Contribution of cAMP-Phosphodiesterase Inhibition and Sensitization of the Contractile Proteins for Calcium to the Inotropic Effect of Pimobendan in the Failing Human Myocardium

Michael Böhm, Ingo Morano, Burkert Pieske, Johann Caspar Rüegg, Michael Wankerl, Rainer Zimmermann, and Erland Erdmann

Previous studies have shown reduced effects of cAMP-dependent positive inotropic agents in the failing human myocardium; thus other cAMP-independent mechanisms of action may be useful to increase force of contraction in this condition. The purpose of this investigation was to determine whether a positive inotropic effect of the cAMP-phosphodiesterase (PDE) inhibitor pimobendan is observed in the failing human myocardium and to study whether other factors, such as an increase in the Ca\(^{2+}\) sensitivity of myofilaments, play a functional role in the increase in force of contraction. Pimobendan produced a positive inotropic effect in isolated preparations from nonfailing donor hearts; however, in moderately (New York Heart Association class II-III, NYHA II-III) and severely (NYHA IV) failing myocardium, this effect was reduced. In addition, in NYHA IV specimens pimobendan inhibited the crude cAMP-PDE (crude PDE) and the isoenzymes I-III (PDE I-III) in a concentration-dependent way. As judged from the IC\(_{50}\) values found in this tissue for the inhibition of PDE III and of crude PDE, the potency of the compound was 18.1 times greater on PDE III. Consistent with a cAMP-PDE–dependent mechanism of action, the positive inotropic effect was potentiated by isoproterenol and inhibited by adenosine in failing myocardium. In failing myocardium, pimobendan also increased the sensitivity of skinned cardiac fibers to Ca\(^{2+}\) and shifted the Ca\(^{2+}\)–tension relation to the left. This sensitizing effect began at 0.01 μmol/l in NYHA II-III and NYHA IV and rose to about 200% at 300 μmol/l in both groups. In contrast, the demethylated metabolite UD-CG 212 Cl failed to produce positive inotropic effects in failing myocardium alone, but in the presence of isoproterenol, it exerted an increase in force of contraction. The potency of UD-CG 212 Cl for PDE III inhibition in NYHA IV was greater than that of pimobendan. The metabolite pronouncedly decreased the sensitivity of skinned cardiac fibers to Ca\(^{2+}\) at 30–300 μmol/l in NYHA II-III and NYHA IV. It is concluded that in the failing human heart pimobendan inhibited PDE III and sensitized contractile proteins for Ca\(^{2+}\). Both effects appear to be involved in the positive inotropic effect of the compound, because its metabolite, UD-CG 212 Cl, had no effect on force of contraction and on the Ca\(^{2+}\) sensitivity of skinned cardiac fibers but inhibited PDE III even more potently than pimobendan. These findings suggest that PDE inhibition alone by the benzimidazole derivatives might not be sufficient to increase force of contraction in the failing human heart, indicating that an additional mechanism, such as a Ca\(^{2+}\)-sensitizing effect of pimobendan, may have functional relevance. (Circulation Research 1991;68:689–701)

In recent years, positive inotropic compounds have been developed as alternative drugs to cardiac glycosides in the treatment of chronic heart failure.\(^1\) Pimobendan (UD-CG 115 BS) is reported to inhibit cAMP-phosphodiesterases (PDEs),\(^2\) to pro-

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Address for reprints: Dr. Michael Böhm, Medizinische Klinik I der Universität München, Klinikum Großhadern, Marchioninistr. 15, D-8000 München 70, FRG.

