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Positive effect of bumetanide on contractile activity of ventricular cardiomyocytes

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Abstract

Although the beneficial effects of loop-diuretics in relieving congestive heart failure and essential hypertension are well established, there has been limited investigation into the direct cardiac effect of these drugs. The aim of the present study was to investigate mechanical and electrophysiological effects of three loop-diuretics, namely, frusemide, bumetanide and torasemide, in ventricular cardiomyocytes isolated from rabbit myocardium. Contractile performance was assessed using a video edge detection method and effects on L-type Ca^{2+} currents were determined using a 'perforated' patch-clamp technique. Unlike frusemide and torasemide, bumetanide produced concentration-dependent (3×10^{-8} to 10^{-5} M) increases in cell shortening and velocity of cell shortening. Neither frusemide, bumetanide nor torasemide exerted any action on peak Ca^{2+} current amplitudes. In summary, the present investigation provides evidence for a direct contractile effect of the loop diuretic, bumetanide, in contrast to absence of effects by frusemide and torasemide, in ventricular cardiomyocytes isolated from rabbit myocardium.

Keywords: Loop-diuretic; Bumetanide; Frusemide; Cardiomyocyte; Contraction

1. Introduction

Diuretics are unquestionably the most widely used of all drugs in treatment of heart failure (Packer, 1992; Taylor, 1993). Interestingly, less is known of their therapeutic impact than with many less frequently used drugs. Improved cardiac function is brought about as a result of a reduction in the pathologically increased circulating blood volume (Andreasen et al., 1993). Although the primary function of diuretic treatment in heart failure is recognised as alleviating the physiological mechanisms, which promote sodium and water reabsorption by the kidney, it is clear that a more complex mode of action exists to achieve therapeutic benefit (Taylor, 1993).

The beneficial effects of loop-diuretics in relieving pulmonary oedema, congestive heart failure and essential hypertension are well established and are generally attributed to their potent diuretic action. These drugs have important cardiovascular haemodynamic effects, which occur even in cases with marked impairment of renal function (Andreasen et al., 1993; Brater, 1992). In addition, clinical relief of symptoms of pulmonary congestion frequently precedes any demonstrable diuretic effect (Biagi and Bapat, 1967; Dikshit et al., 1973), suggesting that extra-renal factors may be involved. Evidence suggests that part of the therapeutic impact of loop diuretics may be mediated by a direct action on the vasculature (Dormans et al., 1996). Indeed, frusemide produces a decrease in vascular resistance that has been attributed to an anti-vasoconstrictor effect (Gerkens, 1987; Stanke et al., 1998). Although the secondary consequences of diuretic treatment on the heart have been widely examined, the direct cardiac effects of these drugs have received limited investigation. The aim of the present study was to examine the direct cardiac cellular actions of the loop-diuretics, frusemide, bumetanide and torasemide using isolated ventricular cardiomyocytes; isoprenaline was used as a positive control in assessing cell shortening.

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2. Materials and methods

2.1. Isolation of ventricular cardiomyocytes

The care and use of animals ($n = 24$) conformed to the UK Home Office regulations in accordance with Guidance on the Operations of the Animals (Scientific Procedures) Act 1986. Ventricular cardiomyocytes were obtained following enzymatic dissociation. New Zealand White male rabbits (16 week; 2.5–3 kg) were anaesthetised using sodium pentobarbitone (50 mg/kg i.v.) following heparinization (400 iu/kg, i.v.). The chest was opened and the heart quickly removed and cannulated through the ascending aorta, on a modified Langendorff perfusion apparatus (Kelso et al., 1995). Blood was flushed from the coronary vasculature with a Ca^{2+}-free modified Krebs–Ringer buffer (KRB), containing (in mM) NaCl 110, KCl 2.6, $NaHCO_3$ 25, $MgSO_4$ 1.2, KH_2PO_4 1.2 and glucose 11 (pH 7.4; 37°C), which had just previously been aerated with 95% O_2/5% CO_2. The perfusate was subsequently supplemented with 0.12% (w/v) collagenase and recirculated for approximately 15 min while maintained at 37°C and continuously aerated with 95% O_2/5% CO_2. Following enzymatic digestion, the hearts were cut at the atrioventricular junction, sliced vertically towards the apex and chopped into cubes of 0.7 mm^3 using a mechanical tissue chopper (McIlwain Chopper, Mickle Laboratory Engineering, Surrey, UK). The minced tissue was placed in the collagenase-containing perfusate, which had been supplemented with 0.2% (w/v) bovine serum albumin, and the mixture triturated using a 10-ml serological pipette for approximately 5 min. The dispersed cells were filtered through a nylon mesh gauze of pore size 200 μm and washed twice. Ca^{2+} was restored by means of centrifugation at $25 \times g$ twice, and the cells were resuspended in modified KRB solutions containing 250 and 500 μM $CaCl_2$, respectively. Finally, the cells were layered onto a solution of 4% (w/v) BSA containing 1 mM $CaCl_2$, and left to settle by gravity, at 37°C. After approximately 5 min, the supernatant was aspirated and the resulting cell material resuspended at a density of 1–2 mg protein/ml in a storage medium (M199 with Earle's salts, containing 5 mM creatine, 5 mM taurine, 2 mM carnitine, 100 iu/ml streptomycin, 100 μg/ml penicillin, pH 7.4) at 37°C. Suspensions of cardiomyocytes were $>70\%$ viable as estimated by their elongated rod-shaped morphology.

