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Positive inotropic and negative chronotropic effects of proton pump inhibitors in isolated rat atrium☆

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Abstract

The effects of three specific H^+/K^+-ATPase inhibitors (omeprazole, lansoprazole and SCH 28080 (2-methyl-8-(phenylmethoxy)-imidazo[1,2-a] pyridine-3-acetonitrile)) were investigated on the mechanical and electrophysiological properties of rat atrium, in vitro. Omeprazole (100–300 μM), lansoprazole (100–300 μM) and SCH 28080 (10–100 μM) increased the amplitude of contractions and decreased the beating rate. These effects are reversible, reproducible and correlated with their order of potency as gastric H^+/K^+-ATPase inhibitors; SCH 28080 > omeprazole = lansoprazole. Cardiac effects of proton pump inhibitors were not inhibited with phentolamine (5 μM), propranolol (15 μM), atropine (1 μM), ouabain (2 μM), theophylline (300 μM) and milrinone (100 μM). Ouabain-induced increase in beating rate and contracture development were antagonized by H^+/K^+-ATPase inhibitors. Ouabain increased the positive inotropic effect of H^+/K^+-ATPase inhibitors. Lansoprazole (300 μM) significantly prolonged the duration of action potentials in rat atrial cells. H^+/K^+-ATPase may play a crucial role in the mechanical and electrophysiological properties of rat atrial myocardium.

Keywords: H^+/K^+-ATPase; Rat atrium; Inotropy; Omeprazole; Lansoprazole; SCH 28080

1. Introduction

H^+/K^+-ATPase, generally known as the proton pump, is widely expressed in various tissues and is responsible for diverse biological effects. Its main function is gastric acid secretion from the parietal cells (Sachs et al., 1976) via active uptake of K^+, in exchange for H^+ (Hershey and Sachs, 1995). H^+/K^+-ATPases were identified in many other epithelial tissues including kidneys (Doucet, 1997; Kone, 1996; Steinmetz and Anderson, 1982; Wingo, 1989; Wingo and Smolka, 1995) and colon (Asano et al., 1998; Codina et al., 1996; Crowson and Shull, 1992; Cougnon et al., 1996; Kaunitz and Sachs, 1986). These nongastric H^+/K^+-ATPases contribute to the regulation of K^+ absorbtion and luminal acidification in the kidneys and colon. Therefore, they are responsible for the homeostasis of serum K^+ as well as acid–base balance.

Functional evidence of H^+/K^+-ATPase was also demonstrated in smooth muscle cells. It has been reported that H^+/K^+-ATPase inhibitors reduce K^+ uptake, K^+ content and intracellular pH in vascular smooth muscle (McCabe and Young, 1992). Proton pump inhibitors induce relaxation of guinea pig and human airway smooth muscles, in vitro (Rhoden et al., 1996). Omeprazole, a specific inhibitor of H^+/K^+-ATPase was reported to cause relaxation and inhibition of spontaneous contractions of human myometrial smooth muscle (Yildirim et al., 2001). Another proton pump inhibitor, SCH 28080 produced reversible relaxation of isolated guinea-pig and human arteries (Rhoden, 2000).

Involvement of H^+/K^+-ATPase in cardiac function was also suggested. A functionally active isoform of H^+/K^+-ATPase was detected in the rat atrial myocytes (Zinchuk et al., 1997). This enzyme was reported to be K^+-dependent, omeprazole-sensitive and ouabain-insensitive. In another study, energy-dependent and omeprazole-sensitive func-

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tional coupling of K^+ uptake and H^+ extrusion was shown in Langendorff-perfused hearts and ventricular myocytes isolated from guinea pigs (Nagashima et al., 1999). Finally, myocardial H^+/K^+-ATPase was identified by combining the sequencing of polymerase chain reaction products, protein analysis using immunocytochemistry and Western blot and functional assessment of K^+ transport in isolated rat cardiomyocytes (Beisvag et al., 2003). Since myocardial tissues have functional H^+/K^+-ATPase activity, we hypothesized that specific inhibitors of H^+/K^+-ATPase are expected to alter the mechanical and electrical properties of cardiac muscle. Inhibition of the myocardial H^+/K^+-ATPase may lead to intracellular acidification (according to a decrease in H^+ extrusion) and membrane depolarization due to a decrease in K^+ uptake.

The purpose of this study is to investigate the effects of three specific H^+/K^+-ATPase inhibitors on the contractile and chronotropic properties of isolated rat atrium. The possible changes in atrial action potential configuration were also studied. Omeprazole (5-methoxy-2-[[[4-methoxy-3,5-dimethyl-2-pyridinyl]-methyl]-sulfinyl]-1H-benzimidazole), lansoprazole (2[[[3-methyl-4-(2,2,2-tri fluoro ethoxy)-2-pyridinyl] methyl] sulfinyl]-1H-benzimidazole) and SCH 28080 (2-methyl-8-(phenylmethoxy)-imidazo[1,2-a] pyridine-3-acetonitrile) were used as specific H^+/K^+-ATPase inhibitors.

