J. Pharm. Pharmacol. 1991, 43: 88–94
Received August 9, 1990

Enhancement of Amrinone-induced Positive Inotropy in Rabbit Papillary Muscles with Depressed Contractile Function: Effects on Cyclic Nucleotide Levels and Phosphodiesterase Isoenzymes

M. SHAHID AND *I. W. RODGER

*Department of Pharmacology, Scientific Development Group, Organon Laboratories Ltd, Newhouse, Lanarkshire ML1 5SH and * Department of Physiology and Pharmacology, University of Strathclyde, 204 George Street, Glasgow G1 1XW, UK*

Abstract—The inotropic activity of amrinone and its effects on cyclic nucleotide levels in rabbit papillary muscles with normal and depressed contractile function have been compared. The effects of amrinone on the cyclic (c) AMP hydrolytic activity of cyclic nucleotide phosphodiesterase (PDE) isoenzymes were also examined. Amrinone ($2{\cdot}4 \times 10^{-4}$–$1{\cdot}2 \times 10^{-3}$ M) produced a relatively weak (maximal increase 11%) positive inotropic effect in papillary muscles stimulated at the near optimal stimulation frequency of 1 Hz. In contrast, large positive inotropic responses (maximal 138–200%) were obtained with amrinone in papillary muscles in which contractile force had been depressed by: (a) lowering stimulation frequency to 0·4 Hz, (b) reducing extracellular Ca^{2+} concentration from $2{\cdot}5 \times 10^{-3}$ M to $6{\cdot}3 \times 10^{-4}$ M, (c) prior addition of sodium pentobarbitone ($6{\cdot}5 \times 10^{-4}$ M). The EC50 values for amrinone under conditions (a), (b), and (c) were $3{\cdot}0 \times 10^{-3}$, $2{\cdot}6 \times 10^{-3}$, and $2{\cdot}8 \times 10^{-3}$ M, respectively. Force-frequency curves in rabbit papillary muscles were compared at normal ($2{\cdot}5 \times 10^{-3}$ M) and low ($6{\cdot}3 \times 10^{-4}$ M) extracellular Ca^{2+} concentration. Contractions at low frequencies of stimulation (< 0·4 Hz) were less sensitive to removal of extracellular Ca^{2+} than higher stimulation rates indicating that in the former situation, recycling of intracellular Ca^{2+} is more important for maintaining contractile force. The positive inotropic effects of amrinone in normal and papillary muscles with depressed contractile force were associated with similar increases in both cAMP (1·6–2·5 fold) and cGMP (2·6–4·0 fold) levels, despite marked differences in the degree of inotropy in these muscles. The cGMP inhibited cAMP-PDE (PDE III) was selectively inhibited by amrinone (IC50 $9{\cdot}9 \times 10^{-5}$ M), whilst the activities of Ca^{2+}/calmodulin-stimulated PDE, cGMP-stimulated PDE and cGMP-insensitive PDE were only weakly affected (IC50 > $2{\cdot}5 \times 10^{-4}$ M). The results show an involvement of cAMP in the mechanism underlying the positive inotropic action of amrinone in cardiac muscle with depressed contractile force. Furthermore, it is suggested that there may be an enhanced effectiveness of cAMP on intracellular Ca^{2+} mobilization in depressed cardiac muscle.

Amrinone has been shown to be a clinically useful cardiotonic agent in patients with severe cardiac failure (Ward et al 1983; Heuer et al 1988). In addition it may prove to be beneficial in providing acute inotropic support to counteract cardiac depression associated with the use of certain cardiovascular drugs (Alousi et al 1985) or surgical anaesthetics (Boncyk et al 1984). Although amrinone has been reported to reverse the negative inotropic effects of a variety of drugs, including sodium pentobarbitone and verapamil, in whole animal preparations (Alousi et al 1985), the cellular mechanisms underlying this action have not been studied. Facilitation of Ca^{2+} transport by amrinone has been suggested to be involved; however, whether this is due to a direct or indirect effect of amrinone (e.g. mediated by cyclic (c)AMP) is not clear. cAMP is known to be involved in the positive inotropic action of amrinone in isolated papillary muscles (Honerjager et al 1981; Endoh et al 1982; Shahid & Rodger 1989). Those studies have shown that the rise in intracellular cAMP produced by amrinone is due to inhibition of cyclic nucleotide phosphodiesterase (PDE) activity, although the relative effects on different PDE isoenzymes were not examined. Thus in normal cardiac muscle, amrinone-induced changes in Ca^{2+} flux appear to be mediated by cAMP. However, it is not known whether amrinone-induced positive inotropy in cardiac preparations with depressed contractile function is also associated with increases in cAMP. Conceivably, in these preparations amrinone may be operating through mechanisms involving direct modification of Ca^{2+} transport or a different alteration of cAMP metabolism when compared with normal tissue. Indeed the stimulatory effects of amrinone on Ca^{2+} transport in dog erythrocytes involve the Na-Ca exchange mechanism (Parker & Harper 1980). The aims of the present study were to examine the inotropic effects of amrinone in rabbit papillary muscles with normal or depressed contractile function and to analyse the involvement of cAMP. The effects of amrinone on the cAMP activity of PDE isoenzymes from rabbit ventricular myocardium were also studied.

Correspondence to: M. Shahid, Department of Pharmacology, Scientific Development Group, Organon Laboratories Ltd, Newhouse, Lanarkshire ML1 5SH, UK.

Materials and Methods

Materials

Sodium pentobarbitone was purchased from May and Baker (UK), amrinone and verapamil were gifts from Sterling Winthrop (UK) and Abbot Pharmaceuticals (UK), respectively. Amrinone ($2{\cdot}7 \times 10^{-1}$ M) was dissolved in 0·5 M lactic acid and dilutions were made in Krebs–Henseleit solution. [8-^{3}H]Adenosine 3′:5′-cyclic monophosphate (25–30 Ci mmol^{-1}), [8-^{3}H]guanosine 3′:5′-cyclic monophosphate (15–

20 Ci mmol^{-1}), [U-^{14}C]adenosine 3′:5′-cyclic monophosphate, [U-^{14}C]guanosine 3′:5′ cyclic monophosphate (Amersham, Bucks, UK). Chromatographic alumina and cyclic nucleotides (Sigma, Poole, Dorset, UK). All other chemicals were obtained either from Sigma or BDH (both of Poole, Dorset, UK) and were of Analar or equivalent grade.

