Dawley rats weighing 180 to 200 g, having *ad lib.* access to food and water, were employed. Atria were removed from decapitated rats and suspended in a modified Krebs-Ringer bicarbonate glucose medium of the following composition (mM)[5]: 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 gassed with 95 per cent O_2–5 per cent CO_2 at pH 7.4 and 30 C. 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 in the above medium was allowed before readings were taken. The experimental values of contractility (peak tension) were compared with those of the control records obtained at zero time (following equilibration) and expressed as per cent change in developed tension. Halothane was administered to the medium by means of the anesthetistat previously described by Paradise and Griffith.[2, 3] Halothane concentration in the medium was determined at 10-to-30-min intervals with a gas chromatograph throughout the experimental period.[2]

In some experiments (fig. 1) the medium was changed to substrate-free (*i.e.*, free of glucose) following the one-hour equilibration period.

In the experiments with bicarbonate-free medium (fig. 2), the procedures were conducted by means of techniques previously described by Ko *et al.*[9] The bicarbonate-free medium was prepared by replacing the sodium bicarbonate in the Krebs-Ringer glucose medium with an equivalent concentration of sodium chloride and bubbling with 100 per cent O_2. The *p*H of the bicarbonate-free medium was initially adjusted to 7.4 with dilute sodium hydroxide, just prior to the experimental procedure.

Results

Effect of Fructose on Substrate-depleted Atria

The effect of fructose on the functional properties of atria had to be established to

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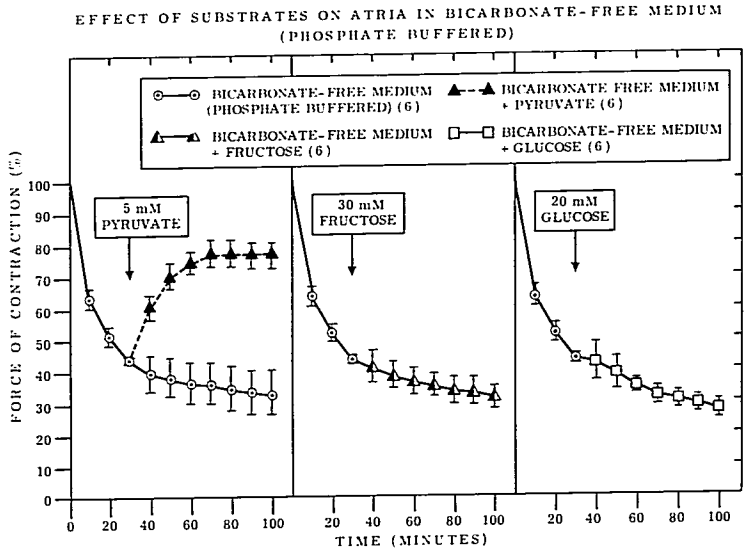

FIG. 2. Effect of substrates on atria in bicarbonate-free medium.

study its action on halothane-depressed atria. Experiments were designed using substrate-depleted medium to determine the contractile behavior of atria in the absence of exogenous substrate, to provide control data with which the responses to fructose might be compared. Results are summarized in figure 1. Developed tension of the atria progressively decreased in the substrate-free medium after the one-hour equilibration period with Krebs-Ringer bicarbonate glucose medium.

After 30 min in the substrate-free medium, fructose was added at a concentration of 10, 20, 30 or 40 mM (fig. 1). The addition of fructose resulted in marked recovery of the force of contraction; the maximally effective concentration of fructose was 30 mM. The same concentration of the nonmetabolized sugar, sucrose, however, had no effect on the substrate-depleted atria, indicating that the action of fructose at this high concentration is a result of its metabolism.

