J. Membrane Biol. 151, 131–138 (1996)

The Journal of
Membrane
Biology

A Ca^{2+}-activated Whole-Cell Cl^- Conductance In Human Placental Cytotrophoblast Cells Activated Via a G Protein

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Received: 9 November 1995/Revised: 18 January 1996

Abstract. Whole-cell patch clamp experiments were performed on cultured human cytotrophoblast cells incubated for 24–48 hr after their isolation from term placentas. Cl^--selective currents were examined using K^+-free solutions. Under nonstimulated conditions, most cells initially expressed only small background leak currents. However, inclusion of 0.2 mM GTPγS in the electrode solution caused activation of an outwardly rectifying conductance which showed marked time-dependent activation at depolarized potentials above +20 mV. Stimulation of this conductance by GTPγS was found to be Ca^{2+}-dependent since GTPγS failed to activate currents when included in a Ca^{2+}-free electrode solution. In addition, similar currents could be activated by increasing the $[Ca^{2+}]$ of the pipette solution to 500 nM. The Ca^{2+}-activated conductance was judged to be Cl^--selective, since reversal potentials were predicted by Nernst equilibrium potentials for Cl^-. This conductance could also be reversibly inhibited by addition of the anion channel blocker DIDS to the bath solution at a dose of 100 μM. Preliminary experiments indicated the presence of a second whole-cell anion conductance in human cytotrophoblast cells, which may be activated by cell swelling. Possible roles for the Ca^{2+}-activated Cl^- conductance in human placental trophoblast are discussed.

Key words: Human placenta — Cl^- channel — Calcium — G protein

Introduction

Although the human fetus accumulates a large quantity of Cl^- during gestation, the mechanisms of Cl^- transport across the placental trophoblast are poorly understood [23]. The main barrier to exchange in human placenta is thought to be the multinucleated syncytiotrophoblast cell layer [23] and transporters for Cl^- have been demonstrated in its maternal-facing microvillous plasma membrane. Studies with vesicles prepared from the microvillous membrane provided data indicative of Cl^- uptake via Cl^-/HCO_3 exchange [7, 12, 18, 28] and also via pathways sensitive to imposed potentials, presumed to be Cl^- channels [7, 11, 18]. Similar experiments with the fetal-facing basal plasma membrane of the syncytiotrophoblast have not yet been reported and little is consequently known about Cl^- transport at this site. However, immunoblotting data do suggest that the anion exchanger is present at this site [32].

In addition to vesicle studies, conventional microelectrode experiments using single placental villi have shown the presence of a Cl^- conductance in syncytiotrophoblast, since extracellular Cl^- replacement caused depolarization of the membrane potential [14]. Using the same preparation, patch clamp experiments have identified the presence of ''maxi'' Cl^- channels in excised patches of the microvillous membrane [6]. Greenwood et al. (1993) [15] have also reported that the maxi Cl^- channel is present in excised patches of cytotrophoblast cells isolated from human term placenta; these cells are similar to those which differentiate to form mature syncytiotrophoblast in vivo [5, 20].

At present there is virtually nothing known concerning the control of ion fluxes across the placenta. Therefore, the aim of the present study was to identify regulated Cl^- conductances in cultured cytotrophoblast cells using the whole-cell variant of the patch clamp technique. Results of these experiments demonstrate, for the first time in placental trophoblast, the presence of a Ca^{2+}-

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activated Cl^- conductance and also provide data to suggest the presence of a volume-activated Cl^- conductance in this tissue. Part of these data have appeared in abstract form previously [19].

Materials and Methods

Cell Isolation and Culture

Cytotrophoblast cells were isolated from human term placentas by methods described in detail elsewhere [15, 20]. For patch clamping, 4 $\times 10^6$ cells were plated in 35 mm culture dishes in 2 ml culture medium which consisted of DMEM/F12 (Gibco) 1:1 with 10% fetal bovine serum (Sigma), 25 mM HEPES, pH 7.4, 0.12% penicillin, 0.2% streptomycin and 0.6% glutamine. Dishes were stored for 24–48 hr in a humidified incubator at 37°C in 5% CO_2/95% air and were thoroughly washed with the experimental bath solution immediately before use in patch clamp experiments.

