Br. J. Pharmac. (1985), **85**, 621–627

Carbachol and dibutyryl cyclic GMP on the vulnerability to ventricular fibrillation in rat isolated hearts

Alan Daugherty[1] & Brian Woodward

Pharmacology Group, University of Bath, Claverton Down, Bath, BA2 7AY

1 The hypothesis that elevation of intracellular guanosine 3':5' cyclic monophosphate (cyclic GMP) concentrations may increase electrical stability of the myocardium was examined by determination of ventricular fibrillation thresholds (VFT) on isolated perfused hearts of the rat. Hearts were paced to circumvent any complicating effects of bradycardia. Using this system, carbachol produced a concentration-related reduction in VFT.

2 The reduction in VFT produced by carbachol was not significantly modified by a high concentration of atenolol (10^{-5} M), indicating that the increased vulnerability to ventricular fibrillation was not an indirect consequence of catecholamine release from intramyocardial stores.

3 Atropine (10^{-6} M) blocked the carbachol-induced reduction in VFT.

4 At the concentrations of carbachol used to reduce VFT, myocardial cyclic GMP concentrations were also elevated. The dibutyryl analogue of cyclic GMP (10^{-4} M) mimicked the effect of carbachol in reducing VFT.

5 Carbachol potentiated the adrenaline (3×10^{-7} M)-induced reduction in VFT.

Introduction

The myocardial tissue content of adenosine 3':5'-cyclic monophosphate (cyclic AMP) has been proposed as an important mediator of ventricular arrhythmias (Podzuweit *et al.*, 1976). Indirect evidence has been drawn from experiments in which cyclic AMP concentrations were correlated with the onset of ventricular arrhythmias in models of acute myocardial ischaemia (Podzuweit *et al.*, 1978). Direct evidence has been presented by the demonstration that the dibutyryl analogue of cyclic AMP lowered the ventricular fibrillation threshold (VFT) in rat isolated hearts and that this effect was potentiated by the phosphodiesterase inhibitor, theophylline (Lubbe *et al.*, 1979).

Although the mechanisms by which cyclic AMP produces ventricular arrhythmias have not been elucidated, it has been speculated that it may be due to enhancement of the slow response (Opie & Lubbe, 1979). Kohlhardt & Haap (1978) have shown that the slow response may be blocked by 8-bromo-guanosine 3':5'-cyclic monophosphate (8-Br-cyclic GMP) thereby implying that cyclic GMP may be acting as a physiological antagonist. On the basis of this finding, Opie *et al.* (1979) suggested that elevated concentrations of cyclic GMP may reduce susceptibility to ventricular arrhythmias. The present study has investigated this suggestion of Opie *et al.* (1979) using ventricular fibrillation thresholds in the rat isolated heart as a model of arrhythmias. Carbachol and adrenaline were used as stimulants of cyclic nucleotide concentrations. In addition, the dibutyryl analogues of cyclic AMP and cyclic GMP were also studied. A preliminary account of this work has been presented previously (Daugherty & Woodward, 1982).

Methods

Heart perfusion

Hearts from Wistar rats (190–260 g, University of Bath strain) were perfused by the Langendorff technique with Krebs-Henseleit solution (composition in mM: NaCl 118.4, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, $CaCl_2$ 2.5, glucose 11.5) gassed with

[1] Present address and correspondence: Cardiovascular Division, Washington University Medical School, 660 South Euclid Avenue, St Louis, MO 63110, U.S.A.

95% O_2 5% N_2 at a constant flow of 10 ml min^{-1} and maintained at 37°C.

Isometric contractions, at a diastolic tension of 2 g, were recorded via a small hook placed in the apex of the left ventricle and attached to a Devices UFI transducer. The resultant signal was also used to trigger a Devices instantaneous ratemeter. Perfusion pressure was monitored with a Bell-Howell pressure transducer. All recordings were displayed on a Devices M19 recorder.

Epicardial electrocardiograms were recorded from two fine wire electrodes placed across the ventricular muscle mass and the signal was fed into a modified Devices 3442 preamplifier. The mute overload in this amplifier was disconnected to avoid its activation during ventricular fibrillation threshold determinations. Electrocardiograms were displayed on a Narco storage oscilloscope.