Isolated papillary muscles

Male New Zealand white rabbits were stunned with a blow to the back of the neck and exsanguinated. The thorax was opened and the hearts rapidly excised and placed in a dish containing warm oxygenated Krebs–Henseleit solution. Papillary muscles were removed from the right ventricle and suspended in an organ bath containing Krebs–Henseleit solution at 32°C. The solution was of the following composition (mM): NaCl 118, KCl 4·7, $MgSO_4$ 1·2, KH_2PO_4 1·2, $CaCl_2$ 2·5, $NaHCO_3$ 25 and glucose 11·7 and gassed with 95% O_2 – 5% CO_2. The papillary muscles were mounted vertically between two platinum wire electrodes so that the base of each tissue was in contact with the bottom electrode whilst the tendon end, attached to a force displacement transducer (Grass FT03C) lay just beneath the upper electrode. The tissues were stretched by adjusting the diastolic tension to 1 g and allowed to stabilise for 60 min (at a driving frequency of 1 Hz with pulses of 1 ms duration at a voltage 50–100% above threshold, from a Grass S88 stimulator). The preparations were rinsed three times with fresh Krebs–Henseleit solution during this period. At the conclusion of the equilibration period, any fall-off in diastolic tension was corrected by resetting the tension to 1 g. Cumulative concentration-effect curves were constructed for amrinone, $CaCl_2$ and sodium pentobarbitone in different preparations. Each drug concentration was allowed to reach peak effect before addition of the next dose. In experiments examining the effects of amrinone in papillary muscles with depressed contractile activity, sodium pentobarbitone or lowering of extracellular Ca^{2+} was used to reduce cardiac force before addition of amrinone. Solvent control experiments showed that lactic acid produced transient negative inotropic responses which were completely and rapidly reversed and there was no significant change in basal tension or in the pH of the Krebs-Henseleit solution. The effects of stimulation frequency on papillary muscle developed tension were determined as described in detail by Rodger & Shahid (1984). Two force-frequency curves were constructed in each preparation at normal ($2·5 \times 10^{-3}$ M) and low ($6·3 \times 10^{-4}$ M) extracellular Ca^{2+} concentration. A 15 min equilibration period was allowed between changes in Ca^{2+} concentration; time-matched controls had shown that there was no significant change in the force-frequency relation over this period.

Cyclic nucleotide analyses

These measurements were made in rabbit papillary muscle according to Rodger & Shahid (1984). Cyclic nucleotide levels were determined at the peak of the positive inotropic response, 15–20 min after drug addition. Control papillary muscles received appropriate amount of solvent over the same period. Only one concentration of amrinone was tested in each preparation and 2–3 identically treated papillary muscles (20–30 mg tissue) were pooled before extraction of cyclic nucleotides.

Preparation of PDE isoenzymes and measurement of activity

Rabbit cardiac PDE isoenzymes were separated by ion-exchange chromatography on DEAE-Sepharose as described by Shahid & Nicholson (1991). PDE activity was measured according to the method described by Shahid & Rodger (1989). The standard assay mixture contained (mM); $MgCl_2$ 3, 5′AMP or 5′GMP 1, ^{3}H-labelled/unlabelled cAMP or cGMP 0.001 (150 000–200 000 d min^{-1}), dithiothreitol 1, 0·05% (w/v) bovine serum albumin in 50 mM Tris HCl (pH 7·4) in a final volume of 200 μL. Samples were incubated at 37°C in a shaking water bath for 15 min and the reaction stopped by placing the test tube in aluminium heating blocks at 105°C for 3 min. The unreacted cyclic nucleotide was recovered by batch chromatography on small alumina columns and counted in a liquid scintillation spectrometer (Packard RCA 2000). [^{14}C]cAMP or [^{14}C] cGMP was used as an internal recovery marker for the alumina columns. Typical recoveries of cAMP and cGMP were 70–80% and 60–70%, respectively. The assays were performed in duplicate and the enzyme samples were diluted to ensure 20–30% substrate hydrolysis. Amrinone was tested on the PDE isoenzymes in the concentration range $1 \times 10^{-7} - 2·5 \times 10^{-4}$ M. In these assays the effects on PDE I and PDE II were examined in the presence of Ca^{2+} (20 μM)/calmodulin (1·5 μg mL^{-1}) and cGMP (1 μM), respectively to boost low level basal PDE activity.

Statistics

The results are presented as mean values ± s.e.m. In isolated papillary muscle experiments the EC50 values were calculated from the cumulative concentration-effect curves, and have been given as mean-log EC50 ± s.e.m. The significance of differences between means was determined by using Student's *t*-test; a *P* value of less than 0·05 was considered significant.

Results

Inotropic activity

The positive inotropic effect of amrinone in rabbit isolated papillary muscles was dependent on the frequency of stimulation (Fig. 1). Amrinone produced concentration-dependent increases in contractile force when papillary muscles were stimulated at 0·4 Hz, giving a 3-fold increase in developed tension at the top concentration and had an EC50 value of $3·0 \times 10^{-3}$ M (−log EC50 2·52 ± 0·08, n = 7). The positive inotropic effect of amrinone was not significantly modified by the presence of propranolol (1×10^{-7} M) indicating the lack of involvement of β-adrenoceptor or noradrenaline release. The EC50 value for amrinone in the latter experiments was $3·0 \times 10^{-3}$ M (−log EC50 2·53 ± 0·05, n = 3). Increasing the frequency of stimulation to 1 Hz dramatically reduced the degree of positive inotropism induced by amrinone (a maximal increase of 11 ± 5% n = 5 over pre-drug value). The absolute increase in developed tension was also greater at 0·4 Hz. The data shown in Fig. 1 are from different papillary muscles; however, the same result was obtained when amrinone was tested at the two stimulation frequencies in the same preparation. Thus the differences in inotropic activity at 0·4 and 1·0 Hz are unlikely to be due to variation in papillary muscle responsiveness.

