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DCPIB is a novel selective blocker of $I_{Cl,swell}$ and prevents swelling-induced shortening of guinea-pig atrial action potential duration

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1 We identified the ethacrynic-acid derivative DCPIB as a potent inhibitor of $I_{Cl,swell}$, which blocks native $I_{Cl,swell}$ of calf bovine pulmonary artery endothelial (CPAE) cells with an IC_{50} of 4.1 μM. Similarly, 10 μM DCPIB almost completely inhibited the swelling-induced chloride conductance in *Xenopus* oocytes and in guinea-pig atrial cardiomyocytes. Block of $I_{Cl,swell}$ by DCPIB was fully reversible and voltage independent.

2 DCPIB (10 μM) showed selectivity for $I_{Cl,swell}$ and had no significant inhibitory effects on $I_{Cl,Ca}$ in CPAE cells, on chloride currents elicited by several members of the CLC-chloride channel family or on the human cystic fibrosis transmembrane conductance regulator (hCFTR) after heterologous expression in *Xenopus* oocytes. DCPIB (10 μM) also showed no significant inhibition of several native anion and cation currents of guinea pig heart like $I_{Cl,PKA}$, I_{Kr}, I_{Ks}, I_{K1}, I_{Na} and I_{Ca}.

3 In all atrial cardiomyocytes ($n=7$), osmotic swelling produced an increase in chloride current and a strong shortening of the action potential duration (APD). Both swelling-induced chloride conductance and AP shortening were inhibited by treatment of swollen cells with DCPIB (10 μM). In agreement with the selectivity for $I_{Cl,swell}$, DCPIB did not affect atrial APD under isoosmotic conditions.

4 Preincubation of atrial cardiomyocytes with DCPIB (10 μM) completely prevented both the swelling-induced chloride currents and the AP shortening but not the hypotonic cell swelling.

5 We conclude that swelling-induced AP shortening in isolated atrial cells is mainly caused by activation of $I_{Cl,swell}$. DCPIB therefore is a valuable pharmacological tool to study the role of $I_{Cl,swell}$ in cardiac excitability under pathophysiological conditions leading to cell swelling.
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Abbreviations: CPAE, calf pulmonary artery endothelial cells; DCPIB, (4-(2-Butyl-6,7-dichlor-2-cyclopentyl-indan-1-on-5-yl) oxybutyric acid); E_{Cl}, chloride equilibrium potential; EGTA, ethylene glycol-O,O'-bis(2-aminoethyl)-N,N,N′,N′-tetraacetic acid; HEPES, N-(hydroxyethyl)piperazine-N′-(2-ethanesulphonic acid); $I_{Cl,Ca}$, calcium-activated chloride current; $I_{Cl,swell}$, swelling-activated chloride current; $[K^+]_o$, external K^+ concentration; RVD, regulatory volume decrease; V_{rest}, resting membrane potential

Introduction

The swelling-induced chloride current $I_{Cl,swell}$ is present in most cells (Nilius *et al*., 1994; Okada, 1997) including that of the mammalian heart (Sorota, 1992; Sakai *et al*., 1995) and it might play an important role in cell volume regulation. The molecular correlate of $I_{Cl,swell}$ is not yet known.

In isolated cardiac cells, activation of $I_{Cl,swell}$ either by positive pressure inflation (Hagiwara *et al*., 1992) or osmotic swelling causes a slight depolarization of the resting membrane potential (V_{rest}) (Akiyama & Fozzard, 1975; Ehara & Hasegawa, 1983; Zhang *et al*., 1993; Du & Sorota, 1997) and a shortening of the action potential (Kawata *et al*., 1974; Ehara & Hasegawa, 1983; Vandenberg *et al*., 1997). Previous studies on ventricular tissue indicate that during ischaemia and reperfusion cell swelling occurs (Tranum-Jensen *et al*., 1981; Jennings *et al*., 1986; Garcia-Dorado & Oliveras, 1993) which, with such a magnitude in isolated cells (Sorota, 1992; Hagiwara *et al*., 1992), could likely activate $I_{Cl,swell}$ (Sorota, 1994; Hiraoka *et al*., 1998). Ischaemia and reperfusion are often associated with cardiac arrhythmias (Janse & Wit, 1989), suggesting that activation of $I_{Cl,swell}$ and its linked APD shortening might participate in the genesis of arrhythmias (Hiraoka *et al*., 1998; Mulvaney *et al*., 2000). Cardiac $I_{Cl,swell}$ has been described in ventricular and atrial cardiomyocytes of several mammalian species including humans, but seems to be predominantly expressed in atrial and sino-atrial node cells (Hagiwara *et al*., 1992; Sorota, 1992; Vandenberg *et al*., 1994; Sakai *et al*., 1995; Li *et al*., 1996). Thus, the activity of $I_{Cl,swell}$ may have a greater impact on atrial, rather than ventricular, electrical activity and it might play a role in controlling the electrical activity of atrial tissue and possibly in pacemaking (Hiraoka *et al*., 1998; Mulvaney *et al*., 2000).

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Whereas several studies investigated the contribution of $I_{Cl,swell}$ to swelling-induced electrical changes in ventricular cells, to our knowledge, no study has analysed the function of $I_{Cl,swell}$ in atrial action potential duration.

Here we investigated the effects of osmotic swelling on the APD of atrial cardiomyocytes isolated from guinea-pig hearts. We show for the first time that the non-diuretic ethacrynic-acid derivative DCPIB is a potent blocker of native $I_{Cl,swell}$ currents present in different cell types like *Xenopus* oocytes, calf bovine pulmonary artery endothelial (CPAE) cells and guinea-pig atrial myocytes. At 10 μM, DCPIB is selective for $I_{Cl,swell}$ since it has no effect on the activity of $I_{Cl,Ca}$ in CPAE cells, on various cloned chloride channels after heterologous expression in *Xenopus* oocytes (CLC-1, -2, -4, -5, CLC-K1 and hCFTR) or on native currents in guinea-pig cardiomyoctes (I_{Ks}, I_{Kr}, I_{K1}, I_{Na}, I_{Ca} and $I_{Cl,PKA}$). In addition, DCPIB did not alter the currents elicited by hKv1.5, hKv4.3, hminK and HERG, which encode the most important repolarizing currents in the human heart (I_{Kur}, I_{to1}, I_{Ks} and I_{Kr},), after their expression in *Xenopus* oocytes. Consistently, DCPIB (10 μM) had no effects on atrial APD under isoosmotic conditions.

This novel selective blocker of $I_{Cl,swell}$ allowed us to specifically determine the contribution of $I_{Cl,swell}$ to swelling-induced changes in atrial APD without interfering with other cardiac currents critically involved in AP generation and shaping. We show that swelling of atrial myocytes by hypotonic perfusion activated $I_{Cl,swell}$ and concomitantly caused a drastic shortening of APD, which was reversed by addition of DCPIB after cell swelling occurred. Moreover, this strong APD shortening was completely prevented by incubating cells with DCPIB prior to hypotonic perfusion, which blocked $I_{Cl,swell}$ but not cell swelling.

Methods

Cell preparation and cell culture

Single cardiac myocytes were obtained from guinea-pig hearts using standard enzymatic techniques. Briefly, guinea-pigs (350–450 g) were killed by cervical dislocation after stunning and the hearts were quickly removed. The aorta was cannulated and the heart was retrogradely perfused for 5 min with a nominally Ca^{2+}-free Tyrode solution at 37.5°C. The Ca^{2+}-free Tyrode solution contained (in mM): NaCl 143, KCl 5.4, $MgCl_2$ 0.5, NaH_2PO_4 0.25, glucose 10, HEPES 5; and pH was adjusted to 7.2 with NaOH (290±5 mOsmol). The solution was aerated with O_2 for 20–30 min. Subsequently the heart was perfused with the same solution which in addition contained 0.33 mg ml^{-1} collagenase (Type CLS II, 270 U mg^{-1}, Biochrom KG) and 15 μM $CaCl_2$. Atrium and ventricle were removed and further dissected into small pieces, and cell dissociation was achieved by gentle mechanical agitation with a pipette in the storage solution. The storage solution contained (in mM): L-glutamine acid 50, KCl 40, taurine 20, KH_2PO_4 20, $MgCl_2$ 1, glucose 10, HEPES 10, EGTA 2; and the pH was adjusted to 7.2 with KOH (260±5 mOsmol). The suspension was filtered through a nylon mesh and stored at room temperature.

Xenopus laevis oocytes were obtained from tricaine anaesthetized animals. Ovaries were surgically removed, cut into pieces and collagenase treated (1 mg ml^{-1}, Worthington, type II) in OR 2 solution containing (in mM): NaCl 82.5, KCl 2 , $MgCl_2$ 1, HEPES 5, pH 7.4 with NaOH for 120 min. Oocytes were stored in recording ND 96 solution containing (in mM): NaCl 96 , KCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5, pH 7.4, additional containing Na^+ pyruvate (275 mg l^{-1}), theophylline (90 mg l^{-1}) and gentamycin (50 mg l^{-1}) at 18°C. Oocytes were injected with approximately 10 ng cRNA. Macroscopic currents were recorded 2–4 days after injection. For measurements of $I_{Cl,swell}$, oocytes were manually defolliculated and stored at 18°C in ND 96.