2. Materials and methods

2.1. Dissection and preparation of atria

The Guiding Principles in the Care and Use of Laboratory Animals together with The Recommendations from the Declaration of Helsinki were strictly adhered to during the execution of all the procedures described within this manuscript. This project was approved by the institutional Experimental Animal Care and Use Ethics Committee of Hacettepe University before the commencement of any intervention. Fifty-two adult male albino Sprague–Dawley rats weighing 150–250 g were anesthetized by ether inhalation. Rats were sacrificed by stunning and exsanguination. The heart was quickly dissected free and transferred into an ice-cold Feigen solution (in mM; NaCl 153.8, KCl 5.6, $NaHCO_3$ 23.8, glucose 11.1, $CaCl_2$ 5.5) which was bubbled with 95% O_2 plus 5% CO_2 (pH 7.4). Atrium was dissected from the heart and suspended in a 50-ml organ bath and kept at 34 °C. Left atria without pacemaker activity were prepared and electrically stimulated in some experiments.

2.2. Measurement of cardiac muscle contractility

The contractile force was measured isometrically, using force displacement transducers (FT10C, Grass Instruments, Quincy, MA, USA) and recorded on a polygraph (Model 7B, Grass Instruments). Beating rate of the spontaneous contractions was monitored simultaneously by a tachograph (Model 7B4H, Grass Instruments). Resting tension was set about 0.5 g which gave maximum contractile force in the rat atrium. Left atria were driven at 2 Hz by electrical field stimulation (square wave pulses of 1 ms duration and about 30 V) using a bipolar platinum electrode via a S44 stimulator (Grass Instruments) and a Grass SIU5 stimulus isolation unit. The preparations were allowed to equilibrate for 60 min before drug administration with replacing the solution every 15 min. After determining a stable basal contractility and beating frequency, concentration–effect curves for H^+/K^+-ATPase inhibitors were constructed by adding increasing amounts of omeprazole, lansoprazole, and SCH 28080.

2.3. Electrophysiological experiments

Atria were excised using the same procedure as contractility measurement experiments. The preparations were fixed on a plexiglass plate covered with Sylgard allowing for continuous perfusion (4 ml/min) with Feigen solution. The temperature of superfusate was 34 °C and the pH was 7.4 when bubbled with carbogene. Preparations were allowed to equilibrate for 60 min before the experiments. Atria were stimulated with rectangular current pulses (having duration of 1 ms and amplitude of 11–15 V). These stimuli were delivered through a pair of platinum electrodes. Transmembrane potentials were recorded with glass microelectrodes having tip resistances of 30–70 MΩ, filled with 3 mol/L KCl. Microelectrodes were electrically coupled to a high impedance amplifier (Axoclamp 2A, Axon Instruments, CA, USA). Biological signals were displayed on an oscilloscope and digitized using a computer-based data acquisition system (MacLab, AD Instruments, Castle Hill, Australia). These data were analyzed with an analyzing program (Scope v3.5.2/s, Castle Hill, Australia) for determination of the resting membrane potential, action potential amplitude, action potential rise time, and duration of action potentials at 10%, 50%, and 80% levels of repolarization.

2.4. Statistical analysis

Results are expressed as arithmetic means ± S.E.M. of n experiments performed on atria from different animals. Comparison of the control and drug groups was performed by Student's t-test for paired or unpaired data, where applicable. When control and multiple concentrations of the same inhibitor were compared, one way analysis of variance (ANOVA) and Tukey's multiple comparison post test were used. $P < 0.05$ was considered to be statistically significant.

2.5. Drugs and reagents

Ouabain (octahydrate), atropine (sulfate) and theophylline were purchased from Sigma Chemical (St. Louis, USA). Milrinone, verapamil, and phentolamine were obtained from Sterling-Winthrop Research Institute (Rensselaer, NY, USA), Knoll (Istanbul, Turkey) and CIBA (USA), respectively. Omeprazole, lansoprazole and SCH 28080 were obtained as gifts from Astra Hassle (Molndal, Sweden), Adilna Sanovel (Istanbul, Turkey) and Schering-Plough Research Institute (Kenilworth, NJ, USA), respectively. Stock solutions of omeprazole and lansoprazole were freshly prepared in 0.1 N NaOH, whereas ethanol was used to dissolve SCH 28080.

