Myocardial protection during ischemic cardiac arrest

Possible deleterious effects of glucose and mannitol in coronary infusates

Cardioplegic protective infusates are designed to induce rapid diastolic arrest and also to reduce or delay the onset of ischemic damage. As this study shows, the use of such infusates can greatly improve postischemic recovery of cardiac function. A number of investigators include glucose, insulin, or mannitol in their infusates in an attempt to increase the amount of protection afforded to the ischemic myocardium. Using an isolated, working rat heart model of cardiopulmonary bypass and ischemic cardiac arrest, we have shown that under certain conditions these additives can be detrimental to tissue protection. The deleterious effects of glucose and mannitol are dose dependent and can be modified by the inclusion of insulin in the infusate. The damaging effects of glucose appear to be both osmotic and metabolic in origin and those of mannitol, purely osmotic. The effects of insulin are complex and may affect a number of cellular processes.

D. J. Hearse, Ph.D., D. A. Stewart, M.Sc., and M. V. Braimbridge, F.R.C.S.,
London, England

The current growth of interest in the use of various cardioplegic and protective infusates during cardiac operations has resulted in the development of a number of different solutions. These solutions are infused into the coronary arteries for a few minutes immediately following aortic cross-clamping. Although the composition of currently used solutions varies, the basic principles underlying their use are similar. First, cardioplegic agents are included in the solution to induce arrest rapidly. Second, protective agents may be included to combat one or more of the deleterious effects of ischemia.

There is an extensive literature which suggests that glucose, in combination with potassium and insulin, may be beneficial to the ischemic or anoxic myocardium during acute myocardial infarction. It is therefore not surprising that glucose, insulin, and potassium have been included in various infusates designed to protect the myocardium during elective ischemic cardiac arrest. Similarly, there is an extensive literature suggesting that mannitol is able to protect the ischemic myocardium during acute myocardial infarction. This suggestion, together with the fact that mannitol was included by Bretschneider and colleagues in some of the first protective coronary infusates that were developed, has resulted in the inclusion of mannitol or other nonmetabolizable carbohydrates such as sorbitol in a number of recently developed coronary infusates.

We have become concerned about the inclusion or exclusion of various compounds in the formulation of infusion solutions. Recently, we suggested that the inclusion of lactate or exclusion of calcium or magnesium from any coronary infusate may markedly reduce the efficacy of the infusate and under certain circumstances may be positively detrimental to tissue survival.

Since the nature of ischemia induced during cardiac operations is different from that occurring during evolving myocardial infarction, we question whether it is reasonable to extrapolate that compounds which are
effective in one situation are necessarily effective in the other. Furthermore, since there have been reports that glucose may be ineffective
coronal perfusion may be simulated by clamping the left atrium at a pressure of 20 cm. H₂O and oxygenated perfusion medium (at 37° C.) enters the
atrial cannula and introducing perfusion fluid at 37° C. into the aorta from a reservoir located 100 cm. above the heart. This preparation, which is essentially that
described in detail, is a left heart preparation in which the entire heart was maintained hypothermically (28° C.) and subjected to ischemic cardiac arrest.

Using our isolated, working rat heart model, we have investigated the effect of various concentrations of glucose and mannitol, both with and without insulin, upon survival during and recovery from ischemic cardiac arrest in hearts which had been subjected to infusion with a protective coronary infusate.

Material and methods

Hearts. Hearts were obtained from male rats (280 to 320 grams of body weight) of the Wistar strain.

The experimental model. The isolated, perfused, working rat heart model, which has already been described in detail, is a left heart preparation in which oxygenated perfusion medium (at 37° C.) enters the cannulated left atrium at a pressure of 20 cm. H₂O and is passed to the ventricle, from which it is spontaneously ejected (electrical pacing was not used in this study) at a rate of 40 to 55 ml. per minute, via an aortic cannula, against a hydrostatic pressure of 100 cm. H₂O. Coronary effluent can be sampled for biochemical analysis or pooled and recirculated with the aortic outflow.

Total cardiopulmonary bypass with maintained coronary perfusion may be simulated by clamping the left atrial cannula and introducing perfusion fluid at 37° C. into the aorta from a reservoir located 100 cm. above the heart. This preparation, which is essentially that described by Langendorff, will continue to beat but does not perform any external work. Ischemic cardiac arrest may be induced in this preparation by clamping the aortic cannula. Short periods of preischemic coronary infusion (at 28° C.) of protective or cardioplegic solutions may be achieved by use of a reservoir (located 60 cm. above the heart) attached to a side arm of the aortic cannula.