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TABLE 1: Diagnoses, Age, and Hemodynamic Measurements of the Patients Studied

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<th>LVEDP (mm Hg)</th>
<th>LVEDV (ml)</th>
<th>EF (%)</th>
<th>CI (l/m²)</th>
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NYHA, New York Heart Association; MRAP, mean right atrial pressure; LVEDP, left ventricular end-diastolic pressure; LVEDV, left ventricular end-diastolic volume; EF, ejection fraction; CI, cardiac index; MI, mitral insufficiency; MS, mitral stenosis; >, predominant valve disease; AS, aortic stenosis; DCM, dilated cardiomyopathy; ICM, ischemic cardiomyopathy.

long the action potential duration,5 and to sensitize guinea pig and dog myofilaments to Ca²⁺.6–8 However, it is unclear whether both cAMP-PDE inhibition and Ca²⁺ sensitization or only one of these mechanisms is causally related to the positive inotropic effect of the compound. Only few data on the positive inotropic effects of pimobendan in failing human myocardium and on the mechanisms involved are available.9 These questions are of importance, since in the failing myocardium the positive inotropic response to PDE inhibitors is reported to be impaired.10–12 The reduced mechanical responses to these agents have been suggested to be due to a reduced basal cAMP formation in the failing heart.10,13,14 An increase in sensitivity of the myofilaments to Ca²⁺ might be useful to lower the energy expenditure for the failing heart while increasing force of contraction.15 Moreover, it is not unreasonable to assume that these agents could produce positive inotropism at lower Ca²⁺ concentrations in the myocardial cell than those agents with cAMP-increasing properties alone.16 It was the aim of this investigation to characterize the positive inotropic effect of pimobendan in human failing myocardium and to study whether both inhibition of cAMP-PDE and sensitization of the contractile proteins are involved. Therefore, the effects on force of contraction of pimobendan were studied in isolated, electrically driven preparations from human hearts of patients with variable degrees of heart failure (nonfailing, New York Heart Association class II-III [NYHA II-III], and NYHA IV) and compared with the inotropic responses to isoproterenol and to an elevation of the extracellular Ca²⁺ concentration. In failing myocardium, the effects on the activity of the crude cAMP-PDE, the PDE isoenzymes I–III, and the Ca²⁺–tension relation of skinned cardiac fibers were also studied and compared with the effects of the demethylated main metabolite UD-CG 212 CI.

Materials and Methods

Myocardial Tissue

All experiments were performed on preparations from human papillary muscles or on isolated, electrically stimulated, human papillary muscle strips. Tissue was obtained during either mitral valve replacement operations or heart transplantations, or from donor hearts. For this study, patients have been grouped as having normal cardiac function or moderate (NYHA II-III) or severe (NYHA IV) heart failure. Two normal hearts from prospective donors (one male, 25 years old; one female, 36 years old)
were studied. Neither heart could be transplanted for technical reasons. Patients with heart failure were classified as NYHA II-III on the basis of clinical symptoms and signs as judged by the attending cardiologist shortly before the operation. This group of patients with moderate heart failure was not further divided into NYHA II and NYHA III. Patients suffering from NYHA heart failure class IV (all cardiac transplant recipients) were studied for comparison. The underlying disease was dilated cardiomyopathy or severe coronary heart disease. All patients gave written informed consent before surgery. The diagnoses, the ages of the patients, and the hemodynamic measurements are summarized in Table 1. Cardiac catheterization was not performed in patients without heart failure. Hence, hemodynamic data cannot be given for them. All patients receiving β-adrenoceptor antagonists or catecholamines were withdrawn from the study. Medical therapy consisted of diuretics, nitrates, and cardiac glycosides in patients with NYHA II-III and NYHA IV. All patients with NYHA IV also received enalapril. The last dose of the medication was given on the evening before the operation. There was no detectable influence of drug pretreatment on the response to the positive inotropic agents in individual patients.

Contraction Experiments

The experiments were performed on electrically driven (1 Hz) papillary muscle strips. Papillary muscle strips of uniform size (diameter, 0.94±0.04 mm; n=114) were dissected in aerated bathing solution (composition see below) at room temperature. The preparations were attached to a bipolar platinum stimulating electrode and suspended individually in 75-ml glass tissue chambers for recording isometric contractions. The bathing solution was a modified Tyrode’s solution containing (mmol/l) NaCl 119.8, KCl 5.4, CaCl₂ 1.8, MgCl₂ 1.05, Na₂HPO₄ 0.42, NaHCO₃ 22.6, Na₂EDTA 0.05, ascorbic acid 0.28,
and glucose 5.0. It was continuously gassed with 95% O₂-5% CO₂ and maintained at 35°C; the pH was 7.4.