Patch Clamp Recording

The conventional whole-cell recording method [16] was applied to measure membrane currents. Patch pipettes were made from hematocrit capillary tubes (Oxford Labware, St. Louis, MO) using a two-stage vertical puller (PB-7; Narishige, Japan). The tip resistances of patch electrodes were 3–5 MΩ. Membrane currents were measured using an Axopatch 1-D amplifier (Axon Instruments, Foster City, CA). Step-voltage pulses were generated by computer using the pClamp software (version 5.5.1, Axon Instruments) and a TL-1 interface (Axon Instruments). Data were stored on the computer hard disk. In all experiments, cell capacitance was maximally compensated using the capacitance compensation facility of the Axopatch 1-D amplifier. The series resistance was not compensated in this study. The ground electrode was a Ag/AgCl pellet. All experiments were carried out at room temperature (19–23°C).

Experimental Design

Three series of experiments were performed with the following objectives: first to identify any spontaneously active conductances under nonstimulated isotonic conditions; second to investigate cell signaling mechanisms responsible for such conductances; third to characterize a Ca^{2+}-dependent whole-cell conductance in more detail.

Series 1. Identification of Spontaneously Active Whole-cell Currents

To investigate whether human cytotrophoblast cells expressed whole-cell Cl^- conductances in the absence of stimulation, K^+-free isotonic solutions (290 mosm/kg_{H2O}) were used. The pipette solution contained (mM) 20 NaCl, 120 Na aspartate, 3 $MgCl_2$, 5 HEPES, 10 glucose, 3 Na_2ATP, 0.5 EGTA-NaOH, pH, 7.2. The bath solution contained (mM) 140 NaCl, 1 $CaCl_2$, 1 $MgCl_2$, 5 HEPES, 10 glucose, 10 mannitol, pH, 7.4. Current-voltage (*I-V*) relationships were determined at 1–2 min intervals to assess the presence of spontaneously active conductances.

Series 2. Effect of GTPγS on Whole-cell Currents

To investigate possible regulation of whole-cell conductances by intracellular second messengers, experiments were performed in which the nonhydrolyzable GTP analogue GTPγS was included in the electrode solution to stimulate plasmalemmal G proteins. In these experiments, the same solutions as in series 1 were used except that 0.2 mM GTPγS (Boehringer Mannheim) was added to the electrode solution. In a further group of experiments, 0.2 mM GTPγS was included in a Ca^{2+}-free electrode solution which contained (mM) 20 NaCl, 110 Na aspartate, 3 $MgCl_2$, 3 Na_2ATP, 5 HEPES, 10 glucose, 10 EGTA-NaOH, pH, 7.2.

Series 3. Characterization of Ca^{2+}-dependent Whole-cell Cl^- Currents

To record Ca^{2+}-dependent Cl^- currents, cells were dialyzed with K^+-free solutions in which the $[Ca^{2+}]$ was buffered at 500 nM. Free $[Ca^{2+}]$ was calculated using a computer program which takes into account the influences of pH, Mg^{2+} and temperature (S. Muallem and D.D.F. Loo, *unpublished*). To avoid development of volume-activated conductances (*see* Results), 40 mM mannitol was added to the bath solution used in previous experiments. To investigate the Cl^- selectivity of the Ca^{2+}-activated conductance, the measured reversal potentials were compared to the Nernst equilibrium potentials for Cl^-. Two groups of experiments were performed in which the pipette $[Cl^-]$ was altered, thereby changing E_{Cl}. One pipette solution contained (mM) 110 Na aspartate, 3 $MgCl_2$, 3 Na_2ATP, 5 HEPES, 10 glucose, 10 EGTA-NaOH, 8.6 $CaCl_2$, pH, 7.2, $E_{Cl} = -46$ mV. The other pipette solution contained (mM) 110 NaCl, 3 $MgCl_2$, 3 Na_2ATP, 5 HEPES, 10 glucose, 10 EGTA-NaOH, 8.6 $CaCl_2$, pH, 7.2, $E_{Cl} = -2$ mV. The sensitivity of Ca^{2+}-activated currents to bath application of 100 μM of the Cl^- channel blocker 4-4′-diisothiocyanostilbene-2,2-disulphonic acid (DIDS) or 0.1% DMSO vehicle was also assessed in some experiments.

Solution Composition

For all solutions used in this study, the expected osmolality was confirmed from the freezing point depression (Roebling Osmometer, Camlab, UK). Concentrations of Cl^- and Na^+ were confirmed using a 925 Cl^- analyzer (Corning, Essex, UK) and a Corning 480 flame photometer respectively.