2.2. Contractile measurement

An aliquot of the cell suspension was placed in a transparent recording chamber (~ 150 μl) mounted on a heated microscope stage (37°C, Zeiss), and allowed to settle for 10 min before being bathed with an oxygenated (95% O_2, 5% CO_2) Krebs–Henseleit solution of the following composition (in mM): NaCl 125, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2, HEPES 10 and glucose 11 (pH 7.4). Cells were field stimulated (Grass stimulator) at 0.5 Hz with biphasic pulses of 0.5-ms duration at 60 V via $Ag/AgCl_2$ wires embedded in the wall of the recording chamber. The cells were visualised at $\times 1280$ magnification and cell shortening was monitored using a video edge detection system (VED 40; Crescent Electronics, USA). The resulting signal was digitised (Digidata 1200, Axon Instruments) and recorded on computer for subsequent analysis (WCP for Windows, Dr. J. Dempster, Department of Pharmacology, University of Strathclyde). This system has a time resolution of 20 ms. Six consecutive contractions were signal averaged to produce data under each discrete set of conditions. Cardiomyocyte contractile function was assessed by measuring absolute cell shortening (μm), time to peak contraction (time taken for the signal to rise from 10% to 90% of peak amplitude, ms), velocity of cell shortening (the maximum rate of rise of the signal measured between 10% and 90% of peak amplitude, μm/s), and relaxation time (time taken for signal to fall from peak amplitude to 10% peak amplitude, ms). Viable cells were used for each experiments as defined by: (1) a rod-shaped appearance without sarcolemmal blebbing, (2) quiescent when unstimulated, and (3) stable baseline contractions to electrical stimulation in Krebs–Henseleit solution. The solution bathing the cells could be changed within 30 s, using a gravity fed, multi-channel solution exchanger consisting of eight reservoirs, a series of solenoid valves and an eight channel input manifold attached to the bath.

2.3. Electrophysiology

An aliquot of cell suspension was placed in a transparent recording chamber and allowed to settle for 10 min before perfusing with a Tyrode's solution containing (in mM) NaCl 137, KCl 5.4, $CaCl_2$ 3, $MgCl_2$ 1.2, HEPES 5, glucose 10, pH 7.4. Action potentials (APs) were elicited by passing a current pulse, of 6–12 ms duration, through the recording patch electrode using an Axopatch 1D patch-clamp amplifier. L-type Ca^{2+} currents were recorded in voltage-clamp mode, and low resistance (1–3 MΩ) electrodes were filled using a standard solution of (in mM): K-gluconate 110, KCl 20, $MgCl_2$ 2, HEPES 10, EGTA 11, $CaCl_2$ 1, GTP 0.1 and creatine phosphate 2.5 (pH 7.2), which was supplemented with 150 μg/ml nystatin (Sigma). A stock nystatin solution (50 mg/ml) was prepared in dimethylsulphoxide and was stable at −20°C for 1 month. Daily aliquots of this solution were prepared following dilution and ultrasonication, resulting in a final concentration of 150 μg/ml in 0.2% dimethylsulphoxide, which was stable for 2 h. This concentration of dimethylsulphoxide applied to the cells had no observable effect on the L-type Ca^{2+} current. Patch electrodes were fabricated from thin-walled borosilicate capillaries with a filament (GC 150TF; Clarke Electrochemical), by means of a horizontal lazer puller (Sutter Instruments, Model P2000), and