Soon after commencement of perfusion the right atrium was removed. Hearts were paced using bipolar electrodes placed on the aortic cannula and in the region of the atrioventricular node. Pacing was performed using square wave pulses of 2 ms duration, 5 Hz frequency and 14 V amplitude, delivered by one channel of a Grass S88 stimulator via a Grass 5A stimulus isolation unit.

Determination of ventricular fibrillation threshold

The ventricular fibrillation threshold (VFT) was determined using a method similar to that described by Lubbe *et al.* (1978). Two fine wire electrodes were placed across the ventricular wall in a position relative to the electrocardiogram electrodes to reduce stimulus artifacts. VFT was established using trains of stimuli which required the use of Grass S88 and S48 stimulators. The Grass S48 stimulator was synchronized with the second channel of the Grass S88, which was itself synchronized with the first channel (i.e. the pacing channel). This arrangement permitted the delivery of a train of pulses from the S48 with a controlled delay after the pacing stimuli. Trains consisted of square wave pulses of 2 ms duration, 200 Hz frequency delivered for 70 ms and were placed 10 ms after the peak of the R wave. Trains were delivered via a Grass 1A constant current unit and a Grass 5A stimulus isolation unit.

Current strength for VFT determinations was started at 1 mA with increments of 1 mA delivered every 20 s until the development of ventricular fibrillation (VF). Once VF had been observed, no extra stimuli were given for 60 s, after which stimulation was recommenced at the same current strength. VFT was taken as the current which produced VF on two successive stimulations.

VFT determinations were performed in 5 series of experiments. In each series the same preparations were used throughout the protocol. Initially VFT was determined following a 15 min stabilization period, then agonist drugs were perfused at the stated concentrations for 5 min before re-assessment of VFT. After each concentration of agonist, a 5 min washout period was permitted and VFT was determined under control conditions. Consequently, VFT control values were obtained after each drug concentration to ensure changes in VFT were specifically related to drug-induced effects and not to changes in sensitivity of the tissue over time. In the case of antagonist drugs, a control VFT was obtained after a 15 min stabilization period, followed by a drug contact time of 15 min before redetermination of VFT. Thereafter, the antagonist was present in the perfusate throughout the course of the experiment. Changes in VFT were statistically analysed by comparing the value obtained at a given drug concentration against the VFT determined under control conditions immediately before perfusion of drug.

In view of the slow rate of transfer of dibutyryl analogues of cyclic nucleotides to the intracellular space, hearts were perfused for 30 min with analogues before VFT assessment. Due to the prolonged time interval for onset of effect and washout of these analogues, this series of experiments was performed using sequential increases in concentration without washout intervals.

Since rat isolated hearts are seldom persistently arrhythmic using this method, a time limit had to be introduced to define VF. VF was diagnosed as the appearance of chaotic electrical activity associated with the loss of contractile activity which persisted for a period greater than 1 s. This is similar to the criteria described by Lubbe *et al.* (1978).

Cyclic nucleotide determinations

Hearts were allowed a stabilization period of 15 min before the administration of carbachol. After a contact period with carbachol of 5 min, hearts were freeze-clamped using Wollenberger tongs which had previously been cooled to the temperature of liquid nitrogen. Five control hearts were perfused for 20 min before freeze-clamping. While maintaining the hearts at the temperature of liquid nitrogen, ventricular muscle was pulverized in a stainless steel pestle and mortar. The finely powdered tissue was extracted in 6% w/v perchloric acid and the resultant solution was neutralized with potassium hydrogen carbonate/Tris buffer.

Cyclic AMP and cyclic GMP were assayed in the neutral solution using standard kits (Amersham Radiochemical Centre TRK 420 and TRK 500 respectively). Tissue concentrations of cyclic nucleotides are expressed as pmol g^{-1} wet weight.

Statistical analysis

Results are expressed as mean ± standard error of the mean (s.e.mean). Paired and unpaired Student's *t* tests (two tailed) were used as appropriate, and values of $P < 0.05$ were considered to be statistically significant.

