Effects of antiarrhythmic agents classified as class III group on ischaemia-induced myocardial damage in canine hearts

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- 1 The cardioprotective effects of antiarrhythmic agents classified as class III, amiodarone, sotalol and E-4031, were investigated in anaesthetized dogs.
- 2 The left anterior descending coronary artery was occluded for 2 h.
- 3 Heart mitochondria were prepared from both the ischaemic and non-ischaemic areas, and their function was estimated polarographically.
- 4 Activities of the lysosomal enzymes, N-acetyl- β -glucosaminidase and β -glucuronidase, were measured in each fraction.
- 5 Two hour occlusion induced ventricular arrhythmias, and amiodarone, sotalol and E-4031 greatly suppressed the development of arrhythmias.
- 6 Amiodarone, sotalol and E-4031 significantly protected mitochondria against ischaemia, and prevented ischaemia-induced leakage of lysosomal enzymes.
- 7 Antiarrhythmic agents classified as class III show cardioprotective effects, which might participate in their antiarrhythmic effect.

Introduction

Of the estimated episodes of acute myocardial infarction, about 50% will cause a patient to die before hospitalization. Patients who die during hospitalization usually succumb to either arrhythmias or pump failure. The introduction and widespread employment of coronary care units designed for continuous electrocardiographic monitoring of patients with acute myocardial infarction have resulted in reduced mortality (Singh *et al.*, 1980). Recent advances in cardiology have shown that substantial quantities of ischaemic myocardium, destined to undergo necrosis, can be salvaged by treatment with several drugs such as β -blockers, nitrates, calcium antagonists and hyaluronidase (Maroko *et al.*, 1972; Smith *et al.*, 1973; Opie *et al.*, 1975; Sugiyama *et al.*, 1983; Nagai *et al.*, 1984). The feasibility of protection of ischaemic myocardium has been demonstrated experimentally by many different techniques in various laboratories and in many different species.

The sudden onset of ischaemia induced by occlusion of the coronary artery is associated with a series of metabolic, functional and ultrastructural changes. The dependence of myocardium on aerobic, in other words, mitochondrial metabolism, is reflected in the fact that about 30% of the volume of a normal left ventricular myocyte is occupied by mitochondria (Page & McCallister, 1973). A persistent decrease in tissue adenosine triphosphate level, based on mitochondrial dysfunction, leads to cellular dysfunction. In addition, lysosomes contain phospholipases and proteinases and leakage of lysosomal enzymes might play an important role in irreversible cellular damage (Hearse, 1979). Indeed, Kennet & Weglicki (1978) showed that in ischaemic myocardium lysosomal enzyme activities were decreased around the 20000 *g* fraction, and these increased in the supernatant of homogenized myocardium. Hence, the estimation of mitochondrial function and of lysosomal enzyme release is a useful biochemical marker to assess myocardial damage following coronary occlusion.

Since an iodinated benzofuran derivative, amiodarone, was introduced, effects of antiarrhythmic drugs of class III on myocardial ischaemia have attracted much interest (Marcus *et al.*, 1981). It is well known that amiodarone has a mild non-

competitive sympathetic antagonistic effect and significant coronary and peripheral vasodilator actions (Mason, 1987). Sotalol is a β -adrenoceptor blocking drug with class III activity (Strauss *et al.*, 1970). It was shown that sotalol prevented ventricular arrhythmias in dogs with coronary artery occlusion (Cobbe *et al.*, 1983). Nevertheless, effects of antiarrhythmic agents classified as class III on ischaemia-induced myocardial damage have been little studied. In the present study, we examined effects of amiodarone, sotalol, and a newly developed class III agent, E-4031 (Sawada *et al.*, 1988), on mitochondrial function and stability of the lysosomal membrane after coronary artery occlusion.

Methods

Animal preparations

Thirty three mongrel dogs of either sex weighing between 8–15 kg were anaesthetized with sodium pentobarbitone (50 mg kg⁻¹) given intraperitoneally. After endotracheal intubation, each animal was mechanically ventilated with a Harvard type ventilator. Lead II of the electrocardiogram was monitored continuously by a VC-640G oscillographic recorder and recorded simultaneously by an ICR-7200 ambulatory electrocardiogram recorder throughout the experiment. The right femoral artery and left femoral vein were cannulated for measurement of arterial blood pressure and for infusion of the drug and physiological saline, respectively.

The chest was opened at the 4th or 5th intercostal space and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery (LAD) was dissected free of fat and connective tissue immediately distal to the first diagonal branch. A silk ligature was placed around the LAD for acute occlusion.

Experimental design

The dogs were divided into 4 groups. Twenty-five minutes before LAD occlusion, physiological saline (25 ml) was administered for 10 min to the control group (*n* = 12). For the amiodarone group (*n* = 7), 25 min before LAD occlusion, amiodarone (3 mg kg⁻¹) dissolved in 5 ml distilled water was administered for 10 min. For the sotalol group (*n* = 7), 25 min before LAD occlusion, sotalol (1 mg kg⁻¹) dissolved in 5 ml of physiological saline was administered for 10 min. For the E-4031 group (*n* = 7), 15 min before LAD occlusion, 2.5 μ g kg⁻¹ min⁻¹ E-4031 (1-(2-(6-methyl-2-pyridyl)ethyl)-4-(4-

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Table 1 Total number of ventricular premature beats, arrhythmia ratio, and total duration time of ventricular tachycardia in all groups

Group	Total number of ventricular premature beats	Arrhythmia ratio (%)	Total duration time of ventricular tachycardia
Control	416.4 ± 275.8	2.57 ± 1.85	34.5 ± 47.8
Amiodarone	11.3 ± 11.2**	0.08 ± 0.07**	0.0 ± 0.0*
Sotalol	87.6 ± 73.1**	0.51 ± 0.39**	0.8 ± 1.4*
E-4031	26.1 ± 29.1**	0.19 ± 0.45**	0.7 ± 1.9*

* $P < 0.05$, ** $P < 0.01$ versus control group.

methylsulphonyl aminobenzoyl)piperidine) was infused throughout the experiment. Two hours after occlusion of LAD. Evans' blue dye was infused via the femoral vein, and then the heart was excised. The ischaemic area of the left ventricle was identified as a non-staining zone and the non-ischaemic area as a blue staining zone.

Analysis of arrhythmias

With the ambulatory electrocardiogram recorder, the number of ventricular premature beats during a 2 h period of occlusion was recorded and analysed by auto analyzer. To express the severity of arrhythmias, the total number of ventricular premature beats was counted and the arrhythmia ratio, defined as the number of ventricular premature beats divided by the total heart beats, was determined. The total duration time of ventricular tachycardia, which was defined as

more than 3 successive ventricular premature beats, was also calculated.

Measurement of mitochondrial function

Heart mitochondria from the ischaemic and non-ischaemic areas of each group were prepared as we have described previously (Sugiyama *et al.*, 1980), and the rate of oxygen consumption in state III (St. III O_2) and the respiratory control index (RCI) were measured immediately after the preparation, by an oxygen electrode and closed cell. The incubation medium contained 0.3 mol l^{-1} mannitol, 10 mmol l^{-1} KCl, 10 mmol l^{-1} potassium phosphate, pH 7.4, 2.5 mmol l^{-1} $MgCl_2$ and 0.25 mmol l^{-1} ethylenediaminetetraacetic acid (EDTA) in a total of 0.7 ml. Respiration was initiated by addition of 0.03 mmol l^{-1} of mitochondrial suspension ($10 \text{ mg protein ml}^{-1}$), and then 0.03 ml of succinate (0.2 mol l^{-1}) as a substrate and 0.03 ml adenosine diphosphate (10 mmol l^{-1}) were added subsequently.

Assays of lysosomal enzymes

According to the methods of Kennett & Weglicki (1978) and Ruth *et al.* (1978), the endocardial tissue from the ischaemic and non-ischaemic areas of each group was immersed in the extraction medium (0.25 mol l^{-1} sucrose, 0.6 mol l^{-1} KCl, 1 mmol l^{-1} adenosine triphosphate, 1 mmol l^{-1} EDTA, and 1 mmol l^{-1} $MgCl_2$, buffered with imidazole at pH 7.2). The tissue was then homogenized with a Teflon pestle tissue grinder. Pellets were obtained by differential centrifugation; the duration in min at the various g -forces were; $1,000g$ for 10 min; $2,500g$ for 10 min; $9,000g$ for 10 min; $20,000g$ for 30 min; $140,000g$ for 60 min. They were resuspended in 0.25 mol l^{-1} sucrose containing 10 mmol l^{-1} imidazole, buffered at pH 7.2. All pellets and the final supernatant were assayed for total activity of N-acetyl- β -glucosaminidase (NAG) and β -glucuronidase (β -gluc) by the methods of Noto *et al.* (1983) and Kato *et al.* (1960).

Statistics

Significance of the results was determined by Dunnett's test except for mortality, for which the Chi-squared test was used. Probability (P) values of less than 0.05 were considered statistically significant. All data in this paper are expressed as mean $\pm$ s.d.

Results

Mortality

Of the 33 dogs that underwent coronary artery occlusion, 5 out of 12 dogs in the control group, died within 2 h of occlusion (4: ventricular fibrillation, 1: pump failure), and were excluded from the subsequent analysis. No dogs died in either the amiodarone, sotalol or E-4031 groups. The mortality in the control group was thus higher than that in the other 3 groups ($P < 0.05$). Data for the remaining 28 dogs formed the basis of the results presented below.

Arrhythmias analysis

Table 1 shows the total number of ventricular premature beats, arrhythmia ratio, and the total duration time of ven-

Figure 1 The rate of oxygen consumption in state III (St. III O_2) in heart mitochondria from the ischaemic (hatched columns) and non-ischaemic areas (open columns) of each group. Two hour occlusion induced a decrease in St. III O_2 . Amiodarone, sotalol and E-4031 lessened the decrease. Each column shows the mean and vertical bars indicate s.d. * $P < 0.01$ versus the non-ischaemic area of the corresponding group. † $P < 0.01$, versus the ischaemic area of the control group.

Figure 2 The respiratory control index (RCI) in heart mitochondria from the ischaemic (hatched columns) and non-ischaemic (open columns) areas of each group. Beneficial effects of amiodarone, sotalol and E-4031 were observed. Each column represents the mean and vertical bars show s.d. * $P < 0.01$, versus the non-ischaemic area of the corresponding group. † $P < 0.01$, versus the ischaemic area of the control group.

Table 2 Total activities of N-acetyl- β -glucosaminidase (nmol g⁻¹ wet weight min⁻¹) in myocardial fractions of each group

Fraction	1,000 g	2,500 g	9,000 g	20,000 g	140,000 g	Supernatant
Non-ischaemic area						
Control	114.6 $\pm$ 29.8	19.9 $\pm$ 5.1	22.4 $\pm$ 3.6	21.5 $\pm$ 3.6	23.3 $\pm$ 2.7	91.1 $\pm$ 16.9
Amiodarone	112.7 $\pm$ 18.7	22.2 $\pm$ 4.5	20.2 $\pm$ 4.2	23.6 $\pm$ 4.8	23.3 $\pm$ 4.5	84.3 $\pm$ 15.6
Sotalol	106.4 $\pm$ 20.9	20.9 $\pm$ 3.6	22.0 $\pm$ 3.0	20.4 $\pm$ 3.2	22.4 $\pm$ 3.3	85.2 $\pm$ 19.3
E-4031	117.0 $\pm$ 30.1	21.1 $\pm$ 5.3	23.7 $\pm$ 2.3	21.5 $\pm$ 4.7	20.4 $\pm$ 3.2	89.5 $\pm$ 8.8
Ischaemic area						
Control	93.2 $\pm$ 34.0	13.8 $\pm$ 6.6	13.8 $\pm$ 5.4**	10.6 $\pm$ 2.0**	11.7 $\pm$ 3.2**	117.3 $\pm$ 14.7**
Amiodarone	113.4 $\pm$ 25.7	21.6 $\pm$ 3.1†	21.5 $\pm$ 6.8†	19.7 $\pm$ 5.5††	19.5 $\pm$ 4.8††	93.2 $\pm$ 18.9†
Sotalol	99.9 $\pm$ 27.7	19.6 $\pm$ 3.4	21.7 $\pm$ 4.4†	20.6 $\pm$ 4.3††	20.5 $\pm$ 2.5††	93.0 $\pm$ 14.7†
E-4031	104.0 $\pm$ 28.3	20.6 $\pm$ 5.2†	21.3 $\pm$ 4.4†	17.5 $\pm$ 3.2††	17.9 $\pm$ 3.3††	94.3 $\pm$ 15.2†

Data shown are means $\pm$ s.d.

** $P < 0.01$ versus the non-ischaemic area in the corresponding group.

† $P < 0.05$, †† $P < 0.01$ versus the ischaemic area in the control group.

tricular tachycardia in each group. In the control group, the total number of ventricular premature beats and arrhythmia ratio were significantly larger than in the drug-treated groups. Furthermore, the total duration time of ventricular tachycardia in the control group was significantly longer than that of the drug-treated groups.

Mitochondrial function

Figure 1 shows St. III O₂ of mitochondria prepared from ischaemic and non-ischaemic areas in each group. In the control group, St. III O₂ from the ischaemic area (161 $\pm$ 38 natoms mg⁻¹ protein min⁻¹) was decreased significantly compared with that from the non-ischaemic area (313 $\pm$ 31). In the amiodarone, sotalol and E-4031 groups, St. III O₂ from the ischaemic area (275 $\pm$ 25, 260 $\pm$ 26, 264 $\pm$ 39, respectively) were lower than that of the non-ischaemic area (respective values of 325 $\pm$ 30, 323 $\pm$ 24, 326 $\pm$ 31). However, this decrease was significantly less than that of the ischaemic area in the control group. Figure 2 shows the RCI in each group.

Figure 3 Time course of changes in systolic and diastolic blood pressure in each group: (○) control group, (●) amiodarone, (▲) sotalol and (■) E-4031-treated groups. Blood pressure was not significantly different between the groups. Each symbol represents the mean and vertical lines show s.d.

Administration of amiodarone, sotalol and E-4031 also lessened ischaemia-induced decrease in the RCI.

Lysosomal enzyme activity

Table 2 shows the changes in total activities of NAG in all particulate and supernatant fractions. In the control group, a significant loss of NAG activity occurred in the post-2,500 g particulate fractions of the ischaemic area, and a significant increase was observed in the supernatant fraction of the ischaemic area compared with those of the non-ischaemic area. However, in the amiodarone, sotalol and E-4031 groups, the decrease in NAG activity of the post-2,500 g particulate fractions and the increase of the supernatant fraction of the ischaemic area were significantly inhibited compared with those of the control group. The changes in total activity of β -gluc in all particulate and supernatant fractions are shown in Table 3. Amiodarone, sotalol and E-4031 also prevented significantly the leakage of β -gluc.

Figure 4 Time course of changes in heart rate in each group. No significant changes in heart rate were observed in the control (○) and E-4031 (■) groups. In the amiodarone group (●), 30 and 45 min after the coronary occlusion, a significant decrease in heart rate was observed. Sotalol (▲) decreased heart rate significantly until 75 min after left anterior descending (LAD) coronary artery occlusion. * $P < 0.05$, ** $P < 0.01$. Each symbol represents mean and vertical lines show s.d.

Table 3 Total activities of β -glucuronidase (μ g g⁻¹ wet weight h⁻¹) in myocardial fractions of each group

Fraction	1,000 g	2,500 g	9,000 g	20,000 g	140,000 g	Supernatant
Non-ischaemic area						
Control	83.9 $\pm$ 28.9	15.1 $\pm$ 5.3	20.0 $\pm$ 3.2	20.3 $\pm$ 2.9	23.0 $\pm$ 3.3	71.3 $\pm$ 17.2
Amiodarone	78.5 $\pm$ 14.3	17.7 $\pm$ 4.3	20.5 $\pm$ 3.5	21.2 $\pm$ 3.4	22.2 $\pm$ 5.2	77.0 $\pm$ 28.2
Sotalol	81.8 $\pm$ 16.3	19.7 $\pm$ 5.1	19.9 $\pm$ 2.3	20.0 $\pm$ 3.1	20.9 $\pm$ 3.3	72.1 $\pm$ 10.8
E-4031	81.0 $\pm$ 10.3	18.2 $\pm$ 2.8	20.1 $\pm$ 3.1	20.5 $\pm$ 3.3	22.7 $\pm$ 3.1	77.0 $\pm$ 14.7
Ischaemic area						
Control	76.1 $\pm$ 14.1	8.1 $\pm$ 4.8*	12.4 $\pm$ 2.8**	10.2 $\pm$ 2.0**	10.9 $\pm$ 3.3**	108.9 $\pm$ 15.5**
Amiodarone	80.5 $\pm$ 14.7	16.6 $\pm$ 3.2††	19.3 $\pm$ 3.7††	20.5 $\pm$ 5.3††	18.2 $\pm$ 2.4††	80.9 $\pm$ 18.3††
Sotalol	77.8 $\pm$ 16.7	15.8 $\pm$ 4.7††	17.4 $\pm$ 3.4†	20.1 $\pm$ 2.3††	19.9 $\pm$ 4.1††	80.2 $\pm$ 14.0††
E-4031	75.6 $\pm$ 10.5	18.5 $\pm$ 4.0††	18.7 $\pm$ 3.7††	18.5 $\pm$ 5.4††	18.9 $\pm$ 3.8††	79.1 $\pm$ 15.8††

Data shown are means $\pm$ s.d.

* $P < 0.05$, ** $P < 0.01$ versus the non-ischaemic area in the corresponding group.

† $P < 0.05$, †† $P < 0.01$ versus the ischaemic area in the control group.

Time course of changes in blood pressure and heart rate

Figure 3 shows the time course of changes in arterial blood pressure. No significant changes in blood pressure were seen between the control and drug treated groups, though in the sotalol group, slight but insignificant decreases in blood pressure were observed. Figure 4 shows changes in heart rate, i.e., the difference between heart rate preinjection (physiological saline or drugs) and that after administration of drugs or saline. No significant changes in heart rate were observed in the control and E-4031 groups. In the amiodarone group, 30 and 45 min after LAD occlusion, a significant decrease in heart rate was observed. Administration of sotalol decreased heart rate significantly until 75 min after LAD occlusion.

Discussion

The general principles of treatment of acute ischaemia are to increase oxygen supply, to decrease oxygen demand, to increase anaerobic metabolism, and to prevent direct cell damage (Kirk & Jennings, 1982). Theoretically, energy transduction in ischaemic tissue might be increased by stimulating anaerobic glycolysis. However, activity of phosphofructokinase, a key enzyme of anaerobic glycolysis, is inhibited due to acidosis in association with ischaemia. Accordingly, anaerobic metabolism cannot contribute to energy production. It is well known that the best way to increase oxygen supply is reperfusion to the ischaemic myocardium. Recent advances in thrombolysis, coronary artery bypass grafting and percutaneous transluminal coronary angioplasty have produced favourable results. However, duration of ischaemia remains a determining factor for the recovery of ischaemic tissue. The most efficient clinical means to decrease oxygen demand is to induce cardiac hypothermias. This is applied to open heart surgery. β -Blockers are also known to decrease oxygen demand. The effectiveness of β -blockers on acute myocardial infarction is widely accepted. Other preventive methods of cellular damage have also been proposed; administration of allopurinol, coenzyme Q₁₀, steroids etc. (Shatney *et al.*, 1976; Nagai *et al.*, 1985). These drugs depend on the identification of the mechanisms responsible for cellular injury associated with ischaemia. With these surgical and/or pharmacological interventions, it is of primary important to prevent or, at least, to delay the onset of irreversible injury until reperfusion.

It is becoming increasingly clear that class III compounds are likely to emerge as the most significant ones for the control of cardiac arrhythmias (Beckers & Kulbertus, 1987). In the present study, we used three class III drugs: amiodarone, sotalol and E-4031. In untreated animals, occlusion of the coronary artery caused ventricular arrhythmias. On the other hand, a significant decrease in the number of ventricular arrhythmias was observed in each drug-treated group. Moreover, indices of mitochondrial function from the ischaemic area were decreased significantly compared with those from the non-ischaemic area in the control group. The leakage of lysosomal enzymes, i.e., instability of lysosomal membranes, was observed in the ischaemic myocardium in the control group, whereas, mitochondrial function from the ischaemic

area in the drug-treated groups was improved significantly compared with that in the control group. Nokin *et al.* (1987) showed a protective effect of amiodarone against ischaemia-induced mitochondrial dysfunction. In the present study, stability of lysosomal membranes was also demonstrated by administration of amiodarone, sotalol or E-4031. These results suggest that class III agents show cardioprotective properties as well as antiarrhythmic effects. The acute effect of amiodarone might not be ascribed to its class III action (Wellens *et al.*, 1984). Hieda *et al.* (1988) showed that propranolol maintained mitochondrial function and prevented the leakage of lysosomal enzymes during coronary occlusion. Sugiyama *et al.* (1988) demonstrated that SUN 1165, a sodium channel inhibitor shows a similar cardioprotective effect to propranolol. Hanaki *et al.* (1989) also found that other sodium channel blockers, classified as group I antiarrhythmic agents, show cardioprotective effects. It is suggested that amiodarone has a potent sodium channel blocking action (Mason *et al.*, 1984). Hence, we cannot rule out the possibility that the protective effects of amiodarone and sotalol might be based on their respective sodium channel blocking action or β -blocking action, irrespective of their class III action. Class I antiarrhythmic drugs, as well as propranolol, have a membrane stabilizing effect (Courtney, 1988). Rhodes & Herbette (1988) suggested that this effect might depend on structural similarity to the lipophilic site in a membrane bilayer. Amiodarone, sotalol and E-4031 might have a similar effect on biomembranes, which might be linked to their cardioprotective effect.

According to the classification of Vaughan Williams (1979), antiarrhythmic compounds are categorized by their effects on (I) the fast sodium current, (II) the sympathetic activity of the heart, (III) the repolarization current, and (IV) the slow inward current. The classifications might be useful for assessment of various types of antiarrhythmic drugs. However, they are controversial in the treatment of arrhythmias associated with ischaemia, because the classification mainly depends on the effect on action potentials (Harrison, 1985). It has been found that counterpulsation reduces ventricular arrhythmias (Hanson *et al.*, 1980). The severity of ventricular arrhythmias has been shown to be related to the extent of myocardial necrosis (Cox *et al.*, 1976). Moreover, cardioprotective drugs, which are not classified as antiarrhythmic drugs such as coenzyme Q₁₀ and phosphocreatine, improve lethal arrhythmias (Nagai *et al.*, 1985; Ruda *et al.*, 1988). Accordingly, it is deduced that not only electrophysiological derangements but also biochemical derangements have been implicated in the genesis of arrhythmias related to acute ischaemia. Muller *et al.* (1986) emphasized the relationship between the antiarrhythmic effect of β -blockers and their cardioprotective effect. The cardioprotective effect of antiarrhythmic agents classified as class III observed here might also participate in their antiarrhythmic effect.

In conclusion, we would like to emphasize that amiodarone, sotalol and E-4031 show a cardioprotective as well as an antiarrhythmic effect in ischaemic heart.

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References

- BECKERS, J. & KULBERTUS, H.E. (1987). Class III drugs: Amiodarone. *Eur. Heart J.*, **8** (suppl A), 53–59.
- COBBE, S.M., HOFFMAN, E., RITZENHOFF, A., BRACHMANN, J., KÜBLER, W. & SENGES, J. (1983). Action of sotalol on potential reentrant pathways and ventricular tachyarrhythmias in conscious dogs in the late postmyocardial infarction phase. *Circulation*, **68**, 865–871.
- COURTNEY, K.R. (1988). Why do some drugs preferentially block open sodium channels? *J. Mol. Cell. Cardiol.*, **20**, 461–464.
- COX, J.R., ROBERTS, R., AMBOS, H.D., OLIVER, G.C. & SOBEL, B.E. (1976). Relations between enzymatically estimated myocardial infarct size and early ventricular dysrhythmia. *Circulation*, **53** (suppl. I), 150–155.
- HANAKI, Y., SUGIYAMA, S., HIEDA, N., TAKI, K., HAYASHI, H. & OZAWA, T. (1989). Cardioprotective effects of various class I antiarrhythmic drugs in canine hearts. *J. Am. Coll. Cardiol.*, **14**, 219–224.
- HANSON, E.C., LEVINE, F.H., KAY, H.R., LEINBACH, R.C., GOLD, H.K.,

- DAGGETT, W.M., AUSTEN, W.G. & BUCKLEY, M.J. (1980). Control of postinfarction ventricular irritability with the intraaortic balloon pump. *Circulation*, **62** (suppl. I), 130-137.
- HARRISON, D.C. (1985). Antiarrhythmic drug classification: New science and practical applications. *Am. J. Cardiol.*, **56**, 185-187.
- HEARSE, D.J. (1979). Cellular damage during myocardial ischaemia: Metabolic changes leading to enzyme leakage. In *Enzymes in Cardiology. Diagnosis and Research*, ed. Hearse, D.J. & Leiris, J. pp. 1-19. Chichester, New York, Brisbane, Toronto: John Wiley & Sons.
- HIEDA, N., TOKI, Y., SUGIYAMA, S., ITO, T., SATAKE, T. & OZAWA, T. (1988). Prostaglandin I₂ analogue and propranolol prevent ischaemia induced mitochondrial dysfunction through the stabilisation of lysosomal membranes. *Cardiovasc. Res.*, **22**, 219-225.
- KATO, K., YOSHIDA, K., TSUKAMOTO, H., NOBUNAGA, M., MASUYA, T. & SWADA, T. (1960). Synthesis of p-nitrophenyl β -D-glucopyranosiduronic acid and its utilization as a substrate for the assay of β -glucuronidase activity. *Chem. Pharmacol. Bull.*, **8**, 239-242.
- KENNETT, F.F. & WEGGLICKI, W.B. (1978). Effects of well-defined ischaemia on myocardial lysosomal and microsomal enzymes in a canine model. *Circ. Res.*, **43**, 750-758.
- KIRK, E.S. & JENNINGS, R.B. (1982). Pathophysiology of myocardial ischemia. In *The Heart*. Fifth edition, ed. Hurst, J.W. pp. 976-1008. New York: McGraw-Hill Book Company.
- MARCUS, F.I., FONTAINE, G.H., FRANK, R. & GROSGOGGAT, Y. (1981). Clinical pharmacology and therapeutic applications of the antiarrhythmic agent, amiodarone. *Am. Heart J.*, **101**, 480-493.
- MAROKO, P.R., LIBBY, P., BLOOR, C.M., SOBEL, B.E. & BRAUNWALD, E. (1972). Reduction by hyaluronidase of myocardial necrosis following coronary artery occlusion. *Circulation*, **46**, 430-437.
- MASON, J.W. (1987). Amiodarone. *N. Engl. J. Med.*, **316**, 455-466.
- MASON, J.W., HONDEGHEM, L.M. & KATZUNG, B.G. (1984). Block of inactivated sodium channels and of depolarization-induced automaticity in guinea pig papillary muscle by amiodarone. *Circ. Res.*, **55**, 277-285.
- MULLER, C.A., OPIE, L.H., HAMM, C.W., PEISACH, M. & GIHWALA, D. (1986). Prevention of ventricular fibrillation by metoprolol in a pig model of acute myocardial ischaemia: Absence of a major arrhythmogenic role for cyclic AMP. *J. Mol. Cell. Cardiol.*, **18**, 375-387.
- NAGAI, S., MIYAZAKI, Y., OGAWA, K., SATAKE, T., SUGIYAMA, S. & OZAWA, T. (1984). The effects of β -blocking agents on mitochondrial function in ischemic myocardium. *Jpn. Circ. J.*, **48**, 32-36.
- NAGAI, S., MIYAZAKI, Y., OGAWA, K., SATAKE, T., SUGIYAMA, S. & OZAWA, T. (1985). The effect of coenzyme Q₁₀ on reperfusion injury in canine myocardium. *J. Mol. Cell. Cardiol.*, **17**, 873-884.
- NOKIN, P., JUNGBLUTH, L. & MOUTON, J. (1987). Protective effects of amiodarone pretreatment on mitochondrial function and high energy phosphates in ischaemic rat heart. *J. Mol. Cell. Cardiol.*, **19**, 603-614.
- NOTO, A., OGAWA, Y., MORI, S., YOSHIOKA, M., KITAKAZE, T., HORI, T., NAKAMURA, M. & MIYAKE, T. (1983). Simple, rapid spectrophotometry of urinary N-acetyl- β -D-glucosaminidase, with use of a new chromogenic substrate. *Clin. Chem.*, **29**, 1713-1716.
- OPIE, L.H., BRUYNEEL, K. & OWEN, P. (1975). Effects of glucose, insulin and potassium infusion on tissue metabolic changes within first hour of myocardial infarction in the baboon. *Circulation*, **52**, 49-57.
- PAGE, E. & MCCALLISTER, L.P. (1973). Quantitative electron microscopic description of heart muscle cells. Application to normal, hypertrophied, and thyroxine-stimulated hearts. *Am. J. Cardiol.*, **31**, 172-181.
- RHODES, D.G. & HERBETTE, L.G. (1988). Discussion of 'Why do some drugs preferentially block open sodium channels?': Consideration of other significant factors in the binding process. *J. Mol. Cell. Cardiol.*, **20**, 571-572.
- RUDA, M. YA., SAMARENKO, M.B., AFONSKAYA, N.I. & SAKS, V.A. (1988). Reduction of ventricular arrhythmias by phosphocreatine (Neoton) in patients with acute myocardial infarction. *Am. Heart J.*, **116**, 393-397.
- RUTH, R.C., KENNETT, F.F. & WEGGLICKI, W.B. (1978). A new technique for isolation of particulate lysosomal activity from canine and rat myocardium. *J. Mol. Cell. Cardiol.*, **10**, 739-751.
- SAWADA, K., NOMOTO, K.-I. & HIRAOKA, M. (1988). Effect of a novel class 3 antiarrhythmic agent, E-4031, on ventricular arrhythmias. *Jpn. Circ. J.*, **52**, 919-920.
- SHATNEY, C.H., MCCARTER, D.J. & LILLEHEI, R.C. (1976). Effects of allopurinol, propranolol and methylprednisolone on infarct size in experimental myocardial infarction. *Am. J. Cardiol.*, **37**, 572-580.
- SINGH, B.N., COLLETT, J.T. & CHEW, C.Y.C. (1980). New perspectives in the pharmacologic therapy of cardiac arrhythmias. *Prog. Cardiovasc. Dis.*, **22**, 243-301.
- SMITH, E.R., REDWOOD, D.R., MCCARRON, W.E. & EPSTEIN, S.E. (1973). Coronary artery occlusion in the conscious dog. Effects of alterations in arterial pressure produced by nitroglycerin, hemorrhage, and alpha-adrenergic agonists on the degree of myocardial ischemia. *Circulation*, **47**, 51-57.
- STRAUSS, H.C., BIGGER, J.T. Jr. & HOFFMAN, B.F. (1970). Electrophysiological and beta-receptor blocking effects of MJ 1999 on dog and rabbit cardiac tissue. *Circ. Res.*, **26**, 661-678.
- SUGIYAMA, S., OZAWA, T., KATO, T. & SUZUKI, S. (1980). Recovery time course of ventricular vulnerability after coronary reperfusion in relation to mitochondrial function in ischemic myocardium. *Am. Heart J.*, **100**, 829-837.
- SUGIYAMA, S., MIYAZAKI, Y., KOTAKA, K. & OZAWA, T. (1983). The effect of verapamil on mitochondrial dysfunction associated with coronary reperfusion. *Jpn. Circ. J.*, **47**, 830-836.
- SUGIYAMA, S., HANAKI, Y., OGAWA, T., HIEDA, N., TAKI, K. & OZAWA, T. (1988). The effects of SUN 1165, a novel sodium channel blocker, on ischemia-induced mitochondrial dysfunction and leakage of lysosomal enzymes in canine hearts. *Biochem. Biophys. Res. Commun.*, **157**, 433-439.
- VAUGHAN WILLIAMS, E.M. (1979). III. Characterisation of new antiarrhythmic drugs. *Prog. Pharmacol.*, **2**, 13-23.
- WELLENS, H.J.J., BRUGADA, P., ABDOLLAH, H. & DASSEN, W.R. (1984). A comparison of the electrophysiologic effects of intravenous and oral amiodarone in the same patient. *Circulation*, **69**, 120-124.

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