Protection of the myocardium during ischemic arrest

Dose-response curves for procaine and lignocaine in cardioplegic solutions

The dose-response curve of procaine or lignocaine (lidocaine) added to the St. Thomas’ Hospital cardioplegic solution was investigated with an isolated working rat heart preparation. In the absence of any cold cardioplegic protection, hearts failed to recover after as little as 30 minutes of ischemia. A single infusion (20°C) of the basic St. Thomas’ Hospital cardioplegic solution allowed hearts to recover to 60% or more of their preischemic control aortic flow after a 120 minute period of ischemia. Addition of procaine to the cardioplegic solution either increased or reduced the apparent protective properties of the solution with a bell-shaped dose-response curve being obtained. The optimum procaine concentration was 0.05 mM/L. At this concentration the protection afforded by the St. Thomas’ Hospital solution was increased by up to two thirds. Substitution of lignocaine for procaine resulted in a similar dose-response curve with its optimum also at 0.05 mM/L. If a similar optimum exists for the human heart, the doses in current clinical use would appear to be too high. These results argue for determining the dose-response characteristics of all substances used in cardioplegic solutions.

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The current growth of interest in the use of various cardioplegic and protective infusion solutions during cardiac operations has resulted in the development of a number of different solutions. Although their composition varies, the basic principles underlying their use are similar: Cardioplegic agents induce arrest rapidly; hypothermia slows metabolic processes and the development of tissue injury; protective agents are included to combat one or more of the deleterious effects of ischemia.

One agent which has been included in many cardioplegic solutions, particularly the early German formulations, is the local anesthetic agent, procaine.

Bretschneider and associates and Hölscher used 7.4 mM/L and Kirsch, Rodewald, and Kalmár used 11.0 mM/L. The inclusion of procaine was based upon its cardioplegic effect, maintaining depolarization by blocking sodium efflux, and upon its antidysrhythmic properties, which were felt to be of value during postischemic reperfusion. Procaine was also added to a number of other European and North American solutions with a view to improving myocardial protection. These solutions, however, induced arrest primarily through their raised potassium content. The procaine was not added in cardioplegic doses, and concentrations in the order of 1 mM/L were usual. The wider use of procaine was limited by three factors: differing surgical experience, the absence of specific characterization or dose-response studies of its effects, and, in the United States, the lack of Food and Drug Administration approval for its intravenous use.

Lignocaine (lidocaine), however, is approved for use in North America and might be expected to exhibit the same spectrum of cardiovascular effects as procaine. Therefore, we have carried out a preliminary study in which the effect of the inclusion of these two agents in...
Fig. 1. Basic cardioplegic protection. Hearts were subjected to 120 minutes of hypothermic (20°C) ischemic arrest: (•) control group; (○) preischemic infusion of St. Thomas’ Hospital cardioplegic solution. Recovery of aortic flow during a 30 minute postischemic reperfusion period is expressed as a percentage of its preischemic control value. Six hearts were used for each group and the bars represent the standard error of the mean. There was no recovery in the control group.

Table I. The St. Thomas’ Hospital cardioplegic solution without procaine

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride (mM/L)</td>
<td>110.0</td>
</tr>
<tr>
<td>Potassium chloride (mM/L)</td>
<td>16.0</td>
</tr>
<tr>
<td>Magnesium chloride (mM/L)</td>
<td>16.0</td>
</tr>
<tr>
<td>Calcium chloride (mM/L)</td>
<td>1.2</td>
</tr>
<tr>
<td>Sodium bicarbonate (mM/L)</td>
<td>10.0</td>
</tr>
<tr>
<td>pH adjusted to 7.8</td>
<td></td>
</tr>
<tr>
<td>Osmolarity = 324 mOsm/kg H$_2$O</td>
<td></td>
</tr>
</tbody>
</table>

various concentrations in the St. Thomas’ Hospital cardioplegic solution has been assessed using an isolated rat heart preparation.

Materials and methods

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

Experimental model. The isolated, perfused, working rat heart model has already been described in detail. It is a left heart preparation in which oxygenated perfusion medium (at 37°C) enters the cannulated left atrium at a pressure of 20 cm H$_2$O and is passed to the ventricle, from which it is spontaneously ejected at 40 to 65 ml/min via an aortic cannula against a hydrostatic pressure of 100 cm H$_2$O. Electrical pacing was not used in this study in order to assess the effect of the local anesthetics on heart rate. 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 any degree of hypothermia) of the cardioplegic solution may be achieved by use of a reservoir (located 60 cm above the heart) attached to a side arm of the aortic cannula.