The force of contraction was measured with an inductive force transducer (W. Fleck, Mainz, FRG) attached to a Hellige Helco Scriptor (Freiburg, FRG) or Gould (Cleveland, Ohio) recorder. Each muscle was stretched to the length at which force of contraction was maximal. The resting force (approximately 10 mN) was kept constant throughout the experiment. The preparations were electrically paced at 1 Hz with rectangular pulses 5 msec in duration (stimulator SD 9, Grass Instrument Co., Quincy, Mass.); the voltage was about 20% greater than threshold. All preparations were allowed to equilibrate in drug-free bathing solution until they were completely mechanically stabilized.

Mechanical effects of isoproterenol, pimobendan, or UD-CG 212 Cl were obtained as follows. The compounds were applied cumulatively to the organ bath, and each concentration was present until equilibration of the inotropic effect (i.e., 16–26 minutes). After each experiment, concentration–response curves of Ca²⁺ (5 minutes of exposure to each concentration) were obtained after a washout period of about 60 minutes. At concentrations higher than 15 mmol/l, Ca²⁺ produced precipitates at the stimulation electrodes, and preparations could not be reliably paced. Therefore, higher concentrations were not studied. In all experiments the bathing solution contained 2% (vol/vol) dimethyl sulfoxide (DMSO) and dimeticone (SAB simplex, Parke-Davis, Freiburg, FRG) as antifoaming agent. These compounds did not change the pH of the medium. The solvent reduced force of contraction by 15% within 2 hours, as measured in control preparations running in parallel to each experiment.

**Phosphodiesterase Assay**

**Enzyme preparation.** Left ventricular myocardium from explanted hearts from patients with severe heart failure (NYHA IV, dilated cardiomyopathy) was prepared in a Potter-Elvehjem homogenizer with 4 vol 0.05 mol/l Tris HCl (pH 7.4) and centrifuged in a Heraeus-Christ Varifuge ST (rotor 5220, Munich) at 6,500g for 15 minutes at 4°C. The supernatant was frozen in aliquots at −30°C. Before the assay procedure of this “crude PDE” preparation, the supernatant was diluted so that 50 µl in the assay system of 150 µl produced 20–30% hydrolysis of substrate within the incubation time. For the separation of PDE isoenzymes, the supernatant was not diluted.

**Separation of phosphodiesterase isoenzymes.** Cardiac PDE (crude PDE) from human left ventricles was separated into three isoenzymes (PDE I–III) by an anionic exchange chromatography method with a fast protein ligand chromatography (FPLC) system with a Mono-Q column (Pharmacia-LKB Biotechnology AB, Uppsala, Sweden) comparable to diethylaminomethyl-cellulose chromatography. After the crude PDE was thawed and centrifuged in a Heraeus Biofuge A at 15,000g, the supernatant was filtered through a sterile filter unit (MILLEX-GS 0.22 µm, Millipore Corp., Bedford, Mass.) and 2 ml of this filtered supernatant was injected into an FPLC system LCC 500 (Pharmacia-LKB) equipped with a prepacked 5-ml Mono-Q anionic exchange column (HR 5/5, Pharmacia-LKB). The separation of the PDE isoenzymes was performed with a nonlinear gradient of NaCl concentration (0–1 mol/l); flow rate was 1 ml/min and maximal pressure was 1.5–2.5 MPa. Fractions of 1 ml were collected, and the cAMP-PDE and cGMP-PDE activities were measured with a substrate concentration of 1.0 µmol/l cAMP or 0.1 µmol/l cGMP, respectively. Three peaks of PDE activity could be identified and pooled as PDE I, PDE II, and PDE III. The activity pattern of the PDE isoenzymes separated by this FPLC method was similar to those described previously.19

**Determination of phosphodiesterase activity.** Inhibition of high-affinity cAMP- and cGMP-PDE in the crude enzymes as well as in PDE I, PDE II, and PDE III isoenzyme preparations of human left ventricles was determined by a modified radioisotope method as described by van Meel et al19 and
first described by Pöch with 1.0 μmol/l [3H]cAMP or 0.1 μmol/l [3H]cGMP as the substrate.