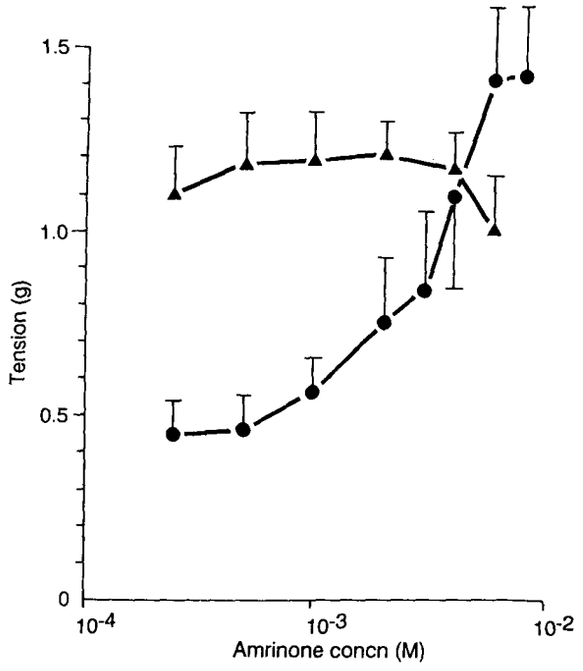

FIG. 1. Cumulative concentration-effect curves for the inotropic effects of amrinone in rabbit papillary muscles stimulated at 0·4 (●) and 1·0 Hz (▲). The basal tension values at 0·4 and 1·0 Hz were 470 ± 90 and 1017 ± 91 mg, respectively. The maximal tension in the presence of amrinone (at 0·4 Hz) was 1430 ± 205 mg. For comparative purposes 0·4 Hz data has been taken from Shahid & Rodger (1989). Each point represents the mean and vertical lines indicate s.e.m. (n = 5–7).

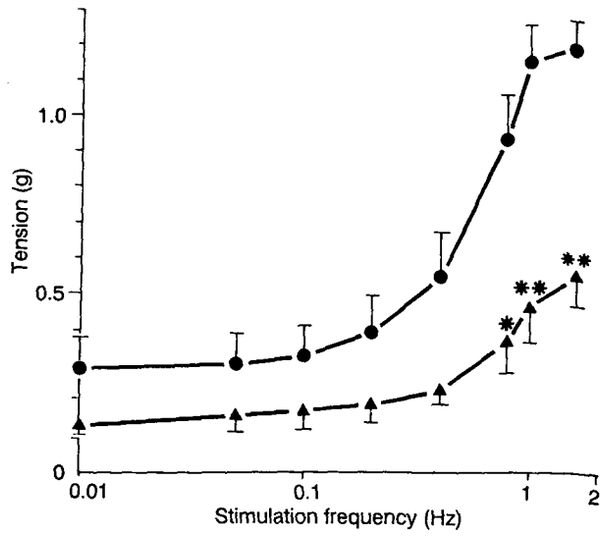

FIG. 2. The effects of lowering extracellular Ca^{2+} concentration on the force-frequency relation of rabbit papillary muscles. Force-frequency curves were constructed at $2{\cdot}5 \times 10^{-3}$ (●) and $6{\cdot}3 \times 10^{-4}$ M (▲) Ca^{2+} concentrations. Each point represents the mean and vertical lines indicate s.e.m. (n = 4). * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$ Student's t-test compared to corresponding values on the $2{\cdot}5 \times 10^{-3}$ M Ca^{2+} force-frequency curve.

To further understand the frequency-dependence of the positive inotropic action of amrinone, the effects of extracellular Ca^{2+} concentration on the force-frequency relationship of rabbit papillary muscles was examined. It is clear from Fig. 2 that as stimulation frequency is raised from 0·01 to 1·6 Hz there is a corresponding increase in developed tension ('positive staircase'). Least tension was developed at 0·01 Hz (pessimal driving frequency) and maximal tension was achieved at 1·6 Hz (optimal driving frequency). The sub-optimal frequency of 0·4 Hz lies one-third of the way up the force-frequency curve at normal ($2{\cdot}5 \times 10^{-3}$ M) extracellular Ca^{2+}. Reduction in extracellular Ca^{2+} concentration to $6{\cdot}3 \times 10^{-4}$ M depressed the force-frequency curve such that contractile force was reduced at each stimulation frequency. However, there was a disproportionately larger decrease in developed tension at higher stimulation frequencies (> 0·4 Hz) than at lower values. Only at stimulation frequencies above 0·4 Hz were the differences in tension values statistically significant.

To examine whether the greater inotropic efficacy of amrinone at 0·4 Hz was related to a reduction in contractile state rather than change in stimulation frequency itself, two methods for reducing contractility were employed. In these experiments the stimulation frequency was maintained at 1 Hz. Either sodium pentobarbitone or lowering of extracellular Ca^{2+} concentration was used to depress cardiac contractile force. Sodium pentobarbitone (2×10^{-5}–3×10^{-3} M) given cummulatively produced concentration-dependent negative inotropic responses that took 1–2 min for onset and 10–15 min to establish a sustained peak response. The compound had an IC50 value of $4{\cdot}3 \times 10^{-4}$ M (−log IC50: 3·37 ± 0·05, n = 5); the mean cumulative concentration-effect curve is shown in Fig. 3. Although the negative inotropic effects of sodium pentobarbitone were stable and well sustained throughout the experimental period they were easily reversed (within 40 min) after washout. The size of

