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Protective effect of Timolol as assessed by energy charge during myocardial ischaemia

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Current evidence suggests that the deleterious effects of myocardial ischaemia are characterized by an early fall in the cellular content of high-energy phosphates such as ATP and creatine phosphate and a rise in the tissue content of AMP, hypoxanthine, xanthine and inosine [1]. At much later stages this damage is evidenced by a major leakage of intracellular enzymes into the extracellular fluid. If the myocardium is reperfused at a time when significant cellular damage has already occurred, then the reperfusion process may cause an acceleration of tissue damage [2] characterized by further ultrastructural disruption and a massive loss of intracellular enzymes and other proteins into the extracellular space [3, 4]. Many therapeutic interventions have been proposed with the aim of reducing or delaying cell damage associated with ischaemia or reperfusion. The beta-adrenoceptor antagonist propranolol has, for example, been suggested to be able to reduce (or at least delay) the development of ischaemia-induced cell necrosis, but whether the protective mechanism involves beta-blockade or some other property of the drug has not been completely resolved [5–8]. In the present study, we have investigated the effect of timolol on ischaemia and reperfusion-induced tissue damage. This beta-adrenoceptor antagonist has recently been shown to reduce mortality and the rate of reinfarction in patients surviving acute myocardial infarction [9]. Other trials with beta-blocking agents have, however, failed to shown any overall benefit upon mortality or reinfarction [10–12]. The exact mechanisms by which timolol exerts its proposed beneficial effects have not yet been defined. In the present study, we have investigated whether timolol has an ability to protect against the fall in cellular high-energy phosphate reserves during early myocardial ischaemia. In particular, we sought to establish whether this compound could influence the 'energy charge' [13] of the myocardium (i.e. balance between ATP-producing and ATP-utilizing pathways) during ischaemia; if so to ascertain whether such energy conservation during ischaemia is beneficial during subsequent reperfusion of the myocardium. This study has been carried out using an isolated heart preparation in order to remove all peripheral effects of timolol and to eliminate any possible actions of the compound on the blood platelet system.

Materials and methods

Perfusion techniques. Male rats (280–320 g body wt) of the Wistar strain were lightly anaesthetized with diethyl ether, the femoral vein was exposed and heparin (200 U) was administered intravenously. One minute later the heart was excised and placed in ice-cold perfusion medium until contraction had ceased (approximately 30 sec). The aorta was cannulated and the heart perfused retrogradely, via the aorta, in the Langendorff mode [14]. Hearts were perfused with oxygenated Krebs–Henseleit medium, pH 7.4, containing 11.1 mM glucose and 1×10^{-7} M isoprenaline (plus 5 μM ascorbate to prevent oxidation) to replace exogenous catecholamine drive which is absent from isolated heart preparations. This concentration of isoprenaline produces submaximal increases in heart rate and contractility in the aerobic, isolated heart [15]. Hearts were perfused aerobically for 15 min and then made globally ischaemic by reducing aortic flow to 0.14 ml/min per g wet wt. In the drug-treated group, timolol was included in the perfusion medium to a concentration of 1.0 mg/l. (2.6 μM) throughout the experimental period. This concentration of timolol was selected, as preliminary experiments showed this concentration to be a potent antagonist of isprenaline-induced increases in cardiac function without causing depression of cardiac function in the absence of isoprenaline. Hearts were divided into two groups: one group was subjected to 20 min of global ischaemia; the other to 3 hr of global ischaemia followed by 15 min of aerobic reperfusion. At the end of the ischaemic period, hearts subjected to 20 min ischaemia were 'freeze-clamped' using stainless-steel tongs cooled to the temperature of liquid nitrogen. The following metabolites were assayed in these hearts: ATP, creatine phosphate, ADP, AMP, creatine and glycogen. ATP, creatine phosphate, ADP, AMP and glycogen were assayed as previously described [1] and creatine was assayed using a modification of the method of Bergmeyer [16]. Contents were expressed as μmole/g dry wt except in the case of glycogen which was expressed as μmole glucose equivalents/g dry wt. The 'energy charge' in each heart was calculated using the following equation:

$$\text{Energy charge} = \tfrac{1}{2}\,\frac{([\text{ADP}] + 2\,[\text{ATP}])}{([\text{AMP}] + [\text{ADP}] + [\text{ATP}])}$$

The leakage of creatine kinase (ATP: creatine phosphotransferase, EC 2.7.3.2) into the coronary effluent was measured in hearts subjected to 3 hr ischaemia and 15 min reperfusion. Creatine kinase was measured by the method of Oliver [17] and was expressed as IU released by the heart. All values were expressed as the mean ± S.E.M. of six experiments in each group. All values of statistical significance were calculated using the Student's *t*-test.