Data Presentation and Statistics

Unless otherwise stated data are mean ± SE of observations of *n* cells isolated from at least three separate placentas in each experimental group. *I-V* relationships are for the maximum current measured at each applied potential. For quantitative comparison current densities were calculated by dividing current amplitude (pA) by cell capacitance (pF). Nonparametric statistical procedures were used to asses the effect of GTPγS, since a Bartlett test [2] revealed significant heterogeneity of variances between data sets. Statistical comparison was achieved using Kruskal-Wallis analysis of variance followed by *post hoc* Mann-Whitney tests to locate inequalities [34]. Results were taken to be statistically significant when $P < 0.05$.

Results

Cell Capacitance and Access Resistance

When placed in culture human cytotrophoblast cells aggregate and fuse over 24–96 hr to form large multinucleated ''syncytial'' islands which are extremely flat [15, 20, 25]. To avoid problems of incomplete dialysis of cells or inefficient voltage-clamp, the present experiments were confined to smaller mononucleate cells prior to completion of this differentiation process after culture for between 1–2 days. Selected cells possessed only small cytoplasmic extensions and did not appear to be aggregated with any neighboring cells. The recorded range of cell capacitance was 12 to 86 pF, with the majority (71%) of cells having capacitances of between 20 to 40 pF. The frequency distribution for cell capacitance was unimodal, though not normally distributed, and had a median of 29 pF. Access resistance ranged from 6–18 MΩ with an overall mean of 11.6 ± 0.4 (N = 59).

Series 1. Spontaneously Active Whole-cell Conductances

Figure 1 shows current profiles recorded under isotonic conditions. In most cells (18/21) no conductances were observed 1–2 min after initiating whole-cell recording (Fig. 1*A*). In a minority of cases (3/21) however, transient activation of an outwardly rectifying whole-cell conductance was observed (Fig. 1*B*). This conductance showed marked time-dependent activation at depolarized potentials, resembling Ca^{2+}-activated Cl^- conductances reported in other cell types [1, 10]. Under these recording conditions, this conductance quickly ran down and had disappeared 2–5 min into whole-cell recording. Extending the recording period beyond 5 min revealed activation of a second whole-cell conductance in 10/21 cells (Fig. 1*C*). This conductance activated gradually and progressively and once activated did not rundown in up to 30 min of recording. The conductance shown in Fig. 1*C* was also outwardly rectifying but displayed little time-dependence in the applied voltage range, resembling volume-activated Cl^- conductances reported in several other cell types [8, 31]. This conductance was observed both in cells which had expressed no conductance initially and in 1 out of the 3 cells which had transiently expressed the conductance in Fig. 1*B*.

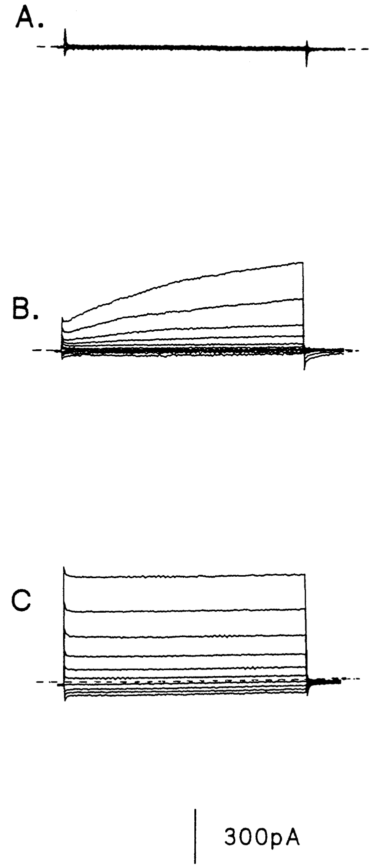

Fig. 1. Representative recordings of spontaneously active whole-cell currents. Conductances were measured using isotonic (290 mosm/kg_{H2O}) K^+-free solutions. Current profiles were obtained by stepping the membrane potential from −120 to +80 mV in increments of 20 mV. Each voltage step was of 1-sec duration. The holding potential was −40 mV. (*A*) the absence of current seen initially in 18 out of 21 recordings. (*B*) time-dependent outward rectifying conductance transiently expressed in 3 out of 21 cells, (*C*) gradually developing time-independent outward rectifying current seen in 10 out of 21 recordings. Dashed lines indicate zero current.