Drugs used

Drugs used were: atenolol (ICI), atropine sulphate, adrenaline hydrogen tartrate, N^6,O^2-dibutyryl adenosine 3′ : 5′-cyclic monophosphate, N^6,O^2-dibutyryl guanosine 3′ : 5′-cyclic monophosphate (Sigma) and carbachol chloride (BDH).

All stock solutions were freshly prepared on the day of experimentation and were dissolved in physiological saline.

Results

Effect of carbachol on ventricular fibrillation threshold

The control value for VFT of 10.7 ± 0.5 mA determined in the present study was in close agreement with that determined by Opie and co-workers. Whilst gaining experience with this model of arrhythmias it became apparent that inter-group control values fluctuated slightly. However, all experiments in this study were performed with hearts acting as their own controls to maintain a well controlled system.

Carbachol reduced VFT in a concentration-related manner (Figure 1). Carbachol is known to release catecholamines from sympathetic nerve terminals (Higgins *et al.*, 1973), and may have exerted its effect on VFT by indirect means. However, indirect release of catecholamines does not explain the carbachol-induced lowering of VFT since the presence of a high concentration of atenolol (10^{-5} M), which was considerably above its pA_2 value for cardiac tissue (Harms, 1976), failed to modify significantly the effect of carbachol on VFT (Figure 2). Atenolol alone did not modify VFT.

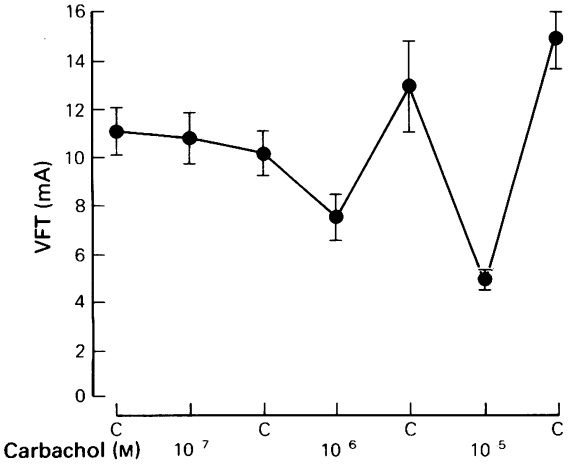

Figure 1 Effects of carbachol on ventricular fibrillation threshold (VFT; mA) in the isolated perfused heart of the rat. VFT was determined under control conditions (C) after perfusion with each carbachol concentration. Points represent mean, and vertical lines s.e.mean, of 6 observations.

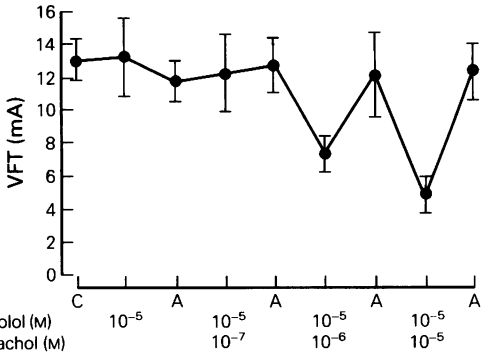

Figure 2 Effects of atenolol on carbachol-induced reduction in ventricular fibrillation threshold (VFT; mA). VFT was assessed under control conditions (C), in the presence of atenolol (10^{-5} M), and during perfusion with both atenolol and carbachol at the stated concentrations. VFT was determined in the presence of atenolol alone (A; 10^{-5} M) after perfusion with each carbachol concentration. Points represent mean, and vertical lines s.e.mean, of 6 observations.

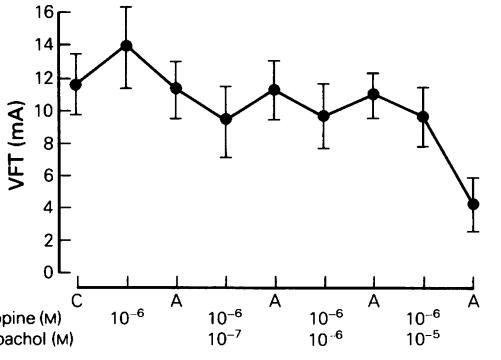

Figure 3 Effects of atropine on carbachol-induced reduction in ventricular fibrillation threshold (VFT; mA). VFT was assessed under control conditions (C), in the presence of atropine (10^{-6} M), and during perfusion with both atropine and carbachol at the stated concentrations. VFT was also determined in the presence of atropine alone (A; 10^{-6} M) after perfusion with each carbachol concentration. Points represent mean, and vertical lines s.e.mean, of 6 observations.

