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*Short communication*

# Differential effects of tamoxifen and $I^-$ on three distinguishable chloride currents activated in T84 intestinal cells

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**Abstract.** The whole-cell mode of the patch-clamp technique has been used to monitor ionic currents in T84 colonic carcinoma cells. The cells were stimulated by either a cAMP cocktail, ionomycin or hypotonicity. Sizeable currents with distinct kinetics were observed after the stimulation with the different agonists. These kinetically distinct $Cl^-$ currents also presented a differential sensitivity to the anti-oestrogen Tamoxifen and to the halide $I^-$. Tamoxifen only inhibits the volume activated $Cl^-$ current without affecting the other two. Substitution of extracellular $Cl^-$ by $I^-$ shifted the reversal potential towards more negative values both in the hypotonicity and ionomycin activated $Cl^-$ currents. The cAMP activated current responded to the $Cl^-$ substitution by $I^-$ with a blockade of both outward and inward currents, in addition to the displacement of the zero current level towards positive values. Thus, the use of these two simple tools, $I^-$ and tamoxifen, allows the distinction of $Cl^-$ channels in epithelial cells.

**Key words:** $Cl^-$ channels, CFTR, calcium-activated $Cl^-$ channels, volume regulated channels, Iodide, Tamoxifen, T84.

## Introduction

Apically located $Cl^-$ channels play a key role in the secretion of fluid and electrolytes across epithelia. Increases in either cAMP or $[Ca^{2+}]_i$ activate airway and intestinal $Cl^-$ channels [reviewed in 2]. $Cl^-$ channels of epithelial cells are also thought to play a role in the maintenance of a constant cellular volume in anisotonic media. These volume activated chloride currents have already been demonstrated in several epithelial cells [8,4]. The studies cited have shown that cAMP, $Ca^{2+}$ and cellular swelling activate $Cl^-$ conductances with characteristic biophysical properties, and it has seemed reasonable to assume that they correspond to separate channel entities. This view has recently been challenged by results obtained in intestinal cells in culture which suggest that all stimuli affect a single channel type [5]. In the present series of experiments we use the whole-cell recording approach to show that three different $Cl^-$ currents can be elicited in the same cell by three different stimuli discussed above, and that these currents can also be distinguished by the selectivity to $I^-$ and tamoxifen blockade.

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## Materials and methods

The T84 cells used in this study were grown and maintained following, in general terms, the methods described previously [3]. Cells were used between 1-3 days after subculturing. Whole-cell currents were recorded by the patch-clamp method following the experimental details given in [4]. The composition of the pipette solution was (mM): 140 NMDGCl, 1.2 $MgCl_2$, 0.1 $CaCl_2$, 5 EGTA, 2 ATP, 0.5 GTP, 10 HEPES, pH 7.2. The free $Ca^{2+}$ concentration of the pipette solution was kept at 1 nM to prevent the spontaneous presence of $Ca^{2+}$-dependent $Cl^-$ currents occasionally seen with higher $Ca^{2+}$ concentrations (unpublished observations). The bathing solution (iso): 140 NaCl, 0.5 $MgCl_2$, 1.3 $CaCl_2$, 10 HEPES, pH 7.2. The hypo solution was obtained by reducing the NaCl concentration to 105 mM. The composition of the cAMP cocktail added to the iso solution was: 200 $\mu$M cAMP, 10 $\mu$M forskolin and 1 mM 3-isobutyryl-1-methylxantine (IBMX). The tonicity of the pipette solution was also hypotonic by about 20 mOsm in respect to the extracellular solution in order to abolish the appearance of swelling-induced $Cl^-$ currents under "isotonic" conditions [8]. Voltage-clamp experiments were performed holding the cell either at 0 mV or at -40 mV and stepping the voltage from -80 mV to 80 mV.

## Results

Figure 1A shows families of currents elicited in a cell bathed in an isotonic solution (Aa). Under these conditions only small currents with a linear current-voltage relation were observed. Exposure of the cell to a cAMP cocktail increased membrane currents with a linear current-voltage relationship, and with no apparent time dependence (Ab). This cAMP-activated $Cl^-$ current was not blocked by the presence of extracellular tamoxifen, a blocker of the Pgp associated $Cl^-$ channel [7], at a concentration of 10 $\mu$M (Ac). Washing with

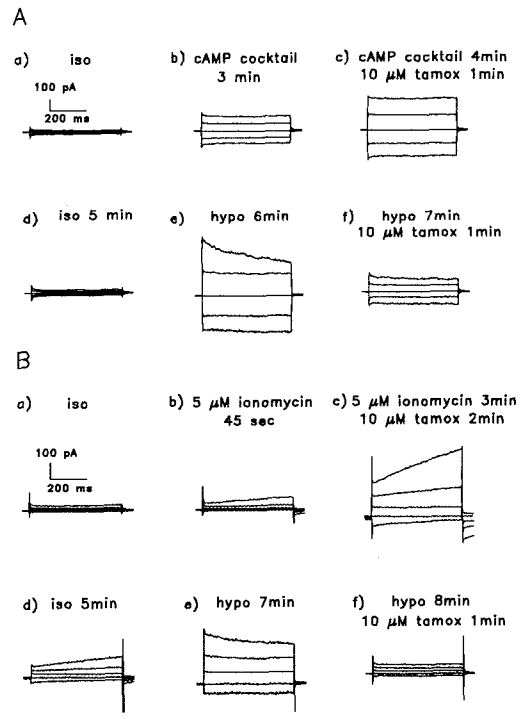


**Fig. 1.** Time-dependent voltage effects on cAMP-, hypotonicity- and ionomycin activated $Cl^-$ currents, and their sensitivity to tamoxifen. A: cAMP- and volume-activated currents elicited in the same cell, hold at 0 mV and stepped from -80 mV to + 80 mV. Cell capacitance 11 pF. B: ionomycin- and volume activated currents obtained in another cell. Ionomycin was added at a concentration of 5 μM. The cell was held at -40 mV and pulsed between -80 and +80 mV. Cell capacitance 20 pF.

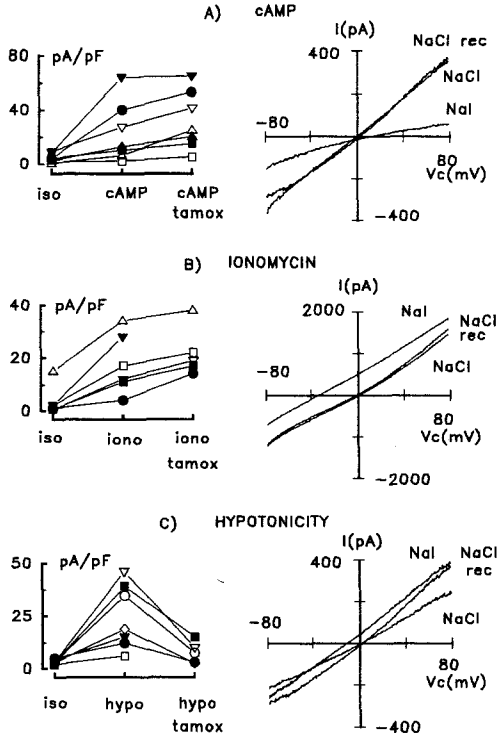


**Fig. 2.** Left-hand panels. Summary of results obtained for the different stimulus applied. $Cl^-$ current measurements taken in isotonic solution (iso), 3 min after adding cAMP cocktail (cAMP), ionomycin (iono) or 5-7 min after giving a hypotonic shock (hypo) and 1 min after the additionof 10 μM tamoxifen (cAMP tamox, iono tamox, hypo tamox). Each set of symbols represent measurements taken in the same cell. Right-hand panels. Effect of anion replacement upon the three different $Cl^-$ currents. The current-voltage curves were obtained by applying a ramp voltage from -80 to 80 mV over a 1 sec period. The solution bathing the cells was changed from a $Cl^-$ containing solution (NaCl) to a $I^-$-containing one (NaI) and back to a $Cl^-$ containing solution (NaCl recovery).

normal isotonic solution after treatment with the cAMP cocktail led to complete recovery of the control currents (Ad, iso). The effect of exposure to a hypotonic condition was tested in the same cell after the recovery of the control currents. The hypotonic shock produced an increase in currents that, at the most depolarised potentials, decayed during the 600 ms pulse (Ae). In contrast to the cAMP-activated $Cl^-$ currents, the swelling-induced $Cl^-$ currents were blocked by the addition of 10 μM tamoxifen to the bathing solution (Af). In a different cell (Figure 1B), the addition of the $Ca^{2+}$ ionophore ionomycin (5 μM) activated $Cl^-$ currents which presented time-dependent activation at depolarised potentials and steady-state outward rectification. This current was already present 45 sec after the addition of the ionophore (Bb) and, similarly to the cAMP-activated $Cl^-$ current, was not blocked by 10 μM tamoxifen (Bc). Membrane currents were greatly reduced after removal of ionomycin (Bd). Under these conditions the cell responded to a hypotonic challenge with an increase in $Cl^-$ current (Be). This current showed characteristics very similar to those of the swell-induced currents recorded in the previous cell (Ae), and a similar blockade by 10 μM tamoxifen (Bf).