Experimental time course. Immediately after exci-
Fig. 2. Procaine dose-response study. The relationship between the concentration of procaine in the cardioplegic solution (mM/L; note the logarithmic scale) and the postischemic recovery of aortic flow (expressed as a percent of the preischemic control value) measured at the end of a 30 minute period of reperfusion following a 120 minute period of hypothermic (20°C) ischemic cardiac arrest. Each point represents the mean of six hearts and the bars indicate the standard error of the mean.

Fig. 3. Lignocaine dose-response study. The relationship between the concentration of lignocaine in the cardioplegic solution (mM/L; note the logarithmic scale) and the postischemic recovery of aortic flow (expressed as a percent of the preischemic control value) measured at the end of a 30 minute period of reperfusion following a 120 minute period of hypothermic (20°C) ischemic cardiac arrest. Each point represents the mean of six hearts and the bars indicate the standard error of the mean.

sion 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 glucose (11.1 mM/L) and gassed with 95% oxygen and 5% 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 3 minute period of coronary infusion (20°C) with the cardioplegic solution under study. Infusion was then terminated and the entire heart was maintained hypothermically (20°C) in an ischemic state for 120 minutes. After this period the hearts were reperfused initially in the Langendorff mode for 15 minutes and then in the working mode for a further 15 minutes. During this latter period the recovery of cardiac function was monitored. Expression of results. During the preischemic working control period the following variables were recorded: heart rate, coronary flow, aortic flow, and aortic pressure. The cardiac output was derived from the sum of aortic and coronary flow and the stroke
Table II. Procaine dose-response study: The effect of procaine concentration in the cardioplegic solution upon the postischemic recovery of various parameters of cardiac function after 120 minutes of ischemia

<table>
<thead>
<tr>
<th>Procaine concentration (mM/L)</th>
<th>Aortic flow</th>
<th>Aortic pressure</th>
<th>Heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (ml/min)</td>
<td>Recovery after 30 min of reperfusion (%)</td>
<td>Control (cm Hg)</td>
<td>Recovery after 30 min of reperfusion (%)</td>
</tr>
<tr>
<td>20.0</td>
<td>53.5 ± 3.8</td>
<td>22.6 ± 2.0*</td>
<td>192 ± 7.2</td>
</tr>
<tr>
<td>10.0</td>
<td>52.0 ± 2.5</td>
<td>40.9 ± 3.4*</td>
<td>198 ± 11</td>
</tr>
<tr>
<td>2.0</td>
<td>52.0 ± 0.8</td>
<td>52.7 ± 7.4†</td>
<td>199 ± 3.9</td>
</tr>
<tr>
<td>1.0</td>
<td>48.3 ± 3.7</td>
<td>68.6 ± 6.0‡</td>
<td>150 ± 1.8</td>
</tr>
<tr>
<td>0.2</td>
<td>49.0 ± 1.7</td>
<td>78.4 ± 5.1</td>
<td>180 ± 6.8</td>
</tr>
<tr>
<td>0.05</td>
<td>52.0 ± 3.1</td>
<td>90.4 ± 4.5</td>
<td>160 ± 7.9</td>
</tr>
<tr>
<td>0.01</td>
<td>50.4 ± 2.1</td>
<td>74.3 ± 4.0§</td>
<td>186 ± 3.7</td>
</tr>
<tr>
<td>0.001</td>
<td>50.0 ± 2.4</td>
<td>78.3 ± 5.3</td>
<td>198 ± 4.6</td>
</tr>
<tr>
<td>0 (control)</td>
<td>52.8 ± 1.9</td>
<td>67.2 ± 3.4*</td>
<td>189 ± 6.9</td>
</tr>
</tbody>
</table>

Statistical analysis: Percent recovery for each concentration group and for each variable has been compared (Student’s t test) with optimal recovery observed. In all instances this involved a comparison of percent recovery with that found in 0.05 mM group.

* p < 0.01.
† p < 0.02.
‡ p < 0.05.
§ p < 0.001.