**Skinned Fibers Technique**

**Skinning procedure.** Fiber bundles were chemically skinned in a solution containing 50% glycerol, 20 mM imidazole, 10 mM NaN₃, 5 mM ATP, 5 mM MgCl₂, 4 mM EGTA, 2 mM dithioerythritol, pH 7.0, at 4°C for 1 hour and then for 24 hours in the same solution that also contained 1% Triton X-100 (Merck, Darmstadt, FRG). Afterward, the fibers were stored at -20°C in the first solution without detergent.

**Isometric tension registration.** The chemically skinned fibers (length, 6–11 mm; diameter, 120–180 μm) were mounted isometrically and connected to a force transducer (AME 801, SensoNOR, Horten, Norway). In relaxation solution, fiber length was adjusted to an extent at which resting tension was just threshold without preload. The relaxation solution contained (in mmol/l) HEPES 50, potassium acetate 80, ATP 5, MgCl₂ 6, creatine phosphate 10, NaN₃ 5, and EGTA 5, as well as creatine kinase (Boehringer Mannheim, Mannheim, FRG) 350 units/ml, pH 7.0. Sarcomere length was measured by laser diffraction (1.95±0.03 μm, n=65) and was maintained during the entire experiment. The desired Ca²⁺ concentration was obtained by stirring the relaxation and contraction solution in the appropriate proportions. The relaxation solution had a pCa>8.0, whereas the contraction solution contained CaEGTA and had a pCa of 4.3. All experiments were calculated by a computer program similar to that reported by Fabiato and Fabiato.

**Compounds and Solutions**

[3H]cAMP and [3H]cGMP were purchased from New England Nuclear, Dreieich, FRG; cAMP and
TABLE 2. EC₅₀ Values (µmol/l) of the Effects of Pimobendan and UD-CG 212 Cl Alone or With Isoproterenol on Force of Contraction of Isolated Cardiac Preparations From Nonfailing Myocardium and From the Hearts of Patients With Moderate (NYHA II-III) or Severe (NYHA IV) Heart Failure

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<th>NYHA III</th>
<th>NYHA IV</th>
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<td>Isoproterenol</td>
<td>0.016 (0.011–0.022)</td>
<td>0.026 (0.017–0.039)</td>
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<td>Pimobendan</td>
<td>33.8 (12.0–95.1)</td>
<td>26 (11.6–58.1)*</td>
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<td>0.4 (0.14–1.1)</td>
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<tr>
<td>UD-CG 212 Cl</td>
<td>...</td>
<td>No effect</td>
</tr>
<tr>
<td>UD-CG 212 Cl + isoproterenol</td>
<td>...</td>
<td>0.06 (0.03–0.11)</td>
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</table>

Values represent the mean and 95% confidence limits of three to 13 experiments. NYHA, New York Heart Association.
*p<0.05 vs. pimobendan plus isoproterenol.

cGMP were from Boehringer Mannheim, FRG. Drugs used were (±)-isoproterenol HCl (Sigma Chemical Co., Munich) and adenosine (Boehringer Mannheim). The racemic mixture (±)-pimobendan (4,5-dihydro-6-(2-[4-methoxyphenyl]-1H-benzimidazole-5-yl)-5-methyl-3(2H)-pyridazinone) and UD-CG 212 Cl were from Dr. Karl Thomae GmbH, Biberach/Riss, FRG. Stock solutions of the compounds were prepared at 10 mmol/l in DMSO. DMSO was from Serva, Heidelberg, FRG. All other chemicals were either of analytical quality or of the best grade commercially available. Twice distilled water was used throughout.

Statistics

The data shown are mean±SEM. Statistical significance was estimated with Student’s t test for unpaired observations or analysis of variance. A value

FIGURE 4. Concentration–response curves for the effect of UD-CG 212 Cl (0.001–1,000 µmol/l) alone and in the presence of isoproterenol (Iso 0.03 µmol/l) on force of contraction of isolated, electrically driven (1 Hz) papillary muscle strips from the hearts of patients with moderate (New York Heart Association class II-III [NYHA II-III], panel A) or severe (NYHA IV, panel B) heart failure. Basal force of contraction was 1.4±0.2 mN (n=6) or 1.3±0.2 mN (n=9) in NYHA II-III and 1.7±0.4 mN (n=5) and 1.9±0.4 mN (n=6) in NYHA IV. Iso (0.03 µmol/l) increased force of contraction by 180.2±41.3% of the predrug value (n=9, NYHA II-III) or by 140.3±54.4% of the predrug value (n=5). The relative maximal increases of force of contraction in the presence of Iso were 46±7% of the value before addition of Iso (n=9, NYHA II-III) and 35±10% of the value before addition of Iso (n=5, NYHA IV).
of $p<0.05$ was considered significant. The EC$_{50}$ was determined graphically in each individual experiment. The IC$_{50}$ and EC$_{50}$ values are given with 95% confidence limits.

**Results**

**Force of Contraction**

The positive inotropic response of both pimobendan and isoproterenol but not of Ca$^{2+}$ was reduced in failing hearts (NYHA II-III) as compared with non-failing hearts (Figures 1A and 1B). Figure 2A shows original tracings of the positive inotropic effect of pimobendan alone and in the presence of isoproterenol. Pimobendan alone produced a positive inotropic effect that reached its maximum after 20–30 minutes. However, after preincubation of the preparations with isoproterenol, the effect of pimobendan was enhanced (Figure 2A, lower tracing). Figure 2B shows a typical original recording of the effect on isometric contraction of pimobendan in a preparation from a moderately failing heart. Pimobendan increased peak tension and rate of force development, but markedly prolonged contraction duration. These effects of the parameters on single contractions were similar in all groups studied (not shown). In this type of preparation, isoproterenol (0.03 µmol/l) shortened contraction duration and increased rate of relaxation and rate of contraction in NYHA II-III and NYHA IV (not shown).

Concentration–response curves summarize the effects of pimobendan alone and in the presence of isoproterenol on force of contraction (Figure 3) in NYHA II-III and NYHA IV. Pimobendan exerted a positive inotropic effect that started at 3 µmol/l in NYHA II-III and at 10 µmol/l in NYHA IV. In the presence of isoproterenol, there was a leftward shift of the concentration–response curve. The positive inotropic effects of pimobendan in the presence of isoproterenol were significantly enhanced at 0.3–30 µmol/l in NYHA II-III and 3–10 µmol/l in NYHA IV. The EC$_{50}$ values are listed in Table 2. The positive inotropic effect of Ca$^{2+}$ did not differ between NYHA II-III and NYHA IV. Neither in nonfailing nor in failing myocardium was evidence of Ca$^{2+}$ overload (i.e., an increase of diastolic tension) observed. To study whether these effects on force of contraction are shared by the in vivo active, demethylated metabolite, the same experiments were performed with UD-CG 212 Cl. Figure 4 summarizes the data in cardiac preparations from NYHA II-III and NYHA IV. UD-CG 212 Cl failed to produce a positive inotropic effect in a concentration range of 0.001–100 µmol/l in both groups. However, when isoproterenol was given before UD-CG 212 Cl, there
was a positive inotropic effect of UD-CG 212 Cl similar to pimobendan under the same conditions. As judged from the EC$_{50}$ values, the effect of UD-CG 212 Cl was more potent in NYHA II-III than in NYHA IV (see Table 2). Again, the effect of Ca$^{2+}$ was not different in either group or under either condition tested.

