Negative Inotropy of the Gastric Proton Pump Inhibitor Pantoprazole in Myocardium From Humans and Rabbits

Evaluation of Mechanisms

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Background—Proton pump inhibitors are used extensively for acid-related gastrointestinal diseases. Their effect on cardiac contractility has not been assessed directly.

Methods and Results—Under physiological conditions (37°C, pH 7.35, 1.25 mmol/L Ca2+), there was a dose-dependent decrease in contractile force in ventricular trabeculae isolated from end-stage failing human hearts superfused with pantoprazole. The concentration leading to 50% maximal response was 17.3±1.3 µg/mL. Similar observations were made in trabeculae from human atria, normal rabbit ventricles, and isolated rabbit ventricular myocytes. Real-time polymerase chain reaction demonstrated the expression of gastric H+/K+-adenosine triphosphatase in human and rabbit myocardium. However, measurements with BCECF-loaded rabbit trabeculae did not reveal any significant pantoprazole-dependent changes of pH. Ca2+ transients recorded from field-stimulated fluo 3–loaded myocytes (F/F0) were significantly depressed by 10.4±2.1% at 40 µg/mL. Intracellular Ca2+ fluxes were assessed in fura 2–loaded, voltage-clamped rabbit ventricular myocytes. Pantoprazole (40 µg/mL) caused an increase in diastolic [Ca2+]i by 33±12%, but peak systolic [Ca2+]i was unchanged, resulting in a decreased Ca2+ transient amplitude by 25±8%. The amplitude of the L-type Ca2+ current (Ica,L) was reduced by 35±5%, and sarcoplasmic reticulum Ca2+ content was reduced by 18±6%. Measurements of oxalate-supported sarcoplasmic reticulum Ca2+ uptake in permeabilized cardiomyocytes indicated that pantoprazole decreased Ca2+ sensitivity (Kd) of sarcoplasmic reticulum Ca2+ adenosine triphosphatase: control, Kd=358±15 nmol/L; 40 µg/mL pantoprazole, Kd=395±12 nmol/L (P<0.05). Pantoprazole also acted on cardiac myofilaments to reduced Ca2+-activated force.

Conclusions—Pantoprazole depresses cardiac contractility in vitro by depression of Ca2+ signaling and myofilament activity. In view of the extensive use of this agent, the effects should be evaluated in vivo. (Circulation. 2007;116:57-66.)

Key Words: calcium ■ contractility ■ heart failure ■ inotropic agents ■ pharmacology

Proton pump inhibitors (PPIs) like pantoprazole, omeprazole, esomeprazole, lanosoprazole, and rabeprazole are the most effective pharmacological means of reducing gastric acid secretion by blocking the final step of proton secretion, ie, the gastric acid pump, H+/K+-adenosine triphosphatase (ATPase). The efficacy of this class of drugs has been demonstrated in the treatment of a number of acid-related gastrointestinal diseases, in particular, gastroesophageal reflux and ulcer disease.1 Hence, there is extensive use of these agents for patients in a variety of settings. In a US Medicaid population, PPIs accounted for 5.6% of the net pharmacy expenditures and ranked first in expenditures among all drug therapy classes during the 12 months before the implementation of the PPI prior-authorization policy.2 Drug shortage bulletins have been issued repeatedly for intravenous PPIs in the United States.3

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It has been shown recently that the expression of H+/K+-ATPase is not limited strictly to gastric tissue. It has also been identified in renal and colonic epithelial cells, vascular smooth muscle cells, and other tissues. In myocardium from rats, the expression of H+/K+-ATPase has been demonstrated at the transcriptional and protein levels. Biochemical evidence and physiological evidence for a myocardial H+/K+-ATPase have also been found. Furthermore, Beisvag and coworkers showed a contribution from the H+/K+-ATPase of oxygen uptake in rat hearts and suggested that the enzyme could contribute significantly to the regulation of myocardial K+ and H+ homeostasis. Inhibition of H+/K+-ATPase might therefore induce cellular acidosis, which is known to depress myocardial contractility mainly at the level of myofilament responsiveness to [Ca2+].