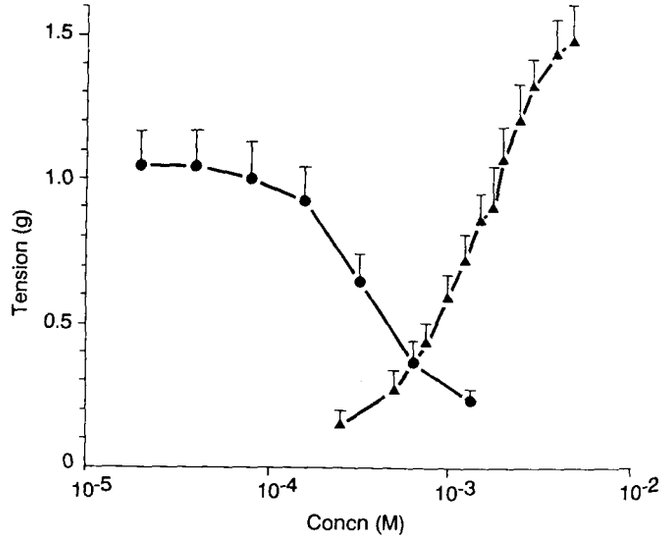

FIG. 3. Cumulative concentration-effect curves for sodium pentobarbitone (●) and $CaCl_2$ (▲) in rabbit electrically stimulated papillary muscles (1 Hz). In the $CaCl_2$ experiments the extracellular Ca^{2+} concentration was lowered to $2{\cdot}5 \times 10^{-4}$ from $2{\cdot}5 \times 10^{-3}$ M and allowed to equilibrate for 15 min before the start of the dose-response curve. The pre-drug tension value in the sodium pentobarbitone experiments was 1075 ± 130 mg. Each point represents the mean and vertical lines indicate s.e.m. (n = 5–6).

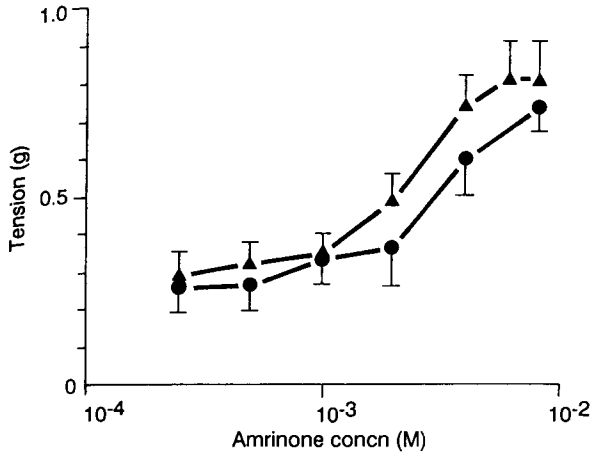

FIG. 4. Cumulative concentration-effect curves for the positive inotropic effects of amrinone in electrically stimulated papillary muscles (1 Hz) in which basal contraction was depressed by sodium pentobarbitone (●; $6{\cdot}5 \times 10^{-4}$ M) or lowering extracellular Ca^{2+} to $6{\cdot}3 \times 10^{-4}$ M (▲). The basal tension values before addition of sodium pentobarbitone or lowering extracellular Ca^{2+} were 720 ± 77 and 915 ± 24 mg, respectively. Each point represents the mean and vertical lines indicate s.e.m. (n = 4).

papillary muscle contractions was directly proportional to the extracellular Ca^{2+} concentration (Fig. 3). Increasing the extracellular Ca^{2+} ($2{\cdot}5 \times 10^{-4}$–5×10^{-3} M) produced concentration-dependent positive inotropic responses that were rapid (15–30 s) in onset and took 5–10 min to reach peak effect. Ca^{2+} concentrations above 5×10^{-3} M produced precipitation.

Equieffective concentrations of sodium pentobarbitone ($6{\cdot}5 \times 10^{-4}$ M) and extracellular Ca^{2+} ($6{\cdot}3 \times 10^{-4}$ M) were selected to depress papillary muscle contractile activity. Amrinone elicited concentration-dependent large positive inotropic responses in these preparation (Fig. 4). The mean decreases in developed tension produced by sodium pentobarbitone or low extracellular Ca^{2+} concentration were $50 \pm 7{\cdot}7$ and $64 \pm 7{\cdot}3\%$ (n = 4) of control value, respectively. Amrinone completely reversed the negative inotropy produced by low Ca^{2+} or sodium pentobarbitone. However, the maximal response in the presence of amrinone was not significantly different from the value before addition of sodium pentobarbitone or lowering of extracellular Ca^{2+}. The tension values before addition of sodium pentobarbitone and the maximal response to amrinone in the presence of sodium pentobarbitone were 770 ± 77 and 744 ± 78 mg (n = 4), respectively. For the low Ca^{2+} experiments, the tension values at $2{\cdot}5 \times 10^{-3}$ M Ca^{2+} and the maximal response to amrinone at $6{\cdot}3 \times 10^{-4}$ M Ca^{2+} were 915 ± 24 and 925 ± 115 mg (n = 4), respectively. The EC50 values for the positive inotropic effects of amrinone in the presence of sodium pentobarbitone or at $6{\cdot}3 \times 10^{-4}$ M Ca^{2+} were $2{\cdot}8 \times 10^{-3}$ M ($-\log$ EC50: $2{\cdot}56 \pm 0{\cdot}07$, n = 4) and $2{\cdot}6 \times 10^{-3}$ M ($-\log$ EC50: $2{\cdot}58 \pm 0{\cdot}04$, n = 4), respectively.

Effects on cyclic nucleotide levels

Fig. 5 shows the effects of a near maximal concentration of amrinone (6×10^{-3} M) on developed tension and cyclic nucleotide levels in rabbit papillary muscles being stimulated at 0·4 and 1·0 Hz. At both stimulation frequencies the positive inotropic effect of amrinone was associated with increases in tissue cyclic nucleotide content. However, the increases in cAMP (1·6–2·0 fold) and cGMP (2·6–3·7 fold) were similar at both stimulation frequencies despite a much greater inotropic change at 0·4 Hz (6·2 fold) than at 1·0 Hz (1·4 fold).

