Effects of 2-Deoxy-D-glucose on Isolated Atria

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SUMMARY

2-Deoxyglucose depresses atrial contractility without altering membrane potentials significantly, although the ATP level is progressively reduced. Pyruvate is able to counteract the 2-deoxyglucose depression only partially, indicating some relation between the Embden-Meyerhof pathway and contraction. Nevertheless, pyruvate elevates the ATP level above the control values. 2-Deoxyglucose depresses more rapidly in the absence of glucose. Addition of glucose restores the developed tension partially, indicating that contractility to some extent depends either on the initial phases of glucose metabolism, or on the operation of a nonglycolytic pathway, since there is evidence that 2-deoxyglucose blocks the Embden-Meyerhof pathway under these conditions.

Atrial rate is not altered by removal of exogenous glucose, is only slightly depressed by 2-deoxyglucose in glucose medium, but is markedly slowed by 2-deoxyglucose in the absence of glucose. Since pyruvate has only a limited ability to restore the depressed rate, the discharge of pacemaker calls is also related to glucose utilization.

INTRODUCTION

Glucose metabolism in the heart has been well studied, but the relation of cardiac function to the operation of the Embden-Meyerhof pathway is not clear. 2-Deoxy-D-glucose (2-DG) interferes with glucose utilization and presumably is much more specific than inhibitors such as iodoacetate. The effects of 2-DG on atrial contractility, rate, and membrane potentials have been investigated in order to establish the nature of the functional dependence on this phase of the metabolism. 2-DG may inhibit certain hexokinases (1) and directly reduce the transport of glucose into cells (2). In addition, 2-DG is phosphorylated to 2-deoxy-D-glucose 6-phosphate (2-DG-6-P), which has been shown to inhibit competitively phosphoglucose isomerase (3–5), and possibly phosphofructokinase or aldolase (4). 2-DG-6-P may also contribute to the suppression of glucose uptake (6, 7). A depletion of ATP also occurs (8–10), perhaps partly by the formation of the nonmetabolizable 2-DG-6-P (11), and this may secondarily reduce glucose phosphorylation. In any event, the inhibitions seem to be exerted specifically on the early phases of glucose utilization.

METHODS

Male rats were decapitated, and the atria were removed and suspended in a modified Krebs-Ringer bicarbonate medium at pH 7.4 with the following composition: Na^+ 145 mM, K^+ 6 mM, Ca^{++} 1.22 mM, Mg^{++} 1.33 mM, Cl^- 126 mM, HCO_3^- 25.3 mM, SO_4^{2-} 1.33

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mM, phosphate 1.2 mM, and glucose 5.5 mM. The medium was gassed with 95% O_2: 5% CO_2 and maintained at 30°. A constant resting tension of 750 mg was exerted on the atria by means of a micrometer head, and the contractile activity was recorded through a strain gauge. The atria were stimulated at a rate of 200/min except in the studies on the spontaneous rate. A 60-min equilibration period was allowed before readings were taken. Transmembrane potentials were obtained with microelectrodes by methods previously described (12). ATP was determined with firefly luciferase and a Farrand fluorometer (13). 2-DG was used at 10 mM and pyruvate at 5 mM.

RESULTS

Effects of Glucose Removal

The behavior of atria in the absence of exogenous glucose was determined to provide control data with which the responses to 2-DG might be compared. The results are summarized in Fig. 1. The developed tension decreases progressively, and by 30 min is —45%.[3] This is not a hypoosmotic effect due to removal of glucose since replacement of the glucose with isosmolar sucrose does not alter the rate of failure. The addition of glucose at 30 min restores the contractile activity completely, whereas pyruvate only restores the contractions to a level of —15%. No changes in resting tension during failure in glucose-free medium could be detected.