Orally applied PPIs are considered safe and have been found advantageous in regard to cardiovascular side effects compared with histamine type 2 (H2) receptor antagonists because of lack of chronotropic and inotropic effects. In contrast to famotidine, omeprazole did not show any changes in cardiac performance in healthy volunteers as measured by impedance cardiography and mechanocardiography after 1-week oral treatment with therapeutic doses. Pantoprazole, lansoprazole, and esomeprazole are currently available as intravenous formulations in the United States. The rationale for use has come primarily with the suggested efficacy in reducing bleeding after endoscopic hemostasis of bleeding peptic ulcers (eg, References 11 and 12). The target goal for gastric pH in these patients has been suggested to be >6 in order to promote hemostasis and minimize clot lysis, in contradistinction to the target pH >4 for treating patients to prevent stress ulcer or heal ulcers or reflux esophagitis.

As such, the dosing amounts of intravenous PPI have been higher than that of oral PPI. Recently, omeprazole and pantoprazole have been dosed at 80 mg followed by 8 mg/h for 72 hours. However, information regarding the cardiac effects of high doses is lacking. Moreover, few data are available regarding the direct effect of such agents on the myocardium.

Hence, because of the abundant use of this drug class and because of the presence of a H+/K+-ATPase in myocardium, we sought to investigate the effects of PPI on contractility of isolated human myocardium using pantoprazole, which is the most commonly used intravenous formulation. In addition, the mode of action in cardiac tissue was further analyzed in isolated cardiac tissue from rabbits.

**Methods**

**Myocardial Tissue**

Experiments were performed in left ventricular muscle strips from 8 end-stage failing human hearts obtained from patients undergoing cardiac transplantation and right atrial trabeculae obtained from 16 patients who underwent cardiac surgery. Additional right ventricular trabeculae were obtained from adult female Chinchilla Bastard rabbits (weight, 2.0 to 2.5 kg; Charles River Deutschland, Kisslegg, Germany). Myocardial trabeculae and adult rabbit ventricular cardiac myocytes were isolated and forces of electrically stimulated preparations were investigated as described previously. Experimental procedures with human tissue were reviewed and approved by the ethical committee of the University Clinics of Goettingen, and the subjects gave informed consent. Procedures with rabbits were performed in accordance with institutional guidelines for the care and use of laboratory animals.

**pH Measurements**

Intracellular pH (pHi) was measured in isolated trabeculae from rabbit hearts as described previously. Briefly, trabeculae were mounted in a cylindrical glass cuvette, connected to an isometric force transducer, and loaded with 2',7'-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF)-AM (15 μmol/L) in Tyrode’s solution by 45-minute incubation at room temperature. Excitation light from a mercury lamp was passed alternately through 2 bandpass filters (450 nm/495 nm) and focused on the muscle strip. Fluorescence emission was collected by a photomultiplier (Scientific Instruments) after passage through a bandpass filter (535±5 nm). Values of pHi were estimated from the ratio of the BCECF fluorescence signals (F1/F0) after subtraction of background fluorescence. At the end of each experiment, the BCECF fluorescence ratio was calibrated in vivo by means of the high K+-nigericin method as previously reported.

**Voltage Clamp and Intracellular [Ca2+] Measurements in Rabbit Cardiomyocytes**

Cardiomyocytes were superfused with a solution consisting of (mmol/L) 144.0 NaCl, 5.4 KCl, 0.3 NaH2PO4, 1.0 MgCl2, 5.0 HEPES, 11.1 glucose, 1.8 CaCl2, 0.1 nifedipic acid, 5.0 4-AP (pH 7.4) at room temperature in a chamber mounted on the stage of an inverted microscope. Voltage clamp was achieved with the use of whole-cell patch-clamp technique with an Axoclamp 2A amplifier (Axon Instruments, Foster City, Calif) in switch clamp (discontinuous) mode. Pipettes were filled with an intracellular solution of the following composition (mmol/L): 20.0 KCl, 100.0 K aspartate (DL), 20.0 TEA Cl, 10.0 HEPES, 4.5 MgCl2, 4.0 Na2ATP, 1.0 Na2CrP, 2.5 EGTA (pH 7.25 with KOH) and were of resistance 3 to 6 mol/L. Intracellular [Ca2+] was measured from fura 2 fluorescence signals by a dual-wavelength spectrophotometer method as previously described. Cytosolic loading of fura 2 was achieved by incubating cardiomyocytes with 5 μmol/L fura 2-AM at room temperature for 15 minutes.