had tip resistances of 1–3 MΩ when filled with the electrode solution. Access to the cell interior was achieved by dialysis of nystatin within 20 min of obtaining a gigaseal. Following stabilization, the Ca^{2+} current was activated by clamping the membrane voltage for 200 ms from a holding potential of −40 mV to test potentials of −40 to +60 mV; the clamp step was induced every 2 s. The amplitude of peak Ca^{2+} current was measured as the difference between the peak of the inward current and the steady state level at the end of the voltage pulse (Varro et al., 1991). Current 'rundown' was not significant over a 60 min time period. All recordings were stored on computer for subsequent analysis using customised software. The various loop diuretics were applied locally to the cell using a gravity fed microperfusion system (150 μl/min).

2.4. Data analysis

Concentration–response curves, for the contractile responses, were normalised to their respective baseline values. Data were expressed as mean ± standard error. Comparisons were made using analysis of variance with repeated measures and Dunnett's multiple comparisons test. Durations of action potential at 90% repolarization (APD) and peak current values were expressed as mean ± standard error. Current–voltage plots were constructed, and peak L-type Ca^{2+} current values were compared at +10 mV. Comparisons were made using analysis of variance and Student's paired *t*-test. Statistical significance was established at $P < 0.05$.

3. Results

3.1. Response of cardiomyocytes to basal contractile stimulation

Rabbit cells were selected only if they showed no signs of spontaneous contraction, when stimulated at 0.5 Hz, during the 10 min equilibration period in 2 mM Ca^{2+}. The video edge detection system was calibrated using a graticule at ×200, ×400 and ×1280 magnification, and a best fit linear regression line was computed having a slope of 93.8 mV/μm and a correlation coefficient of 0.9998. Mean cell length was 111 ± 2 μm (82 cells). Cell shortening, time to peak contraction, velocity of cell shortening, and relaxation time did not change, over a period of 60 min, from basal values of 7.85 ± 0.66 μm, 184 ± 14 ms, 125 ± 18 μm/s, and 235 ± 25 ms, respectively (Fig. 1).

3.2. Contractile response in the presence of frusemide and torasemide

Frusemide did not affect cell shortening, over the concentration range of 3×10^{-8} to 10^{-5} M (Fig. 2a), from a control value of 7.85 ± 0.66 μm; however, there was an apparent decrease (5%) in cell shortening in the presence of frusemide at a maximum concentration (10^{-4} M). Moreover, frusemide did not affect time to peak contraction (Fig. 2b) and velocity of cell shortening (Fig. 2c) from control values of 160 ± 10 ms and 155 ± 9 μm/s, respectively. Frusemide increased the relaxation time (Fig. 2d) at

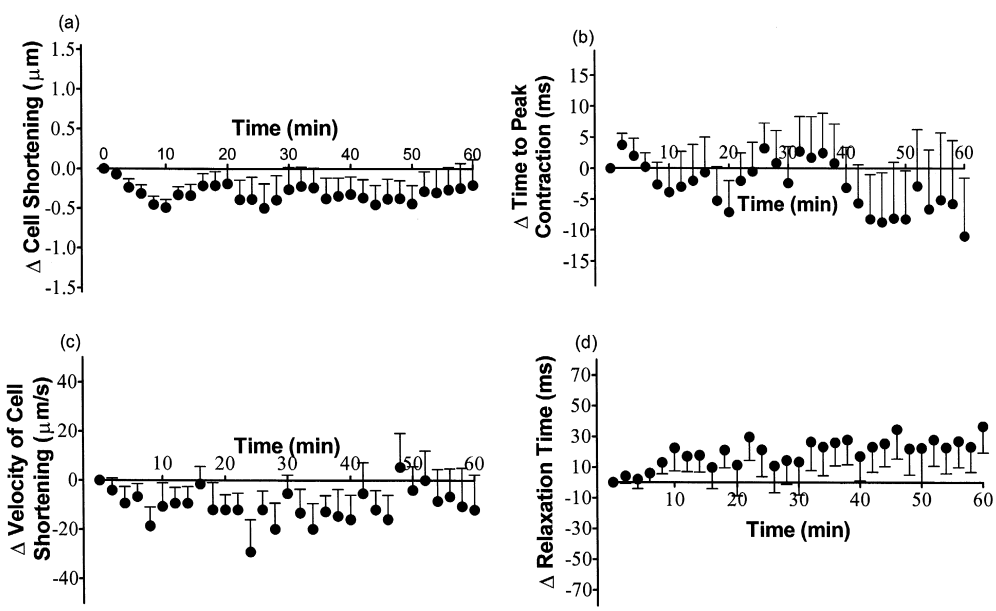

Fig. 1. Contractile performance of ventricular cardiomyocytes, isolated from rabbit myocardium, monitored over a time period of 1 h. Contractile parameters are expressed as an absolute change relative to a mean basal value. Data are presented as mean ± standard error of eight heart cell preparations.