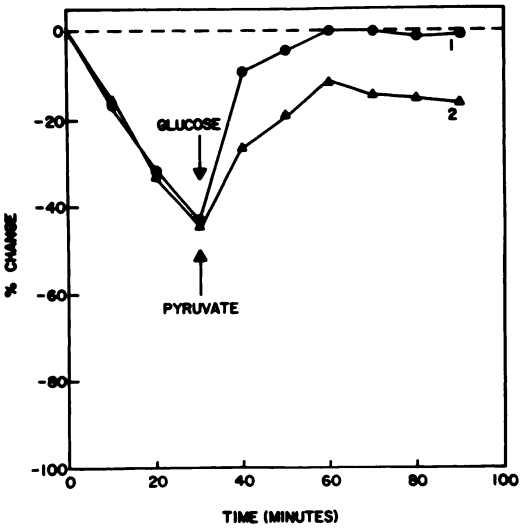

FIG. 1. *Contractile depression in glucose-free medium, and the effects of glucose and pyruvate*

After the equilibration period the atria were washed 4 times with glucose-free medium at 0 time; 5.5 mM glucose (curve *1*) and 5 mM pyruvate (curve *2*) were added at arrows. Each curve is a mean of 6 atria.

Effects of 2-DG in Glucose Medium

2-DG causes a progressive decline in developed tension, the levels being —38% at 30 min and —67% at 90 min (Fig. 2, curve *1*). The addition of pyruvate at 30 min rapidly stops the contractile decline and slowly restores the developed tension to a level of —27% (curve *2*). If 2-DG and pyruvate are added together initially, the

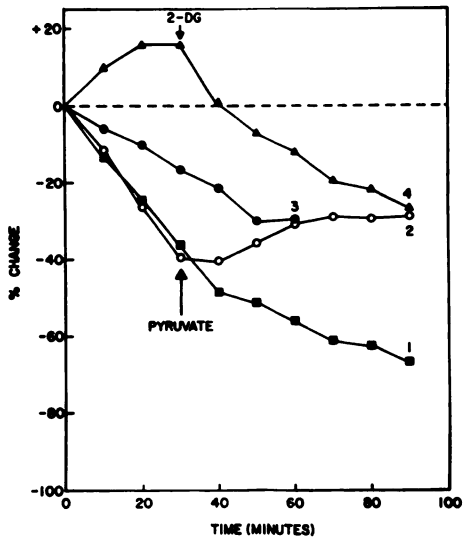

FIG. 2. *Effects of 2-DG on atrial contractility in glucose medium*

Curve *1*: 10 mM 2-DG added at 0 time (3 atria). Curve *2*: 10 mM 2-DG added at 0 time and 5 mM pyruvate added at arrow (7 atria). Curve *3*: 10 mM 2-DG and 5 mM pyruvate added together at 0 time (11 atria). Curve *4*: 5 mM pyruvate added at 0 time and 10 mM 2-DG added at arrow (8 atria).

[3] Contractile depression is indicated by per cent changes from the control initial postequilibration level.

contractile depression occurs more slowly than in the absence of pyruvate and the final developed tension reached is —29% (curve *3*). The addition of pyruvate initially leads to a moderate elevation of the developed tension; 2-DG added at 30 min causes contractile depression which reaches a value of —26% (curve *4*). It is seen that 2-DG in the presence of pyruvate brings about essentially the same final contractile depression (—26% to —29%) whatever the order of addition. The resting tension is usually increased somewhat during the action of 2-DG in the absence of pyruvate, indicating a tendency for the atria to go into contracture.

Effects of 2-DG in Glucose-Free Medium

Atria in glucose-free medium are depressed by 2-DG more rapidly and markedly than in glucose medium (Fig. 3). At

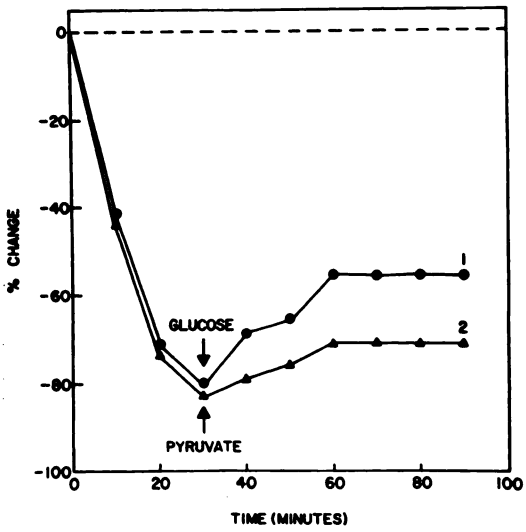

FIG. 3. *Effects of 2-DG on atrial contractility in absence of glucose*

10 mM 2-DG added at 0 time; 5.5 mM glucose (curve *1*) and 5 mM pyruvate (curve *2*), added at arrows. Each curve is a mean of 6 atria.