**Electrophysiological Protocols**

Isolated rabbit cardiomyocytes were held at −80 mV, and the voltage was stepped to −40 mV (50 ms) to inactivate the inward Na+ current before stepping to 0 mV (150 ms). Tetrodotoxin 3×10−3 mol/L was also used to block IaK. This protocol was repeated 40 times at a rate of 1 Hz to achieve steady state Ca2+ transients. Sarcoplasmic reticulum (SR) Ca2+ content and Na+/Ca2+ exchanger (NCX) activity were then estimated by rapidly switching to 10 mmol/L caffeine to cause SR Ca2+ release. In the continued presence of caffeine (20 seconds), the SR is unable to reaccumulate Ca2+, and therefore Ca2+ removal is mainly via NCX. The time course of the decay of [Ca2+]i and the NCX-mediated inward current (IInox) represent rates of extrusion of Ca2+ from the cell predominantly by NCX. These signals were fitted to exponential decays over >80% of their amplitude. The magnitude of non-NCX Ca2+ removal mechanisms was estimated from the Ca2+ decay obtained by rapidly switching to 10 mmol/L caffeine in the presence of 10 mmol/L NiCl2 (which blocks NCX), in which the absence of a current indicated that the current obtained without NiCl2 was solely due to NCX.

**Simultaneous Measurements of Myocyte Shortening and Intracellular [Ca2+]**

Shortening and [Ca2+]i were measured simultaneously as reported previously. Cells were loaded with 10 μmol/L fluo 3-AM (Molecular Probes, Carlsbad, Calif) for 15 minutes. Fluorescence was excited at 480 nm, and fluorescence was measured at 535 nm. The field-stimulation frequency was 1 Hz (37°C; pH 7.35). Normalized amplitude of calcium transients (F/F0) was calculated by dividing
fluorescence F by the baseline fluorescence F₀ after subtraction of the background fluorescence (IonWizard, IonOptix Corp). Cells were treated with 0/10/40 μg/mL pantoprazole followed by a washout. Fluorescence and shortening were analyzed at steady state conditions.

Measurements of SR Ca²⁺ Uptake Characteristics
Measurements of SR Ca²⁺ uptake were performed as described previously.³ Ventricular myocytes freshly dissociated from adult rabbit hearts were permeabilized with 0.1 mg/mL β-escin and kept in mock intracellular solution of the following composition (mmol/L): 100 K⁺, 40 Na⁺, 25 HEPES, 100 Cl⁻, 0.05 EGTA, 5 ATP, 10 CrP (pH 7.0). To test the influence of pantoprazole on SR Ca²⁺ uptake, myocytes were divided into 3 groups, and 0 (control), 10 μg/mL, and 40 μg/mL pantoprazole were added into the cuvette. Oxalate (10 mmol/L) was included to maintain low and constant intra-SR Ca²⁺ conditions.

Skinned Fibers
Fibers dissected from rabbit right ventricles were skinned by incubation with Triton (1%) for 24 hours at 4°C. Paired measures of calcium sensitivity and force development of the myofilaments were performed with the use of 1 relaxation and 10 activation measurements were performed with the use of 1 relaxation and 10 activation steps with NaCl 0.9%. Each exposure to pantoprazole was preceded and followed by 1 control step with NaCl 0.9%.

Transcription of H⁺/K⁺-ATPase in Human Myocardium
RNA was isolated from myocardial tissue and gastric corpus mucosa from humans and rabbits with the use of RNeasy Kits (Qiagen). Quantitative reverse transcription polymerase chain reaction (RT-PCR) was performed by real-time PCR as described earlier.¹⁹ Primer pairs for H⁺/K⁺-ATPase and β-actin are summarized in the Table. Each sample was analyzed in duplicate. The investigated number of cDNA molecules was detected in the same sample to normalize for the quantity of RNA extracted and the efficiency of cDNA synthesis. The relative expression was then calculated as described.¹⁹

**Table 1. Primer Pairs Used in Real-Time PCR**

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<th>Gene</th>
<th>Sequence</th>
<th>Gene Bank Accession No.</th>
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<tr>
<td></td>
<td>Antisense: 5'-CCGATCCACACGAGATCTTG-3'</td>
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![Figure 1](http://circ.ahajournals.org/)