Table III. Lignocaine dose-response study: The effect of lignocaine concentration in the cardioplegic solution upon the postischemic recovery of various parameters of cardiac function after 120 minutes of ischemia

<table>
<thead>
<tr>
<th>Lignocaine concentration (mM/L)</th>
<th>Aortic flow</th>
<th>Aortic pressure</th>
<th>Heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (ml/min)</td>
<td>Recovery after 30 min of reperfusion (%)</td>
<td>Control (cm Hg)</td>
<td>Recovery after 30 min of reperfusion (%)</td>
</tr>
<tr>
<td>20.0</td>
<td>54.8 ± 2.7</td>
<td>38.9 ± 6.9*</td>
<td>202 ± 4.2</td>
</tr>
<tr>
<td>2.0</td>
<td>47.3 ± 2.7</td>
<td>65.5 ± 8.1$</td>
<td>176 ± 4.2</td>
</tr>
<tr>
<td>0.05</td>
<td>54.6 ± 1.9</td>
<td>86.2 ± 3.0</td>
<td>206 ± 4.7</td>
</tr>
<tr>
<td>0.01</td>
<td>61.6 ± 3.2</td>
<td>79.3 ± 4.3</td>
<td>193 ± 8.8</td>
</tr>
<tr>
<td>0 (control)</td>
<td>52.8 ± 1.9</td>
<td>67.2 ± 3.4</td>
<td>189 ± 6.9</td>
</tr>
</tbody>
</table>

Statistical analysis: Percent recovery for each concentration group and for each variable has been compared (Student’s t test) with optimal recovery observed. In all instances this involved a comparison of percent recovery with that found in 0.05 mM group.

* p < 0.001.
† p < 0.01.
‡ p < 0.02.
§ p < 0.05.

Results

Basic cardioplegic protection. To ascertain the degree of protection afforded by the unmodified St. Thomas' Hospital cardioplegic solution, we undertook a series of studies in which the postischemic recovery of function was related to the duration of ischemia and the presence or absence of the cardioplegic solution. The objective of this series of studies was to define a duration of ischemic arrest at which tissue injury was sufficient to prevent complete postischemic recovery but was not so severe as to prevent any recovery. In other words, conditions were established to permit a 60% to 70% recovery of pump function. In this way any protective or injurious effects arising from the inclusion of procaine or lignocaine in the cardioplegic solution by dividing cardiac output by heart rate. During

Cardioplegic solution. The basic St. Thomas' Hospital cardioplegic solution with the omission of procaine was used; the composition is shown in Table I. To this was added various concentrations of procaine hydrochloride or lignocaine hydrochloride.
solution could be readily detected and quantitated.

The results (Fig. 1) revealed that 120 minutes was the ideal ischemic interval. Hearts (n = 6) subjected to ischemic arrest without preischemic cardioplegic infusion failed to recover any pump function upon reperfusion and were observed to be in a state of contracture. By contrast, hearts subjected to preischemic coronary infusion (3 minutes) with the St. Thomas’ Hospital cardioplegic solution (without procaine) recovered 67.2% ± 3.4% of their preischemic aortic flow and 79.5% ± 2.7% of their preischemic cardiac output.

Inclusion of procaine. Procaine hydrochloride was added to the St. Thomas’ Hospital cardioplegic solution to a final concentration of 1.0 mM or 10.0 mM. Hearts (n = 6 for each group) were infused for a 3 minute period with the cardioplegic solution at 20° C. The hearts were then subjected to 120 minutes of hypothermic ischemic arrest. There were no secondary infusions of cardioplegic solution. Upon reperfusion a surprising result was obtained. At 1.0 mM, procaine addition increased postischemic functional recovery when compared with a procaine-free control, whereas at 10.0 mM, procaine addition reduced postischemic recovery. These results indicated that procaine may exhibit a complex dose-response profile and that concentrations hitherto used may not have been in the correct range for optimal protection. A dose-response curve for procaine was therefore constructed. Hearts (n = 6 for each group) were given a 3 minute infusion at 20° C of the St. Thomas’ Hospital cardioplegic solution to which procaine had been added in the following concentrations: 0, 0.001, 0.01, 0.05, 0.2, 1.0, 2.0, 10.0, and 20.0 mM/L. The hearts were then subjected to 120 minutes of ischemia at 20° C followed by 30 minutes of reperfusion. The final recovery of function in relation to the concentration of procaine in the cardioplegic solution is shown in Table II and Fig. 2.

The results showed a bell-shaped dose-response
curve. As the procaine concentration increased from 0 to 0.05 mM/L, there was a progressive increase in protective properties (p < 0.001 with respect to control). Beyond this point, recovery decreased with increasing procaine concentrations, and above 2 mM procaine reduced the apparent efficacy of the cardioplegic solution (p < 0.001 with respect to control). The results indicate that when procaine is included in the cardioplegic solution at the noncardioplegic concentration of 0.05 mM/L, the postischemic recovery of aortic flow is increased from less than 70% to greater than 90% of its preischemic value. At procaine concentrations of 10 mM/L and above, there was a significant reduction in heart rate during the reperfusion period. However, calculation of the stroke volume, rather than aortic flow, made little difference to the shape of the dose-response curve (Table II), though tending to flatten it somewhat.