Calf pulmonary artery endothelial cells were grown in DMEM with 2 mM L-glutamine, 100 μg ml^{-1} streptomycin, 100 U ml^{-1} penicillin and 20% foetal bovine serum. Cultures were maintained at 37°C in 5% CO_2. Cells were detached by exposure to 0.05% trypsin in a Ca^{2+}- and Mg^{2+}-free solution, reseeded, and kept in culture for 2–4 days before use. Only non-confluent cells were used.

Solutions

$I_{Cl,swell}$ *in CPAE cells* The standard external solution was a modified Krebs solution containing (in mM): NaCl 150, KCl 6, $MgCl_2$ 1, $CaCl_2$ 1.5, glucose 10, HEPES 10, adjusted to pH 7.4 with NaOH. The osmolarity was adjusted to 320±5 mOsmol using a vapour pressure osmometer (Vogel, OM 801). At the beginning of the recording the modified Krebs solution was replaced by an isotonic Cs^+ solution (320±5 mOsmol) containing (in mM): NaCl 105, CsCl 6, $MgCl_2$ 1, $CaCl_2$ 1,5, D-mannitol 90, glucose 10, HEPES 10, adjusted to pH 7.4 with NaOH. $I_{Cl,swell}$ was activated by exposing the cells to a 25% hypotonic solution (240±5 mOsmol) by omitting 90 mM D-mannitol. The standard pipette solution contained (in mM): CsCl 40, Cs-aspartate 100, $MgCl_2$ 1, $CaCl_2$ 1.93, EGTA 5, Na_2ATP 4, HEPES 10, adjusted to pH 7.2 with CsOH (290±5 mOsmol).

$I_{Cl,Ca}$ *in CPAE cells* The modified Krebs solution was replaced by a slightly hypertonic Krebs-Cs^+ solution (345±5 mOsmol) containing (in mM): NaCl 150, CsCl 6, $MgCl_2$ 1, $CaCl_2$ 1.5, glucose 10, D-mannitol 25, HEPES 10, adjusted to pH 7.4 with NaOH, to prevent co-activation of $I_{Cl,swell}$. $I_{Cl,Ca}$ was activated by loading the CPAE cells *via* the patch pipette with 1 μM free Ca^{2+} as described previously (Nilius *et al*., 1997a,b). The standard pipette solution contained (in mM): CsCl 40, Cs-aspartate 100, $MgCl_2$ 1, $CaCl_2$ 4.33, EGTA 5, Na_2ATP 4, HEPES 10, adjusted to pH 7.2 with CsOH (290±5 mOsmol).

$I_{Cl,swell}$ *in* Xenopus *oocytes* The isoosmotic solution (220±5 mOsmol) was a mannitol containing ND 48 of the following composition (in mM): NaCl 48 , KCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1, mannitol 110, and HEPES 5; pH was adjusted to 7.4 with NaOH. The hypotonic solution to evoke $I_{Cl,swell}$ was ND 48 without mannitol (110±5 mOsmol).

$I_{Cl,swell}$ *in gp cardiomyocytes* The isoosmotic external solution (290±5 mOsmol) contained (in mM): NaCl 70, sucrose 140, $MgCl_2$ 2, $BaCl_2$ 2, HEPES 5, and the pH was adjusted to 7.5 with CsOH. 2 μM nicardipine was added to block Ca^{2+}-channels and 20 μM ouabain to block swelling-induced Na^+/K^+ pump currents (Whalley *et al*., 1993). The

standard hypoosmotic solution (160±5 mOsmol) was as above except that sucrose was omitted. The internal pipette solution (285±5 mOsmol) contained (in mM): CsCl 58, Cs-aspartate 52, tetraethylammonium (TEA) chloride 20, EGTA 10, Mg_2ATP 5, Na_3GTP 0.2, HEPES 5; pH was adjusted to 7.3 with CsOH.

Action potential recordings The standard isoosmotic external solution (290±5 mOsmol) was (in mM): NaCl 100, sucrose 80, KCl 5.4, $MgCl_2$ 0.5, $CaCl_2$ 1.8, NaH_2PO4 0.33, glucose 11, HEPES 5, pH was adjusted to 7.4 with NaOH. The hypoosmotic solution was similar to the isoosmotic solution except that sucrose was omitted (205±5 mOsmol). The internal pipette solution contained (in mM): K^+-aspartate 100, KCl 20, EGTA 0.2, Na_2-phosphocreatine 5, MgATP 5, $MgCl_2$ 1, Na_3GTP 0.2, and HEPES 5, pH was adjusted to 7.3 with NaOH.

I_{Ks}, I_{Kr}, I_{K1} *in gp cardiomyocytes* The standard solution contained (in mM): NaCl 140, KCl 4.7, $CaCl_2$ 1.3, $MgCl_2$ 1, glucose 10, and HEPES 10; pH was adjusted to 7.4 with NaOH. The solution for I_{Kr} additionally contained 5 μM nifedipine to block Ca^{2+}-channels. The internal pipette solution contained (in mM): KCl 140, NaCl 10, $MgCl_2$ 1.1, K_2ATP 1, EGTA 1, and HEPES 10; pH was adjusted to 7.2 with KOH.

I_{Ks} *in gp cardiomyocytes during cell swelling* Recordings of I_{Ks} during cell swelling were performed as previously described (Rees *et al*., 1995). Myocytes were perfused with an isoosmotic solution containing (in mM): NaCl 100, sucrose 80, KCl 5.4, $MgCl_2$ 0.5, NaH_2PO_4 0.33, glucose 5.5, and HEPES 5.0; pH was adjusted to 7.4 with NaOH (290±5 mOsmol). In the hypotonic solution (200±5 mOsmol) the sucrose was omitted. The solutions contained 0.2 μM dofetilide to block I_{Kr}. The internal pipette solution contained (in mM): K-aspartate 140, $MgCl_2$ 5, K_2ATP 5, EGTA 10, and HEPES 5; pH was adjusted to 7.4 with KOH (310±5 mOsmol).

I_{Na} *in gp cardiomyocytes* The external solution contained (in mM): NaCl 20, CsCl 30, tetramethylammonium chloride 20, $CoCl_2$ 2, $MgCl_2$ 1, 4-aminopyridine 5, sucrose 80, glucose 5, and HEPES 5, pH was adjusted to 7.4 with HCl. The internal solution contained (in mM): NaCl 16, CsCl 115, $MgCl_2$ 1, $CaCl_2$ 0.3, EGTA 10 and HEPES 5; pH was adjusted to 7.2 with CsOH.

I*Ca in gp cardiomyocytes* The bath solution contained (in mM): NaCl 132, CsCl 4.8, $MgCl_2$ 1.2, $CaCl_2$ 1, glucose 5, and HEPES 10; pH was adjusted to 7.3 with CsOH. The internal pipette solution had the following composition (in mM): CsCl 140, $MgCl_2$ 2, $CaCl_2$ 1 EGTA, 11, and HEPES 10; pH was adjusted to 7.3 with CsOH.

$I_{Cl,PKA}$ *in gp cardiomyocytes* The external solution was the same as the isotonic solution for $I_{Cl,swell}$ recordings, except that it contained NaCl 140 mM and sucrose was omitted. To evoke $I_{Cl,PKA}$ isoprenaline, prepared as 1 mM stock solution in water containing 100 mM ascorbic acid, was added to superfusion solutions to a final concentration of 1 μM, or alternatively 3-isobutyl-1-methylxanthine (IBMX), prepared as 1 M stock solution in DMSO, was added to a final concentration of 500 μM. The internal pipette solution was the same as for $I_{Cl,swell}$.

CLCs and CFTR expressed in Xenopus *oocytes* The standard solution was ND 96. For activation of hCFTR the solution contained 1 mM IBMX and 10 μM forskolin; prepared from 1 and 0.1 M stock solutions in DMSO, respectively.

Voltage clamp protocols

During two-electrode voltage clamp experiments, the holding potential in *Xenopus* oocytes experiments was −30 mV. Inhibition values are given at +40 mV, except for rCLC-2 with −100 mV and hCLC-4 and hCLC-5 with +60 mV. For hCLC-1 a voltage step protocol from −120 to +80 mV with 40 mV increments for 500 ms was used. In rCLC-2 recordings voltage steps from −160 to +40 mV in 20 mV increments for 5 s were performed and inhibition data were obtained by stepping to −120 mV and subsequent to −100 mV for 10 s, using a sweep interval of 60 s. hCLC-4 and hCLC-5 were measured by a protocol stepping from −140 to +100 mV in 20 mV increments for 500 ms. CLC-K1 and hCFTR were analysed with a protocol that ranged from −100 to +60 mV in 20 mV steps. hCFTR was recorded for 500 ms and CLC-K1 for 2 s.