**Figure 1.** Dose dependence of isometric twitch force from pantoprazole (A to C) and esomeprazole (D) in different types of myocardium as indicated and partial reversibility after washout of the drug. The number of trabeculae and hearts used for each experiment is indicated in parentheses. *P<0.025 (only concentrations with potential clinical relevance have been tested).
Statistical Analysis
Data are expressed as mean±SEM. Student paired t test (Figures 3 and 5) or repeated-measures ANOVA (Figures 1, 4, 6, and 7) were performed to test for statistically significant differences between different interventions. In general, a value of \( P<0.05 \) was accepted as statistically significant; for post hoc analysis, probability values were adjusted according to Bonferroni (Figures 1 and 4), or the Tukey test was used (Figure 6).

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results
Dose-Response Relationship of PPIs in Isolated Trabeculae From Human and Rabbit Myocardium
Figure 1A and 1B demonstrates a dose-dependent reduction of isometric twitch force of electrically stimulated trabeculae from nonfailing human atrial and failing ventricular myocardium under the influence of pantoprazole. Negative inotropy was at least partially reversible after washout of the drug. The pantoprazole-dependent negative inotropy was also present in ventricular myocardium from healthy adult rabbits (Figure 1C). Moreover, a similar dose-dependent effect was found with esomeprazole in human atrial myocardium (Figure 1D). The effect was highly reproducible, and the maximum effect usually occurred within a few minutes after exposure to pantoprazole. To yield EC\(_{50}\) values of the negative inotropic effect, additional dose-response experiments with maximum pantoprazole concentrations of 160 \( \mu \)g/mL have been performed that induced nearly complete suppression of contractile force. Data points have been fitted with the use of logistic curve fit with OriginPro 7 scientific software (OriginLab Corp). The EC\(_{50}\) was 30.6±1.8 \( \mu \)g/mL in atrial human myocardium (Figure 2B) and 17.3±1.3 \( \mu \)g/mL in ventricular human myocardium (Figure 2D). Compared with control, at doses of 6.25 and 12.5 \( \mu \)g/mL of pantoprazole, the contractile forces in human ventricular myocardium were 3.0±0.4 versus 4.2±0.7 mN/mm\(^2\) and 2.4±0.3 versus 4.0±0.6 mN/mm\(^2\) (\( P<0.025 \), each). This corresponds to a depression of contractile forces by 27±9% and 42±8%, respectively. In human atrial myocardium, forces in the presence of 12.5 \( \mu \)g/mL pantoprazole were 5.1±0.8 versus 5.7±0.4 mN/mm\(^2\) compared with control (\( P<0.025 \), depression by 12±14%). Preincubation of trabeculae with ouabain (0.2 \( \mu \)mol/L) in-

Figure 2. Determination of EC\(_{50}\) for contractile depression of pantoprazole in human myocardium. Single twitches of original recordings of typical experiments in human atrial (A) and ventricular (C) trabeculae are shown. Mean force values in atrial (B) (\( n=8 \) trabeculae from 4 hearts) and ventricular (D) (\( n=6/3 \) trabeculae) are shown. Curves have been fitted to yield EC\(_{50}\) values as detailed in Results.

![Graph A](image1)

![Graph B](image2)

![Graph C](image3)

![Graph D](image4)
duced an increase in contractile force by 31.1%. After exposure to pantoprazole (40 μg/mL), force decreased by 65.2% compared with ouabain treatment and by 54.4% compared with baseline values (P<0.05 each).

Expression of H⁺/K⁺-ATPase in Myocardium and Gastric Mucosa From Humans and Rabbits

H⁺/K⁺-ATPase mRNA expression was detectable in both gastric corpus mucosa and ventricular myocardium from humans and rabbits. However, the expression was very low in ventricular myocardium from both species. The relative expression of H⁺/K⁺-ATPase in corpus mucosa compared with ventricular myocardium was 1.2×10⁶ times higher in humans (1.3±0.8 versus 1.1×10⁻⁶±0.7×10⁻⁶; n=3) and 2.2×10⁶ times higher in rabbits (1262±673 versus 580×10⁻⁶±217×10⁻⁶; n=3), respectively.

