Electrophysiological studies with repeated episodes of ischaemia on isolated rat heart

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In order to know the beneficial effect of preconditioning electrocardiography recording were used as tool to assess myocardial malfunction and for this perfusion apparatus was setup. Electrophysiological changes for each heart were recorded during perfusion at 1, 2, 3, 5, 10, 20, 30 and 60 min of global ischaemia and also during the equal period of reperfusion. Recordings demonstrate that the normal rate was about 240 beats/min with an "R" amplitude of 4mV. During the first ischaemic episode of 1 min the rate was 180 ± 15 beats/min (counted as per "R" wave deflection), at 2 mins it was 60 ± 6 beats/min, at 3 min the rate was 40 ± 2 beats/min, at 5 mins of ischaemia it was 90 ± 6 beats/min, at 10 min 20 ± 2 beats/min, at 20 min the rate was 60 ± 4 beats/min, and at 30 mins there were nil beats/min. The recovery during all the periods of reperfusion was restored to between 120 and 180 beats/min in all episodes. Further after a 60 min of ischaemia the heart stopped to elicit any mechanical response. It is concluded that short term ischaemia can induce a resilient effect on the beating of the heart after a few episodes as seen subsequent to 1 and 2 min of ischaemia. Further, preconditioning was beneficial up to 30 min, beyond which the heart showed signs of fatigue and irreversible injury.

Perfusion of isolated heart has gained the importance in assessing factors responsible for myocardial damage and recovery during ischaemia and reperfusion respectively. Research in this line has been going on for several years with the firm belief that brief periods of ischaemia would ultimately lead to irreversible damage even from the very first episode, leading to collapse. However during the course of study on isolated hearts, scientists discovered that brief periods of ischaemia followed by reperfusion, instead of causing permanent damage, gave rise to a condition of resistance to further attacks of ischaemia which they termed as preconditioning1. Repeated coronary artery occlusion and reperfusion limited infarct size2. Another study demonstrated that brief periods of ischaemia could protect the heart against the deleterious effects of more prolonged ischaemia3.

Thus the subject of repeated ischaemia and beneficial/harmful effect of reperfusion has become a topic of study which invited our attention to design this experiment.

Materials and Methods

In order to test the phenomenon of preconditioning and to record the electrical activity of an isolated heart during repeated periods of ischaemia and reperfusion, an apparatus was set up which was a modified version4. The main features as shown in the Fig.1 are as follows:

1. A manometer is provided to measure and note the pressure build up in the reservoir (a) and the buffer (b) which passes through two double jacket condensers (d, e) before perfusing the heart (g).

2. An additional double jacket condenser (j) is provided for any test solution. The three way taps (S4, S5) when opened allows the test fluid for coronary perfusion, at the same temperature, pressure and the level of oxygenation as that of the buffer because of the three way tap (S2, S3).

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3. Oxygen or carbogen (95% \( \text{O}_2 \) and 5% \( \text{CO}_2 \)) is made to bubble by turning the three way stopper (S1, S2) so that it is directed into the separate condenser.

The isolated heart is connected to the ECG electrode (h) by a junction box with the positive (+) terminal to the apex and the negative (-) terminal to the base of the heart by the needle electrodes made into hooks corresponding to Lead II connections. It has been found that the arrangement does not interfere with the heart rate and the myocardium continued to beat for a minimum period of one hour and a half if the \( \text{pH} \) and the temperature remained steady.

Adult Wistar strain albino rats of either sex, weighing between 150 and 200 g were obtained from the Department of Laboratory Animal Sciences, Madavaram. The rats were acclimatized to animal house condition for a month and were fed on a commercial pelleted rat chow (Hindustan Lever Limited, Bangalore, India) and water ad libitum. The animals were divided into two groups of six animals each. The animals were anaesthetized by subcutaneous injection of heparinised thiopentone sodium (Intraval sodium, Rhone-Poulenc, India Limited, Bombay) at the rate of 40 mg/kg body weight.

The abdomen was opened and the heart exposed by cutting through the diaphragm and the sternum. The descending aorta was cannulated by means of a blunted 18 gauge metallic needle. Immediately, Krebs-Henseleit (KH)\(^5\) buffer with a composition of (mM/L): NaCl, 118; KCl, 4.7; KH\(_2\)PO\(_4\), 1.2; NaHCO\(_3\), 25; MgSO\(_4\), 1.2; glucose, 9.9 and CaCl\(_2\) 2.5 made up to 4L of deionized water, filtered and adjusted to a \( \text{pH} \) of 7.35 - 7.40 by bubbling CO\(_2\) was infused gradually from heparinized syringe retrogradely so as to flush blood from the heart and prevent clots in the coronaries. The heart was rapidly excised and fixed to the outlet of the perfusion apparatus as explained above. The KH buffer was then allowed to pass through the heart at a constant pressure of 60 mmHg and a flow rate of 30 ml/min determined by the graduated cylinder and stopcock method (bucket method) with carbogen bubbled into the reservoir continuously. Enough time was allowed for the heart to stabilize to a rate of 180-200 beats/min.