At the beginning of each measurement in guinea-pig cardiomyocytes an *I-V* relationship was obtained by a triangular ramp protocol; first hyperpolarizing to −140 mV within 40 ms, subsequent depolarizing to +80 mV in 500 ms and within 30 ms back to the holding potential. Inhibition data of $I_{Cl,swell}$ and $I_{Cl,PKA}$ are given at +40 mV. Voltage clamp protocols are illustrated within the figures.

Current measurements and data analysis

Standard two-electrode voltage clamp recordings in *Xenopus* oocytes were performed with a Turbo Tec 10CD (NPI) amplifier and an ITC-16 interface. Patch clamp recordings were amplified with an EPC 9 Series D (HEKA). Both recording methods were combined with Pulse software (HEKA) and Igor for data acquisition on Pentium II PC. Patch clamp recordings were performed in whole-cell mode at 35±1°C *via* a thermocouple feedback circuit. Patches of CPAE cells and Na^+ currents were recorded at room temperature (21±1°C). In Na^+ current experiments linear capacitative and leakage currents were subtracted by the P-P/*n* (P, test voltage; $n=4$) method (Bezanilla & Armstrong, 1977) and series resistance compensation was used at 40–60%. Cells were visualized with an inverted microscope (Zeiss, Axiovert 35) at a magnification of ×400. Changes in cell size were monitored by a camera (Panasonic, WV-CD 22) combined with a VHS video recorder (Panasonic, AG-7355). Photos were obtained by connecting a camera to the microscope (Contax, 167). Volume changes in ventricular myocytes are proportional to changes in cell width (Roos, 1986; Drewnowska & Baumgarten, 1991). In some experiments the cell width was monitored as an indicator of cell volume, as previously reported (Sorota, 1992). Changes in cell width were analysed using a reticle in the occular and the photographs, where the average width at three different

locations was calculated. In experiments with cardiomyocytes tip resistances of 2.0–3.5 MΩ were used. For $I_{Cl,swell}$ recordings tip resistances were 1.5–2.5 MΩ and after membrane rupture the suction port of the electrode holder was opened to the atmosphere to ensure that no pressure was applied to the back of the pipette. For action potential recordings electrodes with tip resistances of 3.0–4.5 MΩ were used. Action potentials were elicited by a 0.8–1 nA current pulse applied for 1–3 ms at a frequency of 0.2 Hz operating in current clamp mode. In all experiments the patch pipette current was zeroed before seal formation. All results are reported as means ± s.e.mean. Statistical differences of resting membrane potential and action potential duration were evaluated by a two population Student's paired *t*-test using Origin software. IC_{50} for block of $I_{Cl,swell}$ was estimated by a Hill equation using Igor software. Data were calculated for significance and indicated by asterisks, with * for $P<0.05$ and ** for $P<0.01$, compared to isotonic conditions unless stated otherwise.

Results

DCPIB is a potent blocker of native $I_{Cl,swell}$

The non-diuretic acylaryloxyacid derivative DCPIB (Figure 1) has been shown to inhibit astroglial swelling (Bourke *et al*., 1981). The related compound IAA-94 has been shown to block epithelial chloride currents (Landry *et al*., 1989) and $I_{Cl,swell}$ (Sorota, 1994). This prompted us to analyse whether DCPIB is able to block $I_{Cl,swell}$. Three different types of cells were used, CPAE cells, *Xenopus* oocytes and guinea-pig atrial cells, which all possess a native swelling-sensitive chloride current. In CPAE cells a large chloride current representing $I_{Cl,swell}$ could be activated by osmotic swelling (Nilius *et al*., 1994). Maximal activation of $I_{Cl,swell}$ occurred within 3–5 min of hypotonic perfusion. Treatment of swollen cells with 10 μM DCPIB inhibited $I_{Cl,swell}$ by 83.7 ± 4% ($n=4$) (Figure 2a–c and Table 1), reflecting an almost complete block of the swelling-induced changes in Cl^--conductance. Maximal block was achieved within 3–5 min. The IC_{50} for block of $I_{Cl,swell}$ by DCPIB was 4.1 μM (Figure 1a). The block was voltage independent (Figure 1b) and fully reversible after washout of DCPIB by hypotonic solution. In *Xenopus* oocytes endogenous $I_{Cl,swell}$ can only be measured if oocytes are follicle enclosed (Arellano & Miledi, 1995; Arellano *et al*., 1996) or if they are manually defolliculated (Ackerman *et al*., 1994). Oocytes were stored in theophyllin-free medium because otherwise the amplitude of the swelling-activated chloride current was reduced (see cyclic AMP dependence: Nagasaki *et al*., 2000). DCPIB also potently inhibited $I_{Cl,swell}$ of *Xenopus* oocytes. The block of $I_{Cl,swell}$ was 53.3 ± 3.0% ($n=4$) at 10 μM.

Next we tested the effect of DCPIB on atrial $I_{Cl,swell}$ using previously reported methods (Vandenberg *et al*., 1994). DCPIB effectively blocked atrial $I_{Cl,swell}$ resulting in an inhibition of 72.9 ± 7.4% ($n=4$) at 10 μM (Figure 2g–i and Table 2). Thus, in all cells examined 10 μM DCPIB was able to potently block native $I_{Cl,swell}$. All cells displayed a basal conductance under isoosmotic conditions. In CPAE cells, the addition of 50 μM DCPIB led to a reduction of the basal current by 15.8 ± 4.2% ($n=4$), whereas the basal current of atrial cardiomyocytes was only reduced by 8.5 ± 3.0% ($n=4$). This is consistent with a low basal activity of $I_{Cl,swell}$ in isoosmotic environment. Therefore, after subtraction of the non-DCPIB sensitive basal conductance, inhibition of the swelling-induced changes in Cl^--conductance of CPAE and atrial cells was almost complete (Figure 2c,i).

DCPIB does not affect cell swelling

Volume changes in isolated cardiomyocytes are proportional to changes in cell width (Roos, 1986; Drewnowska & Baumgarten, 1991). In some experiments the cell width was monitored as an indicator of cell volume. Representative experiments are illustrated in Figure 3. In all atrial cells ($n=6$) a detectable increase in cell width was observed during hypotonic perfusion. The average cell width increased by a factor of 1.31 ± 0.04 ($n=6$) (Figure 3b) after 10 min of hypotonic perfusion. Chloride currents increased over the time course of the experiments. Addition of DCPIB to swollen cells in the hypotonic medium did not reverse swelling of atrial cardiomyocytes. After 5 min of perfusion with a 10 μM DCPIB

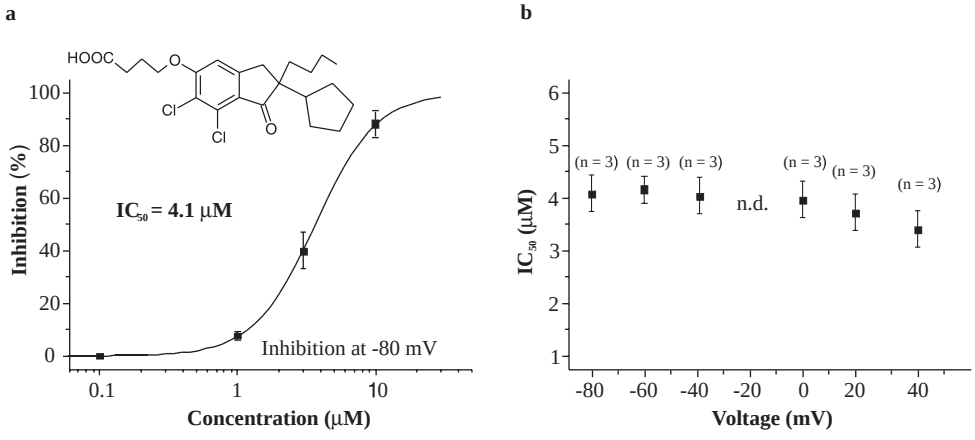

Figure 1 Chemical structure and dose response curve of DCPIB on $I_{Cl,swell}$ in CPAE cells. (a) DCPIB blocked $I_{Cl,swell}$ in CPAE cells with a half-maximal concentration (IC_{50}) of 4.1 μM at −80 mV. Inhibition values were calculated without subtraction of the basal current. If the basal non-DCPIB sensitive current is subtracted the resulting IC_{50} is 2.5 μM at −80 mV. (b) IC_{50} values of DCPIB determined at different voltages without subtraction of the basal current (n.d. = not determined).