Pantoprazole Does Not Affect pHᵢ

pHᵢ is an important modulator of contractility. Therefore, we tested whether pantoprazole induced changes in pHᵢ that might explain the observed negative inotropic effect. Figure 3A shows pHᵢ changes of a BCECF-loaded rabbit ventricular muscle strip before and in the presence of pantoprazole (40
Figure 5. Depolarization-induced Ca\textsuperscript{2+} transients recorded from rabbit ventricular cardiomyocytes. A. Records of membrane voltage (E m), membrane current, and [Ca\textsuperscript{2+}] from single cardiomyocytes (average of 4 sequential signals) during control conditions (i) and after perfusion with pantoprazole (40 \mu g/mL) (ii). B. Mean±SEM of the percent change in diastolic [Ca\textsuperscript{2+}], systolic [Ca\textsuperscript{2+}], and Ca\textsuperscript{2+} transient amplitude (*P<0.01). C. Recordings of [Ca\textsuperscript{2+}], and membrane current recorded on rapid application of 10 mmol/L caffeine (as indicated above the records) during control conditions (i) and after perfusion with pantoprazole (40 \mu g/mL) (ii). D. Mean±SEM of the percent change in \( I_{\text{ncx}} \cdot \) time integral diastolic [Ca\textsuperscript{2+}], Ca\textsuperscript{2+} transient amplitude, and rate constant for the decay of the Ca\textsuperscript{2+} transient. *P<0.05.

\[ \text{Ca}^{2+} \text{ Homeostasis in Isolated Rabbit Myocytes} \]

Similar to the findings in isolated trabeculae, pantoprazole induced a dose-dependent reduction of isotonic shortening of isolated rabbit myocytes by 32.3±5.8% at 10 \mu g/mL and 65.4±3.4% at 40 \mu g/mL. This was paralleled by a depression of Ca\textsuperscript{2+} transient amplitude measured by fluorescence of fluo 3–loaded myocytes (F/F0) by 9.0±2.6% and 10.4±2.1% at 10 and 40 \mu g/mL, respectively (Figure 4). Effects of pantoprazole on intracellular Ca\textsuperscript{2+} cycling were further investigated in voltage-clamped and fura 2–loaded rabbit myocytes (Figure 5A and 5B). On addition of 40 \mu g/mL pantoprazole, diastolic [Ca\textsuperscript{2+}], was increased by 33±12% (P<0.05; n=13), with no significant change in peak systolic [Ca\textsuperscript{2+}] (4±7%; n=14). As a result of these changes, Ca\textsuperscript{2+} transient amplitude was reduced by 25±8% (n=14; P<0.05) compared with control cells. These changes were paralleled by a reduction in \( I_{\text{cal}} \), amplitude (by 35±5%; P<0.05; n=14). To measure SR and NCX function, the intracellular Ca\textsuperscript{2+} signals and the associated \( I_{\text{ncx}} \) were analyzed on rapid application of 10 mmol/L caffeine (Figure 5C and 5D). A reduction in both the amplitude of the caffeine induced Ca\textsuperscript{2+} release (by 18±6%; n=10; P<0.05) and the associated time integral of \( I_{\text{ncx}} \) (by 21±6%; n=10; P<0.05) on exposure to pantoprazole (40 \mu g/mL) indicated that pantoprazole decreased SR Ca\textsuperscript{2+} content. No changes in the rate of decay of the caffeine induced Ca\textsuperscript{2+} transient (1±8%; n=10) and \( I_{\text{ncx}} \) (2±7%; n=10) were
observed after pantoprazole administration, indicating that the sarcolemmal extrusion processes (particularly NCX) were unaffected by the drug.

**SR Ca\(^{2+}\) Uptake Data**

Figure 6 demonstrates the effects of pantoprazole on oxalate-supported Ca\(^{2+}\) uptake in permeabilized rabbit ventricular cardiomyocytes. In the presence of either carrier solution or low (10 \(\mu g/mL\)) or high (40 \(\mu g/mL\)) concentrations of pantoprazole, the \([Ca^{2+}]\) at which half maximal Ca\(^{2+}\) uptake occurred (Kd) and the value of the maximum rate of Ca\(^{2+}\) uptake (Vmax) were estimated with a fura 2–based SR Ca\(^{2+}\) uptake assay. Neither low nor high concentration of pantoprazole influenced the Vmax value. However, the Kd values of SR Ca\(^{2+}\) uptake were increased significantly at both concentrations of pantoprazole (nmol/L: control, 358±15; low pantoprazole, 406±15; high pantoprazole, 395±12; \(P<0.05\)).