3. Results

3.1. Effects of H^+/K^+-ATPase inhibitors on contractility and intrinsic rhythmic properties of spontaneously beating isolated rat atria

All three H^+/K^+-ATPase inhibitors used in this study increased contraction amplitudes and reduced the beating rate. These effects

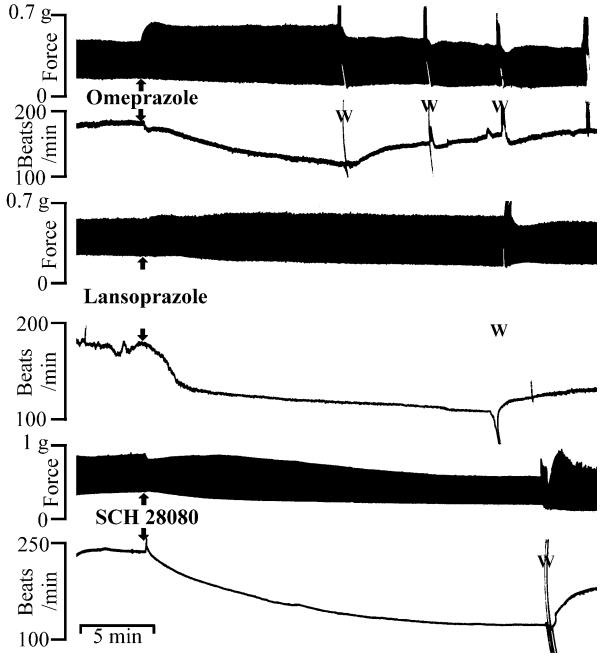

Fig. 1. The figure represents original tracings of single experiments displaying the effects of omeprazole, lansoprazole, and SCH 28080 on spontaneously beating rat atria. Omeprazole (300 μM) and lansoprazole (100 μM) increased the amplitude of atrial contractions and decreased the beating rate. SCH 28080 (100 μM) did not increase contraction amplitudes, but reduced beating rate. After wash out of the inhibitors, drug effects were removed.

occurred immediately after drug administration, they were concentration dependent and reversed completely upon wash out of the drugs. Reapplication of the inhibitors led to same responses with a similar magnitude.

Omeprazole and lansoprazole significantly increased contraction amplitudes and decreased the basal beating frequency at concentrations between 30 and 300 μM (Figs. 1 and 2). NaOH (0.1 N), the solvent of omeprazole and lansoprazole did not alter alter contractility, heart rate and the pH of the medium when applied corresponding to the highest concentration of both inhibitors (0.3%).

SCH 28080 was found to be more potent in altering contraction amplitudes and the beating rate. SCH 28080 exerted negative

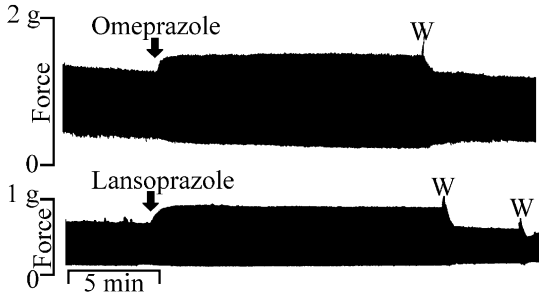

Fig. 3. The figure represents original tracings of single experiments displaying the effects of omeprazole (200 μM) and lansoprazole (100 μM) on electrically driven rat left atria.

chronotropic effect at 50 and 100 μM ($P<0.01$, Fig. 2, $n=6$) and increased atrial contraction amplitudes at 10 μM. However, at 50 and 100 μM, the amplitude of atrial contractions were slightly depressed. SCH 28080 was dissolved in ethanol and at a final bath concentration of 0.2%, corresponding to 100 μM SCH 28080, ethanol inhibited atrial contraction amplitudes by 37.5±11.6% ($n=4$) without altering their beating rate (Fig. 2, inset). Thus, we concluded that inhibitory effect of ethanol masked SCH 28080-induced positive inotropic action.

3.2. Effects of H^+/K^+-ATPase inhibitors on contractility of electrically-driven left atria

To examine whether the positive inotropic effect of H^+/K^+-ATPase inhibitors is secondary to their negative chronotropic effect, they were applied to electrically-driven isolated left atria. Omeprazole and lansoprazole (100–300 μM) significantly ($P<0.05$, $n=5$ for each drug) increased atrial contraction amplitudes in a reversible manner (Fig. 3) and this increase was comparable to those obtained in spontaneously beating preparations. Thus, the positive inotropic effect of H^+/K^+-ATPase inhibitors are independent from their negative chronotropic actions.

3.3. Role of Na^+/K^+-ATPase inhibition in the effects of H^+/K^+-ATPase inhibitors

High concentrations of H^+/K^+-ATPase inhibitors have been reported to inhibit Na^+/K^+-ATPase (Beil et al., 1986; Keeling et al.,

