Effects of Monosaccharides, Pyruvate, Acetate, and Butyrate on the Developed Tension and ATP Levels of Hypodynamic Isolated Rat Atria[1] (35494)

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Inasmuch as myocardial utilization of metabolic substrates can be associated with tissue energy production processes, it is not unlikely that the ability of substrates to restore the contractile tension of isolated cardiac preparations, made hypodynamic by means of their omission from the external medium, could be related to their role on myocardial metabolic reactions required for muscle contraction.

Isolated rat atria suspended in bicarbonate medium suffer a distinct contractile decay after inhibition of glycolysis, as well as in the absence of external glucose (1). Substrates such as pyruvate or lactate, readily metabolizable by the myocardium (2–7), can bring about only a partial recovery of the developed tension of hypodynamic rat atria preparations; whereas glucose restores the isometric contractile tension to the levels observed in control nonhypodynamic conditions (1, 8). These, as well as other evidence (9, 10–11), suggested to us that glycolysis has a unique importance in the regulation of the developed tension of isolated rat atria.

In the present study, we have compared the ability of glucose, mannose, fructose, pyruvate, acetate, and butyrate to modify the peak contractile tension of rat atria previously depressed by incubation in substrate-free medium. Simultaneously, the effects of substrate removal and readmission on atrial ATP levels, were also explored.

Material and Methods. Male rats, weighing 120–130 g, were decapitated and their hearts were removed. The atria were separated from the ventricles and suspended in a modified Krebs–Ringer–bicarbonate medium (K-R-B) containing (m*M*): Na^+, 145; K^+, 4; Ca^{2+}, 1.22; Mg^{2+}, 1.33; Cl^-, 126; HCO^- 25.3; SO_4^{2-} 1.33; PO_4^{2-}, 1.2. The substrate for this medium in control conditions was glucose at 5.5 m*M*. The solution was gassed through a fritted glass disc with a mixture of 95% O_2, 5% CO_2 (at a flow rate of 150 ml/min) and maintained at pH 7.4 and 30° throughout the experiments.

The right atrium was attached to a glass holder carrying platinum stimulating electrodes. The left atrium was connected to a transducer (Statham S. G., model UC3) with a silk thread. A constant resting tension of 750 mg was applied to the atria by means of a micrometric device and the isometric developed tension (peak tension) was recorded on direct writing oscillographs (Dynograph, type RB or Sanborn model 321). Atria were driven electrically with square waves from a stimulator (Grass, model SIU-4) at a constant rate of 200/min; the stimulus being of 5–7 V and 1-msec duration. The equilibration time in K-R-B–glucose medium was 60 min. The recorded developed tension at the end of this period was considered as the initial control developed tension. Adequacy of oxygenation, stability of the level of initial control developed tension with time, functional and metabolic characteristics of these atrial preparations, as well as the effects of substrate addition on osmotic pressure and pH of the medium have been previously reported (1, 8, 10).

The hypodynamic condition of atria was

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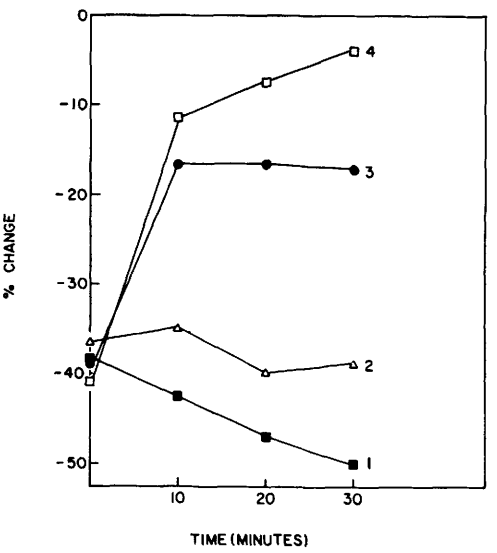

FIG. 1. Effects of 5.5 m*M* glucose, fructose, or mannose on the developed tension of hypodynamic rat atria suspended in substrate-free medium. After the equilibration period, the initial control developed tension level was recorded; then the atria were made hypodynamic by washing 3 times with substrate-free medium. The developed tension levels, indicated on the ordinates at 0 time, represent the percentage of change from the initial control developed tension levels 30 min after substrate omission. Substrates were added at this moment. (1) controls in substrate-free medium with no additions (6 atria); (2) fructose (6 atria), (3) mannose (6 atria); and (4) glucose (6 atria).

induced after the equilibration period by washing the atria three times with substrate-free medium. The contractile decay after this moment was followed for 30 min; different substrates were added at this time, and their effects were explored for the next 30 min. Contractile depression in substrate-free medium was also controlled in further preparations for a period of up to 60 min.