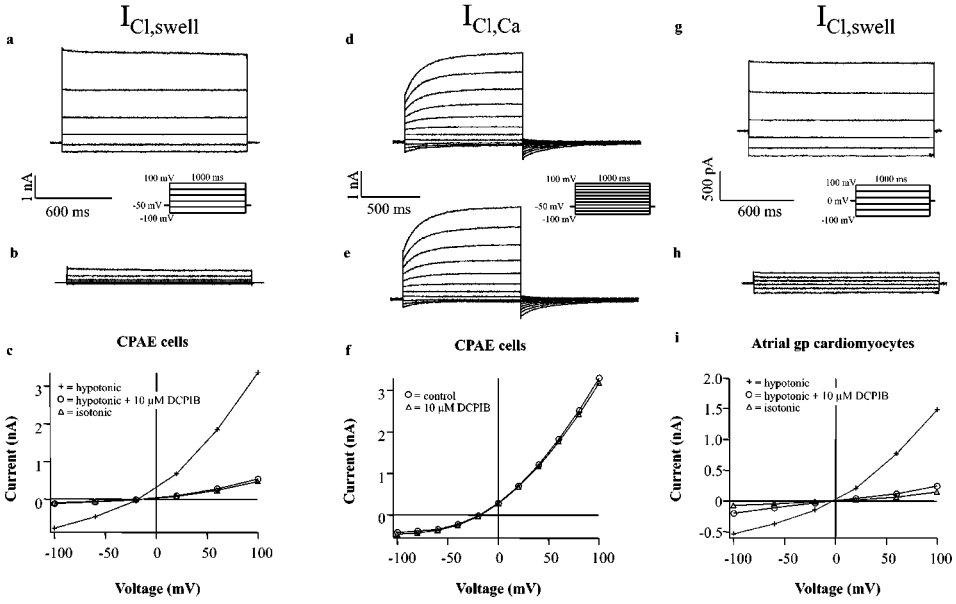

Figure 2 Effects of DCPIB on native $I_{Cl,swell}$ and $I_{Cl,Ca}$ currents. (a) $I_{Cl,swell}$ current traces from CPAE cells after hypotonic solution was applied for 3–5 min in the absence and (b) in the presence of 10 μM DCPIB. (c) $I-V$ curves of $I_{Cl,swell}$ obtained with the illustrated step pulse protocol, in isotonic medium (isotonic), after cell swelling in hypotonic medium (hypotonic) and following the treatment of swollen cells with 10 μM DCPIB (hypotonic + 10 μM DCPIB). (d) $I_{Cl,Ca}$ currents recorded from CPAE cells in response to voltage steps applied every 3 s from a holding potential of −50 mV. (e) $I_{Cl,Ca}$ currents in the presence of 10 μM DCPIB. (f) $I-V$ relationships from currents in (d) and (e). Currents were measured at the end of the voltage steps. (g) $I_{Cl,swell}$ currents of guinea-pig atrial cardiomyocytes. Hypotonic solution was applied for 5–10 min. Atrial currents were measured starting from a holding potential of 0 mV using the illustrated step pulse protocol. (h) $I_{Cl,swell}$ currents in presence of 10 μM DCPIB in the hypotonic bath solution. (i) Corresponding $I-V$ curves.

Table 1 Inhibitory effects of DCPIB on native currents in CPAE cells and heterologously expressed currents in *Xenopus* oocytes

Current/Clone	*% Inhibition* (10 μM DCPIB)	n
*$I_{Cl,swell}$	83.7±4.0	$n=4$
†$I_{Cl,\ swell}$	98.7±5.1	$n=4$
$I_{Cl,Ca}$	4.1±0.3	$n=3$
hCFTR	0.0±0.0	$n=4$
hCLC-1	0.0±0.0	$n=4$
rCLC-2	1.6±1.6	$n=3$
hCLC-4	0.0±0.0	$n=3$
hCLC-5	0.0±0.0	$n=3$
rCLC-K1	5.9±3.4	$n=4$
hKv4.3	1.3±1.5	$n=5$
hKv1.5	2.2±1.2	$n=5$
HERG	1.3±1.0	$n=5$
hminK (I_{Ks})	1.9±0.7	$n=5$

Data given in % inhibition ± s.e.mean at a blocker concentration of 10 μM (n=number of cells). Inhibition values were calculated without (*) and after (†) subtraction of the non-DCPIB sensitive basal conductance. Native $I_{Cl,swell}$ and $I_{Cl,Ca}$ were recorded from CPAE cells. hKv4.3, hKv1.5, HERG, hminK, the hCFTR, and the CLC-chloride channels were expressed in *Xenopus* oocytes.

containing hypotonic solution (Figure 3c) the average increase in cell width was 1.41±0.07 fold ($n=7$) compared to the original isotonic value. Since DCPIB did not decrease cell size, an inhibition of $I_{Cl,swell}$ through attenuation of the hypotonicity-induced cell swelling is very unlikely. Moreover, preincubation of cardiomyocytes with DCPIB did not attenuate subsequent swelling in hypotonic medium. Average cell width after 5 min of preincubation with 10 μM DCPIB was 1.01±0.02% ($n=4$) (Figure 3e) and subsequent cell swelling for 10 min was of the same magnitude as for the swelling in the absence of DCPIB. In DCPIB containing hypotonic solution the cell size increased by a factor of 1.35±0.07 (Figure 3f).

Specificity of DCPIB

To test the specificity for $I_{Cl,swell}$, we first analysed the effects of DCPIB on several Cl^- currents, namely on native $I_{Cl,Ca}$ in CPAE cells, on several members of the CLC-chloride channel family and on human CFTR after heterologous expression in *Xenopus* oocytes. While we could not functionally express hCLC-3 in *Xenopus* oocytes, as has been previously reported (Borsani *et al*., 1995), injection of cRNA encoding the other chloride channels generated currents as previously reported (Bear *et al*., 1991; Drumm *et al*., 1991; Thiemann *et al*., 1992; Uchida *et al*., 1994; Steinmeyer *et al*., 1994; 1995; Friedrich *et al*., 1999). As illustrated in Figure 2d–f, DCPIB (10 μM) did not alter $I_{Cl,Ca}$ in CPAE cells. DCPIB also had no effects on CLC-1, -2, -4, -5, -K1 and hCFTR expressed in *Xenopus* oocytes (Table 1 and Figure 4).

To determine the specificity of DCPIB for cardiac ion currents involved in AP generation and shaping, isolated guinea-pig cardiomyocytes of the atrium and ventricle were treated with 10 μM DCPIB (Figure 5). DCPIB did not affect amplitudes of the following cationic currents: I_{Kr}, I_{Ks}, I_{K1}, I_{Na} and I_{Ca} (Table 2). In addition, we did not observe a shift in $I-V$ relationships of these currents which would also alter APD (data not shown). Similar to hCFTR expressed in *Xenopus* oocytes (Figure 4), $I_{Cl,PKA}$, which is thought to represent the

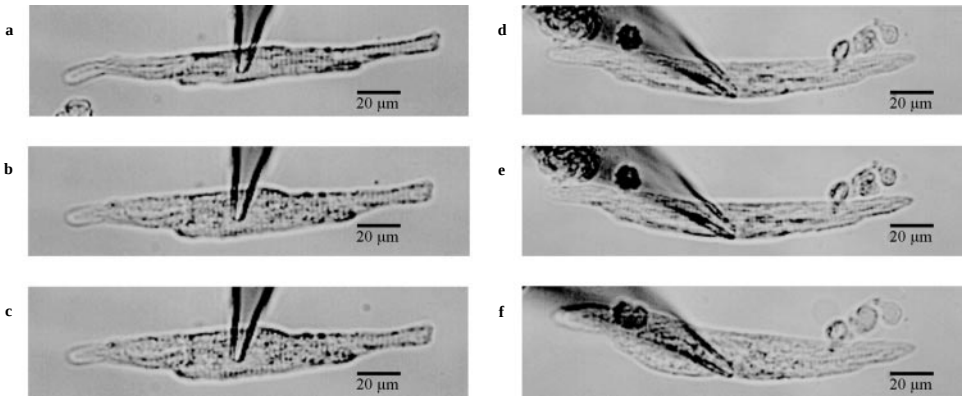

Figure 3 Photographs of guinea-pig atrial cardiomyocytes during whole cell patch clamp recording. Cell width was analysed by averaging the cell width at three different places of the myocyte. (a) In isotonic solution. (b) Following 10 min of hypotonic perfusion and (c) after perfusion (5 min) with 10 μM DCPIB of swollen cells from (b). The figure illustrates that DCPIB did not decrease the cell swelling during hypotonicity. (d) Atrial cardiomyocyte in isotonic solution and (e) after 5 min of preincubation with 10 μM DCPIB and (f) after perfusion with hypotonic medium containing 10 μM DCPIB. Preincubation with DCPIB did not inhibit subsequent cell swelling in hypotonic medium.