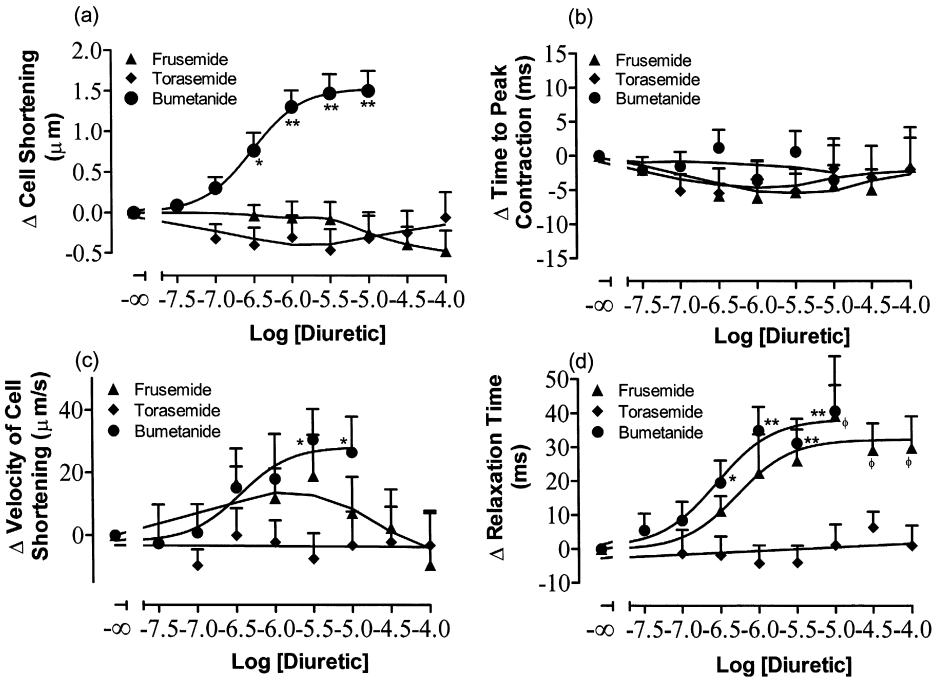

Fig. 2. Effects of cumulative concentrations of the loop-diuretics, frusemide, bumetanide and torasemide on the contractile parameters: (a) cell shortening, (b) time to peak contraction, (c) velocity of cell shortening, and (d) relaxation time. Cells were exposed to each concentration of frusemide for 3 min. Contractile parameters are expressed as an absolute change relative to a mean basal value. Data are presented as mean ± standard error of 9–12 heart cell preparations. $^{*}P < 0.05$ and $^{**}P < 0.01$ bumetanide versus a basal values; $\Phi P < 0.05$ frusemide versus basal values.

concentrations of 10^{-5} to 10^{-4} M; at 10^{-5} M, the response was increased by 16% from a control value of 224 ± 12 ms. Torasemide, on the other hand, did not affect cell shortening, time to peak contraction, velocity of cell