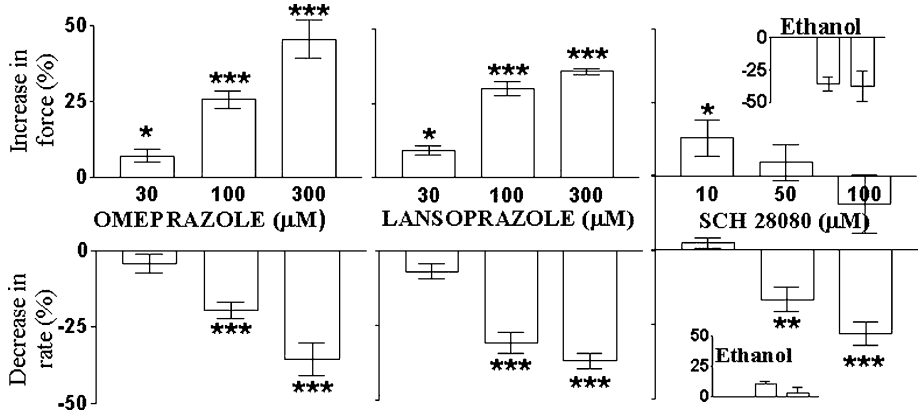

Fig. 2. Effects of omeprazole, lansoprazole, and SCH 28080 on spontaneously beating rat atria. Each bar represents % changes (mean±S.E.M., $n=5–15$) from the baseline. Solvent of SCH 28080 (Ethanol, inset) produced negative inotropic effect without altering beating rate. The effects of each concentration of the inhibitors were compared with their pre-drug values by using one way analysis of variance and Tuckey's post test (*$P<0.05$, **$P<0.01$, ***$P<0.001$).

1985). To determine whether Na^+/K^+-ATPase inhibition contributes to the positive inotropic effect of proton pump inhibitors, we examined their effects in the presence of selective Na^+/K^+-ATPase inhibitor, ouabain. Ouabain-induced effects are contrary to that observed with H^+/K^+-ATPase inhibitors with the exception of positive inotropic action. Ouabain (2–5 μM) caused a transient increase in contractility and increased beating rate (Fig. 4, $n=9$). These responses were followed by an increase in diastolic tension with oscillations in tachograph recordings (arrhythmia) and resulted in development of "contracture" in all of the atria tested. These effects were previously demonstrated in the isolated guinea-pig atria (Watano et al., 1999). After the effects of ouabain were established, we administered H^+/K^+-ATPase inhibitors. Ouabain-induced increase in beating rate and diastolic tension were reversed with omeprazole ($n=5$), lansoprazole ($n=5$), and SCH 28080 ($n=4$). They also regulated ouabain induced dysrhythmic atrial contractions.

Ouabain pretreatment significantly potentiated positive inotropic effects of omeprazole and lansoprazole. Omeprazole when given alone at 100 and 300 μM increased the atrial contraction amplitudes by 25.6±9.4% ($n=11$) and 45.2±16.8% ($n=8$), respectively. However, the same concentrations (100 and 300 μM) of omeprazole led to 64.5±11.0% and 113.4±8.4% ($n=5$) increase, respectively, when they were applied in the presence of ouabain (2 μM). These values were significantly different from those observed with the same concentrations of omeprazole alone ($P<0.05$). Similar potentiation was also observed with lansoprazole. Lansoprazole, when applied alone at 100 μM and 300 μM increased atrial contractions by 29.9±9.1% ($n=16$) and 35.8±2.6% ($n=8$), respectively. In ouabain pretreated atria, contraction amplitudes were increased by 95.5±8.6% and 100.8±2.5%, respectively ($n=5$, $P<0.05$ compared with the effect of lansoprazole alone). This potentiation was not observed when the sequence of drug administration was reversed.

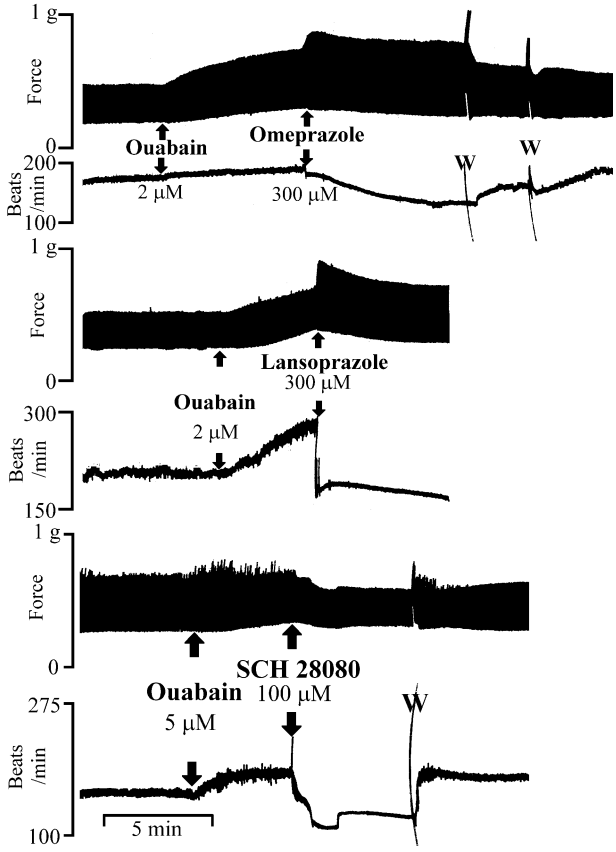

Fig. 4. Effects of omeprazole, lansoprazole and SCH 28080 on ouabain induced changes of spontaneously beating rat atria. The figure represents original tracings of single experiments. Ouabain (2–5 μM) increased contraction amplitude, beating rate and diastolic tension. Omeprazole (300 μM) and lansoprazole (300 μM) further increased atrial contractions, reduced diastolic tension and beating rate after ouabain treatment. SCH 28080 also reduced ouabain-induced increase in diastolic tension and beating rate. Note that ouabain-induced oscillations (dysrhythmic contractions) in tachograph recordings were also inhibited by proton pump inhibitors.