To parallel the experiments examining the inotropic activity of amrinone in papillary muscles with depressed contractile function the effects of amrinone on developed tension and cyclic nucleotide levels in the presence of sodium pentobarbitone ($6{\cdot}5 \times 10^{-4}$ M) or Ca^{2+} ($6{\cdot}3 \times 10^{-4}$ M) were

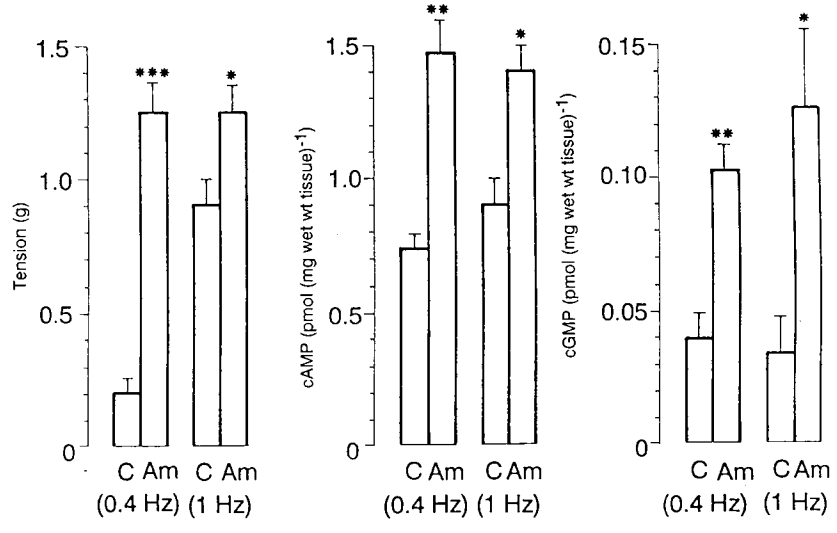

FIG. 5. Comparison of the effects of amrinone on tension and cyclic nucleotide levels in rabbit papillary muscles paced at 0·4 or 1 Hz. Papillary muscles were frozen in liquid nitrogen at the peak of the positive inotropic response (15–20 min after addition of amrinone) and processed for cAMP and cGMP measurements. The bars indicate control (C) values and responses in the presence of a submaximal concentration of amrinone (Am; 6×10^{-3} M). For comparative purposes the 0·4 Hz data have been taken from Shahid & Rodger (1989). Each bar represents the mean and vertical lines indicate s.e.m. (n = 4). * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$ Student's t-test compared with corresponding control value.

Table 1. Effects of amrinone on tension responses and cyclic nucleotide levels in rabbit isolated papillary muscles with depressed contractile function.

			(pmol mg^{-1} wet wt tissue)	
Treatment	Amrinone ($\times 10^{-3}$ M)	Tension (mg)	cAMP	cGMP
Ca^{2+} ($6{\cdot}3 \times 10^{-4}$ M)	Solvent control	364±47	0·60±0·08	0·013±0·005
	0·6	368±40	0·81±0·08	0·023±0·003
	3	637±109*	1·27±0·12**	0·027±0·006
	6	1181±97***	1·46±0·13**	0·051±0·008*
Sodium pentobarbitone ($6{\cdot}5 \times 10^{-4}$ M)	Solvent control	211±27	0·80±0·08	0·014±0·003
	0·6	337±58	0·96±0·08	0·019±0·005
	1·5	387±87	1·12±0·05*	0·027±0·005
	3	728±132**	1·20±0·07**	0·022±0·007
	6	710±128**	1·49±0·05**	0·037±0·005*

* $P<0{\cdot}05$, ** $P<0{\cdot}01$, *** $P<0{\cdot}001$ vs control values (Student's t-test). Each value is the mean±s.e. mean (n=4).

analysed. Papillary muscles, stimulated at 1 Hz, were allowed to stabilize for 60 min before the introduction of Krebs-Henseleit solution containing $6{\cdot}3 \times 10^{-4}$ M Ca^{2+}. This produced an immediate negative inotropic response which reached a stable level within 15 min. At this point, either amrinone or an appropriate volume of solvent was added. Once the inotropic response to amrinone had reached maximum, muscles were frozen in liquid nitrogen and cyclic nucleotide content analysed. Lowering extracellular Ca^{2+} produced, in addition to the fall in developed tension, a significant ($P<0{\cdot}05$) reduction in the basal levels of cAMP (33%) and cGMP (62%). Amrinone produced large increases in developed tension which were associated with concentration-dependent elevations in the levels of cAMP (2·5 fold at 6×10^{-3} M) and cGMP (4 fold at 6×10^{-3} M) as shown in Table 1.

Amrinone also increased cAMP (2 fold at 6×10^{-3} M) and cGMP (3·5 fold at 6×10^{-3} M) content in sodium pentobarbitone-depressed papillary muscle (Table 1). The degree of contractile depression was kept constant (~50% of pre-drug contraction) in every preparation and sodium pentobarbitone alone lowered basal cAMP (11%) and cGMP (59%).