The developed tension values were recorded in milligrams, and their variations after substrate removal and readmission were expressed as percentage of changes from the levels of initial control developed tension.

For ATP analysis, the atria were frozen with wide flat-jawed copper clamps precooled in liquid nitrogen. ATP was determined by the method of Kornberg (12), the analysis being made: (a) at the end of the equilibration period in normal K-R-B–glucose medium, (b) at 30 or 60 min after glucose removal, and (c) at 30 min after the readmission of different concentrations of several substrates.

The parameters explored under the various experimental conditions were compared using the Student's t test. Differences were considered significant if $p = 0.05$ or less.

Results. Effects of glucose, mannose, or fructose on the developed tension (peak tension) *of isolated rat atria made hypodynamic by substrate omission.* Substrate removal caused a progressive decline of the developed tension of isolated rat atria (Fig. 1, curve 1). The effects of substrate readmission at 5.5 m*M* are summarized in Fig. 1, curves 2, 3, and 4. Glucose (Fig. 1, curve 4) restored the initial control developed tension levels; mannose also stimulated peak tension but significantly less than glucose (Fig. 1, curve 3) and fructose only prevented a further decrement of contraction (Fig. 1, curve 2). At a higher concentration (11.0 m*M*), glucose stimulated atrial peak tension to levels above those of initial controls (Fig. 2, curve 4). Mannose at 11.0 m*M* also produced contractile augmentation but the levels of developed tension were smaller than those elicited by glucose (Fig. 2, curve 3). Fructose at 11.0 m*M* only produced a small improvement of atrial peak tension (Fig. 2, curve 2).

Inasmuch as it has been shown that increased osmolarity is able to induce inotropic effects on cardiac preparations (13), the contractile response of atria after the addition of high concentrations of substrates could have been related to osmotic changes. To explore this possibility, the experiments depicted in Fig. 2 were repeatd under the following conditions: the concentration of substrates (glucose, mannose, or fructuse) were maintained at 5.5 m*M* but in addition 5.5 m*M* sucrose was also admitted. Under these conditions, the levels of developed tension were identical to those observed with 5.5 m*M* of the substrates, therefore it seems unlikely that the stimulation of atrial developed tension produced by 11.0 m*M* glucose or mannose is associated with hyperos-

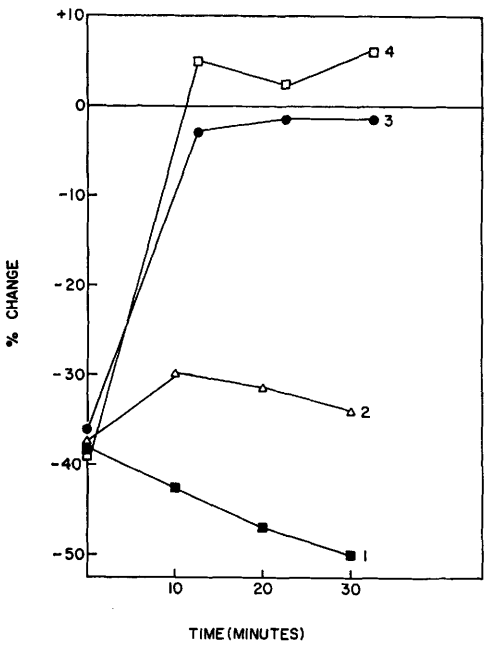

FIG. 2. Effects of 11.0 m*M* glucose, fructose, or mannose on the developed tension of hypodynamic rat atria suspended in substrate-free medium. Conditions and details as described for Fig. 1. (1) controls in substrate-free medium with no additions (6 atria); (2) fructose (7 atria), (3) mannose (6 atria); and (4) glucose (8 atria).

molarity of the medium. On the other hand, the increased atrial peak tension induced by glucose or mannose also occurred in atria from reserpinized animals (5 mg/kg, 24 hr before sacrifice), in atria exposed to tyramine to the point of tachyphylaxis, and in atropinized (0.01 m*M* atropine) atria. These findings would indicate that the action of substrates on atria is not mediated by adrenergic or cholinergic mechanisms.

Effects of acetate, butyrate, or pyruvate on the developed tension (peak tension) *of isolated rat atria made hypodynamic by substrate omission.* Figure 3 shows the inotropic effects elicited by three readily oxidizable substrates (acetate, butyrate, or pyruvate) on hypodynamic rat atria suspended in substrate-free medium. As shown, the addition of 5.5 m*M* acetate (Fig. 3, curve 2), butyrate (Fig. 3, curve 4) or pyruvate (Fig. 3, curve 3) produced a smaller stimulation of atrial peak tension than that induced by an identical concentration of glucose (Fig. 1, curve 4). Furthermore, the contractile augmentation observed with 11.0 m*M* acetate, pyruvate, or butyrate (Fig. 4, curves 2, 3, and 4, respectively) was not only less marked than that with 5.5 m*M* but also significantly smaller than that observed after 11.0 m*M* glucose or mannose.