The experimental time course (Fig. 1). Immediately after excision of the heart, the aorta was connected to the aortic cannula and Langendorff perfusion was initiated for a 5 minute washout and equilibration period. During this 5 minute period, left atrial cannulation was completed. During this and subsequent perfusion periods, the circulating fluid was Krebs-Henseleit bicarbonate buffer, pH 7.4, containing

| Table I. Composition of the coronary infusate |
|-----------------|-----------------|-----------------|
|                | Grams per liter | Millimoles per liter |
| NaCl            | 5.26            | 90.0             |
| NaHCO₃          | 2.10            | 25.0             |
| KCl             | 1.10            | 14.8             |
| MgSO₄·7H₂O      | 0.29            | 1.2              |
| MgCl₂·6H₂O      | 3.01            | 14.8             |
| KH₂PO₄         | 0.16            | 1.2              |
| CaCl₂·2H₂O     | 0.18            | 1.2              |
| Glucose        | 0.0-9.01        | 0.0-50.0         |
| Mannitol        | 0.0-9.11        | 0.0-50.0         |
| Insulin         | 0 or 10 IU/L.   | 0 or 10 IU/L.    |

Legend: For clarity, the composition is expressed in both grams per liter and millimoles per liter, degrees of hydration are stated, and all reagents are of the highest grade of purity.

11.1 mM of glucose per liter and gassed with 95 percent oxygen and 5 percent carbon dioxide. The heart was then converted to a working preparation by terminating the retrograde aortic perfusion and initiating left atrial perfusion. During a 15 minute period, control values for aortic and coronary flow rates, peak aortic pressure, and heart rate were recorded. At the end of this control period, the atrial and aortic cannulas were clamped and the heart was subjected to a 2 minute period of coronary infusion (28° C.) with the protective solution under study. Infusion was then terminated and the entire heart was maintained hypothermically (28° C.) in an ischemic state for a fixed period of time. Although the hearts were perfused at 37° C. during the control
Table II. Recovery of aortic flow, heart rate, and aortic pressure after 70 minutes of ischemia at 28° C.

<table>
<thead>
<tr>
<th>Coronary infusion</th>
<th>Aortic flow</th>
<th>Heart rate</th>
<th>Aortic pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (ml/min.)</td>
<td>Recovery after 30 min. of reperfusion (percent)</td>
<td>Control (beats/min.)</td>
</tr>
<tr>
<td>Noninfused control animals</td>
<td>44.4 ± 1.5</td>
<td>293 ± 10</td>
<td>185 ± 6</td>
</tr>
<tr>
<td>Unmodified infusate</td>
<td>52.4 ± 1.4</td>
<td>276 ± 5</td>
<td>91.6 ± 1.8</td>
</tr>
<tr>
<td>Infusate containing 10 mM of glucose per liter</td>
<td>45.3 ± 1.6</td>
<td>277 ± 12</td>
<td>95.6 ± 4.5</td>
</tr>
<tr>
<td>Infusate containing 10 mM of glucose per liter plus 10 I.U. of insulin per liter</td>
<td>51.7 ± 1.2</td>
<td>293 ± 10</td>
<td>94.8 ± 2.2</td>
</tr>
<tr>
<td>Infusate containing 20 mM of glucose per liter</td>
<td>54.1 ± 1.7</td>
<td>270 ± 6</td>
<td>89.2 ± 2.5</td>
</tr>
<tr>
<td>Infusate containing 20 mM of glucose per liter plus 10 I.U. of insulin per liter</td>
<td>49.5 ± 1.5</td>
<td>271 ± 11</td>
<td>92.5 ± 4.1</td>
</tr>
<tr>
<td>Infusate containing 50 mM of glucose per liter</td>
<td>47.6 ± 1.7</td>
<td>282 ± 8</td>
<td>21.7 ± 13.7</td>
</tr>
<tr>
<td>Infusate containing 50 mM of glucose per liter plus 10 I.U. of insulin per liter</td>
<td>52.4 ± 1.9</td>
<td>269 ± 7</td>
<td>42.8 ± 16.3</td>
</tr>
<tr>
<td>Infusate containing 20 mM of mannitol per liter</td>
<td>47.7 ± 1.5</td>
<td>287 ± 8</td>
<td>88.1 ± 2.7</td>
</tr>
<tr>
<td>Infusate containing 20 mM of mannitol per liter plus 10 I.U. of insulin per liter</td>
<td>56.5 ± 0.4</td>
<td>278 ± 12</td>
<td>87.2 ± 2.2</td>
</tr>
<tr>
<td>Infusate containing 50 mM of mannitol per liter</td>
<td>47.5 ± 2.2</td>
<td>284 ± 10</td>
<td>83.1 ± 2.4</td>
</tr>
<tr>
<td>Infusate containing 50 mM of mannitol per liter plus 10 I.U. of insulin per liter</td>
<td>50.8 ± 2.5</td>
<td>276 ± 12</td>
<td>86.0 ± 5.8</td>
</tr>
</tbody>
</table>