To study whether the positive inotropic effect of pimobendan is involved in cAMP elevation, the effect of a high concentration of adenosine in the presence of pimobendan was studied in NYHA II-III. In Figure 5A, an original recording illustrates the effect. After equilibration of the positive inotropic effect of pimobendan, 1,000 µmol/l adenosine was applied. Note that a negative inotropic effect of adenosine, peaking at 3 minutes, was observed in the presence of pimobendan. Figure 5B summarizes the data. Adenosine at 1,000 µmol/l markedly antagonized the positive inotropic effect of pimobendan. However, the increase of force of contraction was not entirely abolished. Adenosine alone had no effect and failed to antagonize the positive inotropic effect of Ca$^{2+}$ (not shown).

Taken together, there were differences between the responses to the parent compound pimobendan and the metabolite UD-CG 212 Cl in failing myocardium. Pimobendan increased force of contraction alone, whereas UD-CG 212 Cl did not. The effects of pimobendan were enhanced, and those of UD-CG 212 Cl were unveiled by prestimulation with isoproterenol.

Figure 6 shows concentration–response curves for pimobendan (panel A) and UD-CG 212 Cl (panel B) on the isoenzymes of the cAMP-PDE (PDE I–III) isolated from failing left ventricles (NYHA IV). Both compounds inhibited PDE III potently. As judged from the IC$_{50}$ values, the potency of PDE III inhibition was 18.1 times greater than the potency of inhibition of the crude PDE for pimobendan. For UD-CG 212 Cl, PDE III inhibition was 121 times...
more potent than the inhibition of crude PDE. UD-CG 212 Cl was 7.7 times more potent at PDE III than pimobendan was. The IC50 values for inhibition of PDE I–III are included in Figure 6.

Ca2+ Sensitivity of the Myofibrils

Figure 7 shows the effect of pimobendan on the Ca2+-tension relation of a skinned fiber from a heart of a patient with terminal heart failure (NYHA IV). Note that the increase in tension at higher pCa values was more pronounced in the presence of pimobendan than without. Figure 7B summarizes the concentration–response curves. There was a significant shift of 0.15 pCa units to the left by the addition of 100 μmol/l pimobendan. Figure 8 shows the data for UD-CG 212 Cl. In the presence of UD-CG 212 Cl, the increase in tension was reduced at low pCa values (Figure 8A), and the concentration–response curve was shifted to the right (Figure 8B), indicating a decrease in the sensitivity of the contractile proteins for Ca2+.

Figure 9 summarizes the concentration–response curves of pimobendan (panel A) and UD-CG 212 Cl (panel B) in NYHA II-III and NYHA IV. Pimobendan increased the Ca2+ sensitivity with a threshold concentration of 0.1 μmol/l to almost 200% of control at 300 μmol/l (Figure 9A). In contrast, UD-CG 212 Cl increased Ca2+ sensitivity only slightly, if any, at 1 μmol/l but desensitized the myofibrils at 100–300 μmol/l. The effects of both compounds were not different in NYHA II-III and NYHA IV.

Discussion

Pimobendan is a pyridazinone benzimidazole derivative that exerts positive inotropic effects in a variety of species.2,4,7 It is a potent vasodilator22 and is reported to produce beneficial effects in patients with heart failure when given orally23,24 or intravenously.15 In patients with heart failure, a steady-state effect of a decrease in pulmonary capillary wedge pressure and of an increase in cardiac index...
was observed after 4 weeks of oral treatment with pimobendan.24 Plasma concentrations of pimobendan and UD-CG 212 Cl peaked 1.5 and 2 hours after oral administration, respectively.24 Moreover, it was observed that the drug was capable of increasing survival time in Syrian hamsters with dystrophic cardiomyopathy.25