Results and discussion

The inclusion of isoprenaline in the perfusion medium increased heart rate submaximally from 274 ± 9 to 322 ± 14 beats/min. The addition of timolol to the perfusion medium prevented some of this increase in heart rate and the mean heart rate in the timolol-treated group immediately prior to ischaemia was 288 ± 12 beats/min. Table 1 shows the tissue content of ATP, creatine phosphate, ADP, AMP, creatine and glycogen after 20 min global ischaemia. In the normal, aerobic heart, ATP and creatine phosphate content would be approximately 25 and 35 μmole/g dry wt, respectively, and glycogen reserves would be in the order of 90–120 μmole glucose equivalents/g dry wt [1]. Thus 20 min of ischaemia has resulted in a severe depletion of all these compounds, particularly glycogen. Treatment with timolol in part prevents this fall of high-energy phosphates and glycogen. Thus ATP content is increased from 8.4 ± 1.3 to 12.9 ± 1.4 μmole/g dry wt ($P < 0.05$) and glycogen stores from 9.1 ± 1.4 to 54.1 ± 6.4 μmole glucose equivalents/g dry wt ($P < 0.001$). When this is expressed as the energy charge (Table 1) this beneficial effect of timolol is further confirmed with a 35% higher value in the drug-treated group.

The leakage of enzymes into the coronary effluent has been used for many years as an index of tissue damage [18]. Figure 1 shows that in the untreated control group substantial myocardial damage occurred during the 3 hr period of severe ischaemia with 4.9 ± 1.1 IU creatine kinase

Table 1. Cellular metabolite levels after 20 min ischaemia

	Control	Timolol
ATP (μmoles/g dry wt)	8.4 ± 1.3	12.9 ± 1.4*
Creatine phosphate (μmoles/g dry wt)	4.9 ± 0.4	5.9 ± 0.7
ADP (μmoles/g dry wt)	4.6 ± 0.6	6.8 ± 0.6*
AMP (μmoles/g dry wt)	3.2 ± 0.3	2.4 ± 0.5
Creatine (μmoles/g dry wt)	16.1 ± 0.5	15.5 ± 0.9
Glycogen (μmoles glucose equivalents/g dry wt)	9.1 ± 1.4	54.1 ± 6.4***
Energy charge	0.54 ± 0.057	0.73 ± 0.06***

* $P < 0.05$; *** $P < 0.001$.

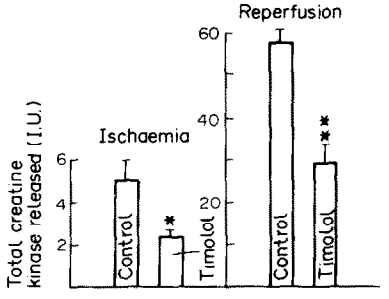

Fig. 1. The leakage of creatine kinase from the isolated, rat heart during ischaemia and reperfusion. Values are shown as the mean ± S.E.M. of six experiments in each group. *Indicates a significant difference at the level of $P < 0.05$ and ** at the level of $P < 0.01$.

being released into the coronary effluent. Upon reperfusion there was a further ten-fold increase in the extent of enzyme leakage (57.5 ± 4.3 IU). The addition of timolol during ischaemia and reperfusion considerably reduced enzyme leakage, which fell by 50% during both ischaemia ($P < 0.05$) and reperfusion ($P < 0.01$).

In this isolated, perfused, rat heart study, timolol has been shown to be capable of reducing cellular damage during a fixed period of ischaemia and reperfusion. The mechanism responsible for this protective effect may be associated with the ability of the compound to maintain the energy charge of the myocardium during ischaemia. This maintenance may be accomplished either by increasing high-energy phosphate production or by decreasing high-energy phosphate utilization during ischaemia. Whatever the mechanism, the beneficial effect demonstrated in the rat heart might well be similar to that operative in the human heart which acts to reduce the incidence of reinfarction in patients who have previously suffered from myocardial infarction.

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