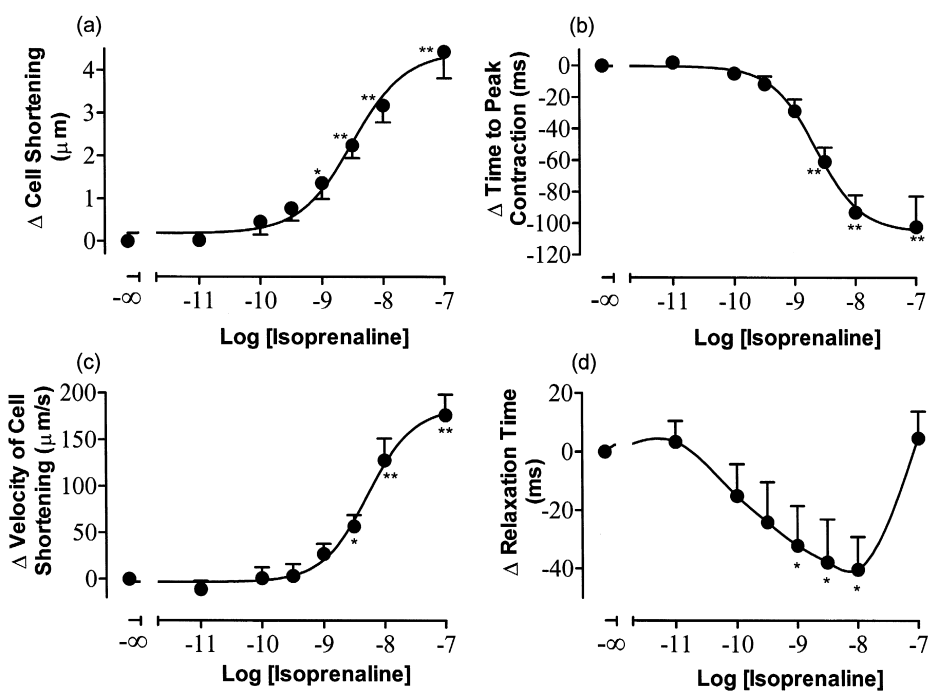

Fig. 3. Effects of cumulative concentrations of isoprenaline on: (a) cell shortening, (b) time to peak contraction, (c) velocity of cell shortening, and (d) relaxation time. Contractile parameters are expressed as an absolute change relative to a mean basal value. Data are presented as mean ± standard error of nine heart cell preparations. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus a basal values.

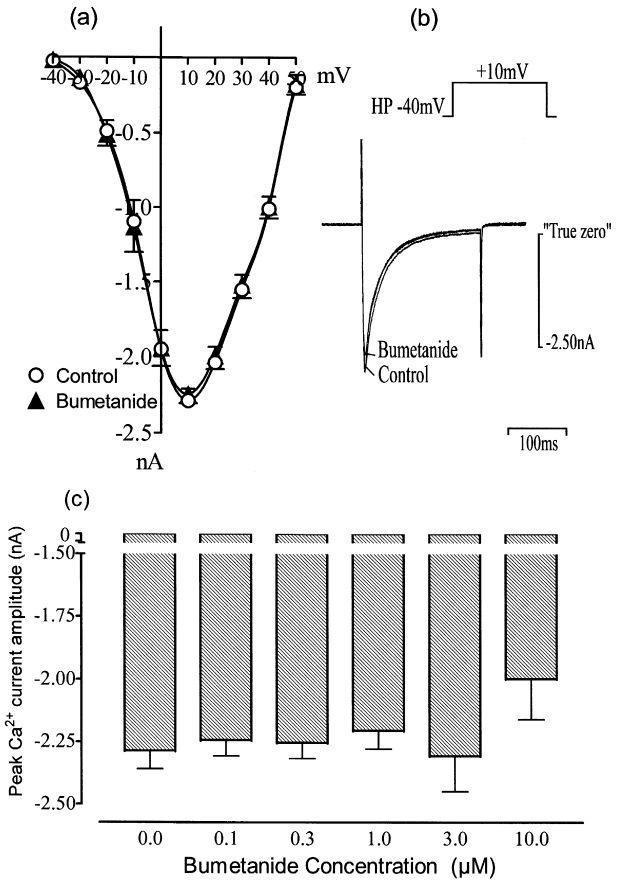

Fig. 4. Effect of bumetanide on L-type Ca^{2+} current. (a) Current–voltage relationship of current before exposure (○) and after a 3 min exposure to bumetanide (▲) (1 μM). The L-type Ca^{2+} current was activated by clamping the membrane voltage for 200 ms from a holding potential of −40 mV to test potentials of −30 to +60 mV. (b) Typical current trace, at +10 mV, recorded before and after exposure to bumetanide (1 μM). (c) Bar-chart comparing the peak current amplitude recorded at +10 mV before and after cumulative exposure to bumetanide over the concentration range, 0.1–10 μM. Data shown represent mean ± standard error ($n = 9$).

shortening, or relaxation time from control values of 7.50 ± 0.5 μm, 215 ± 9 ms, 102 ± 13 μm/s, and 208 ± 10 ms, respectively (Fig. 2).