Table 2 Inhibitory effects of DCPIB on native currents of guinea-pigs

Cardiac current	*% Inhibition* (10 μM DCPIB)	n
$^{*}I_{Cl,swell}$	72.9±7.4	$n=4$
$^{\dagger}I_{Cl,\ swell}$	85.3±6.9	$n=4$
I_{Ks}	2.1±1.5	$n=4$
I_{Kr}	2.2±1.2	$n=5$
I_{K1}	1.3±1.0	$n=4$
I_{Na}	1.3±0.7	$n=4$
I_{Ca}	2.9±1.8	$n=4$
$I_{Cl,PKA}$	2.2±1.4	$n=4$

Data given in % inhibition ± s.e. mean at a blocker concentration of 10 μM (n=number of cells). Inhibition values were calculated without (*) and after (†) subtraction of the non-DCPIB sensitive basal conductance. Native $I_{Cl,swell}$ was recorded from atrial myocytes. Since $I_{Cl,PKA}$ is more abundant in the ventricle of gp cardiomyocytes (Vandenberg *et al*., 1994) it was analysed using ventricular cells. Other currents were recorded from atrial cardiomyocytes. Similar results were also obtained from ventricular cells.

native cardiac CFTR chloride current, was not inhibited by 10 μM DCPIB (Table 2 and Figure 5). To confirm the data obtained with native currents of guinea-pig cardiomyocytes, we tested the effects of DCPIB (10 μM) on the currents elicited by hKv1.5, hKv4.3, hminK and HERG, which underly the most important repolarizing currents in the human heart (I_{Kur}, I_{to1}, I_{Ks} and I_{Kr}). Again, DCPIB did not alter these currents after expression in *Xenopus* oocytes (Figure 6).

These results demonstrated that DCPIB, at a concentration sufficient to almost completely block $I_{Cl,swell}$, does not affect the major currents involved in the cardiac action potential. Therefore, DCPIB at this concentration is selective for $I_{Cl,swell}$ and is a valuable pharmacological tool to study the role of Cl^- currents in swelling-induced action potential changes.

Effects of cell swelling on $I_{Cl,swell}$, V_{rest} *and APD*

Atrial cells are more sensitive to cell swelling than cardiomyocytes isolated from the ventricle (Vandenberg *et al*., 1994). In agreement with this, $I_{Cl,swell}$ could be activated in 100% ($n=7$) of atrial cells when perfused with 29% hypotonic solution for 5–10 min maximum. In contrast, only 25% ($n=8$) of ventricular cells in hypotonic solution elicited an $I_{Cl,swell}$. Within this time range activation of $I_{Cl,swell}$ was fully reversible on return to isotonic conditions and sensitive to DCPIB (Table 2). Under isotonic conditions the resting membrane potential of atrial cardiomyocytes was −62.9±0.8 mV ($n=13$). APD_{90} was 138.3±13.3 ms ($n=13$) and APD_{50} was 97.8±14.7 ms ($n=13$). Consistent with its ineffectiveness on all tested cardiac currents, DCPIB showed no significant effect on isotonic atrial APD (APD_{90} 105.5±3.3% ($n=6$), APD_{50} 96.5±1.9% ($n=6$)), (Table 3b and Figure 7b). Also, the resting membrane potential was not significantly different when 10 μM DCPIB was present in isoosmotic medium (Table 3b).

Hypotonic perfusion, lasting for the same time period required for maximal activation of $I_{Cl,swell}$, significantly shortened atrial APD (Figure 7a). APD_{90} was shortened to 70.2±3.4% ($n=7$; $P<0.01$) and APD_{50} to 61.3±3.5% ($n=7$; $P<0.01$) of the isotonic value (taken as 100%). Addition of 10 μM DCPIB to hypotonic medium abolished APD shortening (Figure 7a) and APD_{90}, as well as APD_{50}, were not statistically different from the original isotonic values (Table 3a). In some experiments APD_{50} tended to remain slightly shortened, whereas APD_{90} appeared to be increased. However, the overall resulting mean values of the APD_{90} and APD_{50} did not differ statistically from the original values (Table 3a). Thus, in all atrial myocytes ($n=7$), APD shortening occurred during cell swelling and was fully recovered by treatment with DCPIB. The resting membrane potential depolarized only slightly from −62.9±1.2 ($n=7$) to −60.9±1.2 mV ($n=7$) ($P<0.05$) during cell swelling. Addition of DCPIB to swollen cells did not result in a significant repolarization and V_{rest} remained at −61.8±1.1 mV ($P>0.05$). In addition to APD shortening, in some cells (two out of seven) the peak voltage of action potentials decreased during cell swelling. This reduction was almost completely antagonised by 10 μM DCPIB (Figure 7a). To exclude that

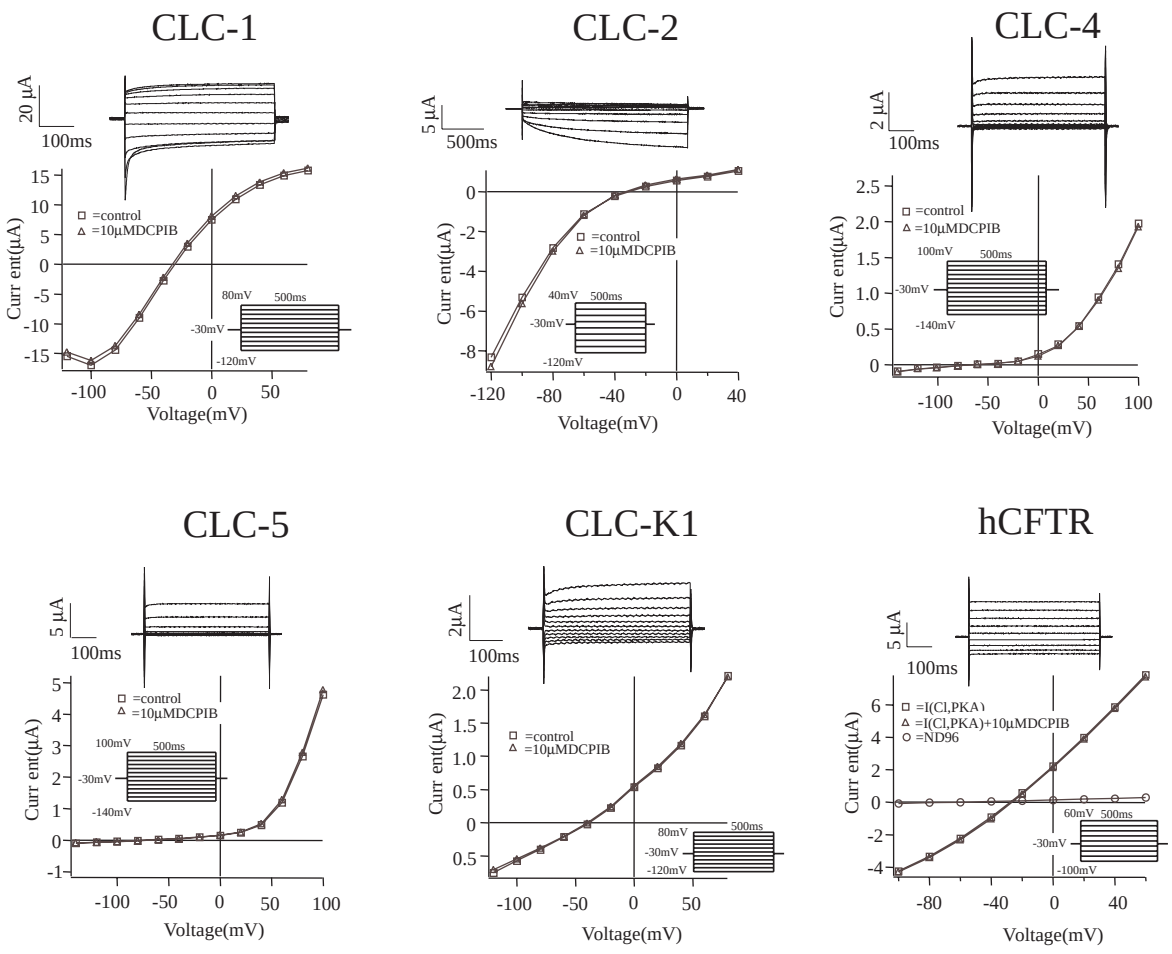

Figure 4 Effects of 10 μM DCPIB on CLC-chloride channels and on hCFTR expressed in *Xenopus* oocytes. hCFTR was activated by 1 mM IBMX and 10 μM forskolin. Currents were elicited starting from a holding potential of -30 mV according to the illustrated step protocols. Original current traces show control currents. $I-V$ relationships were determined from control currents and currents in the presence of DCPIB.

the reduction of the action potential duration was due to the opening of K_{ATP} channels, we applied a triangular ramp pulse protocol to record K_{ATP} currents after cell swelling. However, $I-V$ relationships of resulting currents did not provide any evidence that K_{ATP} channels opened during osmotic cell swelling (data not shown).

In recordings from ventricular cells, initial prolongation of APD was routinely observed during hypoosmotic challenge (Vandenberg *et al*., 1997). This effect was not seen in atrial cells.

Perfusion of atrial myocytes with 10 μM DCPIB in isotonic solution for 5 min completely prevented activation of $I_{Cl,swell}$ (data not shown) and action potential shortening following the switch to hypotonic DCPIB-containing solution (Figure 7b). Even 10 min after the shift to hypotonic solution, a time sufficient to strongly activate $I_{Cl,swell}$, APD was not shortened. In contrast, a slight prolongation was observed and APD_{90} was $128.3\pm7.5\%$ ($n=4$) and APD_{50} was $122.8\pm6.5\%$ ($n=4$) as compared to isotonic conditions ($P=0.03$ for APD_{90}, $P=0.04$ for APD_{50}) (Table 3b). However, in the prolonged presence of DCPIB, the resting membrane potential was not significantly changed during osmotic swelling of cardiomyocytes.

Effects of DCPIB on I_{Ks} *during cell swelling*

To rule out that DCPIB blocks general mechanisms of ion channel activation triggered by cell swelling, we tested whether DCPIB inhibits the swelling-enhanced I_{Ks} currents (Rees *et al*., 1995). We also tested whether DCPIB affected I_{Ks} under hypotonic conditions, since a stronger inhibition of I_{Ks} during cell swelling could also have contributed to the observed APD lengthening. The I_{Ks} measurements during cell swelling were performed as reported previously (Rees *et al*., 1995). Cells were swollen under Ca^{2+}-free conditions to shift activation of I_{Kr} to more negative potentials and activation of I_{Ks} to more positive values (Sanguinetti & Jurkiewicz, 1992; Jurkiewicz & Sanguinetti, 1993). In addition, 0.2 μM dofetilide was added to block remaining I_{Kr}. Control I_{Ks} currents measured at 4.9 s of the voltage step were 0.71 ± 0.08 nA ($n=6$) and cell swelling caused an increase by $137.1\pm35.6\%$ ($n=3$) to 1.68 ± 0.31 nA (Figure 8), similar to previous findings (Rees *et al*., 1995). DCPIB (10 μM) was not able to block I_{Ks} after cell swelling occurred (Figure 8a).

In the presence of DCPIB, I_{Ks} also increased during swelling by $140.3\pm31.2\%$ ($n=3$) (control current of

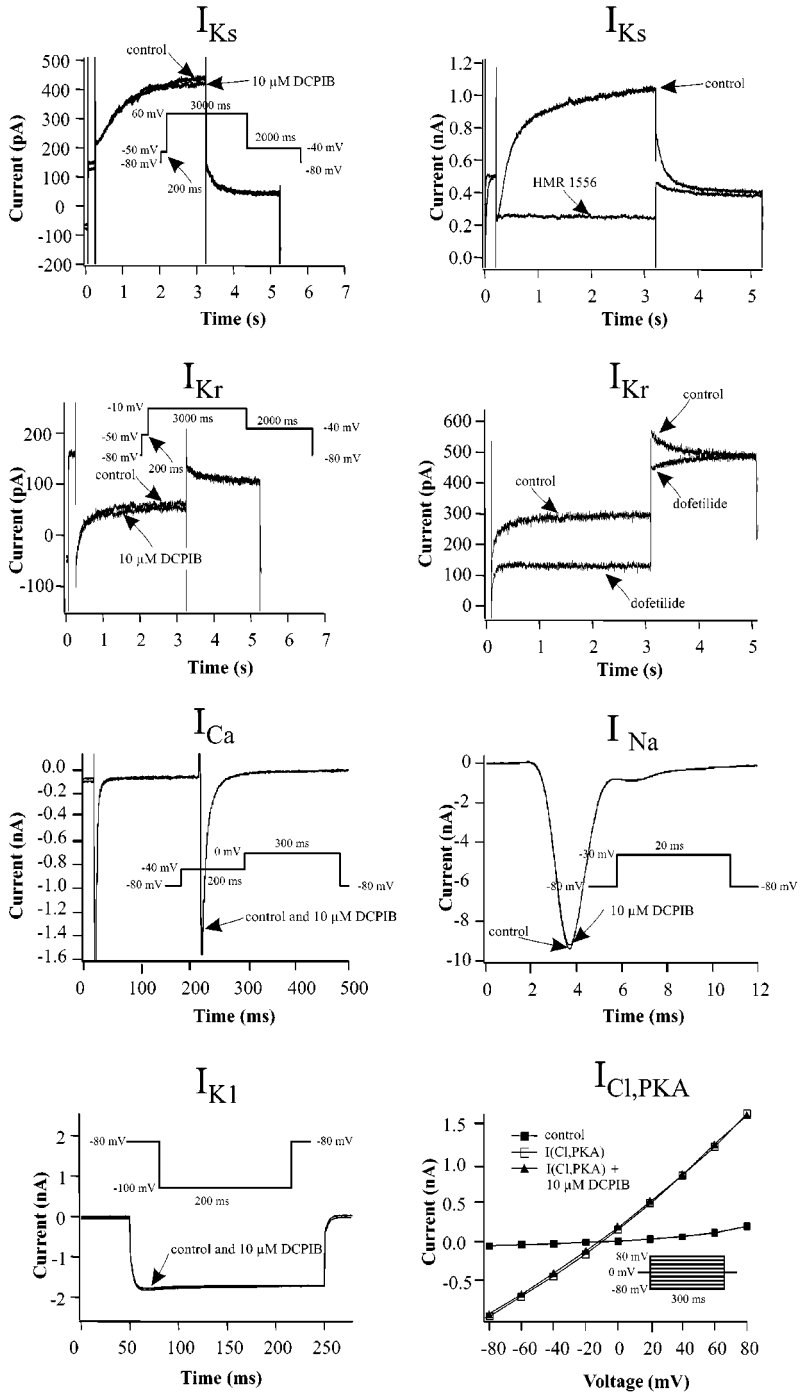

Figure 5 Influence of 10 μM DCPIB on currents of guinea-pig cardiomyocytes. Holding potential in whole cell patches was routinely -80 mV, but 0 mV for measurement of $I_{Cl,PKA}$. Inhibition of I_{Kr} was analysed from the tail currents at -40 mV. I_{Ks} inhibition data were obtained from the steady state current at $+60$ mV. To show that the used voltage protocols (Bosch *et al.*, 1998) can separate I_{Kr} and I_{Ks} we tested their inhibition by 0.2 μM dofetilide and 1.0 μM HMR 1556, respectively. I_{K1} inhibition values were obtained at -100 mV. I_{Na} inward currents were analysed by stepping from the holding potential to -30 mV for 20 ms, with a frequency of 1 Hz. I_{Ca} was studied by a step protocol to analyse its peak inward current. The pulses were applied every 15 s to minimize rundown. $I_{Cl,PKA}$ was activated by isoprenaline (1 μM) or alternatively IBMX (500 μM).

0.68 ± 0.12 to 1.63 ± 0.35 nA). Thus, DCPIB was not able to inhibit the swelling-induced increase in I_{Ks} current amplitude (Figure 8b). The exact mechanism of $I_{Cl,swell}$ block by DCPIB is not yet known. These results, together with the cell size measurements, speak in favour of a blockade of $I_{Cl,swell}$ itself and against inhibition of a general swelling-induced mechanism of ion channel activation.