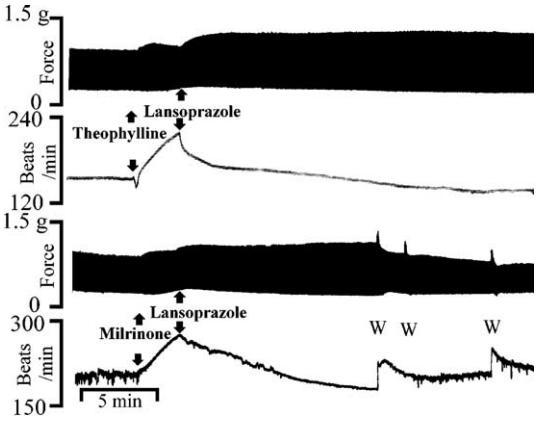

Fig. 5. The figure represents original tracings of single experiments displaying the effects of lansoprazole on theophylline (300 μM) or milrinone (100 μM)-induced contractile alterations in the rat atria. Phosphodiesterase inhibitors increased the amplitude of atrial contractions, beating rate and diastolic tension. Lansoprazole (100 μM) further increased atrial contractions, reduced diastolic tension and beating rate after theophylline or milrinone.

3.4. Role of phosphodiesterase inhibition in the effects of H^+/K^+-ATPase inhibitors

In order to investigate the possible contribution of phosphodiesterase inhibition in the positive inotropic effects of H^+/K^+-ATPase inhibitors, theophylline and milrinone were used. The positive inotropic and positive chronotropic effects of these drugs, were previously demonstrated (Goyal and McNeill, 1986; Zavecz, 1989). Theophylline (300 μM) and milrinone (100 μM) both increased the amplitude of atrial contractions in our experiments by 12.5±3.4% ($n=5$) and 13.6±4.9% ($n=5$), respectively. They also increased atrial beating rate and induced an increase in the basal tone (Fig. 5) which are contrary to the effects of H^+/K^+-ATPase inhibitors and these effects were completely reversed with lansoprazole (100 μM). However, after theophylline (300 μM) and milrinone (100 μM), lansoprazole (100 μM) increased atrial contraction amplitudes by 45.8±4.8% and 43.7±7.2%, respectively ($n=5$ for both). These values were greater but not statistically different from the inotropic effect of lansoprazole itself at 100 μM. (29.9±9.1%, $n=16$).

3.5. Role of adrenoceptors, muscarinic receptors and L-type Ca^{2+} channels in the effects of H^+/K^+-ATPase inhibitors

We tested the contribution of α- and β-adrenoceptors and muscarinic receptors on the effects of H^+/K^+-ATPase inhibitors on

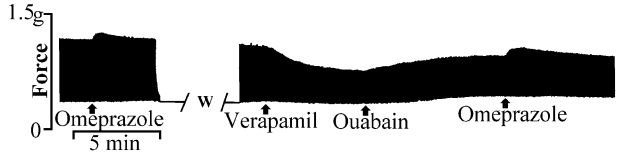

Fig. 6. Inotropic effect of omeprazole (200 μM) did not change after sequential administration of verapamil (2.5 μM) and ouabain (50 μM) in electrically-driven rat left atrium. The figure represents original tracings of a single experiment. Omeprazole was reapplied to the same atrium after the wash out period, which was pretreated with verapamil followed by ouabain. In the presence of verapamil, ouabain still increased diastolic tension which was antagonized by omeprazole.

rat atria by using specific antagonists ($n=5$ for each). Prior treatment of the atria by phentolamine (5 μM), propranolol (15 μM) or atropine (1 μM) did not appreciably alter positive inotropic and negative chronotropic effects of omeprazole (100–300 μM).

We also evaluated the effects of proton pump inhibitors in the rat atria in which L-type Ca^{2+} channels are blocked with verapamil. Verapamil (2.5 μM) decreased the contraction amplitudes of electrically driven left atria by 40.4±8.0% ($n=4$). Administration of omeprazole (100 μM) completely restored verapamil-induced negative inotropic action (data not shown). In the presence of verapamil, ouabain at 2–5 μM did not increase contraction amplitudes, however, at 50 μM it exerted positive inotropic effect with increasing diastolic tension (Fig. 6). The positive inotropic effect of omeprazole was not inhibited in the presence of verapamil and ouabain. In addition, ouabain still elevated diastolic tension in the presence of verapamil, which was reversed by omeprazole. This interaction was observed in each of the two atria tested.