Series 2. Activation of Whole-cell Conductance by GTPγS

To investigate possible intracellular signaling pathways responsible for activation of the whole-cell conductances shown in Fig. 1, plasmalemmal G proteins were stimulated by inclusion of 0.2 mM GTPγS in the electrode solution. When added to a nominally Ca^{2+}-free electrode solution containing 0.5 mM EGTA, GTPγS caused activation of a conductance (*see* Fig. 2*A*) which closely resembled that shown in Fig. 1*B*. In all cases, the activated conductance showed characteristic time-dependent activation at potentials of +20 mV and above. The reversal potential (I_{REV}) of currents activated by GTPγS was close to E_{Cl} (E_{Cl} = −43 mV, I_{REV} = −35.9 ± 5.2 mV, n = 7), indicating that they were principally Cl^--selective. Under these recording conditions, a variable degree of current rundown was observed, though rundown was incomplete after 5 min of whole-cell recording. In a separate group of experiments, 0.2 mM GTPγS was included in an electrode solution containing 10 mM EGTA. In these experiments GTPγS failed to activate whole-cell conductance in any of 9 cells. These data are summarized in Fig. 2*C* which shows current densities for maximal currents recorded at +80 mV 2 min after achieving the whole-cell configuration. Considering every cell in each group, GTPγS in the presence of 0.5 mM EGTA caused a significant activation of outward current

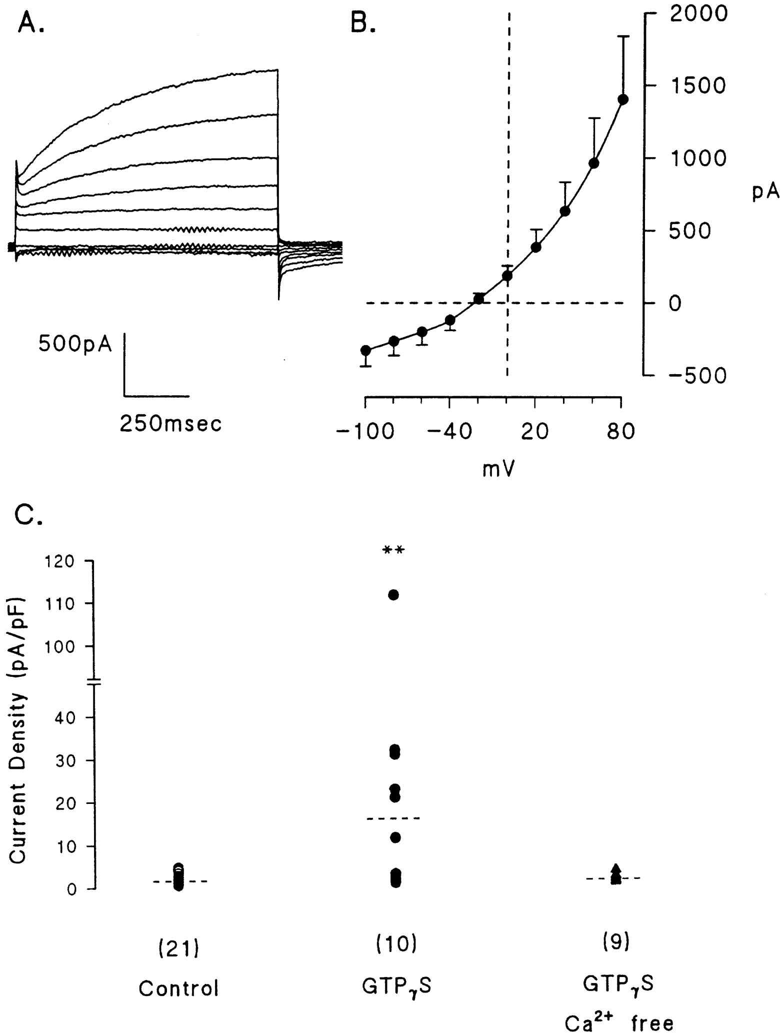

Fig. 2. Ca^{2+}-dependent activation of whole-cell conductance by GTPγS. (*A*) profile of current activated by GTPγS, generated using the same protocol as described in Fig. 1 (*B*) current-voltage (*I-V*) relationship for GTPγS-activated conductance, constructed using the mean ± SE of maximum currents recorded at each applied potential for $n = 7$ recordings, (*C*) outward current density measured at +80 mV 2 min after initiating whole-cell recording (○) control recordings, (●) 0.2 mM GTPγS in the presence of 0.5 mM pipette EGTA, (▲) 0.2 mM GTPγS added to an electrode solution containing 10 mM pipette EGTA. Dashed horizontal bars indicate group medians. *denotes statistically significant difference in outward current density with respect to control (Kruskal-Wallis ANOVA + Mann-Whitney $P < 0.05$).

compared to controls (Kruskal-Wallis + Mann-Whitney $P < 0.05$). However, when using an electrode solution containing 10 mM EGTA, outward current density was not significantly different from control in the presence of GTPγS. The simplest interpretation of these data is that activation of whole-cell conductance by GTPγS is dependent in intracellular Ca^{2+}. More specifically, the Ca^{2+} buffering afforded by 0.5 mM pipette EGTA was presumably insufficient to prevent a physiological Ca^{2+} signal evoked by GTPγS, whereas 10 mM pipette EGTA prevented such a response. In support of this idea, Kotera and Brown (1993) [21] also showed that activation of similar Ca^{2+}-dependent Cl^- currents in lacrimal acinar cells during hypo-osmotic stress was prevented by 10 mM pipette EGTA.

Gradual activation of the conductance shown in Fig. 1*C* was observed after 5 min in 4/9 recordings when GTPγS was included in the Ca^{2+}-free electrode solution. The gradual time course of activation for this conductance was similar to that seen in control cells. It seems unlikely, therefore, that current activation was a consequence of GTPγS, but rather that it occurred spontaneously. These currents apparently represent a separate conductance, since their appearance in this group shows them to be Ca^{2+}- independent.

In preliminary experiments (*data not shown*), similar currents to those in Fig. 1*C* were activated by diluting the bath solution to 220 mosm/kg_{H2O} ($n = 3$) or by applying positive pressure to the back of the electrode ($n = 2$). Conversely, the development of these currents was eliminated by performing experiments in a bath solution made hypertonic (330 mosm/kg_{H2O}) by addition of mannitol ($n = 12$). These data are consistent with the presence of a volume-activated conductance in human cytotrophoblast cells. Indeed the gradual and spontaneous development of similar currents during whole-cell recording has been reported in other studies and shown to be due to volume-activated Cl^- conductances [27, 33]. The properties of this conductance were not investigated further in the present study.

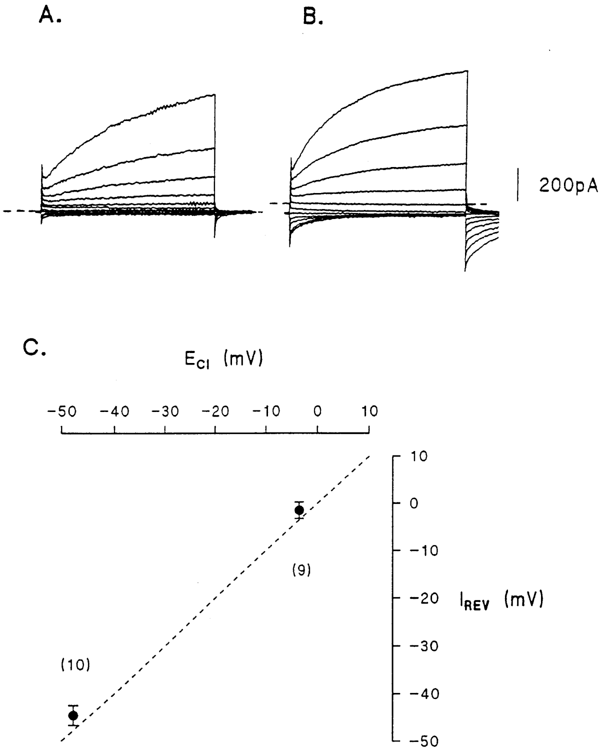

Fig. 3. Activation of whole-cell conductance by 500 nM pipette [Ca^{2+}]. Currents were activated either in the presence of (*A*) low (23 mM) pipette [Cl^-] or (*B*) high (133 mM) pipette [Cl^-]. Use of higher pipette [Cl^-] increased the size of inward currents, revealing time-dependent inactivation at hyperpolarized voltages and was associated with larger inward tail currents following depolarizing voltage pulses. Current profiles were generated as described in Fig. 1. Broken lines indicate zero current. (*C*) The relationship between current reversal potentials (I_{REV}) and E_{Cl} for different initial pipette [Cl^-]. The broken line indicates the relationship between I_{REV} and E_{Cl} calculated from the Nernst equation for a perfectly selective Cl^- conductance.

Series 3. Characterization of Ca^{2+}-dependent Whole-cell Cl^- Conductance

To prevent development of volume-activated Cl^- currents in these experiments, a hypertonic (330 mosm/kg_{H2O}) bathing medium was used throughout. To record Ca^{2+}-dependent Cl^- currents, cells were dialyzed with K^+-free solutions in which the Ca^{2+} concentration was buffered at 500 nM. The ability to activate whole-cell currents with the characteristic time- dependence seen previously (e.g., Fig. 2A) by simply elevating intracellular Ca^{2+} (Fig. 3*A* and *B*) confirms that this conductance is Ca^{2+}-dependent. Similar currents could also be activated in control cells by adding the Ca^{2+} ionophore A23187 (10–20 μM) to the bathing medium ($n = 6$, *data not shown*).

When using 500 nM Ca^{2+} in the electrode solution, currents were generally activated within 2 min and did not show significant rundown in up to 10 min of recording. Two groups of experiments were performed in which the pipette [Cl^-] differed initially (*see* Materials and Methods). Current profiles for low (23 mM) and high (133 mM) pipette [Cl^-] are shown in Fig. 3*A* and *B* respectively. As expected for a Cl^--selective conductance, the size of inward currents was increased when pipette [Cl^-] was elevated, presumably due to the greater concentration of permeant charge carriers inside the cell. The increased size of inward currents in this group allowed a pronounced time-dependent inactivation of currents at hyperpolarized potentials to be seen more clearly. The use of higher pipette [Cl^-] was also associated with more prominent slowly inactivating inward tail currents. Increased tail current amplitude in this group was probably a consequence of E_{Cl} (−2 mV) being positive to the holding potential (−40 mV), providing a driving force for Cl^- efflux (inward current) through channels remaining open following depolarizing voltage steps.

When the reversal potential (I_{REV}) of Ca^{2+}-activated currents was compared to E_{Cl} (Fig. 3*C*), a highly predictive relationship was observed, suggesting that this conductance is Cl^- selective. Since E_{Cl} was manipulated by altering the pipette [Cl^-] and I_{REV} was changed predictably, these experiments also indicate that mononucleate cytotrophoblast cells are adequately perfused by the electrode solution during whole-cell experiments.

Figure 4 shows the effect on the Ca^{2+}-activated Cl^- conductance of the anion channel inhibitor DIDS. Bath application of 100 μM DIDS caused substantial inhibition of this conductance (compare Fig. 4*A* and *B*), an effect which was largely reversible (Fig. 4*C*). Inhibition by DIDS was not obviously voltage dependent causing for example 86.1 ± 5.2% inhibition of outward currents measured at +80 mV and 60.3% ± 10.9% inhibition of inward current measured at −120 mV (Mann-Whitney test, $P > 0.05$, $n = 5$). Addition of 0.1% DMSO vehicle caused no detectable inhibition of the Ca^{2+}-activated Cl^- conductance ($n = 4$).

Discussion

The present study has identified, for the first time in placental cytotrophoblast cells, expression of Ca^{2+}-activated whole-cell Cl^- currents. This conductance could be activated by stimulating plasmalemmal G proteins with GTPγS, a nonhydrolyzable analogue of GTP. Activation of the conductance by GTPγS was not observed when cells were dialyzed with a Ca^{2+}-free pipette solution. Currents were also reliably activated by using pipette solutions with [Ca^{2+}] buffered at 500 nM. The conductance was judged to be Cl^--selective based on the predictive relationship between E_{Cl} and the reversal potential. Currents were almost entirely abolished by application of the anion channel inhibitor DIDS (100 μM).

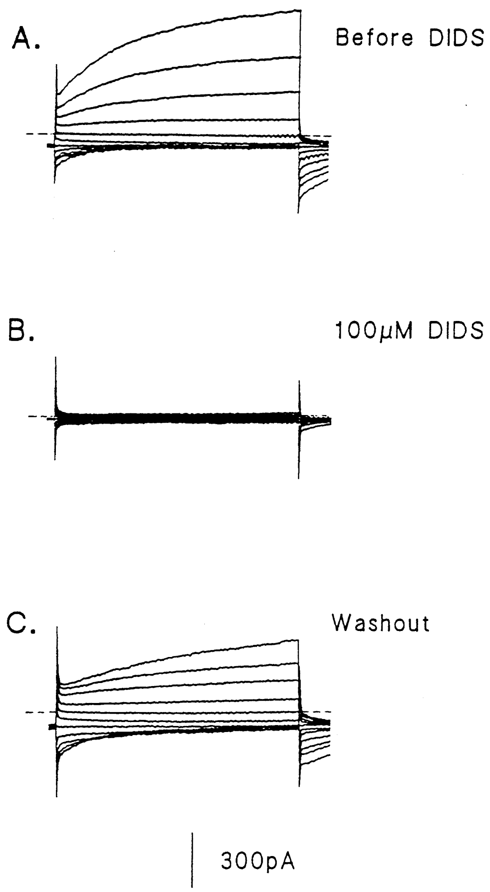

Fig. 4. Inhibition of the Ca^{2+}-activated Cl$^-$ conductance by DIDS. Current profiles were generated as described in Fig. 1. (*A*) activation of whole-cell conductance by 500 nM pipette [Ca^{2+}], (*B*) current profile recorded in the same cell 30 sec after addition of 100 μM DIDS to the bath solution, (*C*) partial reversal of inhibition 1 min after washout of DIDS.

The Ca^{2+}-activated Cl$^-$ conductance described in this study shows marked time-dependent activation at depolarized potentials and inactivation at hyperpolarized potentials, properties which are characteristic of Ca^{2+}-dependent Cl$^-$ conductances reported in other cell types [1, 9, 10, 17].

Comparison with other Placental Cl$^-$ Conductances

Several studies have previously shown the presence of Cl$^-$ conductances in placental trophoblast. In mature syncytiotrophoblast, both vesicle [7, 12, 18] and microelectrode [14] studies have shown conductive pathways for Cl$^-$ in the microvillous membrane, though their precise nature and regulation is unknown. Since vesicle experiments were performed using solutions without added Ca^{2+} and microelectrode impalements were made under ''resting'' conditions, the Ca^{2+}-activated Cl$^-$ conductance described here may not have contributed to the macroscopic conductances reported in those studies. In particular, Ca^{2+}-activated Cl$^-$ currents were seen rarely and only transiently under nonstimulatory conditions in the present study. Rather than contributing to the basal Cl$^-$ permeability, therefore, these channels offer the potential for a regulated increase in Cl$^-$ conductance. It is interesting to note that despite the demonstration of a resting Cl$^-$ conductance in mature placental villi [14], virtually no conductance was initially observed in 18 out of 21 control recordings (*see* Fig. 1A). The reason for this is not clear, but may reflect a difference between cytotrophoblast cells and mature syncytiotrophoblast (i.e., the stage of cell differentiation). Alternatively, methodological differences may be important, for example the loss of cytoplasmic factors during whole-cell recording which may be required for channel activities and would be retained during microelectrode impalements.

Previous patch clamp studies have identified single Cl$^-$ channels in both syncytiotrophoblast [6] and cytotrophoblast cells [15]. These studies showed the presence of a high conductance (~300 pS) ''maxi'' Cl$^-$ channel and, in the latter study, a lower conductance (20 pS) channel which may also be Cl$^-$ selective. However, neither of these channels apparently displayed the voltage-dependent changes in channel open-state probability thought to account for the time-dependent behavior of macroscopic Ca^{2+}-activated Cl$^-$ currents [10]. Thus, these channels probably do not constitute the unitary basis of whole-cell currents. Indeed, single channel studies are unlikely to assist in further characterizing this conductance, since noise fluctuation analysis of similar Ca^{2+}-activated whole-cell Cl$^-$ currents in lacrimal acinar cells suggested an extremely small single channel conductance of around 1–2 pS [22]. Recent data from our laboratory have shown the expression of another low conductance Cl$^-$ channel in human cytotrophoblast cells, the Cystic Fibrosis transmembrane conductance regulator (CFTR) [24]. Unlike the conductance shown in the present study however, CFTR is known to have voltage-independent gating properties [e.g., 13], such that its presence cannot account for the currents described here. Indeed, until the molecular identity of the Ca^{2+}-activated Cl$^-$ channel is discovered, it will be difficult to localize the channel and fully deduce its physiological role.