The added protection resulting from the inclusion of procaine in a concentration of 0.05 mM/L may appear relatively small, but, as the recovery of the procaine group approaches 100%, the full potential for the additional protection might not have been revealed. In order to test this last point, we carried out a series of experiments in which hearts (n = 6 for each group) were subjected to 150 minutes of ischemic arrest with procaine included in the cardioplegic solution at concentrations of 0, 0.01, and 0.05 mM/L. In the procaine-free group, the final postischemic recovery of aortic flow was 33.1% ± 7.8% of its preischemic value. Inclusion of procaine at 0.01 mM/L improved this figure to 50.1% ± 7.8% and at 0.05 mM/L to 53.6% ± 4.9%. Thus it would appear that the inclusion of procaine at its optimal concentration increased the protective properties of the St. Thomas' Hospital cardioplegic solution by approximately two thirds.

Inclusion of lignocaine. In order to ascertain whether lignocaine (lidocaine) affords a similar improvement in the protective properties of the St. Thomas' Hospital cardioplegic solution and also to investigate its dose-response characteristics, we conducted the following studies. Lignocaine hydrochloride was included in the cardioplegic solution at the following concentrations: 0, 0.01, 0.05, 2.0, and 20.0 mM/L. Hearts (n = 6 for each group) were subjected to 120 minutes of ischemia (20°C) and 30 minutes of reperfusion. The results for the postischemic recovery of function in relation to the concentration of lignocaine in the cardioplegic solution are detailed in Table III and Fig. 3.

As with procaine, a bell-shaped dose-response curve was obtained with an optimal protective concentration of 0.05 mM/L. At this concentration, protection was significantly (p < 0.01) improved, with aortic flow increasing from 67.2% ± 3.4% to 86.2% ± 3.0%. At concentrations above 2.0 mM/L, lignocaine reduced the apparent protective properties of the cardioplegic solution, so that at 20.0 mM the recovery of aortic flow fell significantly (p < 0.001) to 38.9% ± 6.9%.

There was a reduction in heart rate at the lignocaine concentration of 20 mM/L, although this reduction was less than that with procaine. Again, calculation of stroke volume tended to flatten the dose-response curve but not to alter it significantly (Table III).

Discussion

The results of this study with the isolated, perfused, working rat heart indicate that the inclusion of local anesthetic agents such as procaine or lignocaine (lidocaine) in the St. Thomas' Hospital cardioplegic solution can have a variable effect upon the ability of the solution to protect the heart against extended periods of ischemia. This variability can be attributed to the complex dose-response characteristics of these agents. Thus, at concentrations in the range of 0.001 to 0.1 mM/L, these agents improved the protective properties of the solution. For both procaine and lignocaine the optimal concentration was 0.05 mM, and at this level the protective properties of the solution could be improved substantially. From a number of studies it appears that the added protection was such that, when the drug was used at an optimal concentration, an additional 20% of preischemic function could be restored after 2 or 2½ hours of ischemia.

At concentrations above 1 to 2 mM, the use of procaine or lignocaine appeared to reduce the protective properties of the cardioplegic solution. Thus, in the 20.0 mM group, postischemic recovery of aortic flow was less than half that of control.

In considering the beneficial and possibly detrimental effects of local anesthetics, it would appear likely that the additional protection at low, noncardioplegic doses is achieved through the ability of procaine to reduce deleterious, ischemia-induced changes in cell membrane activity, possibly through the so-called "membrane stabilizing" action of the drug. The apparent depressive effects of the drugs at high concentrations are less readily explained but may be attributable to some direct membrane action during ischemia or to some residual activity during reperfusion. This latter possibility might be supported by the observation of a sustained depression of heart rate that occurred in the high-dose groups for as long as 30 minutes after reperfusion (this reduction in rate did not significantly alter...
the dose-response curves beside flattening them somewhat). Since it is conceivable that extended periods of reperfusion might have reversed a depression of function, it is not possible in these studies to conclusively equate the depression of function with the induction of damage.

Although these results were obtained in the isolated rat heart, they stress the importance of determining the dose-response characteristics of any agent included in a cardioplegic solution. The observation of bell-shaped dose-response curves is not unusual; for example, we have obtained one for the concentration of magnesium in cardioplegic solutions. Although the optimum concentration of local anesthetic may vary between species, our results suggest that the currently used clinical dosage should be reduced in man. Our results also suggest that lignocaine (lidocaine) is an acceptable alternative to procaine as a component of cardioplegic solutions.

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REFERENCES
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