3.6. Electrophysiological experiments

To investigate the mechanism(s) underlying H^+/K^+-ATPase inhibitor-induced effects on rat atrial myocardium, we recorded resting membrane and action potentials in the presence of 300 μM lansoprazole which induced maximum inotropic and chronotropic responses. Lansoprazole altered action potential configuration without changing resting membrane potential and action potential amplitude (Fig. 7, Table 1). Both depolarization and repolarization phases were significantly retarded following lansoprazole administration. Rise time of the action potential was increased by 66%. Prolongation of action potential duration occurred at all levels.

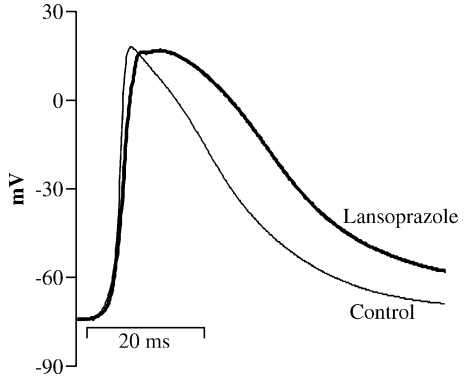

Fig. 7. Effect of lansoprazole (300 μM) on action potential configuration of rat atria. Recordings were obtained from 6 atria, each before and 20–30 min after drug perfusion. Lansoprazole prolonged both depolarization and repolarization phases.

Table 1
Lansoprazole (300 μM) induced changes in rat atrial action potential

Parameters	Control	Lansoprazole
Resting membrane potential (mV)	−74.4±1.6	−74.2±1^{ns}
Action potential amplitude (mV)	92.5±1.7	91.4±1.7^{ns}
Action potential rise time (ms)	0.9±0.1	1.5±0.1^{b}
Action potential duration$_{10}$ (ms)	2.9±0.4	6.3±0.7^{b}
Action potential duration$_{50}$ (ms)	13.3±1.4	21.5±2.6^{a}
Action potential duration$_{80}$ (ms)	30.4±3.5	49.2±6.9^{a}

Depolarization and repolarization phases are significantly delayed without altering resting membrane potential and action potential amplitude. Values indicate mean±SEM, Student's t-test for unpaired data, 6 rats, a, $P<0.05$; b, $P<0.01$; ns, nonsignificant; 10, 50, 80, duration of action potentials measured at % level of repolarization.

However, this prolongation was more profound in the early phase of repolarization. Action potential duration measured at 10% level of repolarization (APD_{10}) was 214% of the control, while APD_{50} and APD_{80} values were increased by 61% and 62%, respectively. Lansoprazole-induced changes in action potential configuration recovered completely after wash out of the drug.

4. Discussion

The results of this study demonstrate that, three different H^+/K^+-ATPase inhibitors induce positive inotropic and negative chronotropic effects in the rat atria, in vitro. These concentration dependent, reversible and reproducible effects seem not to be mediated by well known pathways that enhance contractility such as inhibition of Na^+/K^+-ATPase and phosphodiesterase type III or activation of β-adrenoceptors. None of the pharmacological agents used in this study attenuated the cardiac effects of proton pump inhibitors. However the only common property of these compounds is to inhibit the gastric and nongastric H^+/K^+-ATPases. Previous studies demonstrated the presence of functional cardiac H^+/K^+-ATPase in rats and guinea pigs which was sensitive to omeprazole (Nagashima et al., 1999; Zinchuk et al., 1997) and SCH 28080 (Beisvag et al., 2003). Therefore, putative myocardial H^+/K^+-ATPase inhibition may be responsible for the changes in rat atrial contractility. In addition, prolongation of action potential duration which was observed with lansoprazole, is consistent with the positive inotropic, negative chronotropic and antiarrhythmic effects of proton pump inhibitors and may elucidate, at least partially their mechanism of action.

H^+/K^+-ATPase inhibitors used in this study are members of two structurally unrelated classes, two benzimidazole derivatives (omeprazole and lansoprazole) and an imidazopyridine derivative (SCH 28080). Their site of interaction with the H^+/K^+-ATPase and the mechanism by which they inhibit the pump activity are also different. Omeprazole (Im et al., 1985) and lansoprazole (Nagaya et al., 1989) inhibit the H^+/K^+-ATPase by oxidizing its essential sulfhydryl groups. SCH 28080 competes with K^+ for the high affinity binding site of K^+ and thereby blocks K^+-stimulated ATPase

activity (Wallmark et al., 1987). Therefore in spite of their chemical diversity and distinct inhibitory mechanisms, they produced the same effects on the rat atria. Thus, the cardiotonic effects observed in this study seem to be mediated by inhibition of the myocardial H^+/K^+-ATPase.