It is not clear which mechanism of action is causally related to the positive inotropic effect and, hence, hemodynamic improvement of patients with heart failure. In guinea pig myocardium, pimobendan inhibited crude cAMP-PDE2 and reduced the activity of the low K_m PDE isoenzyme PDE III,4 but it had no effect on adenylate cyclase activity.2 It has been shown that the positive inotropic effect of pimobendan was accompanied by an increase of the cAMP content of intact electrically driven guinea pig papillary muscles.5 Moreover, pimobendan potentiated the positive inotropic effects of the adenylate cyclase stimulators isoproterenol and histamine but had no effect on the concentration–response relation for the positive inotropic effect of dihydropyridine.2 These findings indicate that an increase of cAMP by inhibition of cAMP-PDE most likely is involved in the cardiotonic action of pimobendan.3 The reduced positive inotropic effect of pimobendan in failing myocardium shows that pimobendan undergoes a loss of effectiveness that also has been observed in this study with isoproterenol and previously with other PDE inhibitors, such as milrinone10,12,14 or isobutylmethylxanthine.13

To study whether an increase of the cellular cAMP level is involved in the positive inotropic effect of pimobendan, the effect of adenosine was studied in the presence of pimobendan. In the human ventricular myocardium, adenosine receptors have been identified26,27 that mediate indirect negative inotropic effects on force of contraction, when the cellular cAMP content is increased. Adenosine receptor stimulation has no effect when force of contraction is
enhanced by a compound that has a cAMP-independent mechanism of action.\textsuperscript{28} In human papillary muscles, adenosine at 1,000 \(\mu\)mol/l in the presence of isoproterenol concentration dependently reduced force of contraction but had no effect or a weak positive inotropic effect alone.\textsuperscript{27} Therefore, the negative inotropic effect of adenosine in the presence of pimobendan can be taken as a functional method to directly demonstrate involvement of cAMP in the positive inotropic effects of pimobendan in the failing human heart. These findings provide evidence that specific inhibition of PDE III by pimobendan is involved in its positive inotropic effects. It is noteworthy that PDE III inhibition with pimobendan occurs at lower concentrations than does the positive inotropic effect. This finding indicates that although PDE III inhibition is one contributing mechanism of action for the inotropic effect of pimobendan, alone it might not be sufficient to produce an increase of force of contraction and, hence, might not be the only mechanism of action of pimobendan.

There is evidence that pimobendan sensitizes myocardial contractile proteins for Ca\(^{2+}\)\textsuperscript{6-8} by enhancing the binding of Ca\(^{2+}\) to troponin C.\textsuperscript{7} It is unclear whether this mechanism contributes to the positive inotropic effect of the compound. For the treatment of human heart failure this might be important, since the positive inotropic effects of PDE inhibitors are suggested to be reduced by a decrease of basal cAMP formation\textsuperscript{10,11} caused by a downregulation of cardiac \(\beta\)-adrenoceptors\textsuperscript{12} and an increase of the \(\alpha\)-subunit of the inhibitory guanine-nucleotide-binding protein G\(_s\).\textsuperscript{13} A decrease of cAMP concentration has recently been observed in intact, freeze-clamped papillary muscle strips from failing explanted human hearts.\textsuperscript{20} It has been shown that the increase of the cellular cAMP content by pimobendan was less pronounced in electrically driven papillary muscle strips from failing than from nonfailing hearts, despite a similar PDE III inhibition by the compound in both groups.\textsuperscript{30} Although experimental data failed to show alterations in the Ca\(^{2+}\) sensitivity of failing compared

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**Figure 9.** Concentration–response curves for the effect of pimobendan (0.01–300 \(\mu\)mol/l, panel A) and UD-CG 212 CI (0.01–300 \(\mu\)mol/l, panel B) on Ca\(^{2+}\) sensitivity of skinned myocardial fibers from left ventricular myocardium of patients with moderate (New York Heart Association class II-III [NYHA II-III]) and severe (NYHA IV) heart failure. ctr., Control.
with nonfailing human ventricular tissue and even an increased Ca\(^{2+}\) sensitivity in hypertrophied human atrial tissue, an accompanying effect on the Ca\(^{2+}\) sensitivity of the contractile proteins could at least partially restore the positive inotropic effect. Moreover, it could lead to less pronounced positive chronotropism despite potent positive inotropism.