Following administration of atropine (10^{-6} M) there was a trend for VFT to increase, although this elevation did not obtain statistical significance. The carbachol-induced lowering of VFT was attenuated by atropine (10^{-6} M; Figure 3). A complication of this series of experiments was a significant reduction in the final control value. This was due to the consistent reduction in VFT during washout after the administration of carbachol (10^{-5} M) in the presence of atropine (10^{-6} M).

The effects of adrenaline, and its interaction with carbachol, on the VFT were examined. Adrenaline 3×10^{-7} M was initially administered alone (Figure 4); this concentration produced a relatively small, but significant, reduction in VFT. The concomitant administration of carbachol (10^{-7} and 10^{-6} M) did not antagonize the adrenaline-induced reduction in VFT. In fact, the highest concentration of carbachol used (10^{-5} M) significantly ($P < 0.05$) reduced the VFT from that seen in the presence of adrenaline alone.

The effect of carbachol on cyclic nucleotide concentrations

To confirm that the concentrations of carbachol used in the VFT experiment were affecting concentrations of cyclic GMP, the myocardial content of both cyclic nucleotides was quantified. Over the concentration range of carbachol used in the VFT study, no significant changes in cyclic AMP concentrations were observed (Figure 5a), although cyclic GMP concentrations were significantly elevated by perfusion with 10^{-5} M carbachol (Figure 5b). These measurements of cyclic nucleotide concentrations were recorded at a time which approximately corresponded to the time of VFT determination, as detailed above.

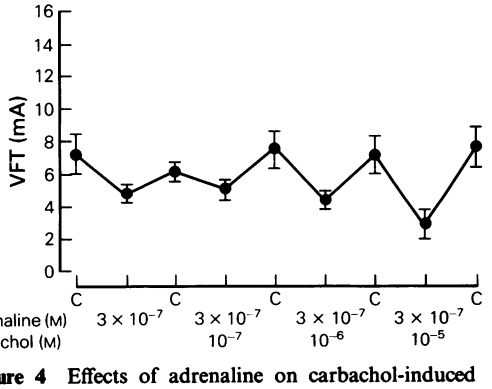

Figure 4 Effects of adrenaline on carbachol-induced lowering of the ventricular fibrillation threshold (VFT; mA). VFT was assessed under control conditions (C), in the presence adrenaline (3×10^{-7} M), and during perfusion with adrenaline and carbachol at the stated concentrations. VFT was determined under control conditions after each agonist concentration. Points represent mean, and vertical lines s.e.mean, of 9 observations.

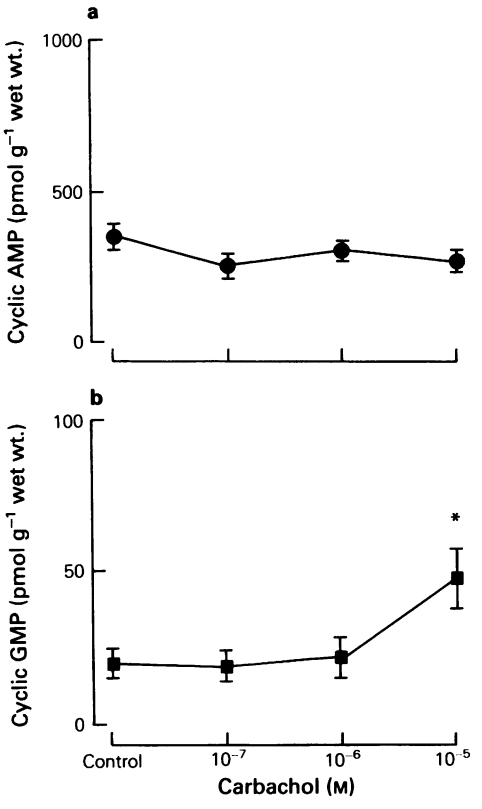

Figure 5 Effects of the various concentrations of carbachol used in the ventricular fibrillation threshold experiments on the myocardial content of: (a) cyclic AMP; (b) cyclic GMP. Each point represents the mean of five observations. * $P < 0.05$, significantly different from control.