The concentrations of omeprazole, lansoprazole and SCH 28080 that changed atrial contractility and intrinsic rhythm seem to be higher than required to inhibit gastric H^+/K^+-ATPase. However, lower expression of H^+/K^+-ATPase in myocardial tissue than in stomach (Beisvag et al., 2003) and relatively basic pH (7.4) in our experiments than in stomach are two main reasons for higher concentrations of these inhibitors to produce cardiac effects. Acidic pH in gastric glands augments the potency of proton pump inhibitors by forming active metabolites (Lindberg et al., 1986; Wallmark et al., 1984; Nagaya et al., 1987, 1989) and traps these basic agents according to the pH gradient. A small change of pH from 7.4 to 6.1, decreased the IC_{50} value of omeprazole by 9 fold (Keeling et al., 1985).

The potency of omeprazole is similar to lansoprazole in our experiments, whereas SCH 28080 is more potent (Fig. 2). Gastric antisecretory potencies of omeprazole and lansoprazole were reported to be similar (Coruzzi et al., 1995; Satoh et al., 1989). The IC_{50} values of omeprazole and SCH 28080 as inhibitors of gastric acid secretion were found to be 36 μM and 1.5 μM at a pH of 7.5 (Keeling et al., 1985; Wallmark et al., 1987). Therefore, the order of potency of these inhibitors, as cardiotonic agents, is similar to their potency as H^+/K^+-ATPase inhibitors. This correlation also supports the hypothesis that the cardiotonic effects of proton pump inhibitors may be related directly to the ability of these compounds to inhibit myocardial H^+/K^+-ATPase.

The effective concentrations of omeprazole and SCH 28080 in our experiments were reported to alter intracellular ionic state of guinea pig and rat myocardial tissue. Omeprazole was shown to inhibit pH and K^+ regulation and L-type Ca^{2+} currents of guinea pig myocardium at a concentration of 300 μM (Nagashima et al., 1999) and 100 μM of SCH 28080 reduced the $^{86}Rb^+$-uptake to 73% of control values in rat cardiomyocytes (Beisvag et al., 2003). The same concentrations of omeprazole and SCH 28080 induced maximum alterations in atrial beating rate and contractility in our experiments.

Beisvag et al. (2003) and Nagashima et al. (1999) both suggested that H^+/K^+-ATPase accounts for K^+-uptake and has a possible role in intracellular pH regulation of cardiomyocytes. Thus, inhibition of this newly described regulatory transport system is expected to decrease intracellular K^+ levels and intracellular pH. However, intracellular acidification is known to reduce force of contraction in cardiac muscle cells (Fabiato and Fabiato, 1978; Vaughan-Jones et al., 1987). If intracellular acidosis reduces force of contraction, inhibition of H^+/K^+-ATPase is expected to decrease the amplitude of atrial contractions.

The experiments performed on electrically-driven left atria indicated that the positive inotropic effect of H^+/K^+-ATPase inhibitors are independent from their negative chronotropic effects. Ineffectiveness of verapamil on omeprazole-induced positive inotropic action indicates an involvement of a mechanism other than Ca^{2+} influx through L-type channels. The positive inotropic and negative chronotropic effects of H^+/K^+-ATPase inhibitors remained unchanged after the blockade of α and β adrenoceptors, as well as muscarinic receptors. Thus, the effects of H^+/K^+-ATPase inhibitors do not appear to be mediated by muscarinic receptors and α or β adrenoceptors.

Although inhibition of Na^+/K^+-ATPase in response to high concentrations of H^+/K^+-ATPase inhibitors was reported (Beil et al., 1986; Keeling et al., 1985, 1991); we observed distinct differences in atrial contractility in response to H^+/K^+-ATPase inhibitors and Na^+/K^+-ATPase inhibitor, ouabain. Contrary to the effects of ouabain, proton pump inhibitors increased contractility without elevating basal diastolic tone, decreased beating rate and exerted antiarrhythmic action and induced these effects in ouabain-treated atria, as well. Thus, Na^+/K^+-ATPase inhibition could not be responsible for the positive inotropic action. Beisvag et al. demonstrated that SCH 28080 did not interfere significantly with the ^{3}H-ouabain binding to the Na^+/K^+-ATPase in rat cardiomyocytes (2003). They concluded that SCH 28080 and ouabain act on different $^{86}Rb^+$-uptake mechanisms. These observations are compatible with our findings that the cardiac effects of proton pump inhibitors operate through a mechanism different from Na^+/K^+-ATPase inhibition.