and subsequent recovery periods, the use of dual temperature circuits permitted the infusion of the protective solutions at 28° C. and also allowed the hearts to be maintained at 28° C. throughout the period of ischemia.

After a suitable period of ischemia, the hearts were subjected to reperfusion at 37° C. Initially, reperfusion was in the Langendorff mode for 10 seconds, during which time nonrecirculating perfusion allowed washout and elimination of the residual protective infusate. Left atrial perfusion was then reinstated for a 30 minute period and the recovery of various parameters of cardiac function was recorded.

**Infusion solution.** The solution used in these studies is shown in Table I. The glucose and mannitol concentrations were varied in the range of 0 to 50 mM and insulin, if present, was 10 International Units (I.U.) per liter.

Precautions were taken to prevent the precipitation of calcium, and all infusates (and perfusion fluid) were filtered through a cellulose acetate membrane (pore size 5 μ) just before used. The solutions were gassed with 95 percent oxygen and 5 percent carbon dioxide immediately before infusion.

**Expression of results.** The absolute recovery values for various parameters of cardiac function in individual hearts were compared and expressed in terms of a percent of those values obtained during the preischemic control period. In addition to eliminating any inherent variability between individual hearts, this approach allowed the recovery of each parameter of function to be expressed as a percent and to be related to the nature of the coronary infusate and the duration of ischemic arrest. At least six hearts were used for each condition studied, and all data were expressed as the mean ± standard error. Comparison between groups was by Student's t test.

**Results**

**Control groups.** One group of 10 hearts was subjected to coronary infusion with a protective solution which did not contain any glucose, mannitol, or insulin (the protected control group). A second group of 10 hearts (the unprotected control group) did not receive...
Fig. 2. The protective effects of the coronary infusate: The postischemic recovery of hearts from 70 minutes of ischemia at 28°C. The recovery of aortic flow is shown as percent of the preischemic control value: (©) these hearts were subjected to a 2 minute period of coronary infusion with the protective solution immediately before the induction of ischemia; (⊗) these hearts were not subjected to coronary infusion. Each point represents the mean of 10 hearts and the standard error of the mean is indicated by the bars.

Myocardial protection during ischemic arrest

The inclusion of 10 mM of glucose resulted in a major reduction in the rate of recovery and also a significant (p < 0.0001) reduction in the final recovery of aortic flow (falling from 80.7 ± 2.4 percent in the infused control group to 59.2 ± 4.7 percent in the glucose group). The inclusion of insulin in this infusate caused a further but small reduction in recovery, but this did not differ significantly from that of the insulin-free group.

When the glucose concentration was increased to 20 mM per liter, there was an additional and substantial decline in the rate of recovery, such that at the end of the 30 minute reperfusion period the aortic flow rate was only 41.3 ± 6.1 percent of the control. Inclusion of insulin in this infusate caused a further large and significant fall in recovery, which after 30 minutes reached only 26.8 ± 6.0 percent of preischemic control values.

When the glucose concentration was increased to 50 mM per liter, then recovery fell to 5.6 ± 3.5 percent, and, when insulin was included, the recovery was 9.5 ± 4.9 percent; there was however no statistical significance between these two groups.