The effect of dibutyryl cyclic nucleotides on ventricular fibrillation threshold

The above results demonstrate that carbachol concentrations which reduce VFT, also increase cyclic GMP concentrations. Thus it was important to determine if this reduction in VFT could be mimicked by directly elevating intracellular cyclic GMP concentrations. The dibutyryl derivative of cyclic GMP was used, as this is considered to mimic the effect of endogenously produced cyclic GMP (Posternak *et al.*, 1962). The effects of dibutyryl cyclic AMP were also examined to confirm the consistency of our study with that of other workers (Lubbe *et al.*, 1979). Dibutyryl cyclic AMP (10^{-4} M) reduced VFT (Figure 6a), in close agreement with the findings of Lubbe *et al.* (1979). Dibutyryl

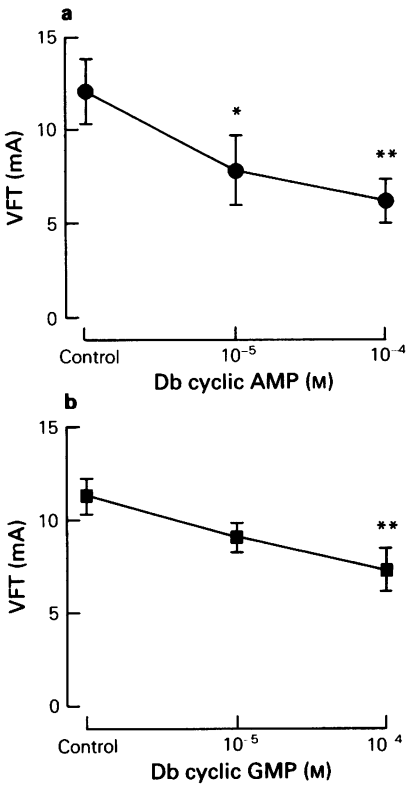

Figure 6 Effects of (a) dibutyryl cyclic AMP (Db cyclic AMP) and (b) dibutyryl cyclic GMP (Db cyclic GMP) on the ventricular fibrillation threshold (VFT; mA). Each point represents the mean, and vertical lines s.e.mean, of 5 observations * $P<0.05$, ** $P<0.01$, significantly different from control.

cyclic GMP (10^{-4} M) also reduced VFT (Figure 6b), which is compatible with the hypothesis that the actions of carbachol on the electrical stability of the heart are due to an effect on the intracellular concentrations of cyclic GMP.

Discussion

The present study was designed to test the speculation of Opie *et al.* (1979) that elevated cyclic GMP concentrations may act in a manner which is opposite to that of cyclic AMP on the electrical stability of the heart. Cyclic AMP increases the vulnerability of the heart to ventricular fibrillation (Lubbe *et al.*, 1979). Several experiments which have implicated the role of cyclic AMP in the production of ventricular arrhythmias were duplicated in the present study to test the consistency of the results with those of other workers. It was found that there was a very good agreement between the present study and those of other authors in the quantities of current required to produce VFT under control conditions (Lubbe *et al.*, 1978), as well as in the effects of adrenaline and dibutyryl cyclic AMP on VFT (Lubbe *et al.*, 1979). However, contrary to the expectations of Opie *et al.* (1979) this study has demonstrated that carbachol, at concentrations which elevated cyclic GMP concentrations, increased vulnerability to ventricular fibrillation. Consistent with this observation with carbachol, the dibutyryl analogue of cylclic GMP also reduced VFT.