**Effects of Pantoprazole in Skinned Fiber Preparations**

The effect of pantoprazole in skinned fibers is shown in Figure 7. There was a reduction of the maximum active tension at saturating Ca\(^{2+}\) concentration by 7.8±0.8% at 10 \(\mu g/mL\) (\(P=NS\)) and 26.5±2.8% at 40 \(\mu g/mL\) (\(P<0.05\)). In addition, a small but significant rightward shift of the Ca\(^{2+}\) response curve indicating a decrease of Ca\(^{2+}\) sensitivity was found. The EC\(_{50}\) was 1.44 mol/L Ca\(^{2+}\) at 0 \(\mu g/mL\) and 1.59 mol/L Ca\(^{2+}\) at 40 \(\mu g/mL\) pantoprazole (\(P<0.05\)).

**Discussion**

The present study shows a negative inotropic effect of pantoprazole in isolated myocardium. This was dose dependent, induced nearly complete inhibition of twitch force at very high doses, and was partially reversible. Negative inotropy of pantoprazole was present in myocardium from different species (human and rabbit) and in myocardium from different origins (atrial and ventricular), and it was found in different myocardial preparations (multicellular and single cells). The EC\(_{50}\) for contractile force depression was 30.6±1.8 \(\mu g/mL\) in nonfailing human atrial and 17.3±1.3 \(\mu g/mL\) in failing human ventricular myocardium, respectively. Moreover, similar results could be obtained with esomeprazole, which is suggestive of a class effect of PPIs. Furthermore, we could reveal 2 underlying mechanisms for the pantoprazole-dependent inhibition of contractile force: (1) reduction in the amplitude of Ca\(^{2+}\) transients as a consequence of impaired SR Ca\(^{2+}\) uptake and reduced Ca\(^{2+}\) influx.
via $I_{Ca,L}$ and (2) reduced Ca$^{2+}$ responsiveness of the myofilaments as a result of a reduced maximal active tension and a slightly lower Ca$^{2+}$ sensitivity. In contrast, despite the expression of the H$^+$/K$^+$-ATPase at the transcriptional level in human and rabbit myocardium, no significant changes in pH, could be detected in the presence of pantoprazole.

Note that the mechanisms underlying the effects of pantoprazole in myocardium are completely different from the mechanisms of the drug in gastric parietal cells and probably do not involve inhibition of H$^+$/K$^+$-ATPase. In regard to gastric proton pump inhibition, all PPIs are prodrugs and require acid to become protonated and converted into the active form.$^1$ After intravenous administration or intestinal absorption, when orally administered the lipophilic unprotonated form readily penetrates cell membranes, including that of gastric parietal cells and myocytes. In parietal cells, as it transverses the cell it is exposed to the acidic environment in the secretory canaliculus of the gastric site and becomes protonated, converting it to a hydrophilic drug that can no longer permeate cell membranes. The drug becomes trapped in the canaliculus of the parietal cell. For this reason, in parietal cells PPIs exhibit a substantial accumulation versus plasma at low pH, eg, 1000-fold for omeprazole and 10000-fold for rabeprazole at a pH of 1. Moreover, protonation of the drug initiates a series of chemical reactions that culminates in covalent binding of the drug with selected cysteine residues of the H$^+$/K$^+$-ATPase.$^1$