Effect of substrates on the ATP concentration of isolated rat atria. The concentration of ATP on atria equilibrated during 60 min in normal K-R-B–glucose medium was 2.81 ± 0.10 μmoles/g (w/w), $n = 6$ (mean ± SEM). This concentration remained unchanged during a further period of 60 min (2.86 ± 0.05 μmoles/g (w/w), $n = 6$). The removal of glucose produced a distinct reduction of ATP concentration, the levels found 30 min after substrate omission being 2.43 ± 0.05 μmoles/g (w/w), $n = 6$. No further change was observed after an additional 30 min period (2.41 ± 0.11 μmoles/g (w/w), $n = 6$). The admission of glucose, mannose, or fructose at 5.5 m*M* (Table I) increased in 30 min the ATP concentration toward control levels. Similarly, pyruvate,

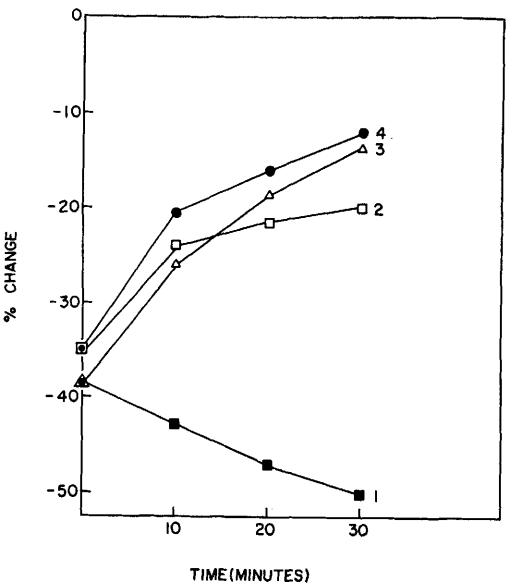

FIG. 3. Effects of 5.5 m*M* acetate, pyruvate, or butyrate on the developed tension of hypodynamic rat atria suspended in substrate-free medium. Conditions and details as described for Fig. 1. (1) controls in substrate-free medium with no additions (6 atria); (2) acetate (6 atria); (3) pyruvate (9 atria); and (4) butyrate (8 atria).

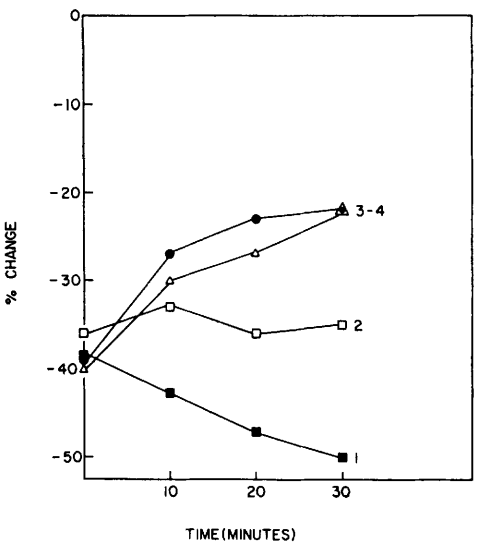

FIG. 4. Effects of 11.0 m*M* acetate, pyruvate, or butyrate on the developed tension of hypodynamic rat atria suspended in substrate-free medium. Conditions and details as described for Fig. 1. (1) controls in substrate-free medium with no additions (6 atria); (2) acetate (7 atria); (3) pyruvate (6 atria); and (4) butyrate (6 atria).

acetate, or butyrate at 5.5 m*M* also increased ATP levels (Table I). The increment of the concentration of all the substrates up to 11.0 m*M* raised ATP concentration up to values comparable to those observed with 5.5 m*M* (Table I).

Discussion. The present study demonstrates that the omission of substrate from the Krebs–Ringer–bicarbonate medium results in a significant reduction of developed tension levels of isolated rat atria. This observation is in agreement with previous reports from our laboratory (1, 8, 10). The rapid reduction of contractile peak tension observed in substrate-free medium suggests that the endogenous energy stores of rat atria are not adequate for the support of the processes involved in the maintenance of the developed tension magnitude. Also, the depression of contraction was accompanied by a significant reduction of atrial ATP concentration. Inasmuch as tissue ATP levels represent a balance between energy production and energy utilization processes, it is interesting that the fall of ATP occurs despite a depressed contractile activity, a situation which would coincide with a diminished ATP utilization.