The actions of cholinomimetic drugs on atrial myocardial tissue are well defined. These effects are generally of a depressant nature, in as much as they cause reduction in heart rate, contractility and excitability. Although the depressant effects of cholinomimetic agonists are extensively characterized in atrial tissue, excitatory effects of these drugs, which lead to atrial fibrillation (Burn, 1979), are known to occur under certain conditions. However, the effects of cholinomimetic agonists on ventricular myocardial excitability and contractility are more controversial (Higgins *et al.*, 1973; Rardon & Bailey, 1983), and direct actions on ventricular arrhythmia production are largely unknown. Several effects of cholinergic stimulation on ventricular myocardium are due to indirect mechanisms via the release of catecholamines from adrenergic neurones. As demonstrated by the adrenaline infusion, release of catecholamines would have reduced VFT in our model, but even at high concentrations of atenolol there was no significant modification of the effect of carbachol on VFT in the isolated heart of the rat. Thus, it is unlikely that a carbachol-induced release of catecholamines is responsible for the lowering of the VFT by this agonist, but rather a direct action of carbachol is implicated. This direct action of carbachol is probably due to an action on cardiac muscarinic receptors since lowering of VFT was abolished by the concomitant administration of atropine. Reductions in VFT were independent of heart rate, since hearts were paced to preclude any indirect effects of interval frequency. Akin to the situation for cyclic AMP, the electrophysiological mechanism by which carbachol reduces VFT cannot be defined, but may be due to cholinergic stimulation producing a shortening of the action potential (Mirro *et al.*, 1979) which has been implicated in arrhythmogenesis in this model (Opie *et al.*, 1979)

It has been proposed that cyclic GMP is an intracellular antagonist of the actions of cyclic AMP (Goldberg *et al.*, 1975). In accord with this hypothesis, excellent correlations have been determined between the ratio of cyclic nucleotide concentrations and contractile performance in frog myocardium (Wollenberger *et al.*, 1973; Singh & Flitney 1980). Despite these results in frog myocardium, intracellular modulation by cyclic nucleotides of contractile state

has not been found in mammalian ventricular tissue (Daugherty & Woodward, 1981; Rodger & Shahab, 1984). In addition, the electro-physiological effects of acetylcholine have been found to be dissociated from the tissue content of cyclic GMP (Mirro *et al.*, 1979). Therefore, it is conceivable that cyclic GMP should not produce opposing effects to those of cyclic AMP in the generation of ventricular arrhythmias, even though antagonistic effects of cyclic nucleotides have been characterized on atrial electrophysiological parameters (Watanabe & Besch, 1975; Kohlhardt & Haap, 1978). Despite the disputed role of cyclic GMP as the intracellular mediator of the effects of cholinomimetic agonists, we were able to demonstrate that dibutyryl cyclic GMP lowered VFT in a manner which was qualitatively similar to the action of carbachol.

Rabkin *et al.* (1982) have also investigated the hypothesis of Opie *et al.* (1979) that an elevation of myocardial cyclic GMP concentration would negate ventricular arrhythmia production. In their study, barium chloride infusions into anaesthetized rabbits were used to initiate ventricular arrhythmias, and 8-bromo-cyclic GMP and sodium nitroprusside were used as stimulants of cyclic GMP concentrations. Although they suggest an antiarrhythmic role for cyclic GMP, two anomalies exist. Firstly, sodium nitroprusside is regarded as an electrophysiologically inert drug (Mirro *et al.*, 1979), and indeed sodium nitroprusside does not effect VFT in the model used in the present study (Daugherty, 1981). Secondly, the rapidity of onset of the effects of 8-bromo-cyclic GMP (<30 s) is surprising in view of the slow transfer of this analogue to the intracellular compartment to effect an increase in cyclic GMP concentrations. Also, this model has more variables than the isolated perfused heart, used in the present study. Further characterization is required to elucidate the interrelationship of their drug-induced changes with the hormonal and haemodynamic changes occuring during barium chloride infusion so as to determine whether these effects were due to a direct action on the myocardium.

It should be noted that vagal stimulation generally reduces the incidence of 'early' post-ligation arrhythmias (Kent *et al.*, 1973; Goldstein *et al.*, 1973) and these arrhythmias are increased by sectioning of the vagal nerves (Harris *et al.*, 1982; Goldstein *et al.*, 1973). Whether these effects are, respectively, due to the indirect protective action of a reduction in heart rate (Chadda *et al.*, 1974) or concomitant sectioning of afferent nerves in the vagus, has not been defined. Currently there is controversy as to the extent and function of vagal innervation to the ventricular myocardium (Rardon & Bailey, 1983). Thus, until the extent and function of ventricular cholinergic innervation is determined, we cannot presently discuss whether our *in vitro* study may be extrapolated to *in vivo* effects.