30 min the developed tension is —80% compared to —38% in the presence of glucose. Since glucose and 2-DG mutually interfere with the uptake and phosphorylation of each other, one might expect 2-DG to enter the atria more rapidly and exert a greater inhibition in the absence of exogenous glucose. However, addition of glucose at 30 min restores the developed tension to —55% (curve *1*), which is probably not significantly different from the level reached after 90 min incubation with 2-DG in the presence of glucose (Fig. 2, curve *1*). Thus, even after 30 min exposure to 2-DG, glucose is able to exert a very significant stimulation of atrial contractility. The addition of pyruvate at 30 min immediately stops the contractile decline but restores the contractions to only a small degree (curve *2*). The small effect of pyruvate here is in contrast to the results obtained in glucose-free medium in the absence of 2-DG (Fig. 1), and it appears that 2-DG in some manner reduces the ability of pyruvate to augment the contractility. 2-DG in the absence of glucose invariably elevates the resting tension simultaneously with contractile depression, although definite contracture does not occur, and this can be reversed by both glucose and pyruvate.

The experiments shown in Fig. 4 illustrate the atrial responses under different conditions. Curve *1* shows the partial re-

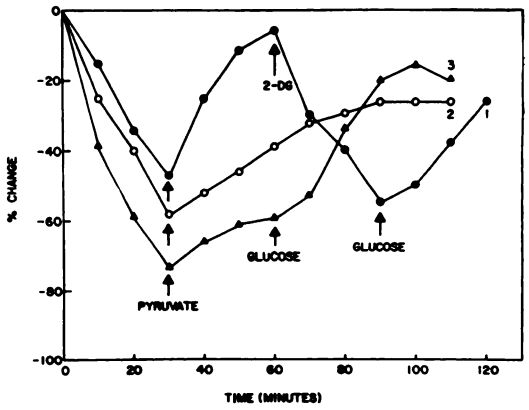

FIG. 4. *Comparison of different treatments on atrial contractility*

Curve *1*: glucose-free medium at 0 time; 5 mM pyruvate at 30 min, 10 mM 2-DG at 60 min, and 5.5 mM glucose at 90 min. Curve *2*: 10 mM 2-DG at 0 time in glucose medium; 5 mM pyruvate at 30 min. Curve *3*: 10 mM 2-DG at 0 time in glucose-free medium; 5 mM pyruvate at 30 min and 5.5 mM glucose at 60 min.

covery produced by pyruvate in glucose-depleted atria, the depression by 2-DG in the presence of pyruvate, and the ability of glucose to restore contractions after incubation with 2-DG. Curve *2* shows the recovery from 2-DG depression produced by pyruvate; it may be noted that the final levels of curves *1* and *2* are the same, glucose, pyruvate, and 2-DG being eventually present in both. Curve *3* shows the slight recovery produced by pyruvate in atria depressed by 2-DG in the absence of glucose; later addition of glucose brings about a much greater recovery than did pyruvate, and again the final level is about the same as in the other experiments.

The inability of 2-DG to suppress markedly the action of glucose, even when the atria are incubated with 2-DG in the absence of glucose, brought up the question whether in rat atria 10 mM is really an inadequate 2-DG concentration. These experiments in glucose-free medium were thus repeated with 30 mM 2-DG. The mean depression in two atria after 30 min was —64%, which is not significantly different from the mean of —82% obtained from 11 atria using 10 mM 2-DG. The addition of glucose brought about recovery to the —42% level, which is actually somewhat more effect than was observed after incubation with 10 mM 2-DG. Increase of the concentration thus does not increase the effectiveness of 2-DG in depressing atrial contractility or preventing the recovery observed with glucose.