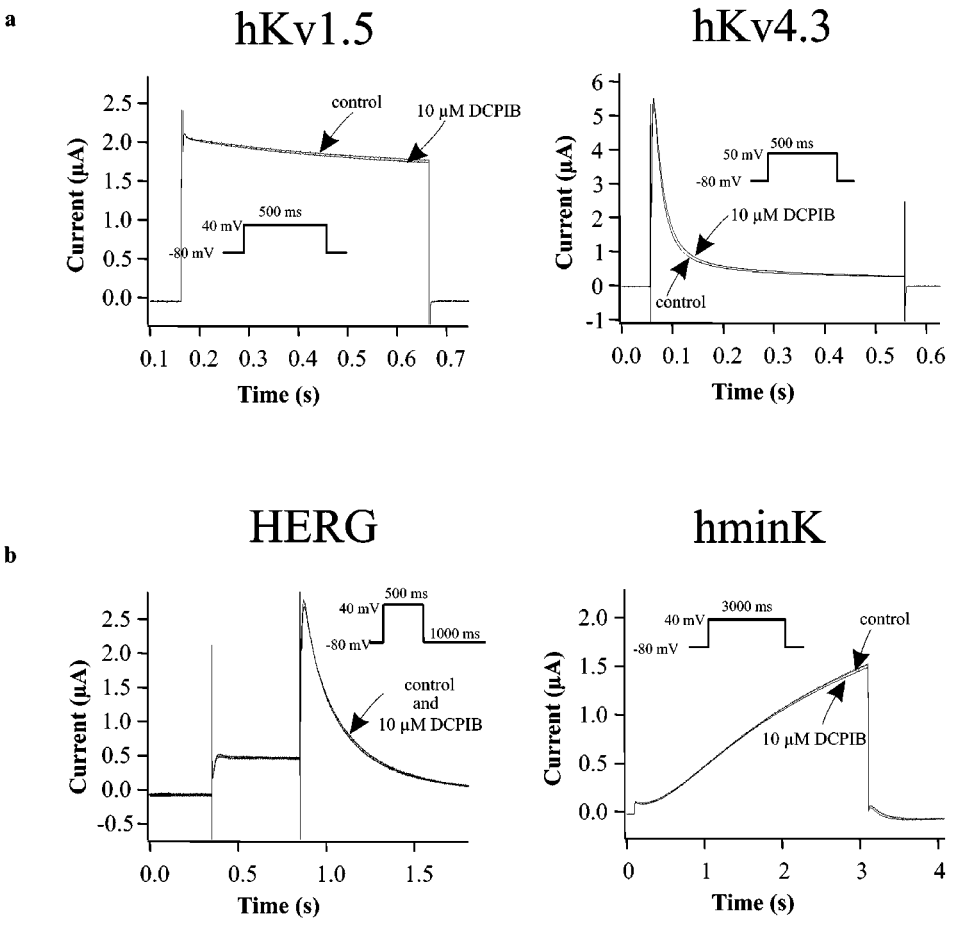

Figure 6 Effects of 10 μM DCPIB on human Kv and delayed rectifier channels, which encode the most important repolarizing K^+ currents in the human heart. The channels were expressed in *Xenopus* oocytes. Holding potential was −80 mV. (a) Current traces of hKv1.5 (I_{Kur}) and hKv4.3 (I_{to1}) before (control) and subsequent to the addition of 10 μM DCPIB. (b) Original current traces of HERG (I_{Kr}) and hminK (I_{Ks}) injected *Xenopus* oocytes in ND96 (control) and in the presence of 10 μM DCPIB.

Table 3 Action potential duration and membrane potential

(a)	*Isotonic*		*Hypotonic*			*Hypotonic + 10 μM DCPIB*		
APD_{90} (ms)	APD_{50} (ms)	V_{rest} (mV)	APD_{90} (%)**	APD_{50} (%)**	V_{rest} (mV)*	APD_{90} (%)	APD_{50} (%)	V_{rest} (mV)
138.3±13.3 $n=13$	97.8±14.7 $n=13$	−62.9±1.2 $n=7$	70.2±3.4 $n=7$	61.3±3.5 $n=7$	−60.9±1.2 $n=7$	112±6.8 $n=7$	101.2±5.2 $n=7$	−61.8±1.1 $n=7$
(b)	*Isotonic*		*Isotonic + 10 μM DCPIB*			*Hypotonic + 10 μM DCPIB*		
APD_{90} (ms)	APD_{50} (ms)	V_{rest} (mV)	APD_{90} (%)	APD_{50} (%)	V_{rest} (mV)	APD_{90} (%)*	APD_{50} (%)*	V_{rest} (mV)
138.3±13.3 $n=13$	97.8±14.7 $n=13$	−62.9±1.0 $n=6$	105.5±3.3 $n=6$	96.5±1.9 $n=6$	−61.9±1.3 $n=6$	128.3±7.5 $n=4$	122.8±6.5 $n=4$	−61.1±1.7 $n=4$

Values are means±s.e. mean (n=number of cells). APD, action potential duration; V_{rest}, resting membrane potential. Changes that are statistically different to the original values in isotonic medium are indicated by an asterisk. *$P<0.05$ compared with isotonic values. **$P<0.01$ compared with isotonic values. (a) APD and V_{rest} in isotonic solution, after exposure of atrial cardiomyocytes for 10 min to a hypotonic solution, and after treatment of swollen cells with 10 μM DCPIB. DCPIB was able to reverse swelling-induced APD shortening. (b) Cells were preincubated with 10 μM DCPIB in isotonic solution for 5 min and subsequently challenged for 10 min by hypotonic medium containing 10 μM DCPIB. DCPIB did not affect APD in isotonic solution and was able to prevent swelling-induced APD shortening.

Discussion

In almost every cell, swelling is linked to the activation of an outwardly rectifying chloride current, $I_{Cl,swell}$, that is possibly involved in subsequent regulatory volume decrease (RVD). In excitable cells activation of this chloride conductance may additionally interfere with their electrical activity. Since chloride equilibrium potential is close to E_{rest}

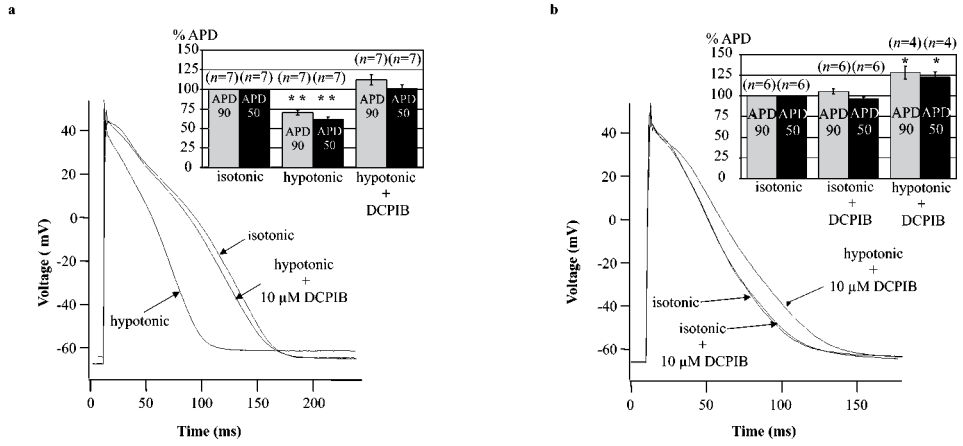

Figure 7 Influence of hypotonicity and 10 μM DCPIB on atrial action potentials. (a) Individual action potential recordings of control cells (isotonic) were compared with AP traces from swollen cells (hypotonic), obtained by perfusion of cells with hypotonic medium for 5–10 min. Hypotonic solution caused a marked reduction in APD. Subsequent addition of 10 μM DCPIB reversed APD shortening (hypotonic + 10 μM DCPIB). Attenuation of the peak voltage under hypotonic medium was only observed in two out of seven cells. *Inset*: The histogram illustrates the results displayed in Table 3a. Action potential durations were normalized and expressed as percentage of the isotonic APD. $^{**}P<0.01$ compared with isotonic values. (b) Prevention of swelling-induced atrial action potential shortening by 10 μM DCPIB. Individual action potentials were recorded from atrial myocytes after perfusion with 10 μM DCPIB in isotonic solution for 5 min. DCPIB did not affect APD under isotonic conditions (compare isotonic *vs* isotonic + 10 μM DCPIB). Subsequent treatment with hypotonic medium containing 10 μM DCPIB for 5–10 min did not result in APD shortening (hypotonic + 10 μM DCPIB). *Inset*: The histogram illustrates the results displayed in Table 3b. Action potential durations were normalized to isotonic conditions and expressed as percentage of the isotonic values. $^{*}P<0.05$ compared with isotonic values.

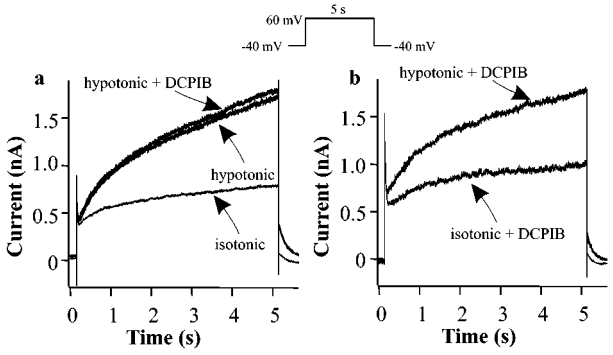

Figure 8 Effects of DCPIB on swelling-enhanced I_{Ks} currents. I_{Ks} was evoked by stepping from a holding potential of -40 to $+60$ mV for 5 s under Ca^{2+}-free conditions. (a) Representative I_{Ks} current traces in isotonic solution (isotonic), after cell swelling caused by hypotonic perfusion (hypotonic) and after addition of 10 μM DCPIB to the hypotonic solution (hypotonic + DCPIB). (b) Representative I_{Ks} current traces in the presence of 10 μM DCPIB in the isotonic solution (isotonic + DCPIB) and after cell swelling in the presence of DCPIB (hypotonic + DCPIB).

and $I_{Cl,swell}$ is an outwardly rectifying current, opening of $I_{Cl,swell}$ should enhance repolarization of the action potential and thereby lead to a shortened action potential duration. The resulting decrease in effective refractory period might favour reentrant arrhythmias (Hiraoka *et al*., 1998; Mulvaney *et al*., 2000). In particular, ischaemia and subsequent reperfusion of affected cardiac tissue cause swelling of cardiomyocytes and therefore will most likely activate $I_{Cl,swell}$, which in turn will result in differences in action potential duration and excitability of ischaemic compared to non ischaemic tissue (Hiraoka *et al*., 1998). The increased dispersion of refractoriness associated with this pathophysiological state might render the heart arrhythmic.