Previous workers have investigated the interaction of cholinergic and adrenergic stimulation on VFT in isolated perfused hearts. In agreement with the present study, Murnaghan (1975) found that carbachol did not prevent the VFT-lowering effect of adrenaline in rabbit hearts. In constrast, it has been found that cholinergic stimulation, using acetylcholine, prevented the adrenaline-induced VFT reduction in rat isolated hearts (Thandroyen *et al.*, 1982). The reason for these contradictory results is not readily apparent, particularly in view of the similarity of the models of arrhythmias used in these studies.

In summary, we have demonstrated that the vulnerability to fibrillation in rat isolated hearts was increased in the presence of carbachol. The concentrations of carbachol used elevated myocardial cyclic GMP concentrations and the dibutyryl analogue of cyclic GMP produced qualitatively similar effects to carbachol. This action of carbachol was mediated by muscarinic receptors and was not due to carbachol-induced release of catecholamines from adrenergic neurones.

Alan Daugherty was supported by a British Heart Foundation Research Fellowship. The gift of atenolol (ICI) is gratefully acknowledged.

References

BURN, J.H. (1979). Excitatory actions of acetylcholine in the heart. *Br. J. Pharmac.*, **67**, 3–12.

CHADDA, K.D., BANKA, V.S. & HELFANT, R.H. (1974). Rate dependent ventricular ectopia following acute coronary occlusion: The concept of an optimal anti-arrhythmic heart rate. *Circulation*, **49**, 654–658.

DAUGHERTY, A. (1981). Myocardial metabolism of calcium and cyclic nucleotides. *PhD. thesis*. University of Bath.

DAUGHERTY, A. & WOODWARD, B. (1981). Calcium and calcium slow channel antagonists on cyclic nucleotide levels in the isolated rat heart *J. mol. cell. Cardiol.*, **13**, 843–854.

DAUGHERTY, A. & WOODWARD, B. (1982). Carbachol-adrenaline interactions on vulnerability to ventricular fibrilloflutter in the isolated rat heart. *Br. J. Pharmac. Proc. Suppl.*, **77**, 464P.

GOLDBERG, N.D., HADDOX, M.K., NICOL, S.E., GLASS, D.B., SANFORD, C.H., KUEHL, F.A. & ESTENSEN, R. (1975). Biologic regulation through opposing influences of cyclic GMP and cyclic AMP: The Yin Yang hypothesis. *Adv. cyclic. Nucl. Res.*, **5**, 307–330.

GOLDSTEIN, R.E., KRASH, R.B., SMITH, E.R., ORLANDO, M., NORMAN, D., FARNHAM, G., REDWOOD, D.R. & EPSTEIN, S.E. (1973). Influence of atropine and of vagally

mediated bradycardia on the occurrence of ventricular arrhythmias following acute coronary occlusion in closed-chest dogs. *Circulation*, **47**, 1180–1190.

HARMS, H.H. (1976). Isoproternol antagonism of cardioselective beta adrenergic receptor blocking agents: A comparative study of human and guinea pig cardiac and bronchial adrenergic receptors. *J. Pharmac. exp. Ther.*, **199**, 329–335.

HARRIS, N., KANE, K.A. & PARRATT, J.R. (1982). Autonomic and hormonal influences on the severity of coronary artery ligation induced arrhythmias in anaesthetised rats. *Br. J. Pharmac. Proc. Suppl.*, **77**, 388P.

HIGGINS, C.B., VATNER, S.F. & BRAUNWALD, E. (1973). Parasympathetic control of the heart. *Pharmac. Rev.*, **25**, 120–155.

KENT, K.M., SMITH, E.R., REDWOOD, D.R. & EPSTEIN, S.E. (1973). Electrical stability of acutely ischemic myocardium: Influences of heart rate and vagal stimulation. *Circulation*, **47**, 291–298.

KOHLHARDT, M. & HAAP, K. (1978). 8-Bromo-guanosine-3′5′-monophosphate mimics the effect of acetylcholine on slow response action potential and contractile force in mammalian atrial myocardium. *J. mol. cell. Cardiol.*, **10**, 573–586.

