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Selective block of swelling-activated Cl^- channels over cAMP-dependent Cl^- channels in ventricular myocytes

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Abstract

The objective of this study on guinea-pig and rabbit ventricular myocytes was to evaluate the sensitivities of swelling-activated Cl^- current ($I_{Cl(swell)}$) and cAMP-dependent cystic fibrosis transmembrane regulator (CFTR) Cl^- current ($I_{Cl(CFTR)}$) to block by dideoxyforskolin and verapamil. The currents were recorded from whole-cell configured myocytes that were dialysed with a Cs^+-rich pipette solution and superfused with either isosmotic Na^+-, K^+-, Ca^{2+}-free solution that contained 140 mM sucrose or hyposmotic sucrose-free solution. Forskolin-activated $I_{Cl(CFTR)}$ was inhibited by reference blocker anthracene-9-carboxylic acid but unaffected by ≤ 200 μM dideoxyforskolin and verapamil. However, dideoxyforskolin and verapamil had strong inhibitory effects on outwardly-rectifying, inactivating, distilbene-sensitive $I_{Cl(swell)}$; IC_{50} values were ≈ 30 μM, and blocks were voltage-independent and reversible. The results establish that dideoxyforskolin and verapamil can be used to distinguish between $I_{Cl(CFTR)}$ and $I_{Cl(swell)}$ in heart cells, and expand the pharmacological characterization of cardiac $I_{Cl(swell)}$.

Keywords: (Guinea-pig); (Rabbit); Verapamil; Dideoxyforskolin; Anthracene-9-carboxylic acid; Stilbene disulphonate

1. Introduction

There are two major types of chloride current (I_{Cl}) in guinea-pig ventricular myocytes. The first type, $I_{Cl(CFTR)}$, is carried by the cardiac isoform of the cystic fibrosis transmembrane regulator (CFTR), and is activated in response to stimulation of protein kinase A (PKA) (for reviews, see Gadsby and Nairn, 1999; Hume et al., 2000; Hume and Horowitz, 1995); the second type, $I_{Cl(swell)}$, is activated in response to hyposmotic cell swelling (for reviews, see Baumgarten and Clemo, 2003; Sorota, 1999). Both $I_{Cl(CFTR)}$ and $I_{Cl(swell)}$ are likely to have important roles in modifying cardiac electrical activity and regulating cell volume under physiological and pathophysiological conditions (Baumgarten and Clemo, 2003; Hiraoka et al., 1998; Sorota, 1999; Vandenberg et al., 1997; Wong et al., 1999).

There are well-characterized differences in the properties of cardiac $I_{Cl(swell)}$ and $I_{Cl(CFTR)}$, including their responses to classical Cl^- channel blockers (Baumgarten and Clemo, 2003; Hiraoka et al., 1998). For example, anthracene-9-carboxylic acid inhibits both $I_{Cl(swell)}$ (Vandenberg et al., 1994) and $I_{Cl(CFTR)}$ (Harvey et al., 1990; Kocic et al., 2001), whereas DIDS (4,4′-diisothiocyanostilbene-2,2′-disulphonic acid) and tamoxifen are selective blockers of $I_{Cl(swell)}$ (Vandenberg et al., 1994). Unfortunately, the actions of the latter two compounds are difficult to reverse unless exposures are kept very short. In a search for reversible selective inhibitors of cardiac $I_{Cl(swell)}$ over $I_{Cl(CFTR)}$, it seemed worthwhile to evaluate the activities of verapamil and dideoxyforskolin because despite the fact that there was no information on possible effects on CFTR channels, they had at least been shown to inhibit $I_{Cl(swell)}$ in some non-cardiac cells (e.g., Anderson et al., 1995; Diaz et al., 1993; Fatherazi et al., 1994; von Weikersthal et al., 1999). Thus, the present study on guinea-pig and rabbit ventricular myocytes was conducted to determine the concentration-dependent effects of verapamil and dideoxyforskolin on $I_{Cl(swell)}$ and $I_{Cl(CFTR)}$.

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2. Materials and methods

2.1. Preparation of ventricular myocytes

All procedures were carried out in accordance with national and university regulations on the care and treatment of laboratory animals. Male guinea-pigs (ca. 250 g) were killed by cervical dislocation, and male New Zealand White rabbits (ca. 1.5 kg) were anaesthetized by injection of sodium pentabarbitone (40 mg kg^{-1}) into a marginal ear vein. Hearts were excised, mounted on a Langendorff column, and perfused through the aorta with warmed (37 °C) normal Tyrode's solution (see below), Ca^{2+}-free Tyrode's, Ca^{2+}-free Tyrode's containing collagenase (0.05–0.1 mg/ml; Yakult, Tokyo, Japan), and storage solution (see below). The ventricles were cut into chunks, and the cells were mechanically dispersed and kept in storage solution at room temperature.

2.2. Electrophysiological recordings

A few drops of cell suspension were placed in a 0.3-ml perfusion chamber mounted on an inverted microscope stage. After the cells had settled on the bottom, the chamber was perfused (~ 4 ml/min) with normal Tyrode's solution and then with test solutions. Whole-cell membrane currents were recorded using an EPC-7 voltage-clamp amplifier (Heka Elektronik, Lambrecht, Pfalz, Germany). Recording pipettes were fabricated from thick-walled borosilicate glass capillaries (Jencons Scientific, Bedfordshire, UK) and had resistances of 2–3 MΩ when filled with solution (see below); liquid junction potentials between external and pipette-filling solution were nulled prior to patch formation. The reference electrode was a flowing 3 M KCl, Ag-AgCl reference electrode located downstream of patched myocytes. Series resistance ranged between 3 and 7 MΩ, and was compensated by 60–80%. Membrane current signals were filtered at 3 kHz and digitized with an A/D converter

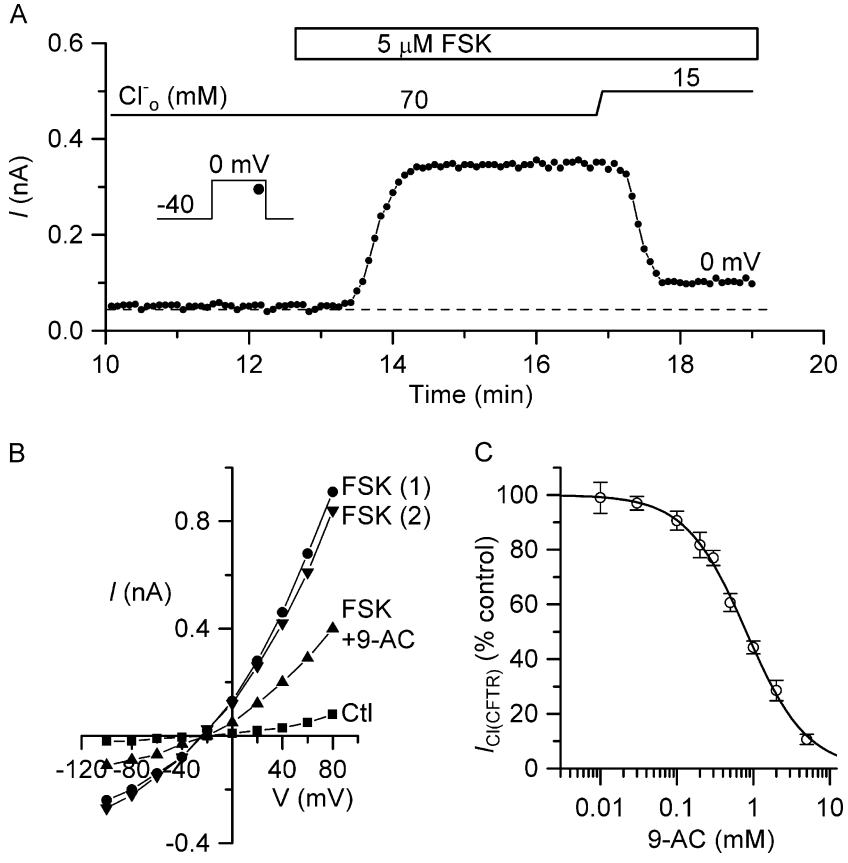

Fig. 1. Properties of forskolin-activated $I_{Cl(CFTR)}$ in guinea-pig ventricular myocytes. The myocytes were superfused with 1 T solutions, and pulsed from −40 to 0 mV for 200 ms except for determination of $I–V$ relations. (A) Effect of lowering external Cl^- (Cl^-_o) from 70 to 15 mM on forskolin-activated current in a myocyte dialysed with 10 mM Cl^- pipette solution. (B) Block of forskolin-activated current by 1 mM anthracene-9-carboxylic acid. The myocyte was dialysed with standard (30 mM Cl^-) pipette solution, and superfused with standard (70 mM Cl^-) external solution; the $I–V$ relations were obtained before (Ctl), during stable activation of $I_{Cl(CFTR)}$ by 5 μM forskolin (FSK (1)), after addition of 1 mM anthracene-9-carboxylic acid (9-AC) for 5 min, and after a 10-min washout with forskolin solution (FSK (2)). (C) Concentration-dependent inhibition of $I_{Cl(CFTR)}$ by anthracene-9-carboxylic acid. Solutions were as in panel B, currents were measured at the end of pulses to 0 mV, and the amplitude of $I_{Cl(CFTR)}$ was determined as forskolin-stimulated current minus pre-forskolin background current. Myocytes were treated with a single concentration of anthracene-9-carboxylic acid for 5–7 min to evaluate inhibition of $I_{Cl(CFTR)}$, and the data ($n = 3–8$ at each concentration) are described by the Hill equation with an IC_{50} of 0.8 ± 0.02 mM and a coefficient of 1.1.

(Digidata 1200A, Axon Instruments, Foster City, CA) and pCLAMP software (Axon Instruments) at a sampling rate of 8 kHz prior to analysis. All experiments were conducted at 36 ± 0.2 °C.

2.3. Solutions

Normal Tyrode's solution contained (in mM) NaCl 140, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, glucose 10, and HEPES 5 (pH 7.4 with NaOH), and $CaCl_2$ was omitted to make Ca^{2+}-free Tyrode's. Storage solution contained (in mM) 30 KCl, 50 KOH, 50 glutamic acid, 30 KH_2PO_4, 3 $MgSO_4$, 20 taurine, 10 glucose, 0.5 EGTA, and 10 HEPES (pH 7.4). After initial equilibration in Tyrode's solution, myocytes were superfused with isosmotic (1 T) Na^+-, K^+-, Ca^{2+}-free solution (290–300 mosM) that contained (in mM) 65 trimethylammonium (TMA)-Cl or *N*-methyl-D-glucamine (NMDG)-Cl, 1.3 $MgCl_2$, 1 $BaCl_2$, 0.2 $CdCl_2$, 10 glucose, 140 sucrose, and 5 HEPES (pH 7.4). In some experiments, the Cl^- in 1 T solution was lowered from 70 to 15 mM (replacement by aspartate). Hyposmotic solution (157 mosM) of approximately one-half (0.5 T) normal osmolarity (1 T) was made by omitting sucrose from the 1 T solution.

Standard pipette-filling solution (290 mosM) contained (in mM) 30 CsCl, 110 Cs aspartate, 5 Mg-ATP, 5 EGTA, and 5 HEPES (pH 7.2). In some experiments, Cl^- was lowered to 10 mM (replacement by aspartate).

2.4. Drugs

Verapamil, anthracene-9-carboxylic acid, DIDS, and dimethyl sulphoxide (DMSO) were obtained from Sigma (St. Louis, MO, USA), and forskolin and dideoxyforskolin were obtained from Calbiochem (La Jolla, CA, USA). Verapamil was prepared as an aqueous stock solution; DIDS was prepared as a 250 mM stock solution in DMSO, stored in a light-proof container, and diluted in the external solution; dideoxyforskolin was also prepared in DMSO

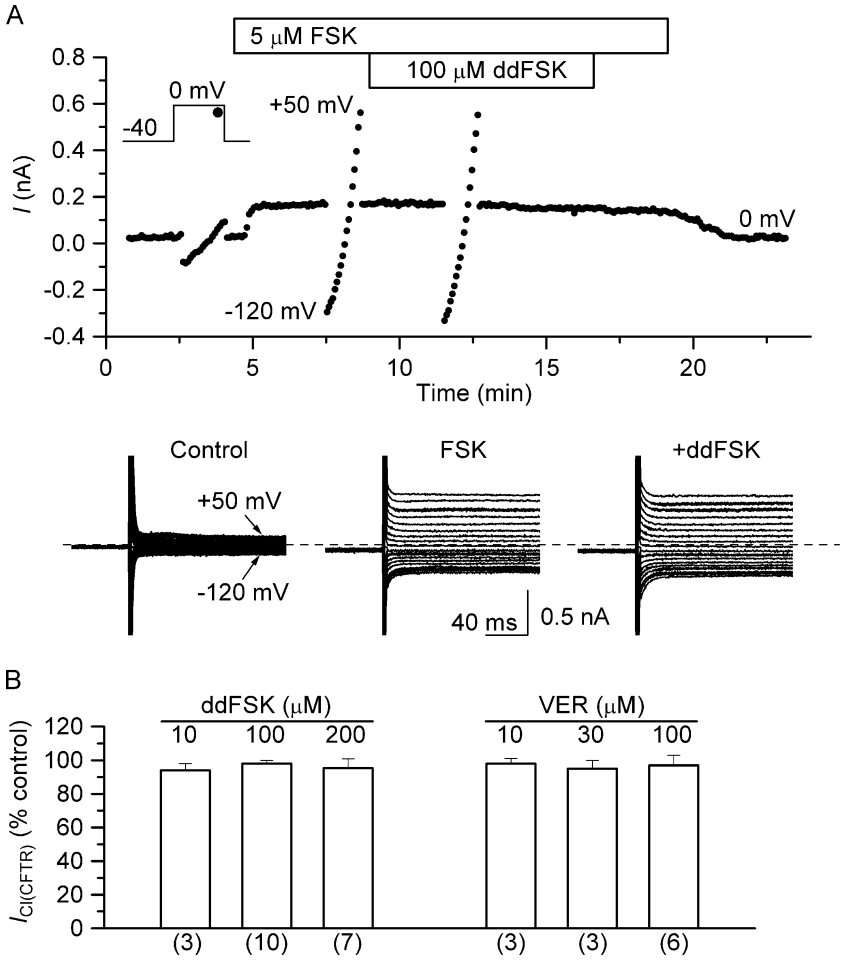

Fig. 2. Effects of dideoxyforskolin and verapamil on forskolin-activated $I_{Cl(CFTR)}$ in guinea-pig ventricular myocytes. Standard solutions were used, and myocytes were pulsed from −40 to 0 mV except for determination of *I–V* relations. Current amplitudes were measured at the end of the 200 ms pulses. (A) Lack of effect of 100 μM dideoxyforskolin (ddFSK) on $I_{Cl(CFTR)}$. Top: time course of changes in current amplitude at 0 mV, as well as readouts of current amplitudes on three *I–V* runs. Bottom: current records obtained on the *I–V* runs. The dashed line indicates zero-current level. (B) Summary of effects of dideoxyforskolin and verapamil (VER) on $I_{Cl(CFTR)}$ measured at 0 mV. Myocytes were treated with a single concentration of drug for 5–8 min. Number of myocytes in parentheses.

such that the final concentration of DMSO in bathing solutions ranged up to 0.2% (200 μM dideoxyforskolin). This concentration of DMSO had no significant effect on $I_{Cl(CFTR)}$ or $I_{Cl(swell)}$ ($n=4$ each) (see also Sakaguchi et al., 1997 for lack of effect of 1% DMSO on $I_{Cl(swell)}$ in guinea-pig atrial myocytes).

2.5. Statistics

The results are expressed as means ± S.E.M.

3. Results

3.1. Effects of anthracene-9-carboxylic acid, dideoxyforskolin and verapamil on $I_{Cl(CFTR)}$

$I_{Cl(CFTR)}$ in guinea-pig ventricular myocytes was investigated under conditions in which cation-carried currents were suppressed by use of isosmotic (1 T) Na^+-, K^+-, Ca^{2+}-free external solution that contained 0.2 mM Cd^{2+}, and Na^+-, K^+, Ca^{2+}-free pipette solution that contained 5 mM EGTA. The myocytes were held at −40 mV, and depolarised to test potential 0 mV for 200 ms at ≈0.1 Hz except for determination of current–voltage (I–V) relations. Forskolin (5 μM) was used to activate cAMP-dependent $I_{Cl(CFTR)}$, and the amplitude of the forskolin-activated current was obtained by subtracting (pre-forskolin) background current from steady-state (forskolin) current. As expected under these experimental conditions, the forskolin-activated current was strongly dependent on the magnitude of the calculated Cl^- equilibrium potential (E_{Cl}). For example, when E_{Cl} in myocytes dialysed with 10 mM Cl^- solution was lowered from −51 to −11 mV by lowering external Cl^- from 70 to 15 mM, the amplitude of forskolin-activated $I_{Cl(CFTR)}$ at 0 mV was reduced by 83 ± 3% ($n=6$) (Fig. 1A).

Anthracene-9-carboxylic acid is known to block $I_{Cl(CFTR)}$ in guinea-pig ventricular myocytes (Harvey et al., 1990; Kocic et al., 2001; Walsh and Wang, 1996), and we have used it as a reference blocker in the present study. Myocytes dialysed with standard (30 mM Cl^-) pipette

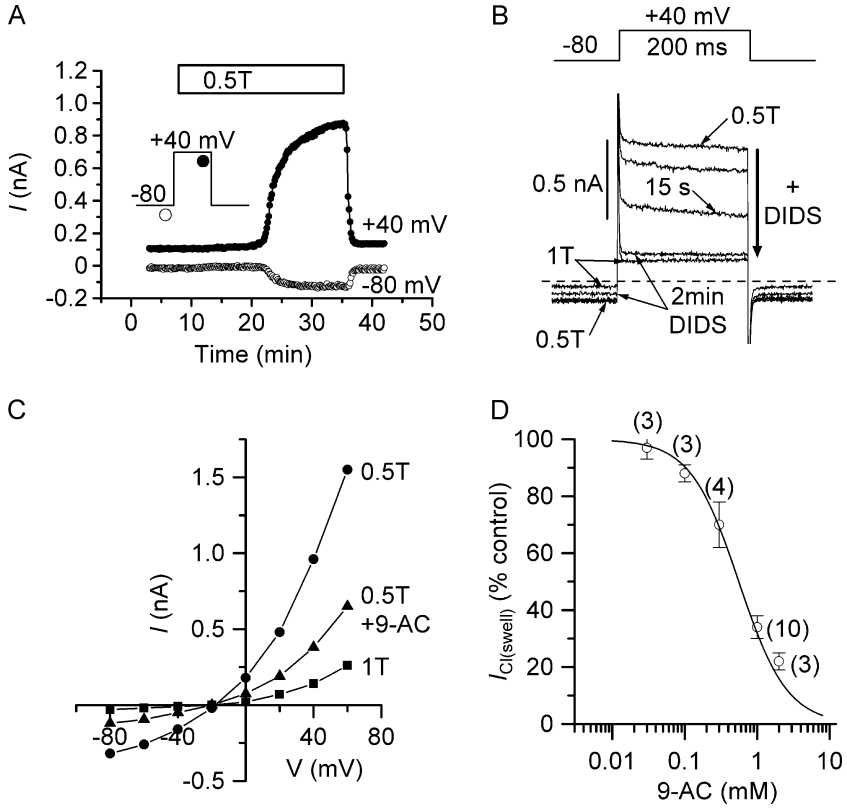

Fig. 3. Properties of $I_{Cl(swell)}$ in guinea-pig ventricular myocytes. The myocytes were dialysed and superfused with standard solutions, and pulsed from resting potential −80 mV (or prepulse potential −40 mV) to +40 mV for 200 ms except for determination of I–V relations. Current amplitude at −80 mV was measured just before test pulses, and current amplitude at test potentials was measured at the end of pulses. (A) Activation of $I_{Cl(swell)}$ upon replacement of control 1 T superfusate with hyposmotic 0.5 T superfusate, and deactivation of the current upon re-admission of 1 T solution. (B) Block of $I_{Cl(swell)}$ by 1 mM DIDS. The records were obtained in the following order: control 1 T; after 22 min superfusion with 0.5 T; during the first 15 s after addition of DIDS (second and third traces from the top at +40 mV; (superimposed) bottom traces at −80 mV); and after 2 min DIDS. Dashed line indicates zero-current level. (C) Voltage-independent block of $I_{Cl(swell)}$ after 5 min exposure to 1 mM anthracene-9-carboxylic acid (9-AC). (D) Dependence of block on the concentration of anthracene-9-carboxylic acid. Myocytes were treated with a single concentration of the drug for ≈5 min; numbers of myocytes in parentheses.

solution and superfused with standard (70 mM Cl^-) external solution were treated with forskolin to activate $I_{Cl(CFTR)}$, and then exposed to the blocker. As indicated by the representative $I–V$ data in Fig. 1B, $I_{Cl(CFTR)}$ was outwardly-rectifying and had a reversal potential (E_{rev}) near the E_{Cl} of −22 mV. Anthracene-9-carboxylic acid (1 mM) decreased $I_{Cl(CFTR)}$ by about 65%, with no apparent effect on E_{rev}; the block was independent of voltage, and reversed by a 10-min wash with forskolin solution. The degree of block measured at 0 mV was dependent on the concentration of the drug, and the overall data are well described by the Hill equation with an IC_{50} of 0.8 ± 0.02 mM and a coefficient of 1.1 (Fig. 1C).

The effects of dideoxyforskolin (10–200 μM) and verapamil (10–100 μM) on $I_{Cl(CFTR)}$ were determined on forskolin-treated myocytes that were dialysed and superfused with standard solutions. The example data in Fig. 2A illustrate that the amplitude of forskolin-activated current at voltages between −120 and +50 mV was unaffected by a 9-min exposure to 100 μM dideoxyforskolin. A similar lack of inhibitory activity was observed in experiments with lower and higher concentrations of the drug, and these results are summarised in Fig. 2B. Verapamil (10, 30, 100 μM) also proved to be an ineffective blocker of $I_{Cl(CFTR)}$ (Fig. 2B).

3.2. Activation of $I_{Cl(SWELL)}$, and block of the current by DIDS and anthracene-9-carboxylic acid

Replacement of external 1 T solution by hyposmotic 0.5 T solution caused visible swelling of guinea-pig and rabbit ventricular myocytes, and, following a variable lag period, provoked marked increases in inward current at negative potentials and outward current at positive potentials. In the example shown in Fig. 3A, the increases began after a lag of 13 min, and the amplitudes of currents monitored at −80 and +40 mV reached near steady-state levels about 10 min later. Subsequent replacement of 0.5 T solution with 1 T solution promptly restored current amplitudes to their pre-0.5 T levels.

DIDS (1 mM), an established blocker of cardiac $I_{Cl(swell)}$ with little effect on $I_{Cl(CFTR)}$ (Baumgarten and Clemo, 2003), had a pronounced inhibitory action on 0.5 T-activated $I_{Cl(swell)}$ in guinea-pig ventricular myocytes, especially at positive potentials (Fig. 3B). Short (≤3 min) applications of the inhibitor blocked 88 ± 3% of outward $I_{Cl(swell)}$ at +40 mV, but a significantly ($P<0.001$) smaller 42 ± 9% of inward $I_{Cl(swell)}$ at −80 mV ($n=7$ myocytes). However, longer applications of the compound resulted in further action on inward current (e.g., block of 73 ± 5% ($n=5$) after 7 min). The marked inhibitory effects of DIDS

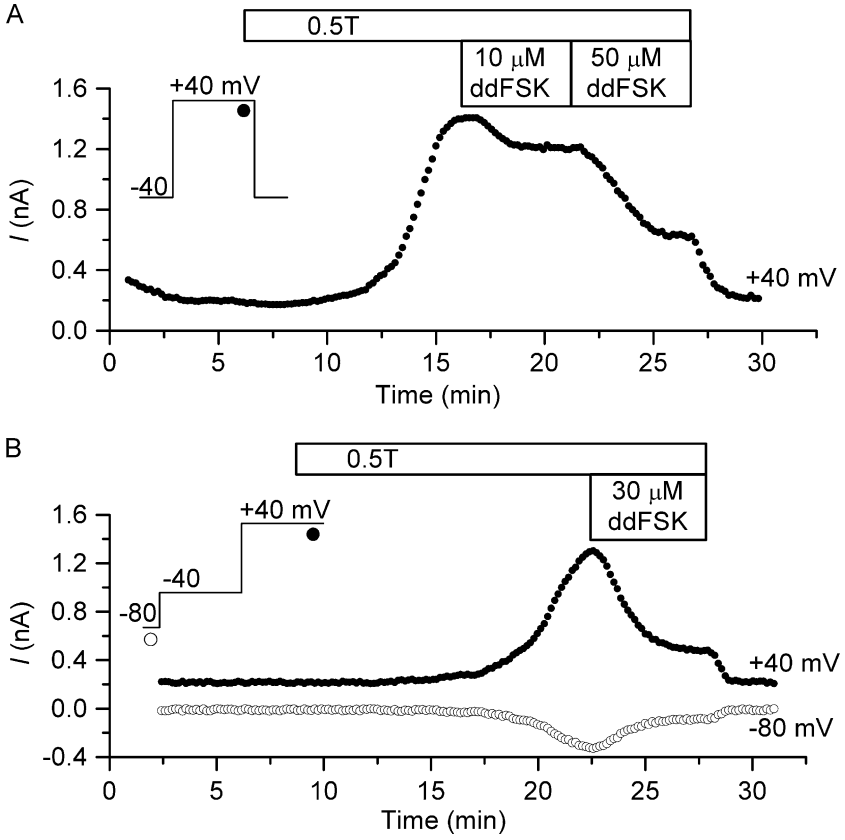

Fig. 4. Inhibition of $I_{Cl(swell)}$ by dideoxyforskolin in representative guinea-pig and rabbit ventricular myocytes pulsed to +40 mV. (A) Inhibition of outward $I_{Cl(swell)}$ at +40 mV in a guinea-pig ventricular myocyte treated with 10 and 50 μM dideoxyforskolin (ddFSK). (B) Inhibition of outward (+40 mV) and inward (−80 mV) $I_{Cl(swell)}$ in a rabbit ventricular myocyte treated with 30 μM dideoxyforskolin.

are in good accord with findings in most (van Borren et al., 2002; Vandenberg et al., 1994; Wong et al., 1999; Yamazaki et al., 1999), though not all (see Kocic et al., 2001), studies on volume-sensitive Cl^- current in guinea-pig ventricular myocytes.

In agreement with earlier studies on $I_{Cl(swell)}$ in guinea-pig ventricular myocytes (Kocic et al., 2001; Vandenberg et al., 1994), anthracene-9-carboxylic acid blocked the 0.5 T-activated current investigated here. In the example shown in Fig. 3C, 1 mM anthracene-9-carboxylic acid blocked about 70% of outwardly-rectifying $I_{Cl(swell)}$ without affecting E_{rev}; in 10 myocytes, the drug blocked 66 ± 4% of $I_{Cl(swell)}$ at +40 mV, and 62 ± 5% at −80 mV. The dependence of inhibition on the concentration of anthracene-9-carboxylic acid was evaluated at +40 mV, and the Hill equation fitting the data has an IC_{50} of 0.6 ± 0.04 mM, and a coefficient of 1.2 (Fig. 3D).

3.3. Inhibition of $I_{Cl(SWELL)}$ by dideoxyforskolin

Fig. 4A shows the effects of sequential application of 10 and 50 μM dideoxyforskolin on $I_{Cl(swell)}$ at +40 mV in a guinea-pig ventricular myocyte bathed in 0.5 T solution. The drug had a strong inhibitory effect on $I_{Cl(swell)}$, with 10 and 50 μM reducing the amplitude of the current by 15% and 65%, respectively. $I_{Cl(swell)}$ in rabbit ventricular myocytes was also sensitive to dideoxyforskolin. In the example shown in Fig. 4B, 30 μM dideoxyforskolin inhibited 74% of $I_{Cl(swell)}$ at +40 mV, and 71% of the current at −80 mV.

A further indication that inhibition of $I_{Cl(swell)}$ by dideoxyforskolin was independent of voltage is provided by the data in Fig. 5A. In this experiment on a guinea-pig ventricular myocyte, 100 μM dideoxyforskolin reversibly blocked approximately 90% of $I_{Cl(swell)}$, with no obvious difference in the time course of block at +40 and −80 mV. An interesting feature of the early phase of block was a speeding up of the decay of outward $I_{Cl(swell)}$ during the 100-ms pulses to +40 mV. Whether this was due to an acceleration of the time-dependent inactivation of $I_{Cl(swell)}$ that occurs at high positive potentials (Shuba et al., 1996), or to a different time-dependent inhibitory mechanism, cannot be resolved from the present data.

The dependence of inhibition of $I_{Cl(swell)}$ on the concentration of dideoxyforskolin was evaluated from measurements on currents recorded at +40 mV (Fig. 5B). The data

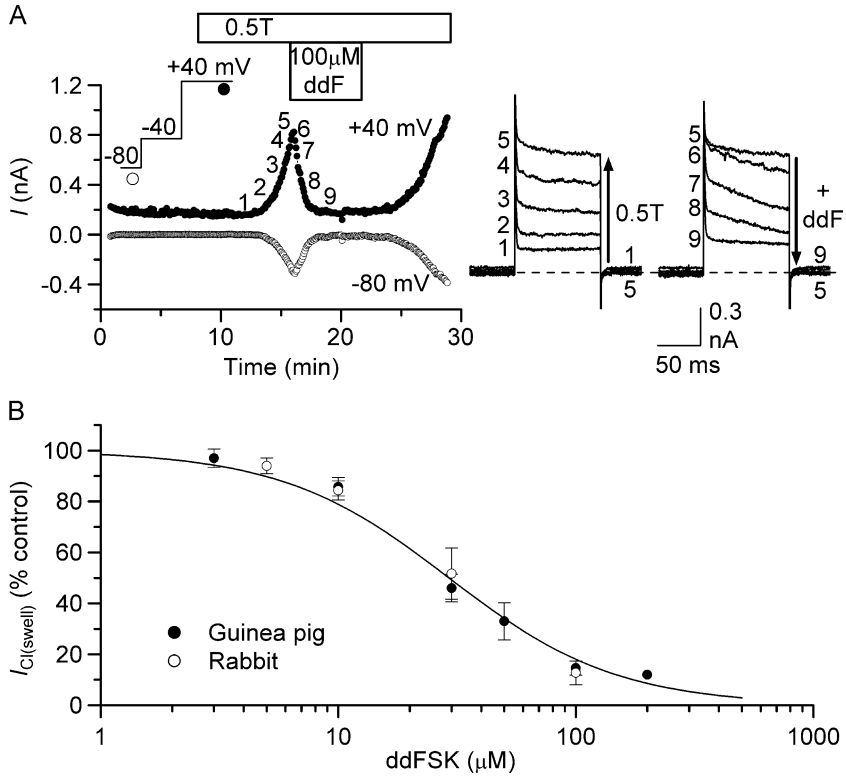

Fig. 5. Concentration-dependent inhibition of $I_{Cl(swell)}$ by dideoxyforskolin. The myocytes were dialysed and superfused with standard solutions, and pulsed from −80 (or −40) to +40 mV for 100 ms. (A) Reversible block of $I_{Cl(swell)}$ by 100 μM dideoxyforskolin (ddF) in a guinea-pig ventricular myocyte. Left: time plot; right: sets of superimposed records obtained on pulses from −40 to +40 mV at the times indicated in the time plot. Note increased time-dependency of the current at +40 mV during development of the block. The dashed line indicates the zero-current level. (B) Dependence of inhibition on the concentration of dideoxyforskolin (ddFSK). $I_{Cl(swell)}$ was measured as (0.5 T minus control 1 T) current at the end of 100 ms pulses to +40 mV, and the degree of inhibition was evaluated after 4–7 min exposure to the drug. The guinea-pig myocyte data (filled circles) are from 33 observations on 30 myocytes (n = 4–5 observations except at 100 μM (n = 16) and 200 μM (n = 1)); the Hill equation fitting these data has an IC_{50} of 29 ± 2 μM and a coefficient of 1.2. The rabbit data (filled circles) are from three to five myocytes at 5, 10, 30 and 100 μM.

obtained from guinea-pig myocytes (filled circles) are described by the Hill equation with an IC_{50} of 29 ± 2 μM and a coefficient of 1.2, and the data obtained from rabbit myocytes (open circles) are consistent with this description. It should be noted that there are uncertainties with the accuracy of the data because they include measurements from experiments in which $I_{Cl(swell)}$ had not yet reached a steady-state (e.g., Fig. 5A).

3.4. Inhibition of $I_{Cl(SWELL)}$ by verapamil

Fig. 6A illustrates the effects of 100 μM verapamil on $I_{Cl(swell)}$ in a rabbit ventricular myocyte. The phenylalkylamine blocked approximately 90% of the current at −80 and +40 mV, with no apparent acceleration of current decay at +40 mV. Although not shown here, block of $I_{Cl(swell)}$ by 50–100 μM verapamil was reversible; in five myocytes, washout with 0.5 T solution for 10 min restored $I_{Cl(swell)}$ amplitude to or above the pre-drug level.

Block of $I_{Cl(swell)}$ by verapamil was dependent on the concentration of the drug, and the data obtained from guinea-pig ventricular myocytes are well-described by the Hill equation with an IC_{50} of 33 ± 4 μM and a coefficient of 1.2 (Fig. 6B). The data obtained from rabbit ventricular myocytes treated with 5, 50 or 100 μM verapamil are in good agreement with this description (Fig. 6B).

4. Discussion

4.1. Selective block of $I_{Cl(SWELL)}$ by verapamil and ddFSK

The principal new finding in this study is that verapamil and dideoxyforskolin selectively inhibit cardiac $I_{Cl(swell)}$ over $I_{Cl(CFTR)}$. This property distinguishes the two compounds from the many that inhibit both $I_{Cl(swell)}$ and $I_{Cl(CFTR)}$ (e.g., anthracene-9-carboxcylic acid (Kocic et al., 2001; present study); glibenclamide (Sakaguchi et al., 1997; Yamazaki and Hume, 1997); niflumic acid (Carpenter and Peers, 1997; Sorota, 1994)), and places them amongst $I_{Cl(swell)}$ inhibitors such as DIDS, tamoxifen, and clomiphene (see below).

The basis of the selective block exerted by verapamil and dideoxyforskolin is unknown. However, based on the likelihood that $I_{Cl(swell)}$ in mammalian cells is carried by ClC-type Cl^- channels (Jentsch et al., 2002; Nilius and Droogmans, 2003; Wang et al., 2003), selective block does not appear to be related to a signature difference in the structures of ClC and CFTR channels. The reason for this view is

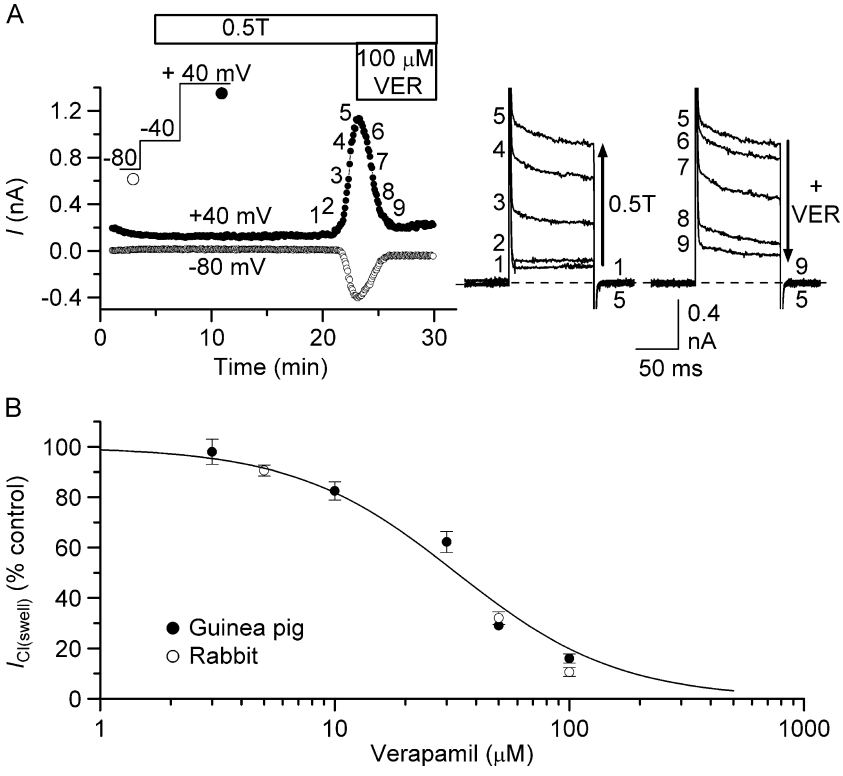

Fig. 6. Concentration-dependent inhibition of $I_{Cl(swell)}$ by verapamil. The experimental conditions were the same as those described under Fig. 5. (A) Strong inhibition by 100 μM verapamil (VER) in a rabbit ventricular myocyte. Left: time plot; right: superimposed records. The dashed line indicates the zero-current level. (B) Dependence of inhibition on the concentration of verapamil. Myocytes were treated with a single concentration of the drug. The guinea-pig myocyte data (filled circles; $n = 5$ except at 50 ($n = 1$) and 100 μM ($n = 15$)) are described by the Hill equation with an IC_{50} of 33 ± 4 μM and a coefficient of 1.2. The rabbit myocyte data (open circles) are from three to five myocytes at 5, 50 and 100 μM.

that not all types of $I_{Cl(swell)}$ are sensitive to inhibition by 50–200 μM verapamil and/or dideoxyforskolin, i.e., substantial block has been evident in some studies (e.g., Anderson et al., 1995; Diaz et al., 1993; Fatherazi et al., 1994; Nilius et al., 1994a; Stutzin et al., 1997; Valverde et al., 1992; von Weikersthal et al., 1999), but not in others (e.g., Ackerman et al., 1994; Botchkin and Matthews, 1993; Dong et al., 1994; Ehring et al., 1994; Gosling et al., 1995; Shuba et al., 2000). In cases where block has been substantial, it was essentially independent of voltage (e.g., Diaz et al., 1993; Nilius et al., 1994a; von Weikersthal et al., 1999). Similarly, block of cardiac $I_{Cl(swell)}$ by verapamil and dideoxyforskolin lacked any significant dependence on voltage.

To our knowledge, this is the first account of a block of cardiac $I_{Cl(swell)}$ by verapamil. However, there have been two earlier reports on the effects of forskolin compounds on $I_{Cl(swell)}$ in dog atrial myocytes. First, Sorota (1994) reported that 100 μM dideoxyforskolin blocked ~80% of outward-directed current; although no examples or statistics were provided, the correspondence with the present results is noteworthy. Secondly, Du and Sorota (1997) reported that 10 μM forskolin had inhibitory effects on $I_{Cl(swell)}$ in approximately 50% of the myocytes they investigated. However, the inhibition was mimicked by PKA-activating isoprenaline, and therefore unlike that found here with (PKA-inactive) dideoxyforskolin.

Verapamil and dideoxyforskolin are P-glycoprotein substrates, and P-glycoprotein may have a modulatory role in the activation of volume-sensitive Cl^- channels (Idriss et al., 2000; Moran et al., 1997). Nevertheless, it seems unlikely that P-glycoprotein is involved in the inhibition of $I_{Cl(swell)}$ in guinea-pig and rabbit myocytes because it has been shown that phenylalkylamines and dideoxyforskolin inhibit hyposmotic-activated I^{125} flux in native NIH/3T3 cells not expressing P-glycoprotein (Moran et al., 1997). Furthermore, $I_{Cl(swell)}$ inhibitor tamoxifen is also a P-glycoprotein substrate, and its blocking action is independent of the complex and most likely due to direct interaction with the channel (Nilius et al., 1994b; Zhang et al., 1994).

4.2. Comparison with other blockers of cardiac $I_{Cl(SWELL)}$

Three compounds that have been shown to distinguish between $I_{Cl(swell)}$ and $I_{Cl(CFTR)}$ are DIDS, tamoxifen, and clomiphene. A drawback with the use of DIDS is that the block of $I_{Cl(swell)}$ is irreversible unless exposures are of very short duration (Sakaguchi et al., 1997; Vandenberg et al., 1994), whereas a potential drawback of the use of tamoxifen (Duan et al., 1997; Vandenberg et al., 1994) and clomiphene (Borg et al., 2002) is that their actions are voltage-dependent. In these respects, verapamil and dideoxyforskolin offer advantages that may be particularly important in studies on $I_{Cl(swell)}$ regulation where concomitant activation of $I_{Cl(CFTR)}$ complicates analysis and interpretation of results (e.g., Du and Sorota, 1997; Nagasaki et al., 2000). Unfortunately, like DIDS (Busch et al., 1994), tamoxifen (Smitherman and Sontheimer, 2001; Thomas et al., 2003), and clomiphene (Borg et al., 2002), both verapamil and dideoxyforskolin have inhibitory effects on cation channels (Asai et al., 1996; Jones et al., 2000; McDonald et al., 1994; Walsh et al., 1989), and these render them unsuitable for studies on cardiac myocytes or tissues that call for exclusive block of $I_{Cl(swell)}$.

4.3. Pharmacological characterization of cardiac $I_{Cl(SWELL)}$

There are various types of $I_{Cl(swell)}$ in mammalian cells (Jentsch et al., 2002; Nilius and Droogmans, 2003), and it has been suggested (Bond et al., 1998; von Weikersthal et al., 1999) that verapamil and dideoxyforskolin selectively block a type characterized by outward-rectification, time- and voltage-dependent inactivation, selectivity $I^->Cl^-$, insensitivity to 0.3 mM Cd^{2+}, and inhibition by tamoxifen, phorbol esters, and DIDS.

$I_{Cl(swell)}$ in guinea-pig myocytes is an outwardly-rectifying, inactivating type of current that is insensitive to 0.5 mM Cd^{2+} (Shuba et al., 1996), has $I^->Cl^-$ selectivity (Shuba and McDonald, 2000; Vandenberg et al., 1994), and is inhibited by tamoxifen (Vandenberg et al., 1994), phorbol esters (Duan et al., 1999), and DIDS (Vandenberg et al., 1997; Yamazaki et al., 1999). The results reported here add inhibition by verapamil and dideoxyforskolin to the list. Whether the current is carried by ClC-3 (Duan et al., 1997; Duan et al., 2000, 2001; Wang et al., 2003) or other type of anion channel, is not yet resolved (see Jentsch et al., 2002; Nilius and Droogmans, 2003; Wang et al., 2003).

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