The Effect of Alpha- and Beta-Adrenoceptor Antagonist Agents on Reperfusion Ventricular Fibrillation and Metabolic Status in the Isolated Perfused Rat Heart

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This study was designed to assess the effect of alpha- and beta-adrenoceptor antagonist agents on reperfusion ventricular fibrillation and myocardial metabolic status preceding the onset of reperfusion ventricular fibrillation in the isolated Langendorff perfused rat heart. Prazosin (alpha$_1$-antagonist), yohimbine (alpha$_2$-antagonist) and phentolamine (alpha$_1$- and alpha$_2$-antagonist) protected against reperfusion ventricular fibrillation, the median effective dose (ED_{50}) values being 2.0, 0.7 and 2.5 μmol/liter, respectively, when given before coronary artery ligation. The ability of alpha-antagonist agents to prevent reperfusion ventricular fibrillation appeared to be the consequence of alpha-adrenoceptor antagonism or the consequence of "membrane-stabilizing activity," or both. Reserpinization before reperfusion reduced but did not eliminate reperfusion ventricular fibrillation. Relative to the reported dissociation constants of myocardial alpha-adrenoceptors, yohimbine (alpha$_2$-antagonist) appeared more active; such antiarrhythmic potential apparently has not been reported previously. The beta-adrenoceptor antagonist agents dl-propranolol (ED_{50} value, 3 μmol/liter) and metoprolol in high concentrations (ED_{50} value, 50 μmol/liter) prevented reperfusion ventricular fibrillation, whereas atenolol was virtually without effect. d-Propranolol (ED_{50} value, 2.5 μmol/liter), the propranolol isomer with modest beta-antagonist activity but with marked membrane-stabilizing activity, evoked protection equivalent to that of racemic propranolol. Beta-adrenoceptor antagonists did not appear to prevent reperfusion ventricular fibrillation as a consequence of receptor antagonism but rather by membrane-stabilizing activity.

Some circumstantial association was evident between improved metabolic status on reperfusion, in preservation of tissue levels of adenosine triphosphate and phosphocreatine, reduction of tissue levels of lactate and cyclic adenosine monophosphate and protection against reperfusion ventricular fibrillation. However, agents affording a similar degree of metabolic preservation were associated with different degrees of protection against reperfusion ventricular fibrillation. Of the four possible protective procedures: alpha-receptor antagonism, beta-adrenoceptor antagonism, membrane-stabilizing activity and metabolic preservation, alpha-receptor antagonism and membrane-stabilizing activity most warrant further evaluation, especially in other species.

Both coronary artery occlusion and subsequent reperfusion of the acutely ischemic myocardium predispose to the development of ventricular arrhythmias, including ventricular fibrillation. Experimental evidence suggests that enhanced adrenergic activity (1,2), in conjunction with metabolic and biochemical sequelae (3) of acute myocardial ischemia, plays an important role in the genesis of ventricular arrhythmias during the period of coronary artery occlusion.

The release of endogenous and systemic catecholamines (2) after coronary occlusion and the accumulation of cyclic adenosine monophosphate (cAMP) (4,5) in the ischemic myocardium are considered to predispose to the occurrence of slow response action potentials (6,7). Therefore, it has been argued that beta-adrenergic receptor stimulation may predispose to the development of ischemic arrhythmias by promotion of the slow response. Alpha-adrenergic receptor stimulation, on the other hand, has only a small effect on the slow response (8), although this may account in part for the proposal that alpha-stimulation enhances the influx of calcium in ventricular muscle (9). The reduction of myocardial high energy phosphate content (10,11) and the increase in lactate concentration (12) in the ischemic myo-

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cardium have also been thought to predispose to ventricular arrhythmias by shortening the action potential duration.

In contrast to ischemic arrhythmias, the role of adrenergic, biochemical and metabolic influences on reperfusion ventricular arrhythmias has not been clearly defined. These factors assume importance because the precise mechanism of reperfusion ventricular fibrillation is unclear and recent evidence (13) suggests that the severity of reperfusion ventricular arrhythmias may be dependent on rhythm disturbances during the antecedent period of coronary occlusion, although the mechanisms might differ.

Therefore, the present study was designed to characterize the metabolic and biochemical status before the onset of reperfusion ventricular fibrillation and assess the role of alpha- and beta-adrenergic influences on reperfusion ventricular arrhythmias and metabolic status. Alpha-adrenergic receptors are classified into subtypes 1 and 2 (14), and because preliminary evidence by Sheridan et al. (15) indicates that alpha$_1$ responses may play an important role in the genesis of reperfusion arrhythmias, we paid special attention to alpha$_1$-adrenoceptor antagonism, comparing it with the effects of alpha$_2$-adrenoceptor antagonism. We also investigated the effect of beta-adrenoceptor antagonism and assessed the importance of the "membrane-stabilizing property" peculiar to some beta-antagonists. The isolated Langendorff perfused rat heart subjected to left main coronary artery ligation and reperfusion was used because of the consistent occurrence and predictable time of onset of reperfusion ventricular fibrillation (16).

Methods

Experimental preparation. Long-Evans rats (weight 275 to 375 g) were anesthetized with ether and their hearts were removed after injection of 100 units of heparin into the femoral vein. The hearts were immediately arrested in ice-cold Krebs-Henseleit solution, mounted on a Langendorff nonfunctioning aortic retrograde perfusion system modified to contain two perfusate reservoirs and perfused at a constant filling pressure of 100 cm water. The heart was enclosed in a glass water jacket maintained at 37°C. The perfusate was modified Krebs-Henseleit solution (potassium ion [K^+] 4.8 mmol/liter; substrate glucose, 11.1 mmol/liter aerated with 95% oxygen and 5% carbon dioxide, and maintained at a constant temperature of 37°C). Lubbe et al. (16) showed that hearts perfused with Krebs-Henseleit solution with a K^+ level of 4.8 mmol/liter are associated with a 100% incidence of reperfusion ventricular fibrillation.

A 5-0 silk suture was placed deep to the left main coronary artery within 2 mm of where it emerged adjacent to the left atrium (17). A thin stainless steel wire was inserted into the right ventricular free wall superficially as one electrode; the other electrode was clamped onto the metal aortic perfusion cannula. The electrocardiogram obtained was used to evaluate heart rate and rhythm. Coronary flow rates were measured by collecting the coronary effluent into a graduated measuring tube over a 1 minute period.

Heart rate and coronary flow were recorded during a 15 minute period of stabilization. Complete coronary artery occlusion was then produced by tightening the previously placed ligature over a short length of polyvinyl tubing that partially encompassed the left main coronary artery. Successful ligation was indicated by a decrease of the coronary flow rate of at least 25% of the basal rate and the darkening of most of the free left ventricular myocardium. With use of these criteria, 40 to 50% of the left ventricle is usually rendered ischemic (17).

After 15 minutes of coronary artery occlusion, the ligature was divided using a surgical blade that was drawn along the polyvinyl tubing to avoid injury to underlying myocardium or coronary artery. The experiment was terminated 10 minutes after the onset of reperfusion. The heart rhythm was monitored by continuous electrocardiographic recording throughout the period of coronary occlusion and on reperfusion.

In a separate series of experiments, hearts were freeze-clamped using precooled Wollenberger tongs (18) for biochemical analysis at the end of the period of coronary artery ligation to assess the metabolic status at this time point, and 10 seconds after the onset of reperfusion. This time sequence was chosen because reperfusion ventricular fibrillation occurs approximately 15 seconds after the onset of reperfusion. In the hearts clamped at the end of the period of coronary artery ligation, the introduction of disulfine blue dye into the aortic perfusion cannula just before clamping enabled separation of ischemic from nonischemic tissue. Disulfine blue dye binds with oxygen and therefore the perfused nonischemic zone is rendered blue while the ischemic zone does not take up the stain.

High energy phosphates, glycogen, lactate and cyclic AMP were analyzed as previously described (19–22). In some experiments, rats were pretreated with reserpine by the procedure of Gaudel et al. (23), which severely depletes the myocardium of both norepinephrine and epinephrine.

Chemical agents. Compounds were obtained in powder form: metoprolol (Ciba-Geigy), dl-propranolol, d- and l-propranolol isomers (ICI), prazosin (Pfizer) and yohimbine (Sigma Corporation Ltd., USA). Phentolamine hydrochloride was obtained as a solution (Boots). Five minutes before and 5 minutes after coronary artery ligation, perfusion was switched from the reservoir containing modified Krebs-Henseleit solution to the other reservoir where the respective pharmacologic agent was present in the respective concentration. Compounds were freshly prepared for each experiment.

Analysis of arrhythmias. Ventricular tachycardia was diagnosed if more than three consecutive morphologically similar ventricular extrasystoles occurred. Ventricular fibrillation was diagnosed if more than six consecutive ventricular complexes showed total irregularity of morphology. During the period of coronary artery occlusion, antiarrhythmic activity was assessed primarily by reduction in the incidence of ventricular fibrillation, ventricular tachycardia and ventricular extrasystoles. Ventricular antiarrhythmic activity after reperfusion was assessed by prevention of the occurrence of ventricular fibrillation and also by reduction of the mean duration of reperfusion ventricular fibrillation and ventricular tachycardia (that is, spontaneous reversion to sinus rhythm during the 600 second period of reperfusion).

Statistical methods. Results are given as mean ± standard error of the mean for the number of experiments. The number (n) of hearts perfused in each series ranged from 10 to 20. Probability [p] values were calculated with Student's *t* test using two-tailed values as corrected for unequal variances. Probability values less than 0.05 indicated significance except when multiple comparisons required Bonferroni's corrections (24). The Fisher's exact test (25) was applied to assess the differences in the incidence of reperfusion ventricular fibrillation between groups. Probability values less than 0.05 again indicated significance.

Criticism of Model

The isolated Langendorff perfused rat heart model has both advantages and limitations.

Advantages of model. a) The consistent occurrence of reperfusion ventricular fibrillation (100%), the predictable time of onset of such fibrillation and the consistent sinus rhythm in the preligation period make this an excellent model to assess antiarrhythmic efficacy. b) The concentrations of pharmacologic agents perfusing the heart are maintained at constant levels. c) The metabolic consequences of acute myocardial ischemia and reperfusion of the acutely ischemic myocardium may be correlated with ventricular arrhythmias.

Limitations of model. a) The isolated heart is devoid of external autonomic nervous control. Nevertheless, there are endogenous catecholamines that are similar in content to the heart in situ and only mildly reduced with coronary ligation (26). b) The isolated heart has an abnormally high coronary flow rate as a consequence of absence of hemoglobin in the perfusion fluid. c)The high preligation heart rate (approximately 300 beats/min) makes it difficult to correctly classify the nature of some tachycardias (up to 800 beats/min) (26). d) The aortic-perfused heart performs no volume work as there is no left atrial filling. e) As in any model, effects may be species-specific, especially in the density and nature of adrenoceptors.

Results

Effect of Coronary Artery Ligation and Reperfusion

Coronary flow. Ligation of the left main coronary artery resulted in a decrease in the mean total coronary flow rate (ischemic and nonischemic flow) in control hearts from 10.3 ± 0.5 to 5.4 ± 0.4 ml/min ($p < 0.001$). On reperfusion, there was an immediate reversal of the coronary flow rate to 12.1 ± 0.9 ml/min ($p < 0.02$), a value significantly higher than that found before coronary ligation. This probably reflects the phenomenon of reactive hyperemia.

In hearts perfused with alpha- or beta-adrenoceptor antagonists, the mean total coronary flow rates during acute myocardial ischemia and on reperfusion were similar or, in some series, significantly lower than the mean coronary flow rate found in the control series (data available on request), suggesting that the antiarrhythmic activity of these agents was not the consequence of improved coronary perfusion. However, any redistribution of coronary flow cannot be excluded because only total coronary flow (ischemic and nonischemic) was measured.

Ventricular arrhythmias. During the 15 minute period of coronary artery occlusion, the following ventricular arrhythmias occurred in the control series (n = 18): 100% of hearts developed ventricular premature systoles (mean number/15 min = 46 ± 10), 89% developed ventricular tachycardia (mean duration 34 ± 6 seconds) and 11% developed ventricular fibrillation. A decrease in the mean heart rate occurred during coronary artery occlusion from 264 ± 10 (preligation) to 214 ± 8 beats/min (postligation; paired *t* test, $p < 0.01$). Within 15 seconds of reperfusion of the acutely ischemic myocardium, ventricular fibrillation occurred in all hearts (100% incidence). Analysis of the heart rhythm during the first 600 seconds of reperfusion showed that the mean duration of ventricular fibrillation combined with ventricular tachycardia over the period was 580 ± 10 seconds.

Effect of Alpha-adrenoceptor Antagonist Agents

Coronary occlusion. Perfusion with yohimbine ($alpha_2$-antagonist), 0.1 to 5 μmol/liter, and phentolamine ($alpha_1$- and $alpha_2$-antagonist), 0.1 to 100 μmol/liter, resulted in a concentration-related decrease in the incidence of ventricular arrhythmias during the phase of acute coronary occlusion. Ventricular fibrillation did not occur in hearts perfused with even the lowest concentrations studied (0.1 μmol/liter) of both phentolamine and yohimbine, whereas with prazosin, ventricular fibrillation was not evident when concentrations of 1 μmol/liter or higher were used. In the control of ventricular tachycardia during ischemia, the ED_{50} values obtained from the log dose-response curves were yohimbine, 0.50 μmol/liter; prazosin, 2.0 μmol/liter and phentolamine, 5 μmol/liter, respectively (Table 1).

Reperfusion. All three alpha-adrenergic antagonist agents produced a concentration-dependent reduction in the incidence of reperfusion ventricular fibrillation, the ED_{50} values obtained from the log dose-response curves were yohimbine, 0.70 μmol/liter; prazosin, 2.0 μmol/liter and phentolamine, 2.5 μmol/liter, respectively (Tables 1 and 2).

Complete protection against reperfusion ventricular arrhythmias occurred with the following concentrations (μmol/liter): yohimbine, 5; prazosin, 20 and phentolamine, 50. The mean heart rate was reduced in all series exhibiting complete protection. Rates were (beats/min): yohimbine, 180 ± 10 ($p < 0.05$); prazosin, 175 ± 8 ($p < 0.01$) and phentolamine, 170 ± 12 ($p < 0.01$) versus control series, 214 ± 8. The reduction in the mean duration of reperfusion ventricular fibrillation and ventricular tachycardia is shown in Figure 1.

Effect of Beta-adrenoceptor Antagonist Agents on Ventricular Arrhythmias

According to the dose-response curve test of the beta-agonist isoproterenol, a concentration of 0.1 μmol/liter exhibited near maximal cardiac effects, that is, an increase in

Table 1. Effect of Alpha- or of Beta-adrenoceptor Antagonist Agents on Ischemic and Reperfusion Ventricular Arrhythmias

Agent (molar)	n	Ischemia		Reperfusion	
		VT + VF Duration	VT Incidence (%)	VT + VF Duration	VF Incidence (%)
		Alpha-adrenoceptor Antagonists			
Phentolamine					
10^{-7}	5	53 ± 17	100	552 ± 15	100
5×10^{-7}	10	27 ± 11	70	407 ± 83	80
10^{-6}	10	26 ± 5	90	301 ± 77	70
5×10^{-6}	8	8 ± 5	50	132 ± 72	38
10^{-5}	13	2 ± 1	38	31 ± 11	28
5×10^{-5}	9	0	0	0	0
Prazosin					
5×10^{-7}	8	35 ± 6	100	551 ± 14	100
10^{-6}	12	16 ± 5	67	424 ± 72	75
5×10^{-6}	9	15 ± 10	33	126 ± 74	22
10^{-5}	11	8 ± 6	27	38 ± 34	18
2×10^{-5}	7	0	0	0	0
Yohimbine					
10^{-7}	9	9 ± 3	67	350 ± 85	78
5×10^{-7}	10	9 ± 4	50	289 ± 89	60
10^{-6}	14	1 ± 1	15	167 ± 64	36
2.5×10^{-6}	9	0	0	0	0
5×10^{-6}	9	0	0	0	0
		Beta-adrenoceptor Antagonists			
dl-propranolol					
10^{-7}	4	27 ± 11	100	593 ± 3	100
5×10^{-7}	4	25 ± 9	75	587 ± 9	100
10^{-6}	7	15 ± 9	57	415 ± 81	86
2.5×10^{-6}	8	12 ± 5	50	171 ± 63	63
5×10^{-6}	9	9 ± 4	44	28 ± 12	11
10^{-5}	8	0	0	0	0
d-propranolol					
10^{-6}	6	13 ± 7	50	385 ± 87	83
2.5×10^{-6}	8	20 ± 8	63	69 ± 33	50
5×10^{-6}	8	8 ± 4	13	15 ± 8	0
10^{-5}	7	0	0	0	0
Metoprolol					
10^{-6}	4	44 ± 20	100	589 ± 5	100
10^{-5}	8	8 ± 3	63	486 ± 57	100
5×10^{-5}	6	15 ± 5	83	150 ± 57	50
10^{-4}	8	0	0	0	0
2×10^{-4}	6	0	0	5 ± 5	0
4×10^{-4}	5	0	0	0	0
Atenolol					
5×10^{-7}	5	39 ± 29	60	600 ± 0	100
10^{-6}	8	60 ± 12	67	596 ± 4	100
5×10^{-6}	4	49 ± 17	75	597 ± 4	100
10^{-5}	6	25 ± 8	100	543 ± 21	100
10^{-4}	9	18 ± 7	78	417 ± 51	100
		Class I Antiarrhythmic Agent			
Lidocaine*					
2×10^{-5}	—	No data	80	No data	100
3.8×10^{-5}	—	No data	50	No data	50
1×10^{-4}	—	No data	5	No data	0

Isolated rat hearts were perfused in retrograde manner via the aorta for 15 minutes before coronary artery ligation, after 15 minutes of coronary occlusion, the ligature was released and the rhythm monitored for a further 10 minutes (600 seconds). Drugs were added 5 minutes before coronary ligation. In control series (n = 18), ventricular arrhythmias were: 1) ischemia VT 89% and VF 11%; and 2) reperfusion VF 100%. Mean duration of reperfusion VT + VF = 580 ± 10 seconds.

n = number of hearts in each group, VF = ventricular fibrillation; VT = ventricular tachycardia; VT + VF = combined duration of these arrhythmias. (For difficulties in distinction, see Didier et al. [26].) (*Adapted from Lubbe et al. [16] with permission.)

Table 2. Comparison of Median Effective Concentration (ED_{50}) of Alpha- or Beta-adrenoceptor Antagonist Agents on the Incidence of Reperfusion Ventricular Fibrillation in Comparison With Published Values for Inhibition or Dissociation Constants (K_I or K_D), Based Chiefly on Displacement of Radioligands From Rat Myocardial and Other Tissues (for relation between K_D and K_I, see Maguire et al. [39])

	ED_{50} (*M*)	K_I for Displacement of Specific α_1 Antagonist (29) (*M*)	K_D for α_2 Radioligand Binding (30) (*M*)	K_D for β_1 Radioligand Binding (*M*)	K_D for Propranolol Binding Site of Dog Heart Measured by Antibody Techniques (42) (*M*)
			Alpha-adrenoceptor Antagonists		
Phentolamine (α_1 and α_2)	2.5×10^{-6}	5×10^{-8}	—	—	—
Prazosin (α_1)	2×10^{-6}	3×10^{-10}	—	—	—
Yohimbine (α_2)	7×10^{-7}	9×10^{-7}	2×10^{-8} (initial) 8×10^{-10} (final)	—	—
			Beta-adrenoceptor Antagonists		
dl-Propranolol	2.0×10^{-6}	—	—	1.6×10^{-9}*	22×10^{-9}
d-Propranolol	2.5×10^{-6}	2×10^{-5}	—	—	4×10^{-6}
Metoprolol	5×10^{-5}	—	—	3×10^{-7}†	—
Atenolol	1×10^{-4}	—	—	7.5×10^{-7}‡	—

* Data for rat heart assuming that dl-propranolol has half the potency of l-propranolol (adapted from Maguire et al [39] with permission), † data for guinea-pig left ventricle (adapted from Hedberg et al. [40] with permission); ‡ data for immature rat erythrocytes (adapted from Palm [41]) with permission).
K_D = values for antibody binding of dl-propranolol presumed to be double that of l-propranolol; *M* = molar, — = no data.

both the heart rate and coronary flow rates. The effects of three beta-antagonists, metoprolol, atenolol and propranolol (added 5 minutes before coronary ligation), on the arrhythmias of coronary occlusion and reperfusion were as follows.

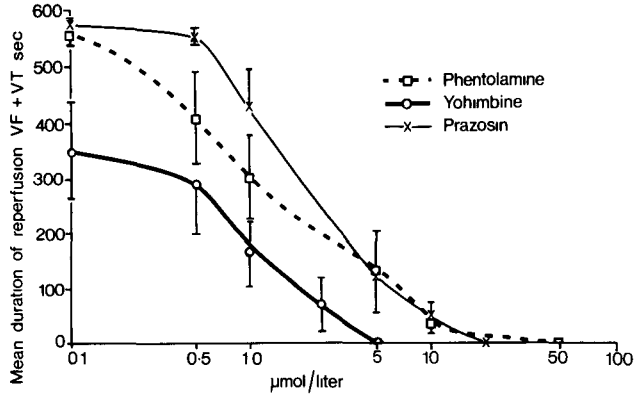
Metoprolol. Metoprolol, 10 μmol/liter, exhibited complete antagonism against the cardiac effects of isoproterenol, 0.1 μmol/liter, as judged by the absence of an increase in heart rate and coronary flow.

Occlusion. Metoprolol, 10 μmol/liter, did not reduce the incidence of ventricular tachycardia but decreased the mean duration of ventricular tachycardia. The median effective dose (ED_{50}) value for reduction in the incidence of ventricular tachycardia was 50 μmol/liter. Ventricular arrhythmias were absent when the perfusate contained metroprolol in a concentration of 100 μmol/liter (Table 1).

Reperfusion. Metoprolol, 50 to 100 μmol/liter, decreased the incidence of reperfusion ventricular fibrillation, and the ED_{50} value obtained from the log dose-response curve was 50 μmol/liter (Fig. 2). The ED_{50} values of metoprolol for reduction in incidence of ventricular tachycardia during occlusion and reperfusion ventricular fibrillation were identical. Reperfusion ventricular fibrillation was not encountered with metoprolol, 100 μmol/liter (Fig. 2). The mean heart rate of hearts perfused with metoprolol, 100 μmol/liter, was 165 ± 7 beats/min. Because metoprolol protected against reperfusion ventricular fibrillation in a much higher concentration (50 to 100 μmol/liter) than that which provided complete beta-adrenoceptor antagonism (Table 2), we compared the antiarrhythmic effects of two other compounds, atenolol and propranolol, with their beta-antagonist effect.

Atenolol. This highly selective $beta_1$-antagonist, which does not have membrane-stabilizing activity when used in a dose of 30 μmol/liter, completely antagonized the cardiac effects of isoproterenol, 0.1 μmol/liter. However, atenolol, 0.5 to 100 μmol/liter, did not protect against ventricular arrhythmias during the period of coronary artery occlusion;

Figure 1. The duration of reperfusion in the experimental protocol was 600 seconds. The control series (not depicted) had a mean duration of reperfusion ventricular fibrillation (VF) and ventricular tachycardia (VT) of 580 ± 10 seconds for virtually the entire period of reperfusion. Antiarrhythmic activity was assessed also by a reduction in the mean duration of these arrhythmias during the 600 seconds of reperfusion. Although lower concentrations of adrenoceptor antagonist may not have reduced the incidence of reperfusion ventricular fibrillation, antiarrhythmic activity was manifest by their ability to cause spontaneous reversion from ventricular fibrillation to sinus rhythm during the 600 second period of reperfusion. Thus, prazosin, yohimbine and phentolamine produced concentration-dependent decreases in the mean duration of reperfusion ventricular fibrillation and tachycardia.



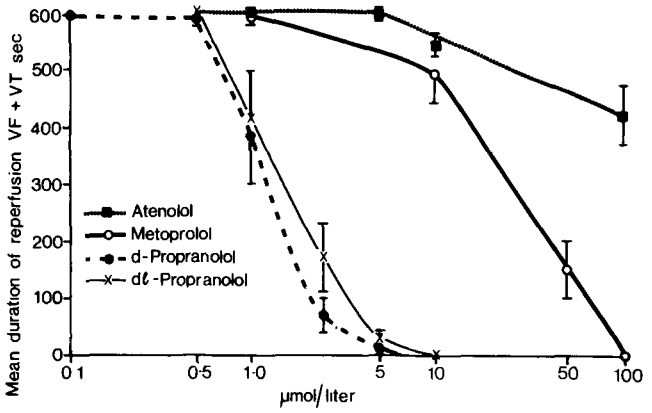


Figure 2. Racemic propranolol, d-propranolol and metoprolol in high concentrations decreased the mean duration of reperfusion ventricular fibrillation and tachycardia. d-Propranolol, the propranolol isomer with only partial beta-antagonist activity but with marked membrane-stabilizing activity, exhibited antiarrhythmic activity similar to that of racemic propranolol. Atenolol was ineffective except at the highest concentration of 100 μmol/liter. Minimal reduction in reperfusion ventricular fibrillation and tachycardia occurred ($p < 0.02$).

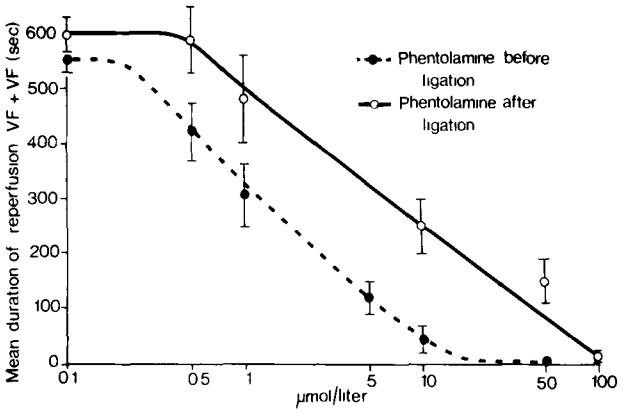


Figure 3. The administration of phentolamine 5 minutes after coronary artery ligation (compared with administration 5 minutes before ligation) shifted the dose-response curve to the right for the reduction in a) incidence of reperfusion ventricular fibrillation, and b) mean duration of reperfusion ventricular fibrillation and ventricular tachycardia. Only data for the latter are shown.

there was a 90% incidence of ventricular extrasystoles (frequency 35 ± 6/15 min) and 83% incidence of ventricular tachycardia (control incidence 89%, mean duration 40 ± 6 seconds). Atenolol, 0.5 to 100 μmol/liter, did not reduce incidence of reperfusion ventricular fibrillation (Table 1).

Propranolol. dl-Propranolol, 0.5 μmol/liter, exerted complete beta-antagonism against the cardiac effects of isoproterenol, 0.1 μmol/liter, but did not protect against reperfusion ventricular fibrillation. dl-Propranolol in a higher concentration of 10 μmol/liter gave complete protection (Fig. 2). d-Propranolol, 10 μmol/liter, the isomer possessing membrane-stabilizing activity (27), and with only little of the beta-blocking activity of the racemic mixture (27), also totally protected against reperfusion ventricular fibrillation (Fig. 2). The reduction in the mean duration of reperfusion ventricular fibrillation and ventricular tachycardia evoked by beta-antagonists is shown in Figure 2.

Effect of Drugs When Added 5 Minutes After Coronary Artery Ligation

Coronary occlusion. Phentolamine, metoprolol and the combination of phentolamine and metoprolol reduced duration and incidence of ventricular tachycardia and also number of ventricular systoles (Table 1).

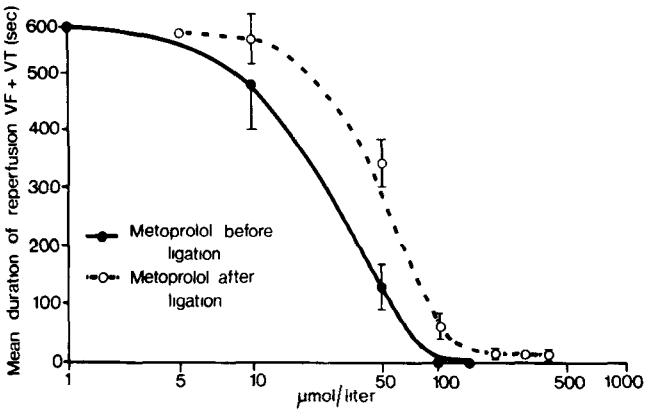
Reperfusion. The ED_{50} value of phentolamine and metoprolol for decreasing the incidence of reperfusion ventricular fibrillation shifted from 2.5 and 50 μmol/liter, respectively (drug added 5 minutes before ligation), to 120 and 150 μmol/liter, respectively (drug added 5 minutes after ligation) (Fig. 3 and 4). The ED_{50} values of both phentolamine and metoprolol closely approximated the concentration of phentolamine (50 μmol/liter) and metoprolol (100 μmol/liter), producing total antiarrhythmic protection in this experimental situation.

Because of the high drug concentration required for an antiarrhythmic effect when added after coronary ligation, the combination of alpha- and beta-antagonism was studied. Metoprolol, 100 μmol/liter, plus phentolamine, 100 μmol/liter, exerted total protection against reperfusion ventricular fibrillation, thereby acting more effectively than either agent in the respective concentrations. However, this combination was associated with marked bradycardia (120 ± 8 versus control 214 ± 8 beats/min, $p < 0.0001$).

Myocardial Metabolism

Coronary occlusion. Fifteen minutes after the onset of acute coronary artery ligation in control hearts, that is, immediately before the onset of reperfusion, the tissue met-

Figure 4. The initiation of metoprolol 5 minutes after coronary artery ligation (compared with initiation 5 minutes before ligation) shifted the dose-response curve to the right for reduction in a) incidence of reperfusion ventricular fibrillation, and b) mean duration of reperfusion ventricular fibrillation and ventricular tachycardia. Only data for the latter are shown.



abolic profile in the ischemic myocardium showed the expected depletion of high energy phosphate and glycogen content and accumulation of lactate and cyclic AMP (data available on request).

The administration of phentolamine, metoprolol or the combination of these two agents (all 100 μmol/liter) begun 5 minutes after coronary artery ligation reduced the marked depletion of phosphocreatine and glycogen content but did not change the extent of accumulation of tissue cyclic AMP in the ischemic myocardium (data available on request).

Reperfusion. Ten seconds after the onset of reperfusion (that is, immediately before the onset of reperfusion ventricular fibrillation), the metabolic changes during the ischemic period were somewhat worsened by the short period of reperfusion (Table 3). Hearts perfused with either metoprolol, 100 μmol/liter, phentolamine, 100 μmol/liter, or the combination, 100 μmol/liter, 5 minutes after the onset of coronary artery ligation were associated with improved metabolic status on reperfusion, such as higher tissue levels of adenosine triphosphate and phosphocreatine and lower levels of lactate and cyclic AMP (Table 3). Metoprolol was least effective in the prevention of reperfusion arrhythmias, but the preservation of adenosine triphosphate content and the reduction in tissue lactate and cyclic AMP content were similar to that of phentolamine or phentolamine plus metoprolol.

All three series were associated with a slower heart rate than that found in the control series (214 ± 8 beats/min). Heart rates were reduced as follows: metoprolol, 164 ± 7 ($p < 0.01$); phentolamine, 140 ± 9 ($p < 0.001$) and phentolamine plus metoprolol, 120 ± 8 ($p < 0.001$) (p values versus control hearts).

Table 3. Effect of Adrenoceptor Antagonist Agents on Metabolic Status of Whole Heart Within 10 Seconds of Reperfusion: Relation to Reperfusion Ventricular Fibrillation and Ventricular Tachycardia

	ATP (μmol/g)	PCr (μmol/g)	Glycogen (μmol/g)	Cyclic AMP (nmol/g)	Lactate (μmol/g)	VF Incidence (%)	VF + VT Duration (seconds)
1. Nonligated hearts	4.5 ± 0.1	4.1 ± 0.2	17.3 ± 1.0	—	0.4 ± 0.02	—	—
2. Ligated hearts	3.1 ± 0.2	2.7 ± 0.1	9.4 ± 0.8	0 42 ± 0.02	—	—	—
p vs 1	<0.001	<0.001	<0.001				
3. Ligated + reperfused hearts	2.8 ± 0.1	1.9 ± 0.1	8.4 ± 1.1	0.49 ± 0 03	8.7 ± 0.4	100	580 ± 10
p vs 1	<0.001	<0.001	<0.001	—	<0.001	—	—
4. Ligated + reperfused hearts + additions							
a. Metoprolol (100 μmol/liter) after CAL	3.1 ± 0.1	3.0 ± 0.1	10.2 ± 1.4	0.39 ± 0.02	7.1 ± 0.7	100	58 ± 12
p vs 3	<0.05	<0.001	NS	<0.05	NS	NS	<0.001
b. Phentolamine (100 μmol/liter) after CAL	3.2 ± 0 1	3.7 ± 0.2	8.8 ± 1.3	0 39 ± 0.02	7 3 ± 0 4	50	8 ± 2
p vs 3	<0.01	<0.001	NS	<0.05	<0 05	<0.05	<0.001
c. Phentolamine + metoprolol (100 μmol/liter) after CAL	3.6 ± 0.1	4.0 ± 0.2	11.7 ± 1.1	0.41 ± 0 02	5.7 ± 0.7	0	1 ± 1
p vs 3	<0.01	<0.001	NS	NS	<0.005	<0.01	<0 005
d. Phentolamine (10 μmol/liter) before CAL	3.4 ± 0.2	3.1 ± 0.2	13.6 ± 1.9	—	6.3 ± 0.8	28	31 ± 11
p vs 3	<0.01	<0.001	<0.01	—	<0.05	<0.01	<0.001
e. Prazosin (10 μmol/liter) before CAL	3.5 ± 0.1	3.4 ± 0.2	12.6 ± 0.8	—	5.6 ± 0 6	18	38 ± 34
p vs 3	<0.01	<0.001	<0.05	—	<0 001	<0.01	<0.001
f. Yohimbine (1 μmol/liter) before CAL	3.9 ± 0.1	3.9 ± 0.2	13.8 ± 1.5	—	4.6 ± 0 6	36	167 ± 64
p vs 3	<0.0001	<0.0001	<0.05	—	<0.001	<0.05	<0.001

Series 1. The nonligated hearts represent hearts perfused for 30 minutes and then clamped for biochemical analysis

Series 2 Ligated hearts represent hearts perfused for 15 minutes, then subjected to left main coronary artery ligation and clamped for biochemical analysis at the end of the 15 minute period of ligation (total duration 30 minutes), i e , immediately before onset of reperfusion

Series 3 Ligated and reperfused hearts represent hearts perfused for 15 minutes, subjected to left coronary ligation for 15 minutes, then reperfused and clamped after 10 seconds (total duration 30 minutes, 10 seconds), immediately before onset of reperfusion ventricular fibrillation that occurs within 15 seconds of reperfusion

Series 4. Ligated and reperfused hearts in which drugs were added either 5 minutes after ligation (4a, b, c) or 5 minutes before ligation (4d, e, f)

Mean ± for 8 to 20 hearts; p values for ligation and reperfusion and drug versus ligation and reperfusion Values represent mean ± standard error of the mean, all values expressed in terms of fresh weight (= dry weight × 5), glycogen expressed as nmol glucose equivalent

AMP = adenosine monophosphate; ATP = adenosine triphosphate; CAL = coronary artery ligation, NS = not significant, p = probability; PCr = phosphocreatine, VF = ventricular fibrillation; VT = ventricular tachycardia, — = absence of data

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Effect of phentolamine, prazosin and yohimbine when added 5 minutes before coronary artery ligation. Hearts perfused with phentolamine, 10 μmol/liter; yohimbine, 1 μmol/liter; and prazosin, 10 μmol/liter, begun 5 minutes before coronary artery ligation were associated with preservation of adenosine triphosphate, phosphocreatine and glycogen; moreover, tissue lactate levels were reduced (Table 3).

Because both yohimbine, 1 μmol/liter, and phentolamine, 10 μmol/liter, were not associated with any significant reduction in the heart rate in comparison with the control series, the improved metabolic status in these series was probably not the consequence of a slow heart rate.

Reserpine pretreatment. In this group, tyramine stimulation of the perfused heart failed to increase the heart rate as in control hearts, showing effective depletion of catecholamines. The duration of reperfusion ventricular tachycardia-fibrillation was reduced from 529 ± 66 (n = 9 control hearts) to 301 ± 68 seconds (n = 12 reserpine-treated hearts, $p < 0.03$).

Discussion

Reperfusion ventricular fibrillation may occur on regression of coronary artery spasm or after revascularization of an acutely occluded coronary artery. The relevance of reperfusion ventricular fibrillation stems from the proposal that it may be the underlying mechanism of sudden death in patients with coronary artery disease who have no evidence of coronary occlusion or acute myocardial infarction.

Role of alpha-adrenergic responses. At present there are discordant observations as to the presence of $alpha_1$- and $alpha_2$-receptor subtypes in the rat myocardium. Williams et al. (28) and Steinberg and Bilezikian (29) have characterized the myocardial alpha-receptors as being primarily of the $alpha_1$ subtype; the apparent inhibitory constants (study of Steinberg) being compatible with the expected order of potency of $alpha_1$- adrenoceptors: prazosin, $3 \times 10^{-10} M$; phentolamine, $5 \times 10^{-8} M$ and yohimbine, $9 \times 10^{-7} M$.

In contrast to these findings, Guicheney et al. (30) demonstrated that the rat myocardium contains an equal number of $alpha_1$- and $alpha_2$-receptors. Their study showed presynaptic binding, resembling $alpha_2$-receptor binding, with initial and final dissociation constants of $2.3 \times 10^{-8} M$ and $8.3 \times 10^{-10} M$ for yohimbine.

$Alpha_1$- and $alpha_2$- mediated response. A mechanism specific to $alpha_1$-receptors, suggested previously by Sheridan et al. (15) and Corr and Crafford (31), appears unlikely because the concentration of prazosin required to exert protection was 10-fold higher than that of yohimbine. According to the dissociation constants for $alpha_1$-adrenergic receptors, prazosin should have been more effective at a much lower concentration than yohimbine in the prevention of reperfusion ventricular fibrillation. It may, therefore, be asked whether the protective mechanism is specific to the $alpha_2$-receptor. If this were so, prazosin, the specific alpha-antagonist, would be expected to be markedly less potent than yohimbine, the specific $alpha_2$-antagonist, which is not the case (Table 2).

Our findings, therefore, suggest a putative role for both $alpha_1$- and $alpha_2$-mediated responses in the genesis of reperfusion ventricular fibrillation. Because the protective concentration of yohimbine, the $alpha_2$-antagonist, approximated more closely its dissociation constant (using the initial value of Guicheney et al. [30], $alpha_2$-responses may be more important than $alpha_1$-responses in the initiation of reperfusion ventricular fibrillation. Although yohimbine has nonspecific effects that could play a major role in this proposed antiarrhythmic effect, our conclusions are of interest as the antiarrhythmic potential of yohimbine has not yet been emphasized.

Relation of protective concentrations of alpha-antagonist agents to the dissociation constants. The concentrations of alpha-adrenergic antagonist agents that protected against reperfusion ventricular fibrillation appear relatively high in comparison with the molar concentrations of the displacement constants of myocardial $alpha_1$- and $alpha_2$-receptors. Factors that may account for this finding are:

a) Displacement constants represent 50% of receptor binding. Protection against ventricular fibrillation may require complete receptor binding, in which case high concentrations of antagonist agents would be necessary.

b) $Alpha_1$-receptors increase in number in the cat heart after the development of acute myocardial ischemia and on reperfusion (32). The concentrations of $alpha_1$-adrenergic antagonist agents required to exhibit receptor antagonism would, therefore, be correspondingly increased. No results are available as to the changes of $alpha_2$-receptors after acute ischemia and on reperfusion.

c) Ligation of the left main coronary artery in the rat results in extensive ischemia of the left ventricular free wall. This is thought to induce a marked local release of endogenous noradrenalin in the ischemic myocardium which supposedly may attain a concentration of 1 μmol/liter (33). To achieve receptor antagonism, a correspondingly high concentration of antagonist agent may be necessary.

