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The Maxi-Chloride Channel in Human Syncytiotrophoblast: A Pathway for Taurine Efflux in Placental Volume Regulation?

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Abstract

Taurine (Tau), the most abundant amino acid in fetal blood, is highly concentrated in human placenta. During pregnancy, Tau is involved in the neurological development of the fetus, and in volume regulation of the placenta. The placenta may release taurine in parallel with K^+ and Cl^- in response to an increase in cell volume. However, the pathway for the volume-activated taurine efflux is unknown. One candidate is a voltage-dependent Maxi-chloride channel from apical syncytiotrophoblast membrane (MVM), with a conductance over 200 pS and multiple subconductance states. Our aim was to study whether this channel could be a Tau conductive pathway in the MVM. Purified human placental MVM were reconstituted into giant liposomes suitable for patch clamp recordings. Typical Maxi-chloride channel activity was detected in symmetrical chloride (Cl^-) solutions, and then taurine (Tau), Aspartate (Asp), and glutamate (Glu) solutions were used in the bath of excised patches to detect single channel currents carried by these anions. The relative permeabilities (P), estimated from the shift in reversal potential of current-voltage curves after anion replacement, were as follows: Chloride > Taurine = Glutamate = Aspartate. In Tau symmetric conditions using equivalent Cl^- concentrations, the slope conductance was 62.4 ± 7.3 pS. The data shows that Tau and other amino acids diffuse through the Maxi-chloride channel, which could be of great importance as part of the mechanism involved in the volume regulation process in human placenta.

Keywords: Chloride channel; Taurine transport; Placenta; Patch clamp; Volume regulation

1. Introduction

In placental syncytiotrophoblast, like in other epithelial cells, membrane channels provide routes for rapid and passive movement of solutes across plasma membranes. It is generally assumed that the major physiological role of membrane channels is to transport inorganic ions for processes such as transepithelial salt absorption and secretion, cell volume regulation, signal transduction, and control of membrane electrical properties. Increasing evidence indicates, however, that channels play an important role in organic solute transport in a wide variety of cell types and organisms. Some of the major physiological roles of organic solute channels include uptake of nutrients, excretion of metabolic waste products, control of mitochondrial metabolism, and volume-regulatory organic osmolyte transport [1].

The placenta, as virtually all cells in a multicellular organism, undergoes swelling or shrinking following changes of intracellular or extracellular osmotic pressure. Although the osmolarity of body fluids, particularly in mammals, is tightly controlled, significant variations may occur in physiological or pathological conditions. Cell-swelling stimulates the release of certain amino acids, in particular the non-protein amino acid taurine, via a pathway which has the characteristics of a channel rather than a carrier [2,3]. Indeed, on the basis of pharmacological inhibition, it was suggested that volume-activated taurine release is via Cl^- channels [4]. In accordance with this suggestion, whole patch

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clamp studies have shown that volume activated Cl^- channels in C6 glioma, Madin-Darby canine kidney (MDCK) and inner medullary collecting duct (IMCD) cells are permeable to taurine, aspartate and glutamate [5–7]. However, in some reports two separate pathways were also proposed for taurine fluxes and chloride ions [8–11].

The syncytiotrophoblast must be able to regulate its volume similarly to other cells. There are several reports of swelling-induced potassium and chloride efflux from freshly isolated placental tissue, suggesting, by the architecture of placental explants, that volume sensitive transport mechanisms are located at the apical membrane [12,13]. In addition Shennan [14] described the properties of volume activated taurine transport in human placental tissue explants and concluded, similarly to previous works [15–17], that placenta may release amino acids in parallel with K^+ and Cl^- in response to an increase in cell volume. Their results confirm that a hyposmotic challenge, hence cell swelling, markedly increases the fractional release of taurine from apical membrane of the syncytiotrophoblast in human placental explants. In the human placenta, taurine is the most abundant amino acid, and its concentration is 100- to 200-fold higher than the concentration in maternal blood [18]. This volume-activated taurine release from human placental tissue was inhibited by a variety of volume-activated anion channels blockers such as DIDS (stilbene derivative 4,4′ diisothiocyanostilbene-2,2′disulphonic acid) suggesting a conductive pathway and also, these results are in favor of a common route for amino acid and anion effluxes. However, one question remains: Is volume-sensitive taurine efflux via a channel?. We believed that an electrophysiological study of placental anion channels would help to resolve the issue of whether cell swelling-induced amino acid transport utilizes anion channels.

A possible molecular candidate for the DIDS-sensitive anion conductance mentioned above in apical syncytiotrophoblast plasma membrane is a Maxi-chloride channel. Maxi-chloride channels have been identified in secreting and absorbing epithelia [19], in non-epithelial cell types [20–23] and also in the apical membrane from human placenta using electrophysiological techniques, by ourselves and others authors [24–26]. This channel may play a role in complex cellular regulation involving volume-dependent processes [27] such as those reported in C127i cells by Sabirov et al. where they conclude that this channel serves as a pathway for swelling-induced ATP release [27]. In several cells this channels is activated by cell swelling or patch excision from non-swollen cells, and there are several indications that the cytoskeleton is involved in its regulation [28].

We have reported a Maxi chloride channel from normal human term placentas reconstituted in giant liposomes [25,26,29]. Human placental apical membrane was purified by differential and gradient centrifugation and fused into small liposomes. Giant liposomes were then generated by the method of cycle dehydration and rehydration of lipid vesicles [30]. The channel is selective for anions over cations, has a large conductance (>200 pS), multiple subconductance states and voltage dependence of its open probability [25]. This channel is inhibited by DIDS and it is inhibited directly by arachidonic acid and other cis unsaturated fatty acids [26]. It is also regulated by annexin 6 [31] and by steroid hormones [29].

Our aim in the current work was to demonstrate, by the patch clamp technique, that taurine is able to permeate the placental Maxi-chloride channel. This finding could be convincing evidence for a shared pathway for volume-activated amino acid and anion effluxes.

2. Materials and methods

2.1. Placenta collection

Placentae obtained from normal pregnancies were collected immediately after delivery from the San José Hospital Maternity Unit and transported to the laboratory on ice.

2.2. Preparation of placental apical membrane (MVM)

The human placental microvillous membranes (MVM) vesicles were prepared by a method we have described [32] that allows simultaneous isolation of apical and basal membranes from the same placenta. This method is a modification of the method described by Illsley et al. [33]. We added one step to isolate plasma membrane free of mitochondrial membranes [34] and have preserved the conditions used in our apical membrane isolation protocol when working with this simultaneous isolation protocol.

The purification method involved precipitation of non-microvillous membrane with magnesium ions, differential centrifugation and a sucrose step gradient. All solutions were buffered with 20 mmol/L Tris-Maleato, pH 7.4. A portion (2–3 ml) of the microvillous-enriched preparation containing about 10–15 mg of protein was overlayed on the sucrose gradient. The band at the 37/45% sucrose interface was collected and diluted tenfold with 20 mmol/L Tris-HEPES, pH 7.4, before centrifugation at 110,000 × *g* for 30 min. The final pellet was resuspended in 300 mmol/L sucrose, 20 mmol/L Tris-maleate, pH 7.4, and stored in liquid nitrogen.

The purity and enrichment of the MVM membrane fraction was determined routinely by assaying for alkaline phosphatase activity, an apical membrane marker and adenylate cyclase as a basal membrane marker and cytochrome-c oxidase/succinate dehydrogenase as mitochondrial membrane markers. Enrichment of alkaline phosphatase activity for MVM was 17–21-fold and was essentially free of basal membranes and mitochondrial membranes.

2.3. Reconstitution of the apical membrane into giant liposomes

Giant liposomes were prepared by submitting a mixture of the isolated apical membrane vesicles and asolectin lipid vesicles to a partial dehydration/rehydration cycle, as reported by Riquelme et al. [30]. An aliquot containing 100–150 μg of membrane protein was mixed with 2 mL of a 13 mmol/L (in terms of lipid phosphorus) suspension of the asolectin vesicles. After the partial dehydration/rehydration cycle, the diameter of the resulting giant multilamellar liposomes ranged from 5 to 100 μm.

2.4. Patch clamp measurements

Aliquots of 1–3 μL of giant liposomes were deposited into an excised Patch chamber (RC-28, Warner Instruments Corporation, USA) mixed with 0.4 ml of the buffer of choice for electrical recording (bath solution). Single-channel recordings were obtained by patch-clamp techniques as described by Hamill et al. [35]. Giga seals were formed on giant liposomes with glass microelectrodes of 5–10 MΩ resistance. After sealing, withdrawal of the pipette from the liposome surface resulted in an excised patch. Current was recorded with an EPC-9 patch-clamp amplifier (Heka Elektronic, Lambrecht/Pfalzt., Germany) at a gain of 50–100 mV/pA and a filter setting of 10 kHz. The holding potential was applied to the interior of the patch pipette, and the bath was maintained at virtual ground ($V = V_{bath} - V_{pipette}$). The bath was grounded via an agar bridge and the junction potential was compensated

for when necessary. The signal was analyzed off-line by means of the TAC (Bruxton Corporation) and Pulse Fit (Heka, Lambrecht/Pfalz.Germany) software. All measurements were made at room temperature.

2.5. Solutions

The pipette and bath solutions had the following composition (in mmol/L): 140 *N*-methyl-D-glucamine chloride (NMDGCl), 2.6 $CaCl_2$, 1.3 $MgCl_2$, 10 Na-HEPES, pH 7.4, unless otherwise stated. Solutions containing 140 mmol/L aspartate or glutamate were used to detect single channel current carried by these anions. Taurine in solution at physiological pH 7.4 is a zwitterionic molecule and exhibits no net charge. To detect single-channel currents carried by taurine, solutions containing 500 mmol/L taurine at pH 8.2 were used, achieving concentrations of negatively charged taurine similar to the other anions (in mmol/L: 140 taurine chloride (TauCl), 2.6 $CaCl_2$, 1.3 $MgCl_2$, 10 Na-HEPES, pH 8.2). The concentration of negatively charged taurine was made by Henderson-Hasselbalch equations ($pH = pK + \log [A-]/[AH]$). The values obtained were similar to those reports by Banderali et al. for the anion taurine at pH 8.2.

2.6. Statistical and data analysis

Results are expressed as means ± SEM. Measures of statistical significance were obtained using Student's *t*-test. A *p*-value of less than 0.05 was considered significant. Also measures of statistical significance were obtained using one-way ANOVA plus Bonferroni multiple comparisons test. A *p*-value of less than 0.01 was considered significant. The *n* for statistical analysis is of the total number of experiments.

Relative permeability of the channel was determined by measuring the shift in V_{rev} upon changing the solutions on one side of the membrane containing 148 mmol/L Cl^- to another with 140 mmol/L *X*, where *X* is the substitute anion and 8 mmol/L Cl^-. The permeability ratio was estimated using the Goldman-Hodgkin-Katz (GHK) equation:

$$V_{REV} = \frac{RT}{F} \ln \frac{P_x[x]_i + P_{Cl}[Cl]_i}{P_{Cl}[PCl]_0} \Rightarrow \frac{P_x}{P_{Cl}} = \frac{[Cl]_0 \, e^{\frac{FV_{rev}}{RT}} - [Cl]_i}{[X]_i}$$

The voltage dependence of the *open channel probability (P_o)* of the Maxi-chloride channel has been obtained as the ensemble-averaged current of *N* consecutive current responses to a voltage ramp pulse applied in independent seals. These P_o values were calculated as:

$$P_0 = \frac{I/V}{G_{max}}$$

Where *I* is the patch current, *V* is the voltage, and G_{max} is the maximal patch conductance near 0 mV. A voltage ramp pulse was usually applied from −120 mV to +120 mV, at a rate of 40 mV/s. Where *F* is the Faraday constant, *R* is the gas constant and *T* is absolute temperature.

3. Results

Single channel current traces were performed by the patch-clamp method in giant liposomes containing reconstituted MVM from normal human placenta. A total of 74 high-resistance excised patches in an ''inside out'' configuration were obtained from cell-size giant liposomes containing MVM from $n = 13$ separate placentas from normal term pregnancies.

To detect single-channel currents carried by taurine, solutions at pH 8.2 were used, achieving concentrations of negatively charged taurine similar to the other anions as mentioned in Section 2.

3.1. A pH of 8.2 does not affect the Maxi-chloride channels from human Placental MVM

The fact that taurine solutions must be at pH 8.2 compelled us to evaluate the effect of this pH on the activity of the Maxi-chloride channel. In agreement with previous results [25,26,29,31], typical Maxi-chloride channel activity was detected in symmetrical chloride solutions at pH 7.4. Similar activity was detected when experiments were performed at pH 8.2.

Different subconductance levels were seen at pH 8.2 as described previously for the Maxi-chloride channel at pH 7.4. Single channel current traces, at the indicated holding potential, for Maxi-chloride channels at pH 7.4 and at pH 8.2, are shown in Figure 1A. Both records include a primary conductance state and also different subconductance levels.

A linear current-potential relationship was obtained with reversal at 0 mV (Fig. 1B) for Maxi-chloride channels at pH 7.4 and at pH 8.2. Only the dominant current level (i.e. the fully open state) was used, ignoring any subconductance levels for current-voltage relationships in each case. These conductances were observed in experiments carried out with bath and pipette solutions containing (in mmol/L): 140 NMDGCl, 2.6 $CaCl_2$, 1.3 $MgCl_2$, 10 Na-HEPES, and pH 7.4 or pH 8.2.

In agreement with previous results [25,26] the single-channel slope conductance was 213 ± 5.7 pS ($n = 26$) for the Maxi-chloride channel at pH 7.4 and slope conductance was 212 ± 6.4 pS ($n = 12$) for the Maxi-chloride channel at pH 8.2. The alkalinization of the solutions did not alter the conductance of the single-channel currents.

The voltage dependence of the open channel probability (P_o) of the Maxi-chloride channel has been obtained as the ensemble-averaged current of 10–25 consecutive current responses to a voltage ramp pulse applied in independent seals as described in the experimental procedures section. Fig. 1C shows the curve P_o versus voltage for these results. In agreement with the characteristics of the open probability reported before for the placental Maxi-chloride channel from normal placenta at pH 7.4 [26,29] the P_o versus voltage relationship could be described by a bell-shaped curve at pH 7.4 and at pH 8.2.

The biophysical characteristic of Maxi-chloride channels at pH 7.4 and pH 8.2 are similar suggesting that pH 8.2 did not affect the electrical activity of the channel.

3.2. Relative taurine permeability of Maxi-chloride channels from human Placental MVM

To determine the taurine selectivity of Maxi-chloride channel, we measured the I–V relationships of excised patches under asymmetric anionic conditions. The I–V relationship was determined by voltage ramps from −120 mV to +120 mV. The pipette contained (in mmol/L): 140 NMDGCl, 2.6 $CaCl_2$, 1.3 $MgCl_2$, 10 Na-HEPES, and pH 8.2. The bath contained the same solution, but 140 mM chloride was replaced with taurine. Measurements with a substitute amino acid were always done under similar concentrations as the measurements in symmetrical chloride solutions. As described in experimental procedures, to increase the percentage of negatively charged taurine

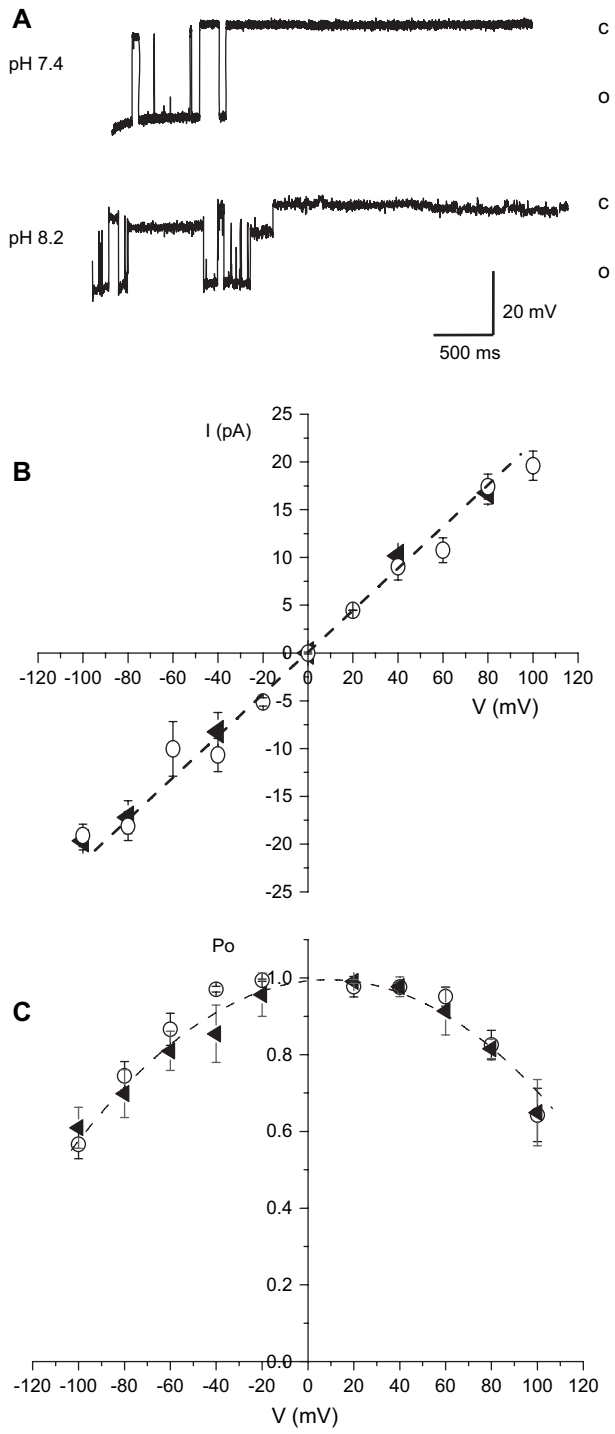

Fig. 1. Comparative activity of the Maxi-chloride channel from human placenta in control conditions at pH 7.4 and in solutions at pH 8.2. Single-channel currents recorded in excised patch from MVM reconstituted into giant liposomes. Currents were recorded in bath and pipette solutions containing (in mmol/L): 140 NMDGCