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The In-vivo Cardiovascular Effects of a Putative Class III Anti-arrhythmic Drug, AM 92016

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Abstract

AM 92016 (1-(4-methanesulphonamidophenoxy)-3-(*N*-methyl-3-4-dichlorophenethylamino)-2-propanol benzoic acid salt), an oxypropanolamine analogue of sotalol, has been shown to possess Class III anti-arrhythmic properties in-vitro at concentrations showing 1000 times more potency than sotalol. The aim of this study was to characterize the effects of AM 92016 in-vivo.

When administered to anaesthetized guinea-pigs, AM 92016 ($10\,\mu g\,kg^{-1}$-$5\,mg\,kg^{-1}$) significantly increased heart rate, systolic arterial blood pressure, left ventricular systolic pressure and the contractile index dp/dt_{max}. AM 92016 also significantly decreased the QT interval of the electrocardiogram from 135 ± 10 to 105 ± 4 ms ($5\,mg\,kg^{-1}$). The time to onset of the first arrhythmia and ventricular fibrillation, induced by intravenous infusion of ouabain, was shortened in the presence of AM 92016. Ouabain-induced ventricular fibrillation occurred at 18 ± 5 and 12 ± 3 min ($P < 0.05$) in control and AM 92016-($1\,mg\,kg^{-1}$) treated guinea-pigs, respectively. An infusion of AM 92016 ($2.5\,\mu g\,kg^{-1}\,min^{-1}$) to anaesthetized pigs significantly increased the total number of arrhythmias occurring following coronary artery occlusion from 266 ● 26 in control pigs to 535 ± 148 ($P < 0.05$) in those receiving AM 92016. The time to onset of ventricular fibrillation was also significantly reduced in anaesthetized pigs from 24 ± 1 to 18 ± 3 min in the presence of AM 92016. The drug did not change haemodynamics in the anaesthetized pig.

We conclude that AM 92016 exhibited proarrhythmic rather than antiarrhythmic activity when administered in-vivo to either guinea-pigs or pigs.

Class III anti-arrhythmic drugs have been characterized by their ability to increase the refractory period of cardiac action potentials by prolonging the action-potential duration (Vaughan Williams 1970). However, the currently available class III agents are not selective for this action. For example, sotalol possesses β-adrenoceptor-blocking activity as well as inhibiting the delayed rectifier potassium current to cause a Class III action.

AM 92016 (1-(4-methanesulphonamidophenoxy)-3-(*N*-methyl-3-4-dichlorophenethylamino)-2-propanol benzoic acid salt), an oxypropanolamine analogue of sotalol, was synthesized in an attempt to separate these two properties of sotalol (Connors et al 1991). The incorporation of *N*-methyl phenylalkyl and 4-phenyl alicylic amine groups separated β-adrenoceptor antagonist activity from inhibition of the delayed-rectifier potassium channel (Connors et al 1991). In-vitro testing of AM 92016 (Connors et al 1991, 1992) demonstrated that in both guinea-pig and rabbit isolated ventricular cells, AM 92016 increased action-potential duration in a concentration-dependent manner, and was 1000 times more potent than sotalol. Prolongation of the action-potential duration was shown to be due to a selective inhibition of the delayed-rectifier potassium channel with no effect on calcium or sodium channels (Connors et al 1992).

Although AM 92016 exhibited class III activity in-vitro experiments there has been no study of its action in-vivo. The aims of the present study were to characterize the effects of AM 92016 on haemodynamic and contractile function in the anaesthetized guinea-pig and to assess its class III anti-arrhythmic activity on ouabain-induced arrhythmias in the anaesthetized guinea-pig and in a model of acute myocardial ischaemia in open-chest anaesthetized pigs.

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Methods

Preparation of guinea-pigs

Male Duncan Hartley guinea-pigs, 300–450 g, were anaesthetized with a combination of Hypnorm (Janssen) ($1\,mL\,kg^{-1}$) and ketamine ($50\,mg\,kg^{-1}$), both administered intramuscularly, and maintained with intravenous injections of Hypnorm ($0.15\,mL\,kg^{-1}$) and ketamine ($15\,mg\,kg^{-1}$) as required. The animals were ventilated throughout the experiment with a Palmer respiration pump (54 strokes min^{-1} and volume of 1 mL per 100 g bodyweight). A plastic cannula was inserted into the left carotid artery and attached to a pressure transducer allowing a continuous recording of arterial blood pressure and heart rate. The calibration of the transducer was checked daily against a mercury column. A second cannula was placed into the left jugular vein for the administration of drugs and anaesthetic. A Millar Micro-Tip catheter pressure transducer was inserted into the right carotid artery and advanced to the left ventricle of the heart allowing contractile parameters (dP/dt_{max}, dP/dt/P and left ventricular pressure) to be recorded. The accuracy of the transducer was checked daily with the internal calibration. A recording of

the electrocardiogram (ECG) was made by inserting standard limb leads subcutaneously. Arterial pressure, left ventricular pressure and ECG were recorded throughout the experiment on a Linton Graphtec Linearcorder WR 3310. An on-line data analysis system (J. Dempster, University of Strathclyde) recorded systolic, diastolic and mean arterial blood pressure, heart rate, systolic, diastolic and mean left ventricular pressure and calculated the derived indices of contractility dP/dt_{max} and dP/dt/P. QT interval was measured from the recording of ECG.

Experimental protocols for guinea-pig studies

Effect of cumulative additions of AM 92016 on haemodynamics and contractile function. Following an initial stabilization period, AM 92016, or equivalent vehicle, was administered intravenously in increasing concentrations at 10-min intervals. The concentrations of drug tested were 10 and 100 $\mu g\,kg^{-1}$ and 1 and 5 $mg\,kg^{-1}$. Heart rate, arterial blood pressure, left ventricular pressure and ECG were recorded for each drug or vehicle addition.

Effect of AM 92016 on ouabain-induced arrhythmias. The animals were prepared as described previously for drug administration and the measurement of haemodynamic and contractile parameters, then, following a stabilization period, 1 $mg\,kg^{-1}$ of AM 92016 or the equivalent vehicle, was administered intravenously as a bolus injection. The effects of the compound or vehicle on haemodynamics and contractile function were monitored for 10 min before the start of the ouabain infusion. Ouabain (80 $\mu g\,mL^{-1}$) was infused continually, by means of a slow infusion pump, at a rate of 70 $\mu L\,min^{-1}$, delivering 5·6 $\mu g\,min^{-1}$ ouabain. The times from the start of the ouabain infusion until the first arrhythmia, ventricular fibrillation and cardiac arrest were recorded.

Preparation of pigs

Large White-Welsh Landrace cross-breed pigs, 28–35 kg, were sedated with azaperone (Stresnil, Janssen) (5–7 $mg\,kg^{-1}$ i.m.) 30–40 min before anaesthesia. Anaesthesia was induced by halothane (4% in oxygen), the animal was intubated and anaesthesia maintained with halothane (1% in air) until an intravenous line had been introduced. The pigs were ventilated with a Palmer respiration pump (16–18 strokes min^{-1}), and the stroke volume and oxygen content of the inspired air were adjusted to maintain arterial CO_2 and O_2 tensions of 45 and 100 mmHg respectively (giving a pH of 7·35–7·40). Catheters were advanced to the aorta and vena cava (via the femoral vessels) for measurement of arterial blood pressure and administration of drugs and anaesthetic respectively. At this point the halothane was discontinued and chloralose (100 $mg\,kg^{-1}$, i.v.) was administered. A fluid-filled catheter was placed in the lumen of the left ventricle (via the left carotid artery) for measurement of left ventricular pressure. A thoracotomy was performed midsternally, from the xyphoid cartilage to the clavicle, and the pericardium opened to gain access to the coronary vessels. The left anterior descending coronary artery (LAD) was dissected free below the second left diagonal branch (to give an area at risk of approximately 25% of free left ventricular wall) and a silk ligature placed around it.

Blood pressures were recorded with Gould transducers and the signals passed through a Buxco haemodynamics analyser (Buxco Electronics, USA) for calculation of systolic, diastolic and mean pressures, heart rate (HR), left ventricular end diastolic pressure (LVEDP) and $LVdP/dt_{max}$ and LVdP/dt/P as two indices of myocardial contractility. Rate-pressure product was calculated as: HR (beats min^{-1}) × blood pressure (mmHg) and employed as an indirect measurement of myocardial oxygen consumption. Pressure traces were simultaneously displayed on a six-channel chart recorder (Gould Instruments). The electrocardiogram was recorded from limb lead II and the signal processed by an ECG analyser (Buxco Electronics, USA) to measure R-R, QT and QRS intervals and alterations in ST-segment. QT_c (i.e. QT-interval corrected for changes in heart rate) was calculated by the formula $QT_{int}/\sqrt{RR_{int}}$. As with the haemodynamic data, the ECG was displayed continuously. Myocardial ischaemia was induced by threading the ends of the ligature through a small piece of polythene tubing and subsequently pulling on the suture to produce a snare which was clamped in place. The subsequent arrhythmias were monitored continuously and later subjected to analysis under the Lambeth Conventions (Walker et al 1988) by counting the number of beats occurring as single ventricular premature beats, salvos (couplets and triplets) and ventricular tachycardia (four or more consecutive arrhythmias). These arrhythmias were counted over 1-min intervals (to give arrhythmia distribution) for a period of 30 min. If ventricular fibrillation developed the heart was restored to sinus rhythm by direct cardioversion within 30 s to allow the experiment to be continued for 30 min.

Experimental protocol for pig studies

All pigs were allowed to stabilize for approximately 20 min following surgery. AM 92016 was infused at a rate of 2·5 $\mu g\,kg^{-1}\,min^{-1}$, 10 min before coronary occlusion and maintained for the duration of the experimental period. Vehicle controls were performed in an identical manner. The total arrhythmia count, percent incidence of ventricular fibrillation (VF) and ventricular tachycardia (VT) and the mean time to onset of VF were noted for each group.

Drugs

AM 92016 was supplied by British Technology Group (London, UK), dissolved in 60% ethanol. Ouabain (Sigma) was dissolved in physiological salt solution.

Statistics

All values are expressed as mean ± s.e.m. of n observations. Changes within groups were compared using either a paired *t*-test or analysis of variance for repeated measures where appropriate. Differences between groups were assessed using either an unpaired *t*-test or Mann Whitney U-test (for non-parametric data). Changes in the incidence of events were analysed by Fischer Irwin (Chi^2 with Yates correction) test. Results were considered to be significant at $P < 0·05$.

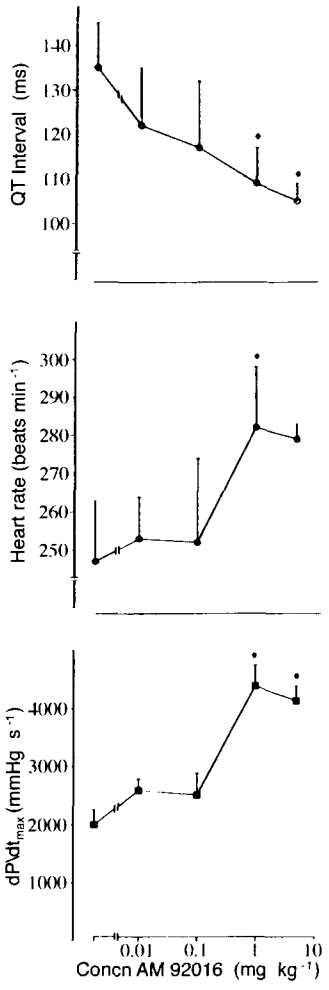

FIG. 1. Effect of cumulative additions of AM 92016 over the concentration range 10 μg kg^{-1} to 5 mg kg^{-1} on QT interval (○), heart rate (●) and dP/dt$_{max}$ (■) of anaesthetized guinea-pigs. All values expressed are means and s.e.m. (n = 4). *$P < 0.05$ compared with control value.

Results

Effect on haemodynamics and contractile function in the anaesthetized guinea-pig

In anaesthetized guinea-pigs resting values of heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), left ventricular systolic pressure (LVSP) and dP/dt$_{max}$ were 247 ± 16 beats min^{-1}; 54 ± 6 mmHg; 36 ± 4 mmHg; 43 ± 2 mmHg and 1998 ± 267 mmHg s^{-1} respectively.

Figure 1 illustrates the effects of AM 92016 (10 μg kg^{-1} - 5 mg kg^{-1}) on HR, dP/dt$_{max}$ and the QT interval. At concentrations of 1 mg kg^{-1} and above, AM 92016 increased both HR, dP/dt$_{max}$ and this was accompanied by increases in SBP (from 54 ± 6 to 76 ± 15 mmHg) and LVSP (from 43 ± 2 to 63 ± 10 mmHg). AM 92016 significantly reduced the QT interval over the concentration range stated. For example, QT interval was 135 ± 10 and 105 ± 4 ms before and following the administration of 5 mg kg^{-1} AM 92016, respectively.

Administration of vehicle alone did not alter haemodynamics or contractile function in these experiments.

Table 1. Times to production of first arrhythmia, ventricular fibrillation and cardiac arrest following infusion of ouabain to guinea-pigs pretreated with AM 92016 (1 mg kg^{-1}).

	Time to onset (min ± s.e.m.)	
	Vehicle control	AM 92016
First arrhythmia	14·3 ± 1·0	10·3 ± 1·0*
Ventricular fibrillation	18·3 ± 2·0	12·1 ± 1·4*
Cardiac arrest	22·5 ± 2·1	17·8 ± 1·4

*$P < 0.05$ compared with control values (n = 6). control, n = 7; AM 92016, n = 6.

Ouabain-induced arrhythmias

In control animals, an infusion of ouabain (5·6 μg min^{-1}) resulted in ectopic beats followed by a period of ventricular tachycardia which ultimately led to VF and cardiac arrest. AM 92016 (1 mg kg^{-1}) significantly reduced the time of onset of the first arrhythmia and VF (Table 1). A reduction in the time to onset of cardiac arrest did not reach significance ($P = 0.096$). The pattern of arrhythmias produced in animals pretreated with AM 92016 did not differ from that observed in control animals, only the time to onset of arrhythmias was altered.

In these experiments, AM 92016 (1 mg kg^{-1}) produced a significant increase in HR (from 248 ± 12 to 264 ± 16 beats min^{-1}), SBP (from 58 ± 4 to 64 ± 4 mmHg), dP/dt$_{max}$ (from 2529 ± 264 to 4612 ± 134 mmHg s^{-1}) and a significant decrease in QT interval from (129 ± 10 to 102 ± 4 ms).

Ischaemia-induced ventricular arrhythmias in the anaesthetized pig

Coronary artery occlusion in control pigs resulted in ventricular ectopic activity within 4–5 min of occlusion. The arrhythmias appeared in two phases between 4–12 (phase 1a) and 22–28 (phase 1b) min. AM 92016 (2·5 μg kg^{-1} min^{-1}) increased the number of arrhythmias in phase 1b but not phase 1a (Fig. 2). AM 92016 significantly increased the total arrhythmia count, primarily as a result of increasing the number of beats appearing as VT (Table 2). The incidence of VF was 100% in control and drug-treated pigs but the time to onset of VF was shorter in the presence of AM 92016 (17·5 ± 2·6 min) compared with control pigs (24 ± 0·7 min, $P < 0.05$). AM 92016 (2·5 μg kg^{-1} min^{-1}) did not significantly modify HR (108 ± 9 before drug administration and 107 ± 7, 10 min after) or dP/dt$_{max}$ (2900 ± 245 before and 2899 ± 229, 10 min after drug administration). However, the drug did significantly increase ST segment height before coronary artery occlusion as illustrated in Fig. 3. Following coronary artery occlusion in control pigs there is a rapid elevation of the ST segment, reaching a sustained peak within 5 min. An increase of similar magnitude was seen in drug-treated pigs following occlusion. The presence of AM 92016 did not modify the change despite the initial increase.

Discussion

AM 92016 was synthesized in an attempt to develop a more potent and selective class III anti-arrhythmic drug than the

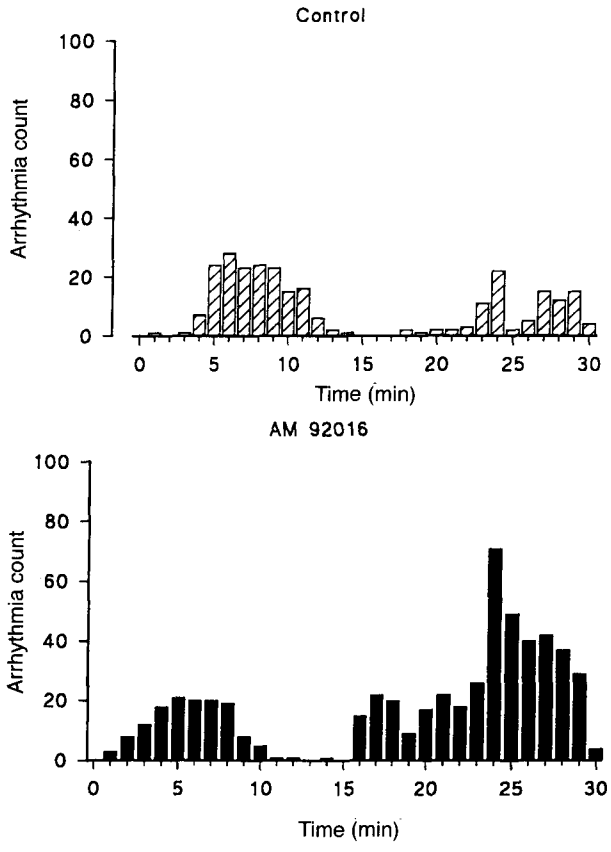

FIG. 2. Distribution of ventricular arrhythmias in controls (upper panel) and pigs given AM 92016 (2·5 μg kg^{-1} min^{-1}-lower panel) over one minute intervals for the thirty minute experimental period (n = 6).

parent compound, sotalol (Connors et al 1991, 1992). The results obtained in this study, however, showed that when tested in in-vivo models of arrhythmia production, AM 92016 was pro-arrhythmic. With the model of ouabain-induced arrhythmias in anaesthetized guinea-pigs, administration of AM 92016 significantly reduced the time to onset of the first arrhythmia and VF, indicating a pro- rather than anti-arrhythmic effect. An infusion of AM 92016 into anaesthetized pigs resulted in a significant increase in the total arrhythmia count following coronary artery occlusion, which was due to an increase in the number of arrhythmias appearing in phase 1b and also the number of beats appearing as VT.

Previously characterized class III anti-arrhythmic

Table 2. Total arrhythmia count (mean ± s.e.m.) in animals receiving AM 92016 (2·5 μg kg^{-1} min^{-1}).

	Count (beats)	
	Vehicle control	AM 92016
Single	175 ± 28	148 ± 52
Salvo	53 ± 8	115 ± 46
Ventricular tachycardia	39 ± 28	344 ± 95*
Total	266 ± 26	535 ± 148*

*$P < 0.05$ compared with control values (n = 6).

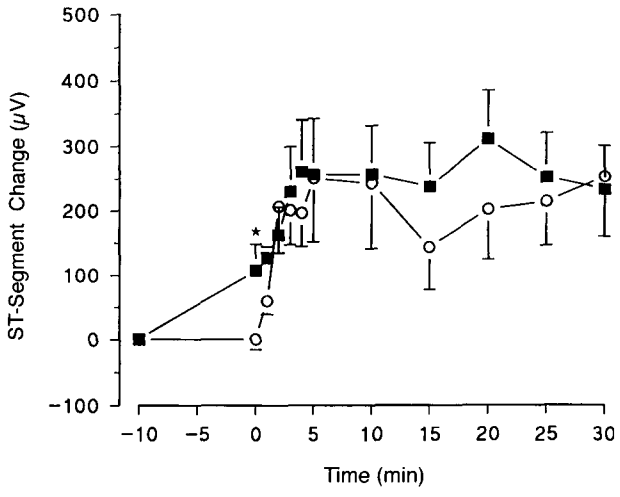

FIG. 3. Changes in ST-segment following commencement of infusion of AM 92016 (2·5 μg kg^{-1} min^{-1}) 10 min before coronary occlusion (performed at time 0) and during the subsequent 30 min period of myocardial ischaemia in control animals (○) and those receiving AM 92016 (■) (n = 6).

compounds have been shown to protect against and reduce the severity of ventricular arrhythmias in animal models of arrhythmia production. Studies carried out by Singh & Vaughan Williams (1970) demonstrated that sotalol could protect against ouabain-induced arrhythmias in anaesthetized guinea-pigs. This anti-arrhythmic effect was thought to be due to sotalol's ability to decrease HR and prolong the QTc interval (QT interval corrected for HR). A recently developed selective class III anti-arrhythmic drug dofetilide (Gwilt et al 1991; Rasmussen et al 1992) has also been shown to prevent the occurence of VF in a model of coronary artery ligation-reperfusion in rats (Maynard et al 1991) and to exhibit antifibrillatory properties in a model of acute myocardial ischaemia in closed-chest pigs (Andersen et al 1992). In the latter case, this anti-arrhythmic activity was also shown to be associated with a prolongation of the QTc interval.

In contrast, in our study, AM 92016 failed to prolong the QT interval in either guinea-pigs or pigs. Indeed, in guinea-pigs, AM 92016 increased HR, SBP, LVSP and dP/dt_{max} and decreased QT interval when added cumulatively over the concentration range 10 μg kg^{-1}–5 mg kg^{-1}. The QT interval was not corrected for the change in HR observed in this model, therefore it is possible that the decrease in QT interval could be attributed to the increase in HR. The QT interval was corrected for HR (QTc) in pigs and no change was observed. AM 92016 did not alter the haemodynamic or contractile parameters measured in the anaesthetized pig, although it produced an increase in ST segment elevation before coronary artery occlusion. Prolongation of the QT interval is indicative of class III anti-arrhythmic activity. The failure to prolong the QT interval in either guinea-pigs or pigs demonstrates a lack of class III anti-arrhythmic activity in-vivo by AM 92016.

A study by Dohadwalla et al (1969) looked at the relevance of β-receptor blockade in ouabain-induced cardiac arrhythmias by comparing the effects of (±)-propanolol with (+)-propranolol. (±)-propranolol is a much

more potent β-receptor-blocking agent than (+)-propranolol. Their study demonstrated that in anaesthetized guinea pigs (±)-propranolol was more potent than (+)-propranolol in protecting against ouabain-induced arrhythmias suggesting a role for catecholamines in the production of cardiac arrhythmias. It is, therefore, possible that the increase in HR and contractility of the heart, as observed in the anaesthetized guinea-pig in this study, is due to increased circulating catecholamines, released directly or indirectly by AM 92016, ultimately resulting in a pro-arrhythmic effect.

An elevation of ST segment of the ECG, as seen in the anaesthetized pig, occurs as a consequence of the development of ischaemia (Wainwright & Martorana 1993; Wainwright et al 1993). Furthermore, agents which suppress this elevation of ischaemia-induced ST segment of the ECG, such as the A_1-adenosine agonist R-PIA, exert a marked antifibrillatory effect in this model (Wainwright & Parratt 1993). This could be possible before coronary artery occlusion if AM 92016 caused a constriction of the coronary vessels. However, no change in arterial pressure was observed throughout the experiment making a direct vasoconstrictor role unlikely. It is conceivable, however, that AM 92016 had a selective effect on the coronary vessels without influencing major arteries. An alternative explanation for the elevated ST segment, is an effect of AM 92016 to increase the heterogeneity of repolarization of the ventricles.

The differences observed in haemodynamic measurements between the two models may be due to the methods employed for administration of AM 92016. The drug was given to guinea-pigs, cumulatively, as bolus injections over a wide concentration range ($10\,\mu g\,kg^{-1}$–$5\,mg\,kg^{-1}$) whereas in the pig model, AM 92016 was infused in a low concentration ($2{\cdot}5\,\mu g\,kg^{-1}\,min^{-1}$) throughout the period of the experiment. In conclusion, AM 92016, a putative class III anti-arrhythmic drug was shown to have pro-arrhythmic effects against both ouabain and ischaemia-induced arrhythmias in guinea-pigs and pigs, respectively. This pro-arrhythmic effect could be explained by the drugs action to increase HR in the guinea-pig and increase ST segment elevation as seen in the pig. The results of this study emphasize that in-vitro electrophysiological studies of anti-arrhythmic drug action may not be a good predictor of activity in-vivo.

Acknowledgements

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References

Andersen, H. R., Wiggers, H. S., Knudsen, L. L., Simonsen, I., Thomsen, P. E. B., Christiansen, N. (1992) Dofetilide suppresses ventricular fibrillation during acute myocardial ischaemia. A randomised study in pigs. Eur. Heart J. 13 (suppl): P804

Connors, S. P., Dennis, P. D., Gill, E. W., Terrar, D. A. (1991) The synthesis and potassium channel blocking activity of some (4-methanesulfonamidophenoxy) propanolamines as potential class III anti-arrhythmic agents. J. Med. Chem. 34: 1570–1577

Connors S. P., Gill, E. W., Terrar, D. A. (1992) Actions and mechanisms of action of novel analogues of sotalol on guinea pig and rabbit ventricular cells. Br. J. Pharmacol. 106: 958–965

Dohadwalla, A. N., Freedberg, A. S., Vaughan Williams, E. M. (1969) The relevance of β receptor blockade to ouabain-induced cardiac arrhythmias. Br. J. Pharmacol. 36: 257–267

Gwilt, M., Arrowsmith, J. E., Blackburn, K. J., Burges, R. A., Cross, P. E., Dalrymple, H. W., Higgins, A. J. (1991) UK 68, 798 is a novel, potent and highly selective class III anti-arrhythmic agent which blocks potassium channels in cardiac cells. J. Pharmacol. Exp. Ther. 256: 318–324

Maynard, A. E., Carpenter, J. F., Decker, G. E., Brooks, R. R. (1991) Efficacy of *d*-sotalol, sematilide and UK 68, 798 in the rat coronary artery ligation-reperfusion (CALR) model of cardiac arrhythmias. Fed. Am. Soc. Exp. Biol. J. 5: N5 A1216

Rasmussen, H. S., Allen, M. J., Blackburn, K. J., Butrous, G. S., Dalrymple, H. W. (1992) Dofetilide, a novel class III anti-arrhythmic agent. J. Cardiovasc. Pharmacol. 20 (suppl. 2): S96–S105

Singh, B. N., Vaughan Williams, E. M. (1970) A third class of anti-arrhythmic action. Effects on atrial and ventricular intracellular potentials and other pharmacological actions on cardiac muscle of MJ 1999 and AH 3474. Br. J. Pharmacol. 39: 675–687

Vaughan Williams, E. M. (1970) The classification of anti-arrhythmic drugs. In: Sandoe, E., Flensted-Jensen, E. (eds) Symposium on cardiac arrhythmias, Astra, Sodertalje, Sweden, pp 449–472

Wainwright, C. L., Martorana, P. A. (1993) Pirsidomine, a novel nitric oxide donor, suppresses ischaemic arrhythmias in pigs. J. Cardiovasc. Pharmacol. 22 (suppl. 7): S44–S50

Wainwright, C. L., Parratt, J. R. (1993) The effects of R-PIA, a selective A_1-adenosine agonist, on haemodynamic and ischaemic arrhythmias in pigs. Cardiovasc. Res. 27: 84–89

Wainwright, C. L., Parratt, J. R., Van Belle, H. (1993) The haemodynamic and antiarrhythmic effects of the nucleoside transport inhibitor R75231 in anaesthetised pigs. Br. J. Pharmacol. 109: 592–599

Walker, M. J. A., Curtis, M. J., Hearse, D. J., Campbell, R. W. F., Janse, M. J., Yellon, D. M., Cobbe, S. M., Coker, S. J., Harness, J. B., Harron, D. W. G., Higgins, A. J., Julian, D. G., Lab, M. J., Manning, A. S., Northover, B. J., Parratt, J. R., Riemersma, R. A., Riva, E., Russell, D. C., Sheridan, D. J., Winslow, E., Woodward, B. (1988) The Lambeth Conventions: guidelines for the study of arrhythmias in ischaemia, infarction and reperfusion. Cardiovasc. Res. 22: 447–455