Cell-signaling Mechanisms Leading to Current Activation

Inclusion of the nonhydrolyzable GTP analogue GTPγS in the electrode solution caused significant activation of the Ca^{2+}-dependent Cl$^-$ conductance. Current activation was presumably a consequence of stimulating G proteins in the plasma membrane, leading to an increase in intracellular [Ca^{2+}]. The inability of GTPγS to activate this conductance when added to a Ca^{2+}-free electrode solution, while demonstrating Ca^{2+} dependence, also appears to rule out direct interaction between G proteins and the underlying channels as a mechanism of activation. Since mechanisms of Ca^{2+} homeostasis in human cytotrophoblast cells are poorly understood, the nature of pathways beyond the G-protein level responsible for such a rise in intracellular [Ca^{2+}] remain to be determined. Ca^{2+} influx pathways are known to exist in cytotrophoblast plasma membrane, since elevation in extracellular [Ca^{2+}] is associated with increased intracellular [Ca^{2+}] [3]. This influx pathway is not thought to be via voltage-operated Ca^{2+} channels (VOCC), as attempts by the same authors to depolarize the cell membrane by increasing [K^+] failed to alter intracellular [Ca^{2+}]. Indeed, preliminary attempts to induce Ca^{2+} entry in the present study by applying conditioning voltage pulses to +20 mV for between 20 msec to 1 sec failed to activate the Ca^{2+}-dependent Cl$^-$ conductance ($n = 6$, *data not shown*), supporting the contention that cytotrophoblast cells may lack VOCCs. Whether G-protein stimulation elevates intracellular Ca^{2+} in cytotrophoblast cells via an IP_3-sensitive intracellular store or perhaps via second messenger operated Ca^{2+} channels remains to be investigated. There are receptors on the microvillous membrane of the syncytiotrophoblast for a number of ligands which could putatively increase intracellular [Ca^{2+}] [29] and determination of which of these are able to regulate the Ca^{2+}-activated Cl$^-$ channel is clearly an important area for further investigation.

Possible Physiological Role for Ca^{2+}-activated Cl$^-$ Channels in Placental Trophoblast

When observed in other cell types the main function ascribed to these channels is vectorial Cl$^-$ transport. For example, the Ca^{2+}-activated Cl$^-$ conductance expressed in pancreatic duct cells is thought to account for continued Cl$^-$ secretion in ducts from transgenic mice which lack the CFTR Cl$^-$ channel [13]. Thus, the absence of expected pancreatic pathology in these animals suggests an important role in transepithelial Cl$^-$ secretion for the Ca^{2+}-activated channel. In another cell type, the lacrimal acinar cell, these channels are thought to be important in secretion of a Cl$^-$-rich fluid following stimulation by acetylcholine [26]. If expression of these channels is conserved during differentiation of cytotrophoblast cells into the transporting syncytiotrophoblast cell layer, which remains to be determined, they may also be important for maternofetal Cl$^-$ flux. At present, the extent to which placental Cl$^-$ transport takes place via a transcellular route involving ion transporters and channels, *vs.* an aqueous paracellular route, is unknown. Since extracellular fetal [Cl$^-$] exceeds that of the mother [4] and the transtrophoblast potential difference is around −4 mV [14], it may be necessary to invoke a transcellular route to explain this apparently uphill Cl$^-$ flux. Further studies are thus indicated to determine the extent to which a Ca^{2+}-activated transcellular Cl$^-$ flux contributes to overall maternofetal Cl$^-$ transport.

If the Ca^{2+}-activated Cl$^-$ channel is not involved in transplacental Cl$^-$ flux, it may be involved in more general cell homeostatis functions. For instance, in lacrimal cells Ca^{2+}-activated Cl$^-$ channels are probably also involved in cell volume regulation, since they are activated by hypotonic challenge [21]. A similar volume regulatory function in cytotrophoblast cells seems less likely because preliminary experiments did not show activation of this conductance during hypotonic shock. Rather, a separate Cl$^-$ conductance was activated (*see* Fig. 1C), which resembled volume-activated Cl$^-$ conductances reported in other cells [8, 31].

Another possible function for the Ca^{2+}-activated Cl$^-$ conductance present in cytotrophoblast cells may relate to stabilization of the membrane potential. Cytotrophoblast cells also express Ca^{2+}-activated K^+ channels [15] and it is feasible that these two Ca^{2+}-dependent channels act cooperatively. For instance, preliminary data showed that addition of the Ca^{2+} ionophore A23187 causes a significant increased in ^{86}Rb efflux from cytotrophoblast cells [30]. Since activation of the K^+ channel in isolation would cause the membrane potential to approach E_K, thus removing the gradient for K^+ efflux, the simultaneous activation of a Cl$^-$ channel could stabilize the membrane potential below E_K, allowing sustained K^+ efflux.

We are very grateful to Dr. P.D. Brown for his advice and comments during this study. The project is supported by the UK Medical Research Council (Grant Number G9209554). Dr. J.D. Kibble is a postdoctoral research fellow funded by the Wellcome Trust (Grant Number 037321/7/92).

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