Effects of 2-DG and Anoxia

Replacement of the oxygen with nitrogen in the gassing mixture causes an 85% depression of developed tension at 10 min and a 96% depression at 30 min (Fig. 5, curve *1*). Reintroduction of oxygen leads to a rapid and almost complete recovery. Anoxic failure is faster and more complete when 2-DG has been present for 15 min, complete loss of contractility occurring within 10 min (curves *2* and *3*). Reintroduction of oxygen at either 15 or 30 min brings about an approximately 60% recovery, the contractions then declining slowly to levels roughly those which would have been reached if no anoxic period had been introduced. Anoxia in glucose-free medium also causes complete failure within 10 min, and addition of glucose with reintroduction of oxygen at 30 min restores contractions almost completely. Comparable experiments, but with 2-DG present, show that failure occurs even more rapidly (within 5 min), and glucose and oxygen reintroduced at 15 min allow 45% recovery.

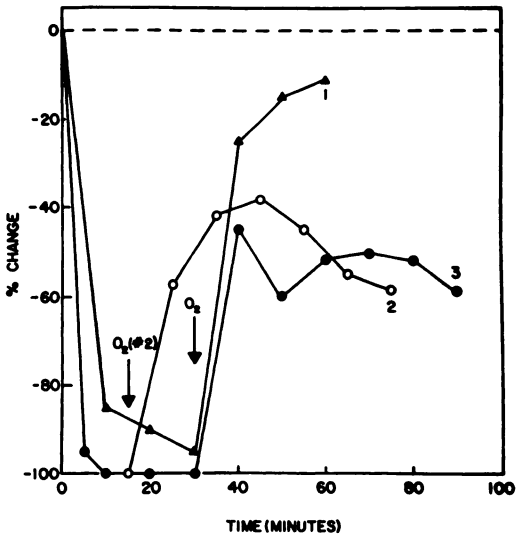

FIG. 5. *Effects of 2-DG on atrial contractility during anoxia*

Oxygen was replaced with nitrogen at 0 time in all. Each curve is a mean of 5 atria. Curve *1*: anoxia alone with oxygen reintroduced at 30 min. Curve *2*: 10 mM 2-DG added at 0 time and oxygen reintroduced at 15 min. Curve *3*: 10 mM 2-DG added at 0 time and oxygen reintroduced at 30 min.

Effects of 2-DG on Atrial Membrane Potentials

2-DG in glucose medium reduces the developed tension 45–50% at 30 min, but no significant changes in the resting potential, action potential magnitude, depolarization rate, or repolarization rate were observed in five atria. The addition of pyruvate at 30 min brings about the partial contractile recovery discussed above, but this is not accompanied by any changes in the membrane potential characteristics. 2-DG is the only inhibitor we

have studied which is able to depress contractility so specifically, all other inhibitors accelerating repolarization and lowering the action potential to varying degrees (12, 14).

Effects of 2-DG on Atrial ATP Levels

The ATP level in atria after 1 hr equilibration in glucose medium is 256 μg/g (mean from 15 atria). 2-DG depressed the contractility of two atria to —54% at 30 min; the mean ATP level at this time was found to be 149 μg/g. In two other atria depressed to a comparable degree by 2-DG, pyruvate addition brought about partial recovery; 30 min after the addition of pyruvate the mean ATP level was 337 μg/g. Despite the elevation of ATP to a level higher than the control, the developed tension was —45% in these experiments. It is interesting that 15 min after addition of pyruvate to normal atria in glucose medium the ATP level was found to be increased to 394 μg/g, at which time the contractility was increased 17%. Two atria were exposed to 2-DG for 30 min, at which time the developed tension was —51%; the glucose concentration was then doubled to 11 mM, and this stopped the contractile decline, although after 30 min only 5% recovery had occurred. The ATP level at this time was 198 μg/g. Elevation of the glucose concentration can thus counteract to some extent the depression by 2-DG and moderately restore the ATP level.

Effects of 2-DG on the Rate of Spontaneously Beating Atria

The atrial rate responds differently than the developed tension to alterations in glucose metabolism (Fig. 6). Rat atria in glucose-free medium exhibit no change in rate over 60 min, this behavior being similar to that of rabbit atria (15), and addition of pyruvate or glucose does not modify the rate (curve *1*). 2-DG in glucose medium reduces the rate to about —10% within 20 min (curve *2*), and this level is apparently maintained. Pyruvate does not appreciably modify this small depression by 2-DG.

Although the rate is not altered in glucose-free medium and is only slightly reduced by 2-DG in the presence of glucose, 2-DG in the absence of glucose depresses the rate rapidly and markedly (curve *3*). The addition of pyruvate brings about a slow and rather poor recovery. The contractile changes in spontaneously beating atria brought about by glucose removal, 2-DG, and pyruvate addition are quite similar to those in the electrically driven atria.

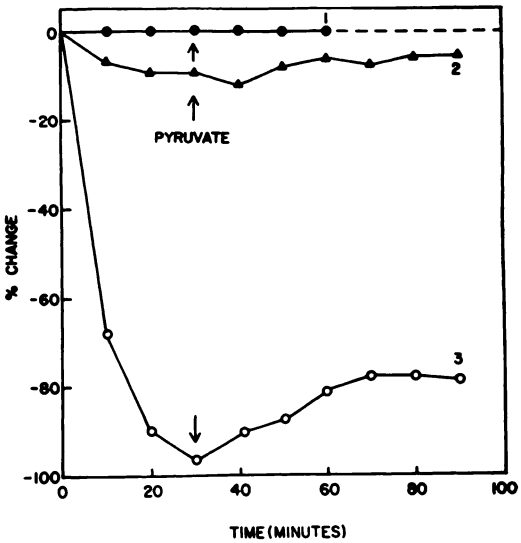

FIG. 6. *Changes in atrial rate due to disturbances in glucose metabolism*

Curve *1*: glucose-free medium at 0 time; 5.5 mM glucose or 5 mM pyruvate added at arrow (4 atria). Curve *2*: 10 mM 2-DG added at 0 time in glucose medium; 5 mM pyruvate added at arrow (6 atria). Curve *3*: 10 mM 2-DG added at 0 time in glucose-free medium; 5 mM pyruvate added at arrow (3 atria).

DISCUSSION

The purpose of this report is to present the basic data on the functional responses of atria to 2-DG. Some of these results are rather surprising and provide interesting possibilities for the metabolic control of contractile activity. Any attempt at this time to explain these results in detail would have to involve too many assumptions. One needs information on the concentrations of intermediates and studies on the metabolic fate of labeled substrates under the conditions described, and this investigation

is now being undertaken. What we believe to be the fundamental problems brought out in this work will be stated, and they will be discussed in general terms.

The atrial responses to glucose-free medium and to 2-DG are different in some respects. The contractile depression is faster with 2-DG. The most obvious explanation is that in the absence of glucose the atria can utilize glycogen, whereas 2-DG blocks this. However, 2-DG depression is not accompanied by changes in the membrane potentials, whereas in glucose-free medium one observes membrane potential changes characteristic of metabolic inhibition (e.g., shortening of the action potential duration) (14). 2-DG in the absence of glucose produces a definite and rapid depression, indicating that glycogen utilization is probably occurring, assuming that 2-DG exerts its direct actions only on the Embden-Meyerhof pathway. In comparing the effects of glucose-free medium and 2-DG one must bear in mind that 2-DG may reduce ATP not only by metabolic block, but also by the formation of 2-DG-6-P.

Previous work with 2,4-dinitrophenol (14) and other metabolic inhibitors (12) had led us to believe that ATP levels regulated those membrane processes concerned with the action potential, but 2-DG can depress contractility over 50% and reduce the ATP level comparably without altering the action potential. The relative lack of effect of 2-DG on cardiac membrane potentials has also been reported for cat papillary muscle (16). Indeed, in anoxic preparations in glucose-free medium, 2-DG at 50 mM temporarily lengthens the action potential duration in a manner similar to glucose, although not as effectively. The most likely explanation for the lack of effect by 2-DG on the action potential is that the ATP is compartmentalized and that the membrane ATP is for some reason not reduced by 2-DG.