Effects of Fructose, Glucose and Pyruvate on Atria Depressed by Bicarbonate-free Medium

Having established the availability of fructose as an energy source for contraction, it was important to determine the pathway by which fructose is utilized. Shaw and Stadie[10, 11] demonstrated the dependence of the phospho-fructokinase reaction in the isolated rat diaphragm on the presence of bicarbonate in the medium. In the absence of bicarbonate neither radioactive glucose nor fructose in the bathing medium could be incorporated into radioactive fructose diphosphate, although earlier products of the metabolism of glucose were found. In the presence of bicarbonate both glucose and fructose could be converted to fructose diphosphate. If bicarbonate were also necessary for the activity of phospho-fructokinase in rat atria, and if fructose were metabolized via this enzyme, we would expect: 1) a fall in contractility of atria incubated in bicarbonate-

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free glucose medium and 2) restoration of contractility with pyruvate (a substrate not metabolized via phospho-fructokinase) but not with added glucose or fructose. Figure 2 demonstrates these findings exactly. Thus, it appears that fructose is metabolized via the phospho-fructokinase enzyme.

Effect of Fructose on Halothane-depressed Atria

Addition of 30 mM fructose 30 minutes after start of administration of halothane resulted in a prompt and sustained increase in force of contraction despite the continued administration of halothane (fig. 3). This effect was similar to that seen with pyruvate, acetate and lactate, but not glucose, on halothane-depressed atria.[5] This antagonism of halothane depression by fructose but not glucose, along with the evidence pointing to the utilization of fructose via the phospho-fructokinase step, suggests that the mechanism of the negative inotropic action of halothane in these atria is blockade of the uptake or utilization of glucose prior to the phospho-fructokinase step.

Effect of Fructose on Normal Atria

Addition of 30 mM fructose to atria bathed in the normal Krebs-Ringer bicarbonate glucose medium resulted in no demonstrable change in cardiac contractility. These results confirm similar observations by Gimeno *et al.*[7] and emphasize the importance of halothane for the positive inotropic effect of fructose.

Discussion

Fructose, when used in high concentration (30 mM) has been shown to serve as an excellent substrate for the maintenance of contractility by the isolated rat atria. Opie *et al.*[6] found fructose (5 mM) to be taken up and metabolized to CO_2 less than ⅓ as rapidly as glucose (5 mM). Gimeno *et al.*[7] demonstrated that fructose, at concentrations of 5.5 or 11 mM, is utilized for contractility but not nearly as efficiently as the corresponding concentrations of glucose. Thus, the uptake of fructose

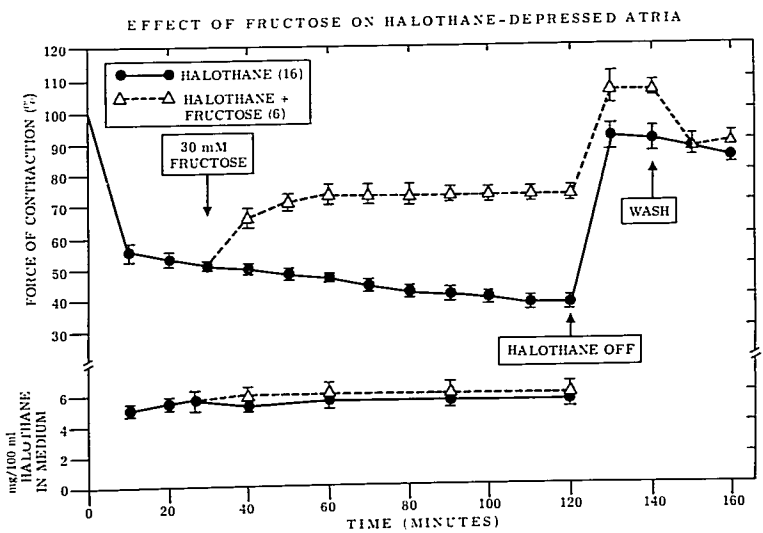

Fig. 3. Effect of fructose on halothane-depressed atria.