The present study is the first one reporting on the expression of H$^+$/K$^+$-ATPase in human and rabbit ventricular myocardium. Recently, it has been suggested that H$^+$/K$^+$-ATPase may contribute to pH homeostasis in rat myocardium.$^7$ We therefore investigated the hypothesis that cellular acidosis subsequent to proton pump inhibition might explain the negative inotropy of PPIs. However, our measurements with the H$^+$-sensitive fluorescence dye BCECF did not reveal any significant influence of pantoprazole on pH.$^1$ Moreover, the absence of acidic compartments in cardiac tissue with pH $<$1 precludes significant accumulation of pantoprazole in the myocardium. In moderately acidic compartments like lysosomes, slow activation of pantoprazole theoretically may occur with an activation half-life of 4.7 hours.$^{20}$ However, negative inotropy in our experiments usually occurred quickly within several minutes. Moreover, the effect was at least partially reversible after washout of the drug, whereas the recovery of the gastric proton pump from pantoprazole has a half-life of $\sim$46 hours$^{21}$ and was suggested to depend mainly on the synthesis of new pump protein.$^{1,22}$ In addition, the expression of H$^+$/K$^+$-ATPase in myocardium was very low compared with gastric mucosa. Therefore, it is unlikely that the negative inotropy of pantoprazole in myocardium is the consequence of H$^+$/K$^+$-ATPase inhibition.

We investigated the effects of pantoprazole on intracellular Ca$^{2+}$ homeostasis and myofilament Ca$^{2+}$ responsiveness, which are the 2 principal physiological mechanisms of the myocardium to alter contractility. We found a reduction in the Ca$^{2+}$ transient amplitude in field-stimulated ventricular myocytes. Interpretation of this result in terms of Ca$^{2+}$ signaling is complicated by possible effects of pantoprazole on the action potential. With fixed depolarizing pulses in voltage-clamped myocytes, pantoprazole caused a rise in diastolic [Ca$^{2+}$], and minimal changes in systolic [Ca$^{2+}$], the net effect being a reduced Ca$^{2+}$ transient amplitude. Independent evidence for pantoprazole-dependent inhibition of SERCA as an underlying mechanism was obtained with a fura 2–based SR calcium uptake assay in permeabilized cardiomyocytes. Moreover, Ca$^{2+}$ influx via $I_{Ca,L}$ was also depressed by pantoprazole. The combination of reduced SR calcium uptake and reduced Ca$^{2+}$ influx would explain the reduction in SR Ca$^{2+}$ content observed and raised diastolic [Ca$^{2+}$].

We also investigated the influence of pantoprazole in skinned fibers. These preparations allow for the investigation of drug effects directly at the myofilament level under well-defined in vitro conditions. Hence, it can be excluded that the observed effects are mediated by changes in [Ca$^{2+}$], or pH. We were able to show a significant reduction in maximal active tension of the contractile proteins at saturating Ca$^{2+}$ concentrations by 26.5$\pm$2.8% at 40 $\mu$g/mL. Moreover, at 40 $\mu$g/mL there was a slight but significant reduction in myofilament Ca$^{2+}$ sensitivity. However, when the marked negative inotropy in multicellular trabeculae is considered, these effects were too faint to explain entirely the mode of action of pantoprazole in myocardium. Therefore, the negative inotropy of pantoprazole observed at 10 $\mu$g/mL mainly results from decreased intracellular [Ca$^{2+}$]. At the higher dose (40 $\mu$g/mL), depression of myofilament responsiveness appears to contribute to the negative inotropic effect. We also suggest that the effects of pantoprazole in myocardium depend on the native unprotonated form of the drug and do not require activation by low pH because all effects occurred at pH 7.3 to 7.4.