Several studies have been dedicated to the possible pathophysiological role of $I_{Cl,swell}$ in the heart. However, conclusions drawn from these studies are hampered by the use of rather unspecific and less potent drugs. Here, we investigated the possibility that DCPIB is a potent and selective blocker of $I_{Cl,swell}$. It is structurally related to indanyloxyacetic acid, a known blocker of chloride channels in epithelial cells (Landry *et al*., 1989). Here we demonstrate for the first time that DCPIB is a potent blocker of $I_{Cl,swell}$ in various cells. DCPIB was very effective in preventing adenosine-stimulated astroglial cell swelling (Bourke *et al*., 1981) and while a possible inhibition of a coupled Cl^--cation transport pathway has been discussed, to our knowledge, the precise target of DCPIB in this experimental setting has never been identified. It also remains unclear whether the corresponding transport protein is present in atrial cardiomyocytes and if so, whether it is able to modulate APD. The finding that DCPIB did not alter isotonic APD does not strictly rule out its presence in cardiomyocytes, since it is possibly only activated during cell swelling. However, in contrast to its effects on astroglial cells, DCPIB did not inhibit swelling of cardiomyocytes, making it rather unlikely that this Cl^--cation transport pathway is present in atrial cardiomyocytes. Moreover, our finding that DCPIB inhibits $I_{Cl,swell}$ suggests that blocking of $I_{Cl,swell}$ might somehow have contributed to the beneficial effects of DCPIB treatment in astroglial cell swelling.

The IC_{50} for block of $I_{Cl,swell}$ in endothelial cells was 4.1 μM. Thus, DCPIB is one of the most potent known inhibitors for this current. More importantly and in contrast to classical chloride channel blockers, like DIDS, NPPB, niflumic acid and 9-AC, DCPIB seems to be selective for $I_{Cl,swell}$ at 10 μM. Even the structurally related compound IAA-94, also a known

blocker of $I_{Cl,swell}$, is not suitable as a pharmacological tool because of its extremely slow time course of inhibition (up to 20 min) and its nonspecific effects on action potential configuration in the absence of cell swelling (Sorota, 1994). Since block by DCPIB is voltage independent, observed changes in APD at different membrane potentials reflect the genuine influence of $I_{Cl,swell}$ on APD, rather than the different effects caused by voltage dependent blockers.

In contrast to known chloride channel blockers, we did not observe effects of DCPIB (10 μM) on various other chloride channels. Since it also showed no effects on several cardiac currents that essentially generate and shape the action potential, we regarded it as a useful tool to analyse action potential alterations during osmotic cell swelling. A further prerequisite was that DCPIB did not affect the cardiac action potential under isotonic conditions. Although we did not individually test all cardiac ion currents involved in action potential shaping, or transporters and exchangers which, under certain conditions, may also modulate the action potential, this result is consistent with the proposed specificity of DCPIB. It also supports the observation that $I_{Cl,swell}$ is only weakly active under isoosmotic conditions in isolated cardiac cells.

Previous studies have already demonstrated that swelling causes slight changes in resting membrane potential and a reduction of APD in ventricular cells (Vandenberg *et al*., 1997). Here we studied the role of $I_{Cl,swell}$ in the AP of atrial cells since $I_{Cl,swell}$ seems to be preferentially and possibly more strongly expressed in atrial cells and atrial swelling was always accompanied by strong action potential shortening. Shortening of APD_{50} was generally stronger than that of APD_{90}, which can be explained in part by the outward rectification of $I_{Cl,swell}$. Furthermore, the APD_{50} is evaluated at a potential positive to the chloride equilibrium potential (E_{Cl}) in cardiomyocytes, whereas APD_{90} emerges at a potential very close to E_{Cl}. Since estimates of the Cl^- activity, a^i_{Cl}, in cardiac muscle place E_{Cl} in the range of -71 to -45 mV under normal physiological conditions (Walker, 1986; Harvey & Hume, 1989), driving force of both inward and outward Cl^- currents near E_{Cl} is very low. This results in an APD shortening over the whole voltage range but to a lesser extent during the late phase of repolarization. The narrow distance of the reported value of E_{Cl} to the recorded V_{rest} of the atrial cardiomyocytes in our experiments partly explains the weak depolarization observed during cell swelling. Harvey & Hume (1989) suggested that chloride currents in cardiomyocytes, which are dialysed with 22 mM Cl^- in a 5.4 mM external K^+ solution, contribute little to the resting membrane conductance because it is dominated by the inward rectifier K^+ current I_{K1}. Consistent with the results by Yamawake *et al*. (1992), the observed effects on the atrial membrane potential might have been more pronounced with a lower $[K^+]_o$ present in our study, since this is expected to reduce the conductance of I_{K1} channels (Sakmann & Trube, 1984). Vandenberg *et al*. (1997) observed a depolarization of ventricular cardiomyocytes during hypotonic perfusion of 3.5 mV (Group *A*), a value which is very close to the 2.0 mV depolarization we measured for atrial cells. However, the depolarization in ventricular cardiomyocytes (Vandenberg *et al*., 1997) occurred also in cells which lacked a DIDS-sensitive component of APD shortening during cell swelling.

Atrial APD shortening was highly sensitive to DCPIB and inhibition of $I_{Cl,swell}$ by DCPIB always caused a reversal of APD shortening, though the achieved effectivity varied slightly from cell to cell. In some cells, a slight shortening of the APD_{50} persisted and a weakly increased APD_{90} remained, in comparison to the original isotonic values, while in other cells, both APD_{50} and APD_{90} were not fully reversed to isotonic APD. These weak differences in the resulting APD might be caused by changes in currents other than $I_{Cl,swell}$ during osmotic swelling. Also, alterations in voltage trajectory or dilutional changes of intracellular K^+ and Cl^- concentrations might influence repolarizing K^+ currents or the E_{Cl}. However, the observed differences in APD were not statistically significant (Table 3a).

In addition to these variations, cell swelling also causes changes in other ionic currents, that also could influence APD: enhancement of I_{Ks} (Rees *et al*., 1995) would lead to decreased APD, while inhibition of I_{Kr} (Rees *et al*., 1995) or I_{Ca} augmentation would lead to prolonged APD. The sum of these opposing changes during cell swelling could explain why action potential shortening in isolated atrial guinea-pig cardiomyocytes due to cell swelling is primarily caused by activation of $I_{Cl,swell}$ and can be fully reversed by DCPIB.

In contrast to our results from atrial cells, full block of $I_{Cl,swell}$ in ventricular cells by DIDS was only capable of reversing 50% of swelling-induced APD shortening (Vandenberg *et al*., 1997), suggesting that in ventricular myocytes activation of $I_{Cl,swell}$ is not the only mechanism contributing to APD shortening during osmotic cell swelling (Vandenberg *et al*., 1997). However, several points must be considered that could explain these different conclusions: (1) DIDS has been shown to activate currents like I_{Ks} and $I_{Cl,PKA}$ (Harvey, 1993; Busch *et al*., 1994), which would also cause APD shortening; (2) Hypotonic perfusion of ventricular cells caused an initial lengthening of the action potential (Vandenberg *et al*., 1997), which was not observed in atrial cardiomyocytes; (3) In these experiments cells were only swollen for 3 min and only showed an APD shortening of 27% of the isotonic value. Considering the slow time course of $I_{Cl,swell}$ activation, its contribution to APD shortening should further increase with time.

However, the contribution of $I_{Cl,swell}$ to APD shortening might differ between atrium and ventricle and also depend on the experimental conditions. Particularly, I_{KATP} has been shown to be the main current causing swelling-induced APD shortening in ventricular cardiomyocytes in the presence of low intracellular ATP (Priebe & Beuckelmann, 1998).

It is known that several currents change during cell swelling (Vandenberg *et al*., 1996), whereas the time course of the changes for each current is not yet known and may also differ from species to species and between atrium and ventricle. The observed increase in atrial APD caused by cell swelling in sustained presence of $I_{Cl,swell}$ blockers (Figure 7b), is similar to that previously observed in ventricular cells (Vandenberg *et al*., 1997). It might in part be due to swelling-induced inhibition of the fast delayed rectifier K^+ current (Rees *et al*., 1995) and an increase in the L-type Ca^{2+} current (Matsuda *et al*., 1996). This increase in APD might only become visible during the block of $I_{Cl,swell}$. In addition, as mentioned above, dilutional changes will also influence many currents and ion transport pathways by altering the reversal potentials of the individual ions. Furthermore, sustained inhibition of $I_{Cl,swell}$ during continued cell swelling might interact with RVD and lead to the activation of unknown compensatory mechanisms. Therefore, several mechanisms could explain the observed deviation of action potential

length during prolonged cell swelling and simultaneous $I_{Cl,swell}$ blockage.

We show that DCPIB is a potent, selective blocker of $I_{Cl,swell}$ in various tissues. Moreover, we provide the first analysis concerning the influence of $I_{Cl,swell}$ in the atrial action potential, from which we conclude that, under the used experimental conditions, swelling-induced atrial AP shortening is primarily caused by activation of $I_{Cl,swell}$. Therefore, DCPIB is a valuable tool for comparative studies of $I_{Cl,swell}$ effects on atrial and ventricular APD and to further our understanding of the role of $I_{Cl,swell}$ in cardiac physiology and pathophysiology.

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