Effects on cyclic nucleotide PDE isoenzymes

Four distinct PDE activities were resolved from rabbit cardiac ventricle after ion-exchange chromatography on DEAE-Sepharose (Fig. 6). These have been labelled PDE I, II, III and IV according to the nomenclature used by Reeves et al (1987) for human cardiac ventricle. The PDE subtypes show different substrate specificity and regulatory properties as well as different sensitivities to a variety of inhibitors (Shahid & Nicholson 1991). Briefly, the distinguishing

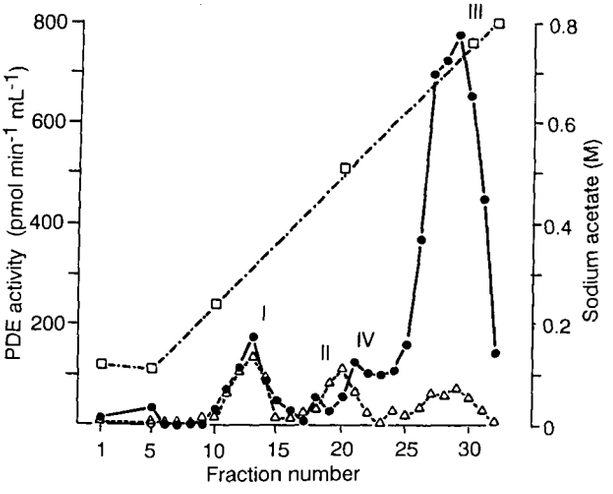

FIG. 6. Elution profile of cyclic nucleotide phosphodiesterase (PDE) activities in rabbit ventricular myocardium after ion-exchange chromatography. A low speed (12000 g) supernatant fraction of the cardiac homogenate was applied to a column of DEAE-sepharose (fast flow) which had been equilibrated in buffer (pH 6·5) containing (mM): Bris Tris 20, dithiothreitol 1, benzamidine 2, ethylenediamine tetraacetic acid 2, sodium acetate 50 and phenylenethanesulphonyl fluoride, 0·1. The bound material was eluted with a linear sodium acetate gradient (0·05 − 1·0 M; □) and collected in 10 mL fractions. These were assayed for PDE activity, using radio labelled cAMP (●) or cGMP (△) at a concentration of 1×10^{-6} M. Four main peaks of PDE activity (labelled PDE I, PDE II, PDE III and PDE IV) were identified.

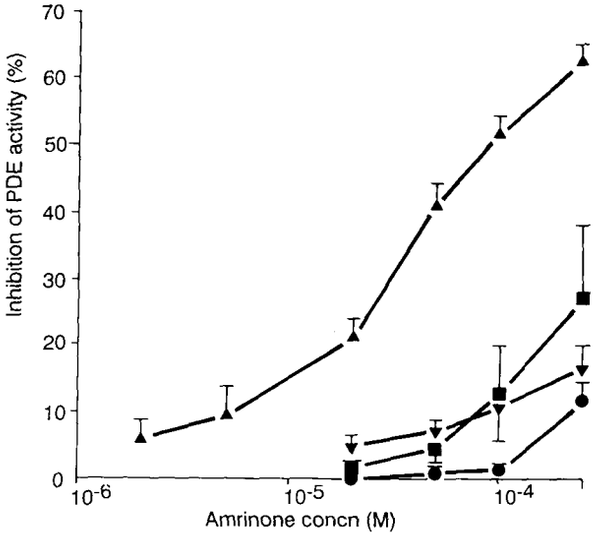

FIG. 7. Effects of amrinone on cAMP (1×10^{-6} M) hydrolysis by rabbit ventricular PDE isoenzymes. The effects on PDE I and PDE II were examined in the presence of the stimulators Ca (20 μM)/calmodulin (1·5 μg mL^{-1}) and cGMP (1×10^{-6} M), respectively. Amrinone produced selective inhibition of PDE III (▲) whilst PDE I (■), PDE II (●) and PDE IV (▼) were only weakly affected.

characteristics of the various PDE isoenzymes are: PDE I is stimulated by Ca^{2+}/calmodulin; the cAMP hydrolysis of PDE II is stimulated whilst that of PDE III is inhibited by low concentrations of cGMP; PDE IV is unaffected by Ca^{2+}/calmodulin or cGMP but is selectively inhibited by rolipram.

Amrinone produced concentration-dependent inhibition of cAMP activity of all PDE isoenzymes (Fig. 7). However, the effects of amrinone were most potent on PDE III (IC50 $98{\cdot}7 \pm 9{\cdot}6$ μM) showing at least a 2·5 fold selectivity when compared with its effects on PDE I (IC50 > 250 μM), PDE II (IC50 > 250 μM) and PDE IV (IC50 > 250 μM).

Discussion

Amrinone exerted a relatively weak positive inotropic effect in papillary muscles stimulated at 1 Hz. In contrast, large positive inotropic responses were obtained with amrinone in papillary muscles in which contractile force was depressed by lowering stimulation frequency to 0·4 Hz or extracellular Ca^{2+} to $6{\cdot}3 \times 10^{-4}$ M or by sodium pentobarbitone ($6{\cdot}5 \times 10^{-4}$ M). The frequency-dependence of the positive inotropic action of amrinone is in agreement with the findings of Komai & Rusy (1984) and Honerjager et al (1981). However, other workers (Onuaguluchi & Tanz 1981; Siegl et al 1984) using rabbit papillary muscles, have reported that amrinone does elicit large increases in contractile force at 1 Hz. The precise reasons for this discrepancy are unclear but it is likely to be related to differences in experimental protocol. Siegl et al (1984) established concentration-effect curves to isoprenaline in each preparation before testing amrinone. It is known that the basal contractile force after an isoprenaline challenge is reduced (Clark & Poyser 1977; Shahid & Rodger 1989). In the study by Onuaguluchi & Tanz (1981) there was a steady decline in contractile force (up to 50%) in control papillary muscles during the experimental period. Thus, there was significant reduction of the basal tension before addition of amrinone, which may explain the greater inotropic response reported in these studies. The inotropic effect of amrinone at 1 Hz is unlikely to be limited by tissue maximum since a maximal concentration of isoprenaline (2×10^{-6} M) can produce a 138% increase in contractile force at this stimulation frequency. However, like amrinone the relative inotropic change elicited by isoprenaline at 0·4 Hz (300%) (Shahid & Rodger 1989) was much greater than at 1 Hz. This is in accord with the suggestion by Mensing & Hilgemann (1981) that agents which act through a cAMP-dependent mechanism cause greater increases in contractile force at low rather than at high stimulation rates. Thus there may be similarities in the mechanisms underlying positive inotropy induced by amrinone or by an increase in stimulation frequency.