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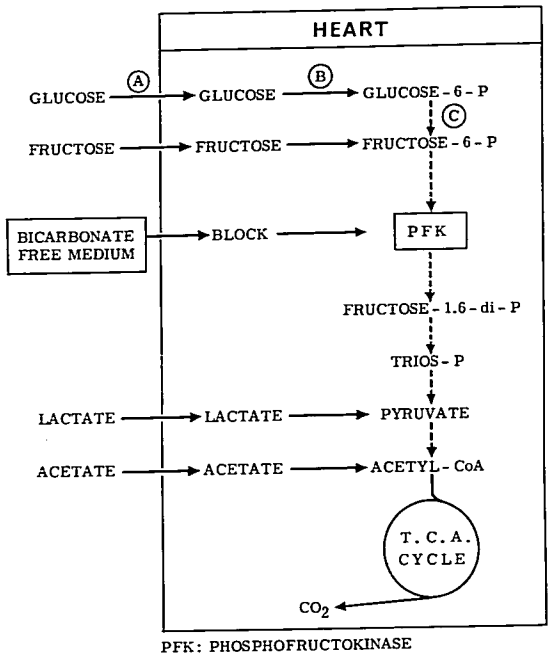

FIG. 4. Schematic representation of glycolysis, showing points at which various substrates enter the scheme. A, B and C represent possible sites of halothane blockade. Lactate, acetate, pyruvate, but not glucose, have been shown to overcome halothane-induced cardiac depression.[5] In this report fructose also was shown to overcome depression by halothane but not by bicarbonate-free medium which inhibits phospho-fructokinase.

or its conversion to fructose-6-phosphate may be rate-limiting, higher concentrations of fructose than glucose being necessary for similar effects.

The lack of a positive inotropic effect of fructose in bicarbonate-free medium is in agreement with the data of Shaw and Stadie, obtained in a study of the rat diaphragm.[10, 11] They showed a failure of conversion of labelled fructose to fructose diphosphate in bicarbonate-free medium, along with other data indicating the importance of bicarbonate for progression of the phospho-fructokinase reaction. Thus, fructose apparently is utilized via phospho-fructokinase in the diaphragm.

A number of studies in the rat heart are consistent with a lack of phospho-fructokinase activity in bicarbonate-free medium. In this medium glucose is relatively ineffective in maintaining the contractile activity of rat ventricle strips.[12-14] Rice and Berman[15, 16] 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,[13] and Hood and Saunders have reported that glucose is rapidly oxidized in this medium.[17]

The positive inotropic effect of fructose on halothane-depressed, but not on normal, atria, along with the previous report[5] showing a lack of positive inotropic effect of additional glucose in halothane-depressed atria, suggest that the negative inotropic effect of halothane is at least partly the result of an interference with glucose uptake or metabolism prior to the phospho-fructokinase step. Figure 4 is a

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schematic representation of the glycolytic pathway. The possible sites of halothane blockade are *A*) uptake of glucose into the heart, *B*) conversion of glucose to glucose-6-phosphate by the enzyme hexokinase, and *C*) conversion of glucose-6-phosphate to fructose-6-phosphate by phosphohexose isomerase. Site B is not very likely since, in addition to glucose, hexokinase catalyzes the conversion of fructose to fructose-6-phosphate, a reaction seemingly unimpeded since fructose is apparently well utilized by the halothane-depressed atria. Site A has interesting implications since, at least in the erythrocyte, fructose and glucose appear to be taken up by different mechanisms: at least, the kinetics for uptake are different.[18] It would be of interest to study the uptake of the nonmetabolizable sugar, 3-0-methyl glucose, in the presence of halothane. This sugar is taken into the heart by the same mechanism as glucose, with which it competes for uptake. This will be the subject of future investigations.

A report by Hoech *et al.*[19] indicates that halothane (5 vol per cent) is without effect on anaerobic glycolysis of rat brain. These studies, however, were done on homogenates, where uptake is not a factor. If glycolysis of homogenized brain and intact atria are similar, this would seem a further indication that uptake is a likely mechanism of halothane blockade.

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