Ouabain and phosphodiesterase type III inhibitor-induced increase in beating rate and diastolic tension were effectively antagonized by the proton pump inhibitors in our experiments. Inhibition of Na^+/K^+-ATPase (Deitmer and Ellis, 1980; Vaughan-Jones et al., 1983) and phosphodiesterase (Kafiluddi et al., 1988) lead to intracellular accumulation of Ca^{2+} through different pathways. Inhibition of Na^+/K^+-ATPase leads to intracellular Na^+ accumulation that activates the Na^+-Ca^{2+} exchanger and results in elevated intracellular Ca^{2+} concentration. Thus a high intracellular Na^+ level is responsible for ouabain-induced Ca^{2+} overload, whereas increased cAMP levels are responsible for phosphodiesterase inhibitors. Overload of intracellular Ca^{2+} results in persistent active myofilament tension development throughout diastole and therefore is known to be responsible for elevated diastolic tension (Lorell et al., 1988). Reversal of this condition by H^+/K^+-ATPase inhibitors implies that a mechanism contributing to intracellular Ca^{2+} handling also may be related to inhibition of H^+/K^+-ATPase activity. Blockade of L-type Ca^{2+} channels with verapamil did not prevent ouabain-induced toxic rise in diastolic tension, whereas this was effectively reversed by omeprazole (Fig. 6). Thus, elevated $[Ca^{2+}]_i$ appeared to be decreased with H^+/K^+-ATPase inhibitor by intracellular handling of this cation.

The pharmacodynamic interaction of ouabain and H^+/K^+-ATPase inhibitors on the rat atrial inotropy may be either potentiation or additive interaction, depending on the order

of application. An additive type of interaction occurred when the former agent was omeprazole or lansoprazole. However, if the sequence of administration was reversed, ouabain significantly potentiated the H^+/K^+-ATPase inhibitor-induced inotropy (Fig. 4). By this interaction, the amplitude of atrial contractions was reached to 200% of the baseline values. A similar supra-additive interaction were also observed with theophylline or milrinone pretreatment (Fig. 5). Thus, inhibition of Na^+/K^+-ATPase or phosphodiesterase which are known to be associated with intracellular accumulation of Ca^{2+} appeared to facilitate the cardiotonic actions of omeprazole and lansoprazole.

Electrophysiological alterations induced by lansoprazole are compatible with our observations on mechanical changes observed in the rat atrium. Lansoprazole at 300 μM, which induced maximum increase in contractile force and caused profound bradycardia, in vitro, significantly prolonged action potential duration. It is well established that there is a positive correlation between the action potential duration and force of contraction (Morad and Trautwein, 1968). Due to prolonged repolarisation, delaying Ca^{2+} channel inactivation, activator Ca^{2+} probably increases within the myocytes (Mitchell et al., 1987; Morad and Trautwein, 1968; Reiter, 1988). Therefore, positive inotropic effect of lansoprazole may be explained by prolongation of action potential duration. Since similar membrane currents are largely responsible for the repolarisation phase in the sinus node cells (Noble, 1985), prolongation of action potential duration also seems to be responsible for the reduction of the heart rate through increasing cycle length. Although, the ionic background of these differences cannot be identified from the present experiments, pronounced lengthening of the early phase of the action potential is probably caused by suppression of K^+ currents. Lansoprazole prolonged the rise time of action potential, indicating that blockade of fast Na^+ channels may also be involved. Therefore, antagonism of ouabain-induced toxicity may be explained by prolongation of the ouabain-induced shortening of action potential duration and a reduction in Na^+ influx. In our study, lansoprazole did not alter atrial resting membrane potential. H^+/K^+-ATPase inhibition is expected to result in a decrease in K^+ uptake into cells; however a decrease in $[K^+]_i/[K^+]_o$ would be associated with a decrease in the membrane potential. It is also possible that changes in intracellular K^+ and H^+ concentrations may trigger secondary changes in other transport pathways which regulate membrane potential. In conclusion, prolongation of action potential duration and refractoriness was demonstrated with lansoprazole, which is in accordance with a class III antiarrhythmic mode of action. Because positive inotropic, negative chronotropic and antiarrhythmic actions are consistently associated with class III antiarrhythmic action (Tande and Refsum, 1990).

Cardiac H^+/K^+-ATPase may play a physiological role and may also contribute to pathological processes such as cardiac failure. Beisvag et al. (2003) demonstrated a significant upregulation of the rat myocardial H^+/K^+-ATPase in heart failure after myocardial infarction. This observation supports our findings that myocardial H^+/K^+-ATPase functions in the physiological properties of cardiac muscle. Although our experimental data cannot be extrapolated directly to the clinical settings, identifying and a more complete understanding of the functional and biological properties of myocardial H^+/K^+-ATPase may provide a new insight into the cardiac pathophysiology.

In conclusion, compounds known to inhibit H^+/K^+-ATPase increased contractility, decreased heart rate and exerted antiarrhythmic actions in isolated rat atria. These effects appear to be mediated by inhibition of myocardial H^+/K^+-ATPase and seem to be a result of prolongation of action potential duration. Our study provides additional evidence for the existence and physiological importance of cardiac H^+/K^+-ATPase.

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