Furthermore, it was also found that the rapid atrial hypodynamic state observed after substrate omission can be modified with different effectiveness by different substrates. Increasing concentrations of readily fermentable substrates, such as glucose or mannose, were more effective than acetate, pyruvate, or butyrate in restoring peak tension levels of hypodynamic rat atria. This finding indicates the unique importance of glycolysis for atrial contraction by a mechanism other than the mere supply of substrates for the tricarboxylate cycle.

It has been demonstrated that when the concentration of glucose is doubled, the rate

TABLE I. Effect of Substrates on the ATP Concentration of Hypodynamic Rat Atria.[a]

Substrate	Conc (m*M*)	ATP[b] (μM/g; w/w)	*p* values[c]
None	—	2.43 ± 0.05 (6)[d]	—
	—	2.41 ± 0.11 (6)[e]	—
	5.5	3.01 ± 0.10 (6)	>0.001
Glucose	11.0	2.84 ± 0.08 (8)	>0.005
	5.5	2.93 ± 0.11 (6)	>0.001
Mannose	11.0	3.03 ± 0.10 (6)	>0.001
	5.5	2.75 ± 0.03 (6)	>0.001
Fructose	11.0	2.99 ± 0.07 (7)	>0.001
	5.5	2.80 ± 0.09 (6)	>0.005
Pyruvate	11.0	2.87 ± 0.07 (6)	>0.001
	5.5	2.79 ± 0.09 (6)	>0.005
Acetate	11.0	2.67 ± 0.07 (8)	>0.020
	5.5	2.67 ± 0.08 (7)	>0.025
Butyrate	11.0	2.92 ± 0.07 (6)	>0.001

[a] The hypodynamic state was considered established 30 min after washing the atria with substrate-free medium.

[b] Mean ± SEM at 30 min after substrate addition. Numbers in the parentheses refer to the number of atria on which ATP determinations were performed.

[c] Values at 30 min after substrate addition vs values at 30 min in substrate-free medium.

[d] Mean ± SEM at 30 min in substrate-free medium. Numbers in parentheses refer to the number of atria on which ATP determinations were performed.

[e] Mean ± SEM at 60 min in substrate-free medium. Numbers in the parentheses refer to the number of atria on which ATP determinations were performed.

of metabolism of this substrate is also almost doubled (14). Therefore, it is likely that the contractile effects of glucose on atria could be associated with an increased metabolic rate. Inasmuch as mannose is also transported and metabolized by muscle cells (15–17), it is reasonable to assume that the effects of mannose on hypodynamic rat atria are also associated with a metabolic mechanism similar to that of glucose. It should be noted that the experimental findings indicate that the contractile influence of these substrates does not appear to be related to osmotic, adrenergic, or cholinergic mechanisms.

The cardiac metabolism of fructose is significantly less than that of glucose and it has been considered doubtful that fructose could serve directly as a fuel for myocardial contraction (14, 18). Our observations indicate that fructose is unable to stimulate developed tension of hypodynamic atria; however, it is also clear that fructose halted the decrement of contraction observed when no substrate was present in the medium.

With regard to the effect of substrates on ATP concentration of hypodynamic rat atria, it is interesting to note that all the substrates tested, despite their different potency in restoring peak tension, produced a comparable increment of ATP levels. This finding is in accord with the observations of Furchgoot and Lee (19) of the lack of a simple and direct correlation between muscle developed tension magnitude and ATP concentration.

Summary. The effects of several substrates on the developed tension (DT) of isolated rat atria, made hypodynamic by the removal of glucose, were investigated. The addition of pyruvate, acetate, or butyrate at 5.5 m*M*, led to a partial recovery of the DT. Nevertheless, when they were incorporated in concentration of 11.0 m*M*, the recovery of DT was significantly smaller than that obtained with lower concentrations. The addition of 5.5 m*M* glucose restored the DT completely; whereas higher concentrations (11.0 m*M*) stimulated the DT above control levels. Mannose, at 5.5 m*M*, produced only a partial recovery of DT; whereas at a higher concentration (11.0 m*M*) it was also able to recover the DT completely. Although fructose at both concentrations did not produce a significant recovery of DT, it halted the depression caused by the absence of substrate. The atrial levels of ATP were decreased by the absence of glucose, and recovered by all the substrate studied, independently of their molarities and of the degree of contractile recovery attained. From the present results, it appears that glycolysis plays a fundamental role in the regulation of DT levels; and, furthermore, that the modification of ATP concentration is not the only mechanism by which different substrates can alter the DT of hypodynamic rat atria.

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