3.3. Positive contractile effect of bumetanide differs from isoprenaline

A maximum positive contractile response of bumetanide was attained following 1–2 min exposure of the cells to the drug. Bumetanide produced a dose-dependent effect on cell shortening over the concentration range 3×10^{-8} to 10^{-5} M (Fig. 2). The maximal effect of bumetanide (3×10^{-5} M) on cell shortening was 9.93 ± 0.7 μm, which was 20 ± 4% ($P < 0.01$) above the basal value of cell shortening of 8.42 ± 0.8 μm; the EC_{50} value was $3.02 \pm 0.33 \times 10^{-7}$ M. The time to peak contraction was unaltered by bumetanide from a basal value of 193 ± 8 ms, over the concentration range studied (Fig. 2b). The velocity of cell shortening also appeared to increase in a concentration-dependent manner, but only reached significance ($P < 0.05$) at a threshold concentration of 3×10^{-6} M (Fig. 2c); the maximum effect, at this concentration, increased to 183 ± 19 μm/s from a basal value of 156 ± 23 μm/s. The relaxation time was also increased ($P < 0.01$) by bumetanide to a maximum of 22 ± 4% greater than a basal value of 223 ± 14 ms (Fig. 2d); the EC_{50} value was $2.82 \pm 0.30 \times 10^{-7}$ M.

In contrast, the maximum increase in cell shortening produced by isoprenaline, which was used as a positive control, was 68 ± 11% ($P < 0.01$) greater than a basal contractile amplitude of 7.0 ± 0.5 μm (Fig. 3a), and was accompanied by a concentration-dependent decrease in time to peak contraction (Fig. 3b), and a concentration-de-

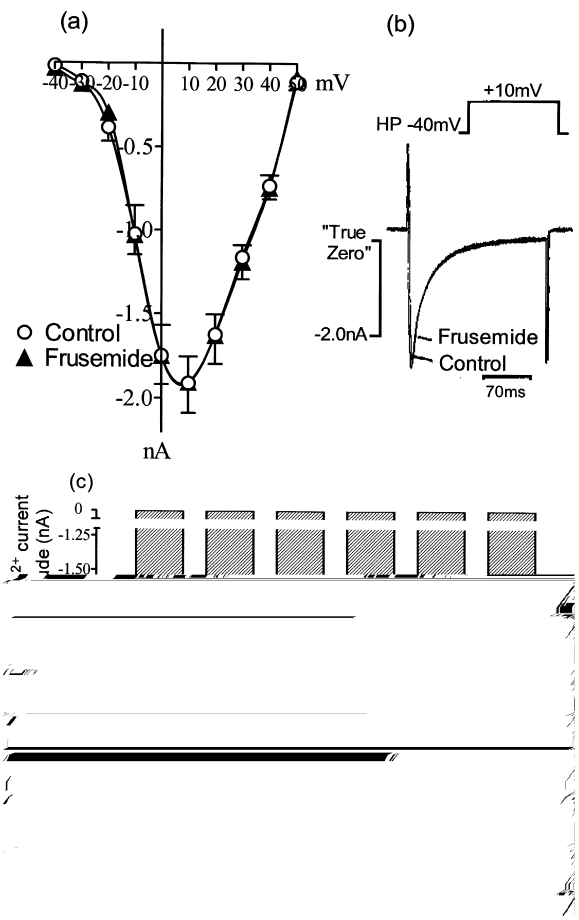

Fig. 5. Effect of frusemide on L-type Ca^{2+} current. (a) Current–voltage relationship of current before exposure (○) and after a 3 min exposure to frusemide (▲) (10 μM). The L-type Ca^{2+} current was activated by clamping the membrane voltage for 200 ms from a holding potential of −40 mV to test potentials of −30 to +60 mV. (b) Typical current trace, at +10 mV, recorded before and after exposure to frusemide (10 μM). (c) Bar-chart comparing the peak current amplitude recorded at +10 mV before and after cumulative exposure to frusemide over the concentration range, 3–300 μM. (d) Temporal relationship of frusemide at 5 s intervals. The drug was applied to the cell using a gravity fed microperfusion system, which allowed the solution surrounding the cell to be changed within 2 s. Data shown represent mean ± standard error ($n = 9$).

pendent increase in velocity of cell shortening (Fig. 3c). The time to peak contraction was maximally decreased, at a concentration of 10^{-7} M, by $38 \pm 2\%$ ($P < 0.01$) to 147 ± 6 ms from a basal value of 239 ± 16 ms (Fig. 3b). Isoprenaline maximally increased the velocity of cell shortening from 93 ± 12 to 265 ± 28 $\mu m/s$, at a concentration of 10^{-7} M. The EC_{50} response of isoprenaline on cell shortening was $3.89 \pm 0.14 \times 10^{-9}$ M. Isoprenaline maximally decreased the relaxation time, at a concentration of 10^{-8} M, by $17 \pm 4\%$ ($P < 0.05$) to 190 ± 14 ms from a basal value of 229 ± 12 ms; this decrease was reversed at the higher concentration of 10^{-7} M and was not different from basal (Fig. 3d).