Needle electrodes were then fixed as described above which remained in situ through out the course of the experiment and were calibrated to give a deflection of 10 mm/mV.

The rats were grouped as follows. Group I comprised normal rats. In this group isolated hearts perfused for 15 min were used as controls. ECG recordings were taken to ensure the stability of the preparation. Group 2 comprised of the ischaemic group of rats. After 15 min of preliminary perfusion in order to stabilize, coronary perfusion was stopped for 1, 2, 3, 5, 10, 20, 30 and 60 min by closing the buffer line leading to the aortic cannula. Each episode of ischaemia was followed by an equal period of reperfusion i.e. 1, 2, 3, 5, 10, 20, 30 and 60 min.

Statistical analysis was carried out by Student’s ‘\( t\)’ test and results were expressed as means ± SD.

Results
The recordings of the isolated heart before ischaemia (normal) are shown in Fig. 2. There was an inverted P wave followed by the narrow QRS complex and a T wave of repolarization. There was a notch in the R wave which may be normal or a conduction abnormality. Further this was an isolated specimen and hence the notch may be taken as normal. The inverted P wave may have indicated a
retrograde conduction through the atria. With retrograde activation of the atria, the activation front is directed cranially i.e. away from the (+) ve poles of standard Leads II and III, and Lead AVF.

These leads consequently reflect negative 'P' deflection. Nevertheless these recordings were taken as standard for this experiment. After 1 min of ischaemia, there was a deepening of S wave. Reperfusion for a period of 1 min reduced the depression and the notch on the R wave disappeared. In 2 min ischaemia there was a deeper notch in the R wave which disappeared after 2 min of reperfusion. A 3 min ischaemia showed bradycardia with second degree type (3:1) block with slight plateau at the apex of R wave which disappeared after reperfusion. The P waves were regular at the rate of 120 beats/min. At 5 min of ischaemia recording showed bradycardia, absence of P wave, widened QRS segment fused with isoelectric line and a ventricular escape rhythm; the amplitude of R wave was 4mV. Reperfusion deepened the P wave. In the 10 min ischaemic episode, there was a block with A:V ratio of 4:1. The R wave amplitude reduced to 3.5 mV.

Reperfusion for 10 min restored the amplitude to 4mV and the rate to 180 beats/min. At 20 min of ischaemia the recording showed a condition of bradycardia with a rate of 60 beats/min with an atrial standstill with no P wave. Reperfusion for 20 min increased the rate but the R wave amplitude did not change. The 30 min ischaemia showed a complete block. On reperfusion the P wave appeared normal and there was a restoration of the rate to 120 beats/min with an increase in 'R' wave amplitude. Ischaemia for a period of one hour showed a complete block. Reperfusion only restored P wave denoting that the heart was dying as there was no sign of conduction to the ventricles. The heart finally underwent a total arrest as the final end point (Table 1).

**Discussion**

In the present study, warm heart retrograde perfusion was performed. Warm heart surgery was developed as a means of preventing ischaemia. Reports indicate that there were beneficial effects of continuous normothermic cardioplegia. The initial ischaemia that could have occurred by the

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**Table 1**—Electrophysiological changes during ischaemia and reperfusion in isolated rat heart. Normal rate @ 240 beats/min 'R' amp 4 mV

<table>
<thead>
<tr>
<th>Ischaemia</th>
<th>Duration (min)</th>
<th>Heart-rate/ min</th>
<th>Ventricular depolarization mv</th>
</tr>
</thead>
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<tr>
<td></td>
<td>1</td>
<td>180±15</td>
<td>3±0.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>60±6</td>
<td>3±0.3</td>
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<tr>
<td></td>
<td>3</td>
<td>40±2</td>
<td>2.5±0.2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>90±6</td>
<td>4.0±0.3</td>
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<tr>
<td></td>
<td>10</td>
<td>20±2</td>
<td>3.5±0.4</td>
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<tr>
<td></td>
<td>20</td>
<td>60±4</td>
<td>4±0.4</td>
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<tr>
<td></td>
<td>30</td>
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<td>-</td>
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<td></td>
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<table>
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<tr>
<th>Reperfusion</th>
<th>Duration (min)</th>
<th>Heart-rate/ Min</th>
<th>Ventricular Depolarization Mv</th>
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<td>3±0.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>120±12</td>
<td>3.5±0.3</td>
</tr>
<tr>
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<td>4±0.4</td>
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<td></td>
<td>60</td>
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<td>0.5</td>
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placing of the excised heart in cold buffer was avoided by the aortic cannulation in situ and the immediate transfer of the cannulated heart to the perfusion unit. Thus the study was done under normothermic conditions from the start in order to maintain the heart at a steady state. The heart rate was ranging between 150 and 220 beats/min. This rate was higher than the constant pacing of 120 beats/min by Montrucchio et al. who studied the effect of platelet activating factor (PAF) in relation to cardiac dysfunction in isolated hearts. The ECG recording in the normal heart showed a dipping of S wave signifying a pre-existing ischaemia as this was an isolated preparation which borders on hypoxia with dissolved carbogen circulating at a pressure of 60 mmHg. In the present study each steady state represented different contractile states and hence no common baseline values could be obtained to which all data could be referred to except the first group which was used for normal recording. The first 1 min of ischaemia followed by reperfusion offered the heart to its first episode of preconditioning. There was no change of rate or amplitude of R wave. In the subsequent period of 2 min ischaemia there was a drastic reduction in the heart rate but the rhythm remained steady with each ventricular wave preceded by a P wave. There was no change in the electric potential either. Reperfusion restored the rate to 120 beats/min which was twice the rate as during ischaemia. The full recovery in this episode could be the phenomenon of reversible myocardial injury as a result of reperfusion in animals models.