These results indicate that the inclusion of glucose or glucose plus insulin in the protective infusate used in these studies causes a dose-dependent reduction in the protective properties of the infusion solution. If glucose

any coronary infusion. Both groups of hearts were then subjected to hypothermic (28°C) ischemia for 70 minutes and then to normothermic reperfusion. The results (Fig. 2 for recovery of aortic flow rate and Table II for recovery of all indices of cardiac function) reveal that, at the end of the 30 minute reperfusion period, hearts in the noninfused control group failed to recover any aortic flow. In contrast and illustrating the protective properties of the infusion solution, the hearts in the protected control group exhibited a good recovery of cardiac function (Fig. 2 and Table II), with aortic flow recovering to 80.7 ± 2.4 percent of its preischemic control value.

Glucose and glucose plus insulin groups. The effects of inclusion at various concentrations of glucose or glucose plus insulin in the cardioplegic protective solution were ascertained by subjecting hearts (six in each group) to a 2 minute period of coronary infusion with a protective solution to which had been added 10, 20 or 50 mM of glucose per liter. In a second series of studies, insulin (10 I.U. per liter) was added to each infusate in addition to glucose. All hearts were subjected to 70 minutes of hypothermic (28°C) ischemia and then to reperfusion for 30 minutes. The aortic flow recovery profiles during this 30 minute period are shown in Fig. 3 and the final recovery values for all indices of cardiac function are shown in Table II.
is included at a concentration of 50 mM per liter, all of the protective properties of the solution are lost. In each instance the inclusion of insulin exacerbates these effects.

In an attempt to learn more about the mechanism of this deleterious effect (in particular whether it is predominantly metabolic or osmotic in its origin) and also to investigate the consequences of the inclusion of mannitol in the infusate, we carried out the following studies:

**Mannitol and mannitol plus insulin groups.** The preceding series of experiments were repeated except that mannitol (20 and 50 mM per liter) was substituted for glucose. Again, two series of studies were carried out in this group; in one series insulin (10 I.U. per liter) was included in the infusate and in the other series it was omitted.

The results (Fig. 4 and Table II) reveal that the inclusion of mannitol (20 mM per liter) or mannitol plus insulin (20 mM per liter and 10 I.U. per liter, respectively) in the protective infusate caused a small reduction in recovery. This was particularly apparent during the first 15 minutes of postischemic reperfusion, but after 30 minutes the reduction in recovery was not statistically different from the control.

However, when the mannitol concentration was increased to 50 mM per liter, there was a substantial reduction of recovery, particularly in the insulin-free group, which had recovered to only 49.5 ± 7.4 percent of control at the end of the 30 minute recovery period.

Although the inclusion of mannitol in the infusion solution reduced the postischemic recovery, it should be noted that the reduction was not as great as that observed with equivalent doses of glucose. Furthermore, although in the glucose group the inclusion of insulin exacerbated the deleterious effects of glucose, the inclusion of insulin in the mannitol group reduced the deleterious effects of mannitol.

**Discussion**

The results presented in this paper reveal that, under the conditions of this study, the infusate detailed in Table I is able to improve substantially the postischemic recovery of cardiac function. However, both glucose and mannitol reduce, in a dose-dependent manner, the protective properties of the infusate; for example,
Fig. 4. The effect of mannitol and mannitol plus insulin in the coronary infusate upon postischemic recovery. The postischemic recovery of hearts from 70 minutes of ischemia at 28° C. The recovery of aortic flow is shown as a percent of the preischemic control value. Hearts were subjected to a 2 minute period of coronary infusion immediately before the induction of ischemia. The infusion solution contained (♀) no mannitol and no insulin; (△) 20 mM of mannitol per liter; (▲) 20 mM of mannitol per liter plus 10 I.U. of insulin per liter; (◇) 50 mM of mannitol per liter; (▽) 50 mM of mannitol per liter plus 10 I.U. of insulin per liter. Each point represents the mean of six hearts and the standard error of the mean is indicated by the bars.

the inclusion of 50 mM of glucose per liter completely negates the protective properties of the infusate. The results also show that, at equivalent concentrations, the effects of mannitol are less detrimental than those of glucose. The effects of glucose are exacerbated by the inclusion of insulin, whereas insulin appears to reduce mannitol-induced impairment of recovery.