3.4. Absence of loop-diuretic effects on the L-type Ca^{2+} current

Neither bumetanide (3×10^{-8} to 10^{-5} M; Fig. 4), frusemide (3×10^{-7} to 10^{-4} M; Fig. 5), nor torasemide (data not shown) had any effect on the L-type Ca^{2+} current, from control peak amplitudes of -2.29 ± 0.08, -2.0 ± 0.19, and -2.65 ± 0.20 nA, respectively. Moreover, frusemide did not demonstrate any transient effect on the L-type Ca^{2+} current, as noted from an absence of change in current recorded at 5 s intervals, from a control value of -2.50 ± 0.31 nA (Fig. 4d).

4. Discussion

The findings of the present study indicate a novel direct effect of the loop-diuretic, bumetanide, on the myocardium, at clinically relevant concentrations (Holazo et al., 1984). In contrast to frusemide and torasemide, which had no effects on contractile parameters, bumetanide produced a concentration-dependent increase in contraction of isolated ventricular cardiomyocytes. However, neither bumetanide, frusemide nor torasemide exerted any action on peak Ca^{2+} current amplitudes. Although the cardiac consequences of diuretic treatment have been widely examined, in terms of the cellular basis of diuretic action and renal adaptation (Brater, 1992), the direct effects of these drugs on the heart have received limited investigation. Indeed, examination of the contractile function of loop diuretics, at the cardiac cellular level, has not previously received attention.

Plasma concentrations of bumetanide are reported to be approximately 3×10^{-7} M following a single dose of 1 mg given to healthy volunteers (Holazo et al., 1984). In ventricular cardiomyocytes isolated from rabbit myocardium, bumetanide (3×10^{-7} to 10^{-5} M) increased cell shortening and velocity of cell shortening, did not affect the time to peak contraction, but increased the relaxation time, thus achieving a contractile response of increased magnitude and slower response. The contractile effect of isoprenaline was assessed as a positive control in the present study and differs from that produced by bumetanide, both in efficacy and temporal nature. The magnitude of the maximum response of bumetanide is approximately one third of the maximum response produced by isoprenaline. Moreover, bumetanide prolonged the duration of contractile response, whereas isoprenaline decreased the duration of the contractile response, as a consequence of decreases in both times to peak contraction and relaxation. Such differences between isoprenaline and bumetanide would suggest different mechanisms of action. A prolonged relaxation could be due to slowing of the Ca^{2+} reuptake mechanisms, and is in contrast to the response by isoprenaline, which enhances Ca^{2+} reuptake (Harding et al., 1988). Kim et al. report an increase, by frusemide, in the slower myosin isoform V_3, a factor, which could contribute to prolonged relaxation time (Gorza et al., 1981; Kim et al., 1996); however, such an alteration is unlikely to explain the prolonged contractile response of bumetanide since this effect was observed in isolated cardiomyocytes within 1–2 min following superfusion with the drug.

The absence of effect of frusemide on contractile activity in isolated ventricular cells is in agreement with previous reports using myocardial tissue preparations (Poole-Wilson et al., 1978; Borchgrevink and Jynge, 1989). Frusemide did not alter mechanical function in the interventricular septum from rabbit (Poole-Wilson et al., 1978) nor ventricular tissue isolated from the ischaemic rat heart (Borchgrevink and Jynge, 1989). However, a negative inotropic effect of frusemide was reported, at elevated concentrations (10^{-3} M), in isolated rabbit heart (Feldman et al., 1987). In the present study, the highest concentration of frusemide studied was 10^{-4} M, at which there was a small apparent decrease in cell shortening. Hence, a significant decrease in cell shortening may well have become apparent at a concentration of 10^{-3} M in isolated cardiomyocytes. However, study of such a high concentration is unlikely to be of clinical relevance; patients with heart failure who receive oral frusemide (40 mg) are reported to have plasma levels of 2 $\mu g/ml$ ($< 10^{-5}$ M) (Vasko et al., 1985; Feldman et al., 1987). The elimination half-life of frusemide ranges from 60 to 178 min, and although intravenous administration increases the bioavailability of a given dose threefold, it is unlikely that the drug concentration would increase by such a degree to produce a significant negative effect on myocardial function in vivo (Brater, 1993).