During global ischaemia calcium in myocardial cells rose for a period less than 20 min after which reperfusion led to recovery of the calcium to pre-ischaemic levels within five minutes. During the first 10 min of subsequent episodes of severe ischaemia preconditioned myocardium used much less ATP than that was utilized in virgin control myocardium and accumulated much less nucleosides and bases and products of anaerobic glycolysis such as lactate. This metabolic slowing, they pointed out, was the most final pathway of the cardioprotective effect.

Preconditioning delayed but did not prevent lethal cell injury, that is, the infarct size was limited if measured after a test episode of 40 to 60 min but if the episode was extended to 90 or more min the protective effect of preconditioning dissipated. In the present study during 3 min ischaemia there was a P:R ratio of 3:1 signifying a bundle block. The R wave amplitude also declined. During reperfusion there was a pick up of the heart rate with an increase in R wave amplitude to 4mV. This phenomenon of full recovery was seen in the other episodes consisting of 5, 10 and 20 min ischaemia followed by reperfusion. This could be due to the initial depletion of ATP but with no further loss during repeated ischaemia for short duration. Adenosine build up was an important mechanism of protection afforded by ischaemic preconditioning. Adenosine was involved in mediating the protective effect of preconditioning against myocardial damage.

The ECG recordings demonstrated the beneficial effect of ischaemia followed by reperfusion where changes in the anaerobic tissue content of glycolytic products like lactate, H+ or NADH took place. In the dog with four periods of ischaemia each lasting 5 min and separated by 5 min of reperfusion and subsequently during a 40 min period of sustained ischaemia there was a marked limiting effect of infarct size. It was also demonstrated that the vulnerability to subsequent reperfusion induced arrhythmia was beneficially influenced by preconditioning. In a number of species this vulnerability to reperfusion arrhythmia was critically dependent on the preceding period of ischaemia. Another factor that could affect heart could be the release of PAF which was shown to exert a direct negative inotropic effect on cardiac muscle coupled with a reduction in the duration of action potential, a contribution of PAF released during reperfusion in the cardiac muscle. The 30 min episode during another "no flow" global ischaemia, although the rate had substantially increased from 0 to 120 beats/min, it remained below controlled level. The contraction amplitude and heart rate decreased to zero, in a 15 min ischaemia but with reperfusion the contraction amplitude and rate recovered slightly but remained well below the controlled value following reperfusion for an equal period of time.

In the final 60 min of ischaemia, the myocardium was rendered irreversibly damaged and the heart finally went into a stand still. Isolated heart was not completely stable and a progressive decline in the functioning occurred during the course of an experiment. The mechanism of this irreversible injury could be multifactorial and includes oxygen free radical, calcium influx and damage from inflammation related to white blood cell infiltration and activity, decline in cardiac function with acidosis (lactic acidosis), and finally that isolated hearts border on...
hypoxia. Further it was found that the locally produced eicosanoids might exert both beneficial as well as deleterious effect on flow deprived and reperfused hearts and that the conversion of arachidonic acid to its biologically active metabolites and the loss of cellular integrity were mutually related.

In addition earlier investigations have shown that chemical degradation of cell membranes was responsible for ischaemia induced loss of cell viability and also membrane destabilisation was due to distinct changes in both the physio-chemical properties of membrane lipids and in the architecture of the lipid bilayer. During the ischaemic insult lipid material was extruded from cardiac membranes and prolonged oxygen deprivation (60 min and longer) induced the extrusion of lipid material from the sarcolemma of cultured neonatal cells.

Thus in the final analysis it is demonstrated that preconditioned heart has its own threshold level of tolerance to ischaemic insults and ultimately fails due to several causes which needs further study.

Acknowledgement
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References