LUBBE, W.F., BRICKNELL, O.L. & MARZAGAO, C. (1978). Ventricular fibrillation threshold and vulnerable period in the isolated perfused rat heart. *Cardiovas. Res.*, **9**, 613–620.

LUBBE, W.F., PODZUWEIT, T., DARIES, P.S. & OPIE, L.H. (1979). The role of cyclic adenosine monophosphate in adrenergic effects on ventricular vulnerability to fibrillation in the isolated perfused rat heart. *J. clin. Invest.*, **61**, 1260–1269.

MIRRO, M.J., BAILEY, J.C. & WATANABE, A.M. (1979). Dissociation between the electrophysiological properties and total tissue cyclic guanosine monophosphate content of guinea pig atria. *Circulation Res.*, **45**, 225–233.

MURNAGHAN, M.F. (1975). The effect of sympathetic amines on the ventricular fibrillation threshold on the rabbit isolated heart. *Br. J. Pharmac.*, **53**, 3–9.

OPIE, L.H. & LUBBE, W.F. (1979). Catecholamine-mediated arrhythymias in acute myocardial infarction. *S. Afr. Med. J.*, **56**, 871–879.

OPIE, L.H., NATHAN, D. & LUBBE, W.F. (1979). Biochemical aspects of arrhythmogenesis and ventricular fibrillation. *Am. J. Cardiol.*, **43**, 131–148.

PODZUWEIT, T., DALBY, A.J., CHERRY, G.W. & OPIE, L.H. (1978). Cyclic AMP levels in ischaemic and non-ischaemic myocardium following coronary artery ligation: relation to ventricular fibrillation. *J. mol. cell. Cardiol.*, **10**, 81–94.

PODZUWEIT, T., LUBBE, W.F. & OPIE, L.H. (1976). Cyclic adenosine monophosphate, ventricular fibrillation and antiarrhythmic drugs. *Lancet*, **1**, 341–342.

POSTERNAK, T., SUTHERLAND, E.W. & HENION, W.F. (1962). Derivatives of cyclic 3′,5′-adenosine monophosphate. *Biochem. biophys. Acta.*, **65**, 558–560.

RABKIN, S.W., OHMAE, M. & KLASS, D.J. (1982). Antiarrhythmic effects of cyclic guanosine 3′5′-monophosphate and sodium nitroprusside on barium chloride arrhythmias in rabbits. *Arch. int. Pharmacodyn.*, **257**, 225–238.

RARDON, D.P. & BAILEY, J.C. (1983). Parasympathetic effects on electrophysiologic properties of cardiac ventricular tissue. *J. Am. Coll. Cardiol.*, **2**, 1200–1209.

RODGER, I.W. & SHAHID, M. (1984). Forskolin, cyclic nucleotides and positive inotropism in isolated papillary muscles of the rabbit. *Br. J. Pharmac.*, **81**, 151–159.

THANDROYEN, F.T., HIGGINSON, L.M., WORTHINGTON, M.G., MULLER, C.A. & OPIE, L.H. (1982). Cholinergic modulation of enhanced vulnerability to ventricular fibrillation by adrenergic stimulation in the isolated rat heart. *Adv. Myocard.*, **3**, 199–204.

SINGH, J. & FLITNEY, F.W. (1980). Adenosine depresses contractility and stimulates 3′,5′ cyclic nucleotide metabolism in the isolated frog ventricle. *J. mol. cell. Cardiol.*, **12**, 285–297.

WATANABE, A.M. & BESCH, H.R. (1975). Interaction between cyclic adenosine monophosphate and cyclic guanosine monophosphate in guinea pig ventricular myocardium. *Circulation Res.*, **37**, 309–317.

WOLLENBERGER, A., BABSKII, E.B., KRAUSE, S.G., GENZ, S., BLOHM, D. & BOGDANOVA, E.V. (1973). Cyclic changes in levels of cyclic AMP and cyclic GMP in frog myocardium during the cardiac cycle. *Biochem. biophys. Acta.*, **55**, 446–452.

(*Received December 5, 1984.*
Revised January 30, 1985.
Accepted February 19, 1985.)