In seeking to understand these results and assess them in the light of the known effects of glucose, mannitol, and insulin on the ischemic myocardium, one must remember that these studies have been carried out using one basic infusate only and under conditions of hypothermic ischemic arrest in a rat heart model of cardiopulmonary bypass and ischemic cardiac arrest.

A comparison of the effects of equimolar doses of glucose and mannitol reveals that glucose produces twice as great an impairment of recovery as mannitol. Since glucose is a metabolizable sugar and mannitol is a nonmetabolizable sugar, it is tempting to attribute the deleterious effects of mannitol to its osmotic properties and those of glucose to a combination of osmotic and metabolic factors. The deleterious effects of cell swelling are widely known\(^3\), for this reason, the basic coronary infusate used in these studies was formulated so as to have an osmolarity of 300 mOsm. per liter and thereby to minimize osmotic shock. The inclusion of increasing concentrations of glucose or mannitol in such a solution creates an increasingly hyperosmolar solution which may lead to excessive cellular water loss and tissue damage. A number of coronary infusates in current clinical use include various concentrations of mannitol or other osmotic agents. Although most of these are included in concentrations designed to keep the osmolarity of the infusate in the range of 280 to 340 mOsm., some, e.g., the Kirsch\(^3\) solution, contain up to 247 mM of sorbitol per liter. In this latter example, it could well be the high osmolarity (463 mOsm. per liter) that contributes to the reported\(^3\) damaging effects of the Kirsch solution.

The fact that glucose is more detrimental than equivalent doses of mannitol may possibly be explained by its metabolic effects in the ischemic myocardium. Through its ability to stimulate and act as a substrate for anaerobic glycolysis, glucose may lead to increased glycolytic activity for a time, at least during the period of ischemic arrest. Although this anaerobic glycolysis
may be considered to be beneficial (because of the associated anaerobic production of adenosine triphosphate which may be used for cellular protection and postischemic recovery), it is also possible that it may be damaging. The stimulation of glycolysis, anaerobic production of adenosine triphosphate, and consequent utilization of adenosine triphosphate may lead to an increase in the number of protons in the cytoplasm. The resulting acidosis, together with the accumulation of potentially toxic metabolites such as lactate, may lead to extensive cellular damage and inhibition of a number of key metabolic pathways which otherwise might have remained functional. The accumulation of protons and the resulting damage would be particularly likely to occur in the model used for these studies, since the model is globally ischemic and does not have any extra cardiac-to-coronary or intercoronary collateral flow to contribute to the cellular washout of protons and other toxic cellular metabolites.

The ability of insulin (which, among its many metabolic effects, can stimulate cellular glucose uptake and metabolism) to exacerbate the detrimental effects of glucose during ischemic arrest reinforces further the proposed metabolic component of the glucose-induced damage. However, the role of insulin is probably more complex, since in the mannitol series of studies its effect is to reduce the mannitol-induced impairment of recovery. Under these conditions, in the absence of any glycolytic substrates, insulin would appear to be beneficial to cellular protection. Although the mechanism for this protection is unknown, it may well be related to the effect of insulin on membrane function, lipolysis, or protein synthesis and degradation. In all probability, insulin exerts a spectrum of effects and the balance between harmful and beneficial ones may depend upon the nature of the other components of the infusate.

These results and discussion serve further to underline our belief that, although coronary infusates are able to exert striking protective effects in the ischemic myocardium, they should be formulated with great care. Any compound included in an infusate should be investigated fully and, until the consequences of its inclusion and interaction with other components are understood, should not be used in high concentrations.

REFERENCES


12 Brachfeld N: Ischemic myocardial metabolism and cell necrosis. Bull NY Read Med 50:26, 1974

13 Hearse DJ, Chain EB: The role of glucose in the survival and recovery of the anoxic isolated perfused rat heart. Biochem J 128:1125, 1972

14 Hearse DJ, Humphrey SM: Enzyme release during myocardial anoxia. A study of metabolic protection. J Molec Cell Cardiol 7:463, 1975


17 Opie LH, Owen P: The effect of glucose-insulin-potassium infusions on arteriovenous differences of glucose and of free fatty acids and on tissue metabolic changes in dogs with developing myocardial infarction. Am J Cardiol 38:310, 1976


33 Langendorff O: Untersuchungen am überlebenden Saugetierherzen. Pflugers Arch 61:291, 1895