Another problem is why pyruvate is unable to counteract completely either the effects of glucose-free medium or 2-DG. One might postulate that pyruvate does not penetrate sufficiently well, but on the other hand pyruvate is able in normal atria to elevate the contractility and ATP levels, and in atria depressed by 2-DG, pyruvate increases the ATP level to values higher than in the controls. Thus pyruvate is able to penetrate readily and generate ATP, but in the absence of exogenous glucose or during inhibition of glycolysis, pyruvate can produce only a partial restoration of contractions. This would lead one to suppose that the contractile activity depends in some manner on the operation of the Embden-Meyerhof pathway, particularly on the phosphorylation of glucose. The ATP arising from pyruvate may be unavailable for some aspect of contraction, but it is quite possible that some factor other than ATP is involved.

It is surprising that after incubation of the atria with 2-DG for 30 min in the absence of exogenous glucose, the addition of glucose is able to produce a marked restoration of contractions, and is indeed more effective than pyruvate. One might assume that 2-DG at 10 mM is not able to block glucose utilization very effectively, but then why does 2-DG depress contractility so readily? Even 30 mM 2-DG is unable to block glucose effects. It is reasonable to suppose that the Embden-Meyerhof pathway is blocked by 2-DG at some early site or sites, and that glucose restores contractions either by its entry into the atrial cells or by its subsequent phosphorylation, rather than by its metabolism through the entire Embden-Meyerhof pathway. A shift in the glucose metabolism through the pentose-phosphate pathway is also a possibility but there is little evidence that this pathway is important in cardiac muscle. Although the pentose-phosphate pathway has been stated to be operative in rabbit ventricle (17), it appears not to be important in rabbit atria (17), guinea pig ventricle (18), rat ventricle (19), or dog ventricle (20). Further studies with rat atria on this point are required. In contrast to these results with rat atria, 2-DG seems to be able to block quite effectively the response to glucose in the cat papillary (16) after incubation in the absence of glucose. Pyruvate restores contractions less readily after 2-DG than after glucose-free

medium, and one must consider the possibility that 2-DG can suppress pyruvate utilization. Such an inhibition has been observed in ascites carcinoma cells (21), but here 2-DG, like glucose, depresses respiration (Crabtree effect), whereas in cardiac tissue glucose does not depress respiration. Furthermore, pyruvate is able to elevate ATP markedly in the presence of 2-DG.

2-DG increases the rate of contractile failure under anoxic conditions, indicating that some energy must be coming from anaerobic glycolysis. Even in the absence of glucose, 2-DG accelerates anoxic failure so that one assumes anaerobic utilization of glycogen occurs. In any event, it is clear that 2-DG is able to act under anaerobic conditions, but from the data one cannot say that the action is either more or less than aerobically.

Finally, one is struck by the very marked difference in the rate response to 2-DG in the presence and absence of glucose (Fig. 6), especially as absence of glucose alone has no effect at all on the rate. One must assume that in glucose-free medium the pacemaker cells are able to derive sufficient energy from the utilization of either glycogen or noncarbohydrate substrates. The fact that 2-DG depresses so markedly under these conditions would point to glycogen being the major source of this energy, unless in atrial tissue 2-DG is able in some manner to reduce the utilization of fatty acids or other substrates. 2-DG in the presence of glucose depresses the rate very little, much less than the contractile activity; this might be explained by only a partial inhibition by 2-DG and a relatively small ATP requirement by the pacemaker cells, but one might also assume that noncarbohydrate substrates are providing the required energy. The failure of pyruvate to bring about much recovery in atria incubated with 2-DG in the absence of glucose would point to a dissociation between pacemaker discharge and tricarboxylate cycle activity, a situation different from that found in rabbit atria (15) where pyruvate is more effective in restoring the rate than the contractility in substrate-depleted tissue.

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