In contrast to our data, Yenisehirli and Onur$^{23}$ found a positive inotropic effect of 3 different PPIs in rat atria that was potentiated by pretreatment with the Na$^+$/K$^+$-ATPase inhibitor ouabain. Moreover, a Lansoprazole induced a prolongation of the action potential. The authors speculated that this could be mediated by inhibition of H$^+$/K$^+$-ATPase promoting altered intracellular Ca$^{2+}$ handling. In our study with human and rabbit myocardium, pantoprazole decreased tension and reduced Ca$^{2+}$ transient amplitude in "unclamped" trabeculae and single cells. When single rabbit myocytes were clamped with a fixed voltage clamp duration, pantoprazole decreased the Ca$^{2+}$ transient amplitude to a degree similar to that seen in "unclamped" preparations. This suggests that in humans and rabbits, action potential duration changes are not instrumental in the decreased Ca$^{2+}$ transient and subsequent mechanical response caused by pantoprazole application. Moreover, a significant contribution of H$^+$/K$^+$-ATPase to the action potential is unlikely because of the low degree of expression. The different responses to PPIs may therefore be related to species. Compared with human and rabbit myocardium, in rats the action potential is short and intracellular sodium is high. In particular, the latter condition favors Ca$^{2+}$ entry by reverse-mode Na$^+$/Ca$^{2+}$ exchange, which contributes to SR Ca$^{2+}$ load and contractility when action potential duration increases.$^9$ In contrast to the study by Yenisehirli and Onur, the negative inotropic effect of pantoprazole in rabbit myocardium observed by us was not influenced by blockade of Na$^+$/K$^+$-ATPase, and the magnitude of the effect was similar to that in experiments without ouabain.
Finally, although this was beyond the scope of the present study, we would like to speculate whether our findings might be of clinical relevance. Because the negative inotropic effect was partially reversible after washout of the drug and significant accumulation in the myocardium is unlikely, the effect of pantoprazole in vivo probably depends on plasma concentrations. Maximal pantoprazole plasma concentrations of 4.6 µg/mL and 10.4 µg/mL (Altana Pharma AG, written communication, July 23, 2004) have been found after administration of common oral (40 mg) and intravenous (80 mg) doses. In the present work, similar concentrations induced a reduction of contractile force of isolated trabeculae by 27±9% (6.25 µg/mL) and 42±8% (12.5 µg/mL), which might indicate a potential clinical relevance of our findings. However, the duration of cardiac side effects is temporally limited because all PPIs are quickly eliminated from blood with plasma elimination half-life periods of ≈1 to 2 hours.20,24 Moreover, cardiac side effects may be attenuated in vivo because the activity of the active free compound may be substantially lower because of high plasma protein binding.20 On the other hand, particular conditions may be attenuated in vivo because of the impaired function of the Na+/K+-ATPase25 or negative force-frequency relationship.26 In addition, the dependence of H+-elimination from H+/K+-ATPase may be increased in heart failure because of the impaired function of the Na+/H+ exchange subsequent to increased [Na+]i.27 Moreover, all PPIs undergo extensive hepatic biotransformation before elimination. In CYP2C19-poor metabolizers that represent 3% to 5% of whites, a similar percentage of blacks, and 12% to 25% of different Asian populations, much higher plasma concentrations and longer elimination half-life periods have been found.1 The same holds true for patients with severe liver impairment.28

Recent American29 and Canadian30 studies have shown that appropriate use of intravenous PPIs was seen in less than half of the patients. In view of our data, PPIs should be prescribed carefully. We have recently initiated a clinical study for the investigation of cardiac side effects of pantoprazole in healthy volunteers. Moreover, we encourage clinical studies to identify individuals with increased intrinsic risk for cardiac side effects of PPIs.

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Disclosures
None.

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20. Schillinger et al Negative Inotropy of Pantoprazole 65

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CLINICAL PERSPECTIVE

The proton pump inhibitor pantoprazole was evaluated for its effects on cardiac contractility in isolated myocardium from humans and rabbits. We found a dose-dependent negative inotropic effect mainly resulting from alterations in intracellular Ca\(^{2+}\) handling. At higher concentrations of the drug, depression of myofilament Ca\(^{2+}\) responsiveness was also observed. However, despite the expression of the gastric proton pump in human and rabbit hearts, no relevant changes in pH homeostasis could be detected. Moreover, expression of the pump was very low in myocardium compared with gastric tissue. A similar effect was observed with esomeprazole. Thus, proton pump inhibitors affect cardiac contractility by intracellular mechanisms that are distinct from their effects in gastric parietal cells. The effects in isolated myocardium were observed at concentrations that might be of potential clinical relevance. Thus, cardiac effects of proton pump inhibitors should be evaluated in vivo because of their extensive use for acid-related gastrointestinal diseases.
Negative Inotropy of the Gastric Proton Pump Inhibitor Pantoprazole in Myocardium
From Humans and Rabbits: Evaluation of Mechanisms
Wolfgang Schillinger, Nils Teucher, Samuel Sossalla, Sarah Kettlewell, Carola Werner, Dirk
Raddatz, Andreas Elgner, Gero Tenderich, Burkert Pieske, Giuliano Ramadori, Friedrich A.
Schöndube, Harald Kögler, Jens Kockskämper, Lars S. Maier, Harald Schwörer, Godfrey L.
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