Torasemide differs from frusemide and bumetanide both in its chemical structure and metabolic profile, and is more potent and more potassium-sparing than frusemide (Luft, 1993; Masereel et al., 1993; Ferrara et al., 1997). The direct effects of torasemide on the myocardium have not previously been investigated, but unlike frusemide, it has been found to increase intracellular levels of cyclic AMP and cyclic GMP in the aorta of the renal hypertensive rat (Yamanaga et al., 1992). It is known that an increase in

intracellular cyclic AMP and cyclic GMP in the aorta causes vasodilation, which may explain the antihypertensive properties of torasemide at non-diuretic doses (Brater, 1993). However, torasemide did not alter contractile function in isolated cardiomyocytes over the concentration range of 10^{-7} to 10^{-4} M; therapeutic doses of torasemide administered to humans result in plasma concentrations in the range of 3×10^{-6} to 1.4×10^{-5} M (Lameire and Dodion, 1987; Brater, 1993).

Frusemide and bumetanide have been reported to inhibit the L-type Ca^{2+} current (5×10^{-7} M) in both isolated atrial and ventricular cells of the rabbit, using a conventional ruptured whole-cell patch-clamp technique (Shimoni, 1991); the response was transient reaching a maximum after 8 s and then declining after 12 s (effects over a longer time period were not documented), and therefore is unlikely to have much clinical relevance. A major disadvantage of the ruptured whole cell patch-clamp technique is that Ca^{2+} current may 'rundown' by as much as 50% over a period of 10 min. This problem is completely eliminated using the perforated patch-clamp technique where the current amplitude is stable for over 45 min (Kelso et al., 1996). Using this technique, neither frusemide, bumetanide nor torasemide altered the L-type Ca^{2+} current amplitudes. Moreover, an early transient effect, which was reported by Shimoni (1991), was not observed in the present study since frusemide (10^{-5} M) did not produce any short-term effect on the Ca^{2+} current nor duration of the action potential and may therefore reflect differences in experimental recording technique. Changes in the L-type Ca^{2+} current have been speculated to contribute to the arrhythmogenic mechanisms often associated with loop diuretic therapy (Lumme and Jounela, 1986; Singh et al., 1992); however, results from the present study would not support this theory.

There are a number of mechanisms of action, which have been implicated in the non-diuretic effects of loop diuretics, including synthesis of prostaglandins (Craven and Derubetis, 1982; Liguori et al., 1999), inhibition/activation of adenylate cyclase (Ebel, 1974; Dawborn et al., 1977; Feldman et al., 1987) and $Na^+/K^+/2Cl^-$ co-transport system (Haas and McManus, 1983; Liu et al., 1987; Mitani and Shattock, 1992). Frusemide has been reported to inhibit the Ca^{2+}-activated chloride current, $I_{Cl(Ca)}$ (Greenwood et al., 1995) in smooth muscle cells, which is thought to contribute to the vasodilator action. Moreover, frusemide was reported to decrease intracellular chloride activity in rabbit papillary heart muscle, possibly by inhibiting chloride influx via the Na^+/Cl^- transport system (Oyama and Walker, 1986). It is possible that bumetanide may inhibit the Na^+/Ca^{2+} exchange, which could modify intracellular Ca^{2+} levels and increase the availability of free Ca^{2+} for the contractile system; such a mechanism of action was responsible for the positive inotropic effect of the potassium sparing diuretic, amiloride in atria (Floreani and Luciani, 1984). Further study is therefore needed to elucidate the mechanism(s) of action responsible for the positive contractile effect of bumetanide in cardiomyocytes.

In conclusion, the present study provides evidence for a direct cardiac effect of the loop diuretic, bumetanide. In the absence of loading conditions and neurohormonal influences in isolated ventricular cardiomyocytes, bumetanide produced a positive contractile effect, which was not associated with an increase in L-type Ca^{2+} current. Such a positive inotropic may be of benefit in the treatment of heart failure, especially at high doses where concerns exist of worsening clinical status associated with administration of high doses of frusemide in patients with decompensated heart failure (Feldman et al., 1987). Frusemide and torasemide had no significant effects on cardiomyocyte function. It is clear that the mechanisms of action of diuretic therapy are indeed multifactorial and include both beneficial diuretic and non-diuretic actions.

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