Pimobendan increased the duration of isometric single contractions not only in human (this study) but also in pig and ferret papillary muscles. Moreover, pimobendan more pronouncedly increased force of contraction in relation to the positive chronotrophic effect compared with isoproterenol. To study whether sensitization to Ca\(^{2+}\) of the contractile proteins plays a role, we studied the effect of pimobendan on Ca\(^{2+}\)-force relations of skinned fibers from failing hearts. Pimobendan increased the Ca\(^{2+}\) sensitivity of myocardial skinned fibers from the hearts of patients with moderate and severe heart failure. Similar effects have previously been observed in guinea pig and dog myocardium. Therefore, it is reasonable to assume that this mechanism contributes to the positive inotropic response in failing human heart muscle.

The demethylated major metabolite of pimobendan, UD-CG 212 Cl, has been shown to inhibit cAMP-PDE and to increase force of contraction in guinea pig myocardium even more potently than the parent compound, pimobendan. Moreover, UD-CG 212 Cl reduced left ventricular end-diastolic pressure and vascular resistance in anesthetized pigs. In the failing myocardium, it inhibited PDE III 7.7 times more potently than pimobendan did (this study). When the preparations were preincubated with isoproterenol, UD-CG 212 Cl produced a positive inotropic effect. As judged from the EC\(_{50}\) values, UD-CG 212 Cl was even more potent than pimobendan in increasing force of contraction under these conditions. These findings indicate that although there was no positive inotropic effect with UD-CG 212 Cl alone in the failing myocardium, PDE inhibition might be involved in the positive inotropic effect in the presence of isoproterenol in failing myocardium. Similar results were obtained with the PDE III inhibitor milrinone, which has no effect on Ca\(^{2+}\) sensitivity of the myofibrils. In one study on preparations from severely failing hearts, milrinone as UD-CG 212 Cl (this study) failed to increase force of contraction alone, but a marked response to the PDE inhibitor was observed in the presence of the adenylyl cyclase stimulator forskolin. The positive inotropic effect of pimobendan and the failure of its metabolite UD-CG 212 Cl to increase force of contraction indicates that an additional mechanism of action is involved in the effect of the parent compound. The desensitizing properties of UD-CG 212 Cl at high concentrations could also contribute to the lack of effect of the metabolite. The data on UD-CG 212 Cl alone show that even strong PDE III inhibition is not capable of producing positive inotropic effects. The results with the metabolite in the presence of isoproterenol suggest that the maximal effect on force of contraction at 10 \(\mu\)mol/l is observed only when PDE III is inhibited by 77% of basal activity.

The data of this study provide evidence that PDE inhibition and sensitization of the contractile proteins to Ca\(^{2+}\) both acting in concert are causally related to the positive inotropic effect of pimobendan. As pointed out by Lee et al., a combined action of cAMP-PDE inhibition and Ca\(^{2+}\) sensitization could have comparative advantages over mechanisms involving only one of these effects. This must be anticipated since an increased binding of Ca\(^{2+}\) to troponin C initiated by its increased affinity for Ca\(^{2+}\) relies on the intracellular concentration of free Ca\(^{2+}\) achieved by a cAMP-dependent increase of the slow Ca\(^{2+}\) inward current or on the cAMP-dependent phospholamban phosphorylation. Therefore, in patients with heart failure in which a permanent \(\beta\)-adrenergic stimulus is imposed on the heart by activation of the sympathetic nervous system and elevated norepinephrine levels, beneficial hemodynamic effects caused by direct inotropic effects on the failing heart by pimobendan are nevertheless possible.

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**References**


Key Words • pimobendan • positive inotropic agents • heart failure • human heart • phosphodiesterase inhibition • contractile proteins • cAMP
Contribution of cAMP-phosphodiesterase inhibition and sensitization of the contractile proteins for calcium to the inotropic effect of pimobendan in the failing human myocardium.

M Böhm, I Morano, B Pieske, J C Rüegg, M Wankerl, R Zimmermann and E Erdmann

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