A summary of the results obtained with the different stimuli applied is shown in Figure 2 (left panels). All the cells treated with the cAMP cocktail (A) responded with an increase of time-independent and linear $Cl^-$ currents (see Fig 1A, b and c) that varied in magnitude between 6 and 65 pA/pF (n = 7). None of these currents was inhibited by the addition of 10 μM tamoxifen to the extracellular solution. Ionomycin treated cells (B) presented time-dependent, outwardly rectifying $Cl^-$ currents (see Fig 1Bb and c) with a normalized magnitude between 14 and 34 pA/pF (n=6). These $Cl^-$ currents, as the cAMP-activated $Cl^-$ currents were not blocked by tamoxifen. Finally, the volume activated currents (C) were elicited in cells after restoring the currents to levels similar to the ones obtained previous the challenge with either cAMP cocktail or ionomycin (in the left panel of Fig 2C only the measurements presented with the symbols ○ and ▽ were obtained from cells without previous treatment with cAMP or ionomycin).

The anion selectivity sequence is another haracteristic that helps to distinguish the different $Cl^-$ channels activated in T84 cells. The selectivity of the different currents (cAMP-, $Ca^{2+}$- and hypotonicity-

activated $Cl^-$ currents) to the halide $I^-$ was examined in anion-replacement experiments. Figure 2 (right hand panels) shows current-voltage relations, obtained with a voltage-ramp, after exposure to the different stimuli. Under our experimental conditions, i.e. NMDG-Cl pipette and NaCl bath solutions, the main charge carrier is $Cl^-$ and the reversal potential was close to 0 mV in all three cases (A, B and C). Replacing $Cl^-$ with $I^-$ moved the reversal potential to +14 mV for the cAMP activated $Cl^-$ current and decreased both outward and inward currents, consistent with a permeability sequence $P_{Cl} > P_I$ and with a blockade by $I^-$ (A). Similar results have been obtained for the CFTR channel by others [6,1]. On the other hand, the same substitution in the ionomycin- and hypotonicity-activated $Cl^-$ currents shifted the reversal potential to -30 mV and -10 mV respectively (B and C), suggesting a sequence $P_I > P_{Cl}$. This is in accordance with the sequence previously reported for the $Ca^{2+}$- and volume-activated $Cl^-$ currents [2,4]. Table 1 shows a summary of the reversal potentials as well as the calculated permeability ratios for the anions used.

**Table 1.** Effect of $Cl^-$ replacement by $I^-$ on $Cl^-$ currents of T84 cells.

| | $E_{rev}$ | $P_I/P_{Cl}$ | n |
|---|---|---|---|
| cAMP | 10±2 | 0.65 | 4 |
| ionomycin | -18±8 | 2 | 4 |
| hypotonicity | -11±3 | 1.6 | 3 |

Results are means ± SEM for the number of experiments indicated. $E_{rev}$ current reversal potential. Relative ion permeabilities ($P_I/P_{Cl}$) were calculated as described in [4].

## Discussion

Our present experiments using the patch-clamp technique to measure whole-cell currents in T84 cells, show that single cells can display kinetically distinct $Cl^-$ currents in response to the cellular swelling or to agonists that elevate the intracellular levels of cAMP or $Ca^{2+}$. Currents with similar kinetics have been observed in different preparations [2], but this is the first report in which the activation of a single current type, with specific kinetic properties, has been linked to a given regulatory stimulus in the same recorded cell. The simplest interpretation to this observation is that the different types of macroscopic currents correspond to the expression of distinct channels, although the possibility that they were the product of the activation of a single population of channels by different stimulus must be taken into account [5]. One possible way to discriminate between these possibilities is to test for inhibitory substances that might have differential effects on the observed $Cl^-$ current patterns. We have chosen the anti-oestrogen tamoxifen, which has been shown recently to block the volume-activated $Cl^-$ current associated to the expression of Pgp [7], as a possible inhibitor of the T84 $Cl^-$ currents. Also, the anion selectivity sequence is a distinguishing fingerprint of individual channels. For that purpose we compared the permeability of the halide $I^-$ to that of $Cl^-$ on the different $Cl^-$ currents. Combining these two approaches we have been able to discriminate between the different kinetic types of $Cl^-$ currents on T84 cells. The cAMP-activated $Cl^-$ current presents a $P_{Cl} > P_I$ and is blocked by $I^-$ but not by tamoxifen. For the $Ca^{2+}$-activated $Cl^-$ current the sequence is $P_I > P_{Cl}$ and this current is not blocked by tamoxifen and, finally, the swelling-induced $Cl^-$ current also presents a $P_I > P_{Cl}$, but it is blocked by tamoxifen. It is tempting therefore to speculate that these macroscopic currents correspond to the operation of three different $Cl^-$ channels present in the plasma membrane of these cells. The differential effects of $I^-$ and tamoxifen observed in the present work might be help to in identify the channels underlying these kinetically distinct types of current.

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