The present study also shows that lowering of extracellular Ca^{2+} has less effect on papillary muscle contractions at low frequencies of stimulation than at high stimulation rates. This is consistent with the idea that at low stimulation frequencies intracellular recycling of Ca^{2+} is more important for maintaining contractile force (Allen et al 1976). Thus it seems likely that at 0·4 Hz the positive inotropic effects of amrinone are mediated mainly by stimulating intracellular Ca^{2+} flux. Sarcolemmal Ca^{2+} transport may also be important in the positive inotropic action of amrinone as suggested by the sodium pentobarbitone and low Ca^{2+} experiments in which papillary muscles were stimulated at 1 Hz. Sodium pentobarbitone is routinely used in models of acute cardiac failure (Alousi et al 1985) and its negative inotropic effect is believed to be due to a reduction in the amount of La^{3+} – displaceable, superficially-bound Ca^{2+} in cardiac cells (Nayler & Szeto 1972). Amrinone completely reversed the cardiodepressant effects of sodium pentobarbitone. Reducing extracellular Ca^{2+} enhanced the positive inotropic effects of amrinone, which is in agreement with the findings of Rendig & Amsterdam (1984) in cat papillary muscles. These results suggest that the positive inotropic effect of amrinone may also involve augmentation of plasmalemmal Ca^{2+} influx via Ca^{2+} channels. This view is supported by the observation that amrinone was able to reverse the negative inotropic effect caused by Ca^{2+} channel blockade using verapamil (Shahid, unpublished results). Thus the mechanisms underlying the positive inotropic effect of amrinone involve facilitation of both Ca^{2+} entry and Ca^{2+} release from intracellular sites.

cAMP is known to regulate myocardial contractility by stimulating Ca^{2+} entry and intracellular Ca^{2+} release (see Opie 1982; Sperelakis 1988 for reviews). The present study shows that the positive inotropic effects of amrinone in normal and depressed papillary muscles were associated with increases in cAMP levels. Whilst this is in agreement with published data (Honerjager et al 1981; Endoh et al 1982; Shahid & Rodger 1989), those studies did not examine the effects of amrinone in cardio-depressed tissues. Thus the mechanism underlying the positive inotropic effect of amrinone in cardiac muscle with depressed contractile function also involves cAMP. However, the greater inotropic activity of amrinone in depressed muscles was not due to a larger increase in cAMP than that obtained in normal preparations. Indeed the effects of amrinone on cAMP levels in both normal and in depressed papillary muscles were similar. This implies that the same rise in cAMP is better able to stimulate Ca^{2+} transport in depressed muscles than in normal preparations; it would be interesting to test this possibility by measuring the effects on intracellular Ca^{2+} and/or levels of cAMP-dependent protein phosphorylation.

Amrinone was selective for inhibiting PDE III and had a relatively small effect on the activities of PDE I, PDE II and PDE IV. Although previous studies (Carpendo et al 1984; Masuoka et al 1990) have shown that amrinone inhibits a high-affinity cAMP-PDE the complete PDE isoenzyme inhibitory profile of the compound was not determined. In particular it was not clear whether PDE III or PDE IV, both of which are high affinity cAMP-PDE, had been used by those workers. It has been suggested that PDE III is important in regulating cardiac force since a number of new positive inotropic agents have also been shown to be highly selective and potent inhibitors of this PDE isoenzyme (Manganiello 1987). However, the large difference in the EC50 value for positive inotropy and the IC50 concentration for PDE III inhibition with amrinone indicates that there is no direct correlation between these two parameters. Similar disparities between EC50 and IC50 values have been reported for other PDE III selective inhibitors (Brunkhorst et al 1989; Silver et al 1989; Shahid & Nicholson 1991). The exact reasons for this discrepancy are not clear but it is

possible that increases in cAMP produced by PDE III inhibition alone may be limited by the relative activities and distribution of PDE I, PDE II and PDE IV. In view of the differences in IC50 and EC50 values it is likely that, at concentrations producing positive inotropy, amrinone elevates cAMP by inhibiting all PDE isoenzymes. The cardiac affects of amrinone were also associated with large increases in cGMP. The rise in cGMP was similar in normal and depressed papillary muscles and therefore cannot explain the difference in the effects of amrinone in these preparations. Nevertheless, since cGMP can counteract the effects of cAMP in ventricular myocardium (Endoh 1980) a moderating effect on amrinone-induced positive inotropy, due to the rise in cGMP, cannot be excluded. The elevation of intracellular cGMP is probably due to inhibition of cGMP-PDE activity as shown previously (Shahid & Rodger 1989). The effects of PDE inhibitors on cardiac cGMP metabolism and the relevance of this second messenger to the myocardial actions of these agents have not been well studied and are poorly understood.

In conclusion the results demonstrate that amrinone shows greater inotropic activity in rabbit isolated papillary muscles in which contractile function was depressed by lowering stimulation frequency or extracellular Ca^{2+} or by the addition of sodium pentobarbitone. However, the positive inotropic effects of amrinone in both normal and papillary muscles with depressed contractile force were associated with similar increases in both cAMP and cGMP. It is suggested that this inconsistency between degree of positive inotropy and change in cyclic nucleotide level reflects an enhanced effectiveness of cAMP on intracellular Ca^{2+} mobilization in tissues with depressed contractile force. Amrinone reduced the cAMP activity of rabbit cardiac ventricle PDE isoenzymes producing selective inhibition of PDE III, indicating this to be the main mechanism underlying its effects on cyclic nucleotide levels and cardiac force.

Acknowledgement

The authors would like to thank Dr R. J. Marshall for helpful comments.

References

Allen, D. G., Jewell, B. R., Wood, E. H. (1976) Studies of the contractility of mammalian myocardium at low rates of stimulation. J. Physiol. (London) 254: 1–17

Alousi, A. A., Canter, J. M., Fort, D. J. (1985) The beneficial effect of amrinone on acute drug-induced heart failure in the anaesthetised dog. Cardiovas. Res. 19: 483–494

Boncyk, J., Redon, D., Rusy, B. (1984) Haemodynamic effects of amrinone in dogs anaesthetised with halothane: comparison with isoproterenol and dobutamine. Res. Commun. Chem. Path. Pharmacol. 44: 347–354

Brunkhorst, D., van der Leyen, H., Meyer, W., Nigbur, R., Schmidt-Schumacher, C., Scholz, H. (1989) Relation of positive inotropic and chronotropic effects of pimobendan, UDCG212 Cl, milrinone and other phosphodiesterase inhibitors to phosphodiesterase III inhibition in guinea-pig heart. Naunyn-Schmiedebergs Arch. Pharmacol. 339: 575–583

Carpendo, F., Floreani, M., Cargnelli, G. (1984) Competitive inhibition of phosphodiesterase activity by amrinone: its implication in the cardiac effect of the drug. Pharmacol. Res. Commun. 16: 969–977

Clark, S. J., Poyser, R. H. (1977) Effect of tazolol on β-adrenoceptors in isolated preparations of the guinea-pig and rat. J. Pharm. Pharmacol. 29: 630–632

Endoh, M. (1980) The time course of changes in cyclic nucleotide levels during cholinergic inhibition of positive inotropic actions of isoprenaline and theophylline in the isolated canine ventricular myocardium. Naunyn-Schmiedebergs Arch. Pharmacol. 312: 175–182

Endoh, M., Yamashita, S., Taira, N. (1982) Positive inotropic effect of amrinone in relation to cyclic nucleotide metabolism in the canine ventricular muscle. J. Pharmacol. Exp. Ther. 221: 775–783

Heuer, H., Frenking, B., Grulker, H. (1988) Effect of amrinone in acute severe cardiac failure. Arzneim-Forsch. (Drug Res.) 38: 567–570

Honerjager, P., Schafer-Korting, M., Reiter, M. (1981) Involvement of cyclic AMP in the direct inotropic action of amrinone. Biochemical and functional evidence. Naunyn-Schmiedebergs Arch. Pharmacol. 318: 112–120

Komai, H., Rusy, B. F. (1984) Inotropic effect of amrinone in rabbit papillary muscle: reversal of the myocardial depressant effect of halothane. Can. J. Physiol. Pharmacol. 62: 1382–1386

Manganiello, V. C. (1987) Subcellular localisation and biological function of specific cyclic nucleotide phosphodiesterse. J. Mol. Cell. Cardiol. 19: 1037–1040

Masuoka, H., Ito, M., Nakano, T., Naka, M., Tanaka, T. (1990) Effects of amrinone and enoximone on the subclasses of cyclic AMP phosphodiesterase from human heart and kidney. J. Cardiovasc. Pharmacol. 15: 302–307

Mensing, H. J., Hilgemann, D. W. (1981) Inotropic effects of activation and pharmacological mechanisms in cardiac muscle. Trends Pharmacol. Sci. 2: 303–307

Nayler, W. G., Szeto, J. (1972) Effect of sodium pentobarbital on calcium in mammalian heart muscle. Am. J. Physiol. 222: 339–344

Onuaguluchi, G., Tanz, D. (1981) Cardiac effects of amrinone on rabbit papillary muscle and guinea-pig Langendorff heart preparations. J. Cardiovasc. Pharmacol. 3: 1342–1355

Opie, L. H. (1982) Role of cyclic nucleotides in heart metabolism. Cardiovasc. Res. 16: 483–507

Parker, J. C., Harper, J. R. (1980) Effects of amrinone, a cardiotonic drug, on calcium movements in dog erythrocytes. J. Clin. Invest. 66: 254–259

Reeves, M. L., Leigh, B. K., England, P. J. (1987) The identification of a new cyclic nucleotide phosphodiesterase activity in human and guinea-pig cardiac ventricle. Biochem. J. 241: 537–541

Rendig, S. V., Amsterdam, E. A. (1984) Positive inotropic action of amrinone: effect of elevated external Ca^{2+}. J. Cardiovasc. Pharmacol. 6: 293–299

Rodger, I. W., Shahid, M. (1984) Forskolin, cyclic nucleotides and positive inotropism in isolated papillary muscles of the rabbit. Br. J. Pharmacol. 81: 151–159

Shahid, M., Rodger, I. W. (1989) Chronotropic and inotropic actions of amrinone, carbazeran and isobutylmethylxanthine: role of phosphodiesterase inhibition. Ibid. 98: 291–301

Shahid, M., Nicholson, C. D. (1991) Comparison of cyclic nucleotide phosphodiesterase isoenzymes in rat and rabbit ventricular myocardium: positive inotropic and phosphodiesterase inhibitory effects of Org 30029, milrinone and rolipram. Naunyn-Schmiedebergs Arch. Pharmacol. In press

Siegl, P. K. S., Morgan, G., Sweet, C. S. (1984) Responses to amrinone in isolated cardiac muscles from cat, rabbit, and guinea-pig. J. Cardiovasc. Pharmacol. 6: 281–287

Silver, P. J., Hariss, A. L., Canniff, P. C., Lepore, R. E., Bentley, R. G., Hamel, L. T., Evans, D. B. (1989) Phosphodiesterase isozyme inhibition, activation of the cAMP system, and positive inotropy mediated by milrinone in isolated guinea-pig cardiac muscle. Ibid. 13: 530–540

Sperelakis, N. (1988) Regulation of calcium slow channels of cardiac muscle by cyclic nucleotides and phosphorylation. J. Mol. Cell. Cardiol. 20 (Suppl. II): 75–105

Ward, A., Brogden, R. N., Heel, R. C., Speight, R. M., Avery, G. S. (1983) Amrinone. A preliminary review of its pharmacological properties and therapeutic use. Drugs 26: 468–502