Antiarrhythmic Action of Labetalol and Its Effect on Adenine Metabolism in the Isolated Rat Heart

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Labetalol, a combined alpha- and beta-adrenergic antagonist, was assessed for antiarrhythmic activity in the isolated perfused rat heart. Inclusion of 5.0 and 7.5 μmol/liter labetalol in the perfusate reduced the fall in ventricular fibrillation threshold and eliminated ventricular arrhythmias during the coronary occlusion period of 15 minutes. In treated hearts, levels of high energy phosphates were significantly higher and lactate, adenosine and hypoxanthine levels were lower in ischemic myocardium. Cyclic adenosine monophosphate levels were reduced in uninvolved myocardium (0.31 ± 0.01 and 0.24 ± 0.02 versus 0.43 ± 0.02 nmol/g fresh weight) and in the ischemic myocardium (0.38 ± 0.02 and 0.34 ± 0.04 versus 0.65 ± 0.05 nmol/g) in hearts treated respectively with 5.0 and 7.5 μmol/liter labetalol versus control hearts. When untreated hearts were perfused with 3.0 mmol/liter potassium in perfusate, all had ventricular tachycardia or fibrillation during coronary ligation and developed ventricular fibrillation after reperfusion. Labetalol, 5.0 μmol/liter, reduced ventricular tachyarrhythmias during coronary occlusion and after reperfusion, whereas labetalol, 7.5 μmol/liter, eliminated tachyarrhythmias during occlusion and reperfusion. Labetalol had potent antiarrhythmic activity in the hearts rendered uniformly prone to arrhythmias by perfusion with a low potassium solution.

Methods

Experimental preparation. The procedures involved in the perfusion and biochemical preparation of these rat hearts have been described in detail (11,12). Male Wistar rats (200 to 300 g) were anesthetized with methoxyflurane and given 200 IU heparin intravenously. The hearts were rapidly excised, placed in ice-cold perfusate and mounted for perfusion by the Langendorff technique. The perfusate was Krebs-Henseleit bicarbonate buffer, continuously gassed with 95% oxygen and 5% carbon dioxide and maintained at 37°C. The substrate was d-glucose, 11 mmol/liter; the perfusate potassium concentrations were 5.9 mmol/liter (normal K⁺) and 3.0 mmol/liter (low K⁺). The perfusion column was 100 cm in height. A 4-0 silk suture was placed deep to the left coronary artery for its ligation later in the experiment. A period of 30 minutes of perfusion was allowed for stabilization before heart rate and coronary flow rates were estimated.
Estimation of ventricular fibrillation threshold. After stabilization, the ventricular fibrillation threshold was obtained by the single stimulus technique. Thin platinum wire electrodes (36 standard wire gauge) were inserted subepicardially at the apex and base of the left ventricle, remote from the myocardium likely to be involved by ischemia after coronary ligation. The electrocardiogram was obtained by inserting thin copper wire electrodes into the right atrium and ventricle. The heart was monitored continuously on a Tektronix memory oscilloscope and a continuous electrocardiogram was recorded at 5.0 mm/s during the period of coronary artery ligation and for 15 minutes after reperfusion (Elema mingograph).

Stimuli were generated by a microprocessor-based stimulus generator (Motorola 6800) programmed to deliver 2.0 ms square wave impulses at variable intervals after the onset of the R wave which was used as a trigger. The ventricular fibrillation threshold (minimal current required to precipitate ventricular fibrillation lasting at least 6 cycles) was estimated by scanning the region of the T wave with stimuli of initial current strength of 3.0 mA. Two stimuli were delivered at 2.5 ms intervals between 10 and 50 ms after the onset of the R wave. The current strength was increased by 0.5 mA per series of stimuli across the T wave. When ventricular fibrillation persisted, the heart was defibrillated with up to two stimuli using the same current that precipitated the arrhythmia; if this was unsuccessful, ice-cold Krebs-Henseleit solution was applied to the heart until arrest occurred. The heart invariably restarted in sinus rhythm. A recovery period of 2 minutes was allowed before stimulation was resumed.

Experimental procedure. Coronary artery occlusion was obtained by abrupt tightening of the previously placed ligature. Estimation of coronary flow rate, heart rate and ventricular fibrillation threshold was performed at 15 minutes after ligation. In the reperfused hearts, the ligature was divided at this stage and the electrocardiogram recorded for another 15 minutes. In hearts destined for biochemical analysis, freeze clamping with liquid nitrogen cooled tongs by the Wollenberger technique was performed 2 seconds after injection of 0.2 ml disulfine blue (an intravascular dye) immediately above the aortic perfusion cannula, as previously described (11,12).

In labetalol-treated hearts, perfusion was switched after stabilization to Krebs-Henseleit solution containing 1.0, 2.5, 5.0 or 7.5 μmol/liter labetalol. The pure hydrochloride salt was a fine white powder manufactured by Glaxo Group Research Ltd. At these concentrations the powder dissolved readily. In control experiments, the procedure was identical except that the labetalol was omitted.

Scoring of spontaneous arrhythmias. The electrocardiograms recorded during the 15 minute period of coronary artery ligation and after reperfusion were analyzed for ventricular arrhythmias, and the maximal score was allocated to each heart as follows: 1 = bigeminal rhythm, couplets or triplets of ventricular origin; 2 = single episode of ventricular tachycardia; 4 = multiple episodes of ventricular tachycardia, but the heart was in sinus rhythm at 15 minutes; 6 = continuous ventricular tachycardia or single episode of ventricular fibrillation; 8 = multiple episodes of ventricular fibrillation, but the heart was in sinus rhythm at 15 minutes and 10 = continuous ventricular fibrillation until 15 minutes after ligation or reperfusion.

Biochemical estimations. After freeze clamping, the ischemic and nonischemic myocardium were separated under liquid nitrogen according to the distribution of the disulfine blue (11,12). The frozen myocardium was powdered in a liquid nitrogen-cooled percussion mortar. Aliquots of tissue (50 to 100 mg) were extracted into 1 ml of ice-cold 6% weight/weight (w/w) perchloric acid using an Ultra Turrax blender (Jancke and Kunkel, Staufen). Extracts were centrifuged for 15 minutes at 15,000 g and the supernatant neutralized with a mixture of four parts 40% (w/w) potassium hydroxide saturated with potassium carbonate and six parts 0.2 M Tris-hydrochloride buffer, pH 7.5. The perchlorate precipitate was removed by centrifugation to yield a clear supernatant and the metabolites measured in these extracts. Adenosine triphosphate, creatine phosphate, adenosine, inosine, hypoxanthine/xanthine and lactate were measured by standard enzymatic degradation techniques and spectrophotometry (13). Tissue cyclic adenosine monophosphate (cyclic AMP) was measured by the competitive protein-binding assay of Tovey et al. (14). Each sample was assayed on three occasions and values differing by less than 10% were used to calculate a mean value. Aliquots of powdered heart tissue were used to determine the dry/wet weight ratio, which was used to obtain the fresh weight (fresh weight = dry weight × 5).

Substrates and enzymes were obtained as analytic grade chemicals from Sigma Chemical Corporation. The cyclic AMP assay kits were obtained from Amersham International Ltd.

Statistical methods. The results obtained in each group of hearts were expressed as the mean value ± the standard error of the mean. Probability (p) values were obtained using Student's t test (two-tailed test to compensate for unequal variances).

Results

Effects of coronary artery ligation in hearts perfused with potassium, 5.9 mmol/liter. Coronary artery ligation in 13 control hearts did not alter heart rate but did reduce coronary flow rate from 9.6 ± 0.6 to 6.3 ± 0.5 ml/min (p < 0.005). The ventricular fibrillation threshold was reduced from 8.0 ± 0.8 mA before ligation to 3.0 ± 0.4 mA at 15 minutes after ligation (p < 0.001). The arrhythmia score during the occlusion period was 1.6 ± 0.5. Ventricular fibrillation did not occur; three hearts had multiple
episodes of ventricular tachycardia, two had single episodes of ventricular tachycardia and eight had no arrhythmias.

**Severe reduction of the high energy phosphates, adenosine triphosphate and creatine phosphate was present in the ischemic myocardium by 15 minutes after ligation (Table 1). The lactate content of ischemic myocardium was increased 20-fold. The adenine metabolites, adenosine, inosine and hypoxanthine/xanthine were also increased about 10-fold in the ischemic myocardium. Cyclic adenosine monophosphate in the uninvolved myocardium was 0.43 ± 0.02 nmol/g; in the ischemic myocardium the level was 0.65 ± 0.05 nmol/g fresh weight (p < 0.001). These values were consistent with those found in similar studies in another laboratory (12).**

**Effects of labetalol in hearts perfused with normal K+.** Inclusion of 5.0 and 7.5 μmol/liter labetalol in the perfusate did not alter the heart rate before or after ligation. The coronary flow rate was increased in hearts treated with labetalol, 5.0 μmol/liter, both before (12.9 ± 1.2 versus 9.6 ± 0.6 ml/min) and after ligation (9.0 ± 0.8 versus 6.3 ± 0.5 ml/min) (p < 0.05 in both instances). With 7.5 μmol/liter labetalol, the coronary flow rate was not significantly altered. The ventricular fibrillation threshold was not altered by labetalol treatment before coronary ligation, but was significantly increased after ligation with labetalol, 5.0 μmol/liter (5.2 ± 0.8 versus 3.0 ± 0.4 mA, p < 0.05) and with 7.5 μmol/liter (9.2 ± 1.8, p < 0.005). All arrhythmias were eliminated during the 15 minute period of coronary occlusion with 5.0 μmol/liter; the arrhythmia score was 0.2 ± 0.2 in the hearts perfused with 7.5 μmol/liter of labetalol. One heart in the treated series had a single episode of ventricular tachycardia.

**The high energy phosphates in uninvolved myocardium were unaltered by labetalol.** In ischemic myocardium, the adenosine triphosphate and creatine phosphate content was increased in hearts treated with both 5.0 and 7.5 μmol/liter labetalol (Table 1). The lower rate of high energy phosphate depletion was supported by the observation that the lactate content of ischemic myocardium was significantly decreased in hearts treated with 5.0 μmol/liter (22.1 ± 1.6 μmol/g) and with 7.5 μmol/liter of labetalol (20.9 ± 1.9 μmol/g) in comparison with the value in the untreated hearts (26.4 ± 1.1 μmol/g fresh weight) (p < 0.05 in both instances). The adenosine, inosine and hypoxanthine/xanthine content of nonischemic myocardium was unaltered by labetalol treatment; the adenine metabolites were significantly decreased in ischemic myocardium of hearts treated with both 5.0 and 7.5 μmol/liter labetalol.

**Major effects of labetalol were seen in the cyclic AMP content of the nonischemic as well as the ischemic myocardium.** The nonischemic myocardial cyclic AMP was reduced to 0.31 ± 0.01 nmol/g fresh weight with 5.0 μmol/liter labetalol, and to 0.24 ± 0.02 nmol/g with 7.5 μmol/liter (p < 0.001 in both instances), compared with the level of 0.43 ± 0.02 nmol/g in the untreated hearts. In ischemic myocardium, the level was reduced from 0.65 ± 0.05 nmol/g fresh weight in the untreated hearts to 0.38 ± 0.02 nmol/g in hearts treated with 5.0 μmol/liter and to 0.34 ± 0.04 nmol/g in hearts treated with 7.5 μmol/liter (p < 0.001 in both instances).

**Antiarrhythmic effects of labetalol in hearts perfused with low K+.** The antiarrhythmic effects of labetalol were further investigated in hearts exposed to a reduced level of K+ (3.0 mmol/liter) in the perfusate. In 32 control hearts perfused without labetalol, the arrhythmia score during the period of coronary occlusion was 6.3 ± 0.5; 20 hearts developed ventricular fibrillation, 15 of which had repeated episodes or persistent ventricular fibrillation. After reperfusion, 28 of the 32 hearts had persistent ventricular fibrillation (arrhythmia score 9.8 ± 0.2). Ventricular fibrillation threshold measurements were not performed in these arrhythmia prone hearts, and because these hearts were subjected to reperfusion, freeze clamping was not performed.

**The antiarrhythmic effect of labetalol in concentrations of 1.0, 2.5, 5.0 and 7.5 μmol/liter in the perfusate is shown in Figure 1.** A concentration-related reduction in arrhythmia score was seen for arrhythmias occurring both during the period of coronary occlusion and after reperfusion.

**Discussion**

**Antiarrhythmic activity.** In the isolated perfused rat heart, inclusion of labetalol in the perfusate exerted a concentration-related protective effect against spontaneously occurring ventricular arrhythmias during the period of coro-

**Figure 1.** Antiarrhythmic action of labetalol in hearts perfused with low potassium (3.0 mmol/liter) in perfusate. During the occlusion phase, arrhythmia scores (mean ± standard error of mean, see text) were significantly reduced by labetalol, 2.5, 5.0 and 7.5 μmol/liter (p < 0.001 in each instance in comparison with the score obtained in the control hearts). Similarly, after reperfusion, the arrhythmia scores of 10 and 9.8 (10 = maximal attainable) in the control hearts and hearts treated with 1.0 μmol/liter labetalol, respectively, were significantly reduced by 2.5, 5.0 and 7.5 μmol/liter (p < 0.001 in comparison with control hearts). Because these hearts were not freeze-clamped, the numbers in parentheses indicate the number of hearts studied by reperfusion.
Table 1. Heart Function and Tissue Metabolic Changes in Control and Labetalol-Treated Hearts

<table>
<thead>
<tr>
<th></th>
<th>Control (13 hearts)</th>
<th>Labetalol</th>
<th>Labetalol</th>
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<tr>
<td></td>
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<td>5.0 µmol/liter (10 hearts)</td>
<td>7.5 µmol/liter (10 hearts)</td>
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<tr>
<td>Heart rate (beats/min)</td>
<td>231 ± 15</td>
<td>248 ± 7</td>
<td>234 ± 16</td>
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<tr>
<td>A</td>
<td>217 ± 17</td>
<td>243 ± 12</td>
<td>223 ± 15</td>
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<tr>
<td>Coronary flow rate (ml/min)</td>
<td>9.6 ± 0.6</td>
<td>12.9 ± 1.2*</td>
<td>8.7 ± 0.8</td>
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<tr>
<td>B</td>
<td>6.3 ± 0.5</td>
<td>9 ± 0.8*</td>
<td>5.3 ± 0.6</td>
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<tr>
<td></td>
<td>1.6 ± 0.6</td>
<td>0±</td>
<td>0.2 ± 0.2±</td>
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<tr>
<td>Arhythmia score (max = 10)</td>
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<tr>
<td>VFT (mA) B</td>
<td>8 ± 0.8</td>
<td>6.0 ± 0.6</td>
<td>10.0 ± 1.3</td>
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<td></td>
<td>3 ± 0.4</td>
<td>5.2 ± 0.8*</td>
<td>9.2 ± 1.8†</td>
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<tr>
<td>ATP (µmol/g) U</td>
<td>3.2 ± 0.2</td>
<td>3.0 ± 0.1</td>
<td>3.4 ± 0.2</td>
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<td>0.44 ± 0.04</td>
<td>0.74 ± 0.08†</td>
<td>0.70 ± 0.05‡</td>
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<td>Creatine phosphate (µmol/g) I</td>
<td>2.3 ± 0.2</td>
<td>2.6 ± 0.1</td>
<td>2.1 ± 0.3</td>
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<td></td>
<td>0.22 ± 0.02</td>
<td>0.46 ± 0.10*</td>
<td>0.38 ± 0.04†</td>
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<tr>
<td>Lactate (µmol/g) U</td>
<td>0.9 ± 0.10</td>
<td>1.6 ± 0.3</td>
<td>1.80 ± 0.42</td>
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<td></td>
<td>26.4 ± 1.10</td>
<td>22.1 ± 1.6*</td>
<td>20.9 ± 1.9*</td>
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<tr>
<td>Adenosine (µmol/g) U</td>
<td>0.04 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.05 ± 0.01</td>
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<td>0.69 ± 0.07</td>
<td>0.54 ± 0.05</td>
<td>0.49 ± 0.06*</td>
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<tr>
<td>Inosine (µmol/g) I</td>
<td>0.91 ± 0.03</td>
<td>0.68 ± 0.04†</td>
<td>0.69 ± 0.04†</td>
</tr>
<tr>
<td>Hypoxanthine/xanthine (µmol/g) U</td>
<td>0.07 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.07 ± 0.01</td>
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<td>0.28 ± 0.02</td>
<td>0.21 ± 0.02‖</td>
<td>0.21 ± 0.01†</td>
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<tr>
<td>Cyclic AMP (nmol/g) U</td>
<td>0.43 ± 0.02</td>
<td>0.31 ± 0.01‡</td>
<td>0.24 ± 0.02‡</td>
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<td></td>
<td>0.65 ± 0.05</td>
<td>0.38 ± 0.02‡</td>
<td>0.34 ± 0.04‡</td>
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All biochemical values are expressed per gram of tissue, fresh weight (mean ± standard error of the mean)
* probability (p) < 0.05; † p < 0.01; ‡ p < 0.001 (difference from control hearts)
A = 15 minutes after coronary ligation, AMP = adenosine monophosphate, ATP = adenosine triphosphate, B = before coronary ligation, I = ischemic myocardium; U = uninvolved myocardium; VFT = ventricular fibrillation threshold.

nary occlusion and also against arrhythmias after reperfusion of ischemic myocardium. The decrease of the ventricular fibrillation threshold after coronary artery ligation was significantly less in hearts exposed to labetalol. Labetalol therefore had antiarrhythmic activity according to each of the criteria tested. The antiarrhythmic activity of labetalol was sufficiently powerful to be effective against the spontaneous arrhythmias in hearts perfused with reduced perfusate K⁺; in those hearts not treated with labetalol, ventricular tachycardia or fibrillation was uniformly prevalent during the period of coronary occlusion and invariably occurred after reperfusion.

Effect on heart rate and coronary flow. Relatively minor or no effects were seen on heart rate and coronary flow rate in the concentrations studied. The intermediate concentration of labetalol (5.0 µmol/liter) increased the coronary flow rate, whereas the highest concentration studied (7.5 µmol/liter) did not. The significance of this diver-
gent concentration-related effect of labetalol on coronary flow is unclear. A possible explanation may reside in differential receptor antagonism at different concentrations. The increase in heart rate and coronary flow rate with 5.0 μmol/liter suggests that with this concentration alpha-receptor antagonism may have been more prominent relative to beta-antagonism, which became more evident with 7.5 μmol/liter causing a return of heart rate and coronary flow toward levels encountered in untreated hearts.

**Biochemical effects.** Labetalol significantly altered the biochemical status of the ischemic myocardium in the hearts with coronary artery ligation. The depletion of the high energy phosphates (adenosine triphosphate and creatine phosphate) and accumulation of lactate in ischemic myocardium were reduced by labetalol. The reduction of lactate accumulation and the lower extent of high energy phosphate depletion encountered in labetalol-treated hearts is consistent with an improvement by labetalol of residual perfusion in the ischemic myocardium. Labetalol effects were also seen on adenosine, inosine and hypoxanthine/xanthine, the content of which was reduced in ischemic myocardium. Labetalol caused a highly significant reduction of the cyclic adenosine monophosphate (cyclic AMP) content in both nonischemic and ischemic myocardium. The levels of cyclic AMP recorded in these labetalol treated hearts were similar to those in hearts treated with amiodarone (11) and dl-propranolol (12). The antiarrhythmic effect of labetalol in the isolated rat heart was, therefore, like that of propranolol and amiodarone in this model, accompanied by a reduction in ischemic myocardial cyclic AMP levels.

**Mechanisms of labetalol's effects.** The possibility that labetalol was antiarrhythmic by virtue of its membrane stabilizing activity cannot be entirely excluded, but is unlikely. Membrane stabilization with labetalol occurs with concentrations in perfusate of about 10 μmol/liter (1). It is considerably less potent than propranolol in this action (1) yet exerts significant antiarrhythmic activity, especially against reperfusion arrhythmias, an effect that we were unable to demonstrate with propranolol.

The powerful inhibitory effect of labetalol on reperfusion arrhythmias resembles that of amiodarone (11) and the alpha-adrenergic antagonists: phentolamine, prazosin (10) and nifedipine (16). The alphalyclic component of labetalol may confer on it this valuable action which has not been demonstrated among the beta-adrenergic antagonists (15). It is of interest that antiarrhythmic action against reperfusion ventricular fibrillation has also been recorded with other agents that share vasodilator properties, that is, amiodarone (11), verapamil (17) and nifedipine (15), even though their mechanisms of action are likely to be different.

**The effect of labetalol on cyclic AMP may be important for its action on ouabain-toxic arrhythmias (1).** Tanz et al. (18) have recently demonstrated that the arrhythmias encountered in ouabain-toxic guinea pig hearts are associated with increased myocardial levels of cyclic AMP. The antiarrhythmic action of labetalol on coronary occlusion arrhythmias (associated with markedly reduced levels of cyclic AMP in ischemic myocardium) provides additional support for the hypothesis linking the antiarrhythmic actions of such agents as propranolol (10) and amiodarone (11) to their actions of reducing cyclic AMP accumulation in ischemic myocardium (19).

**Clinical implications.** These observations on labetalol in the isolated rat heart may have important clinical implications. With its actions of increasing coronary perfusion in dogs (5) and in patients with coronary artery disease (4), its improvement of exercise tolerance in patients with ischemic heart disease after intravenous administration (2) and reduction of angina pectoris in hypertensive patients (7,8) labetalol may have an important therapeutic role in ischemic heart disease. The antiarrhythmic effects and preservation of ischemic myocardial high energy phosphate content seen in the present study support the observations in which labetalol limited ischemic damage in rats with coronary ligation (6). The potent antiarrhythmic effect shown in the hearts perfused with a reduced potassium concentration, and therefore highly prone to ventricular fibrillation, may have important implications for patients developing myocardial ischemia in the presence of potassium depletion: for example, after energetic diuretic therapy. Two uncontrolled clinical studies (20,21) have suggested that labetalol may have effective antiarrhythmic activity. Intravenous infusion of labetalol restored sinus rhythm in 9 of 11 patients with life-threatening arrhythmias and also caused a decrease in blood pressure. This agent clearly deserves controlled clinical investigation in patients with ischemic heart disease.

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## References


cardial cell necrosis after coronary arterial occlusion in rats. Am J Cardiol 1980;46:249–54