d) The protective effect of alpha-antagonist agents may be due to a nonspecific effect. Thus, Rosen et al. (34) showed that phentolamine in concentrations of 10 μmol/liter or greater produces consistent electrophysiologic changes in canine cardiac Purkinje fibers similar to those manifested by class I antiarrhythmic agents that possess membrane-stabilizing activity, namely, decreased automaticity and membrane responsiveness and increased conduction time, action potential duration and effective refractory period, respectively (phentolamine, 1 μmol/liter, did not consistently produce these changes). Further evidence that phentolamine may exert a primary electrophysiologic effect stems from the findings (35) that phentolamine prevented the reduction in

effective refractory period during coronary occlusion as well as the overshoot in the effective refractory period on reperfusion in the dog. These electrophysiologic changes were associated with protection against reperfusion arrhythmias in that dog model.

In our study, the concentration of phentolamine producing a 50% reduction in the incidence of reperfusion ventricular fibrillation was 2.5 μmol/liter, with complete protection occurring at 50 μmol/liter. Therefore, it is not unlikely that the protective effect of alpha-adrenergic antagonist agents in our study may be dependent partly or completely on a nonspecific effect, namely, membrane-stabilizing activity. This proposal is strengthened by the finding that pretreatment with reserpine reduced the duration of reperfusion ventricular tachycardia-fibrillation.

Comment. The propensity of alpha-adrenergic antagonist agents to protect against reperfusion ventricular fibrillation is complex and may be dependent either on $alpha_2$- and less so on $alpha_1$-receptor antagonism or on membrane-stabilizing activity, or both.

Role of beta-adrenergic-mediated responses. Three beta-adrenoceptor antagonists, atenolol, 30 μmol/liter; metoprolol, 10 μmol/liter, and propranolol, 0.5 μmol/liter, displayed complete beta-antagonism against the cardiac effects of the $beta_1$-agonist, isoproterenol, 0.1 μmol/liter. However, in these concentrations, all three agents were ineffective in the prevention of ventricular fibrillation. In higher concentration, atenolol, 100 μmol/liter, displayed no protection, whereas metoprolol, 10 μmol/liter, and propranolol, 10 μmol/liter, respectively, exhibited marked protection. The common property shared by these high concentrations of metoprolol and propranolol (27) and lacking in atenolol is membrane-stabilizing activity. The much higher concentration of metoprolol required reflects its much lower membrane-stabilizing activity. d-Propranolol, the propranolol isomer with dominant membrane-stabilizing activity but with only some of the beta-antagonist activity of the racemic mixture, showed protection against reperfusion-induced fibrillation equivalent to that of equimolar racemic dl-propranolol.

Comment. Our findings suggest that it is not the beta-adrenergic antagonist activity itself that mediates protection against reperfusion arrhythmias, but rather the membrane-stabilizing activity possessed by some beta-adrenergic antagonists.

Role of time of administration of drug in the prevention of reperfusion ventricular fibrillation. Initiation of antiarrhythmic therapy 5 minutes after coronary artery ligation (compared with initiation 5 minutes before coronary ligation) shifted the dose-response curve of phentolamine and metoprolol to the right. The relative inaccessibility of drugs to receptor or membrane due to impaired coronary perfusion may explain the necessity for high concentrations when drug administration was begun after coronary ligation.

Major side effect. Drug concentrations of 10 μmol/liter or greater resulted in a 25 to 40% reduction in heart rate. Thus, in secondary antiarrhythmic prophylaxis (drug administration after the onset of ischemia), optimally protective concentrations against reperfusion ventricular fibrillation may not be achieved because of marked hemodynamic impairment. Administration before the onset of ischemia, therefore, would be a more desirable approach in the prevention of reperfusion ventricular fibrillation because a lower drug concentration is then effective.

Relation between ventricular arrhythmias during coronary occlusion and reperfusion. The incidence of reperfusion ventricular fibrillation appeared to depend in part on the incidence or severity of ventricular arrhythmias during the antecedent period of coronary artery occlusion. a) The ED_{50} values of alpha- and beta-antagonist agents for reduction in the incidence of ventricular tachycardia during coronary occlusion and reperfusion ventricular fibrillation, respectively, were similar. b) The concentrations of alpha- and beta-antagonist agents that exerted maximal antiarrhythmic activity during occlusion and on reperfusion were similar.

Thus, although disparate electrophysiologic mechanisms (36) have been considered to underlie ventricular arrhythmogenesis during occlusion and after reperfusion, this study demonstrates that a single mode of antiarrhythmic therapy with phentolamine or metoprolol may be effective on arrhythmias in both settings.

Effects on myocardial metabolism. We have proposed that biochemical sequelae of acute myocardial ischemia may predispose to arrhythmogenesis (3). Thus, shortening of the action potential duration may arise from the reduction in the glycolytic (10) and mitochondrial (11) production of adenosine triphosphate, whereas slow response action potentials are hypothetically related to the accumulation of cyclic AMP in the ischemic tissue. In our study, some circumstantial association was evident between improved metabolic status on reperfusion and protection against reperfusion ventricular fibrillation (Table 3). Preservation of high energy phosphate compounds may exhibit antiarrhythmic activity by maintenance of action potential duration and cell membrane integrity, thereby preventing the proposed arrhythmogenic effect of lysophosphoglycerides (37). In addition, the lower levels of cyclic adenosine monophosphate 10 seconds after reperfusion may possibly be a factor in preventing reperfusion arrhythmias (38). However, in our study, agents affording a similar degree of metabolic preservation were associated with varying degrees of antiarrhythmic activity (compare metoprolol, 100 μmol/liter added 5 minutes after ligation and phentolamine and 10 μmol/liter added 5 minutes before coronary ligation). If preservation of metabolic status plays a role in the mediation of the antiarrhythmic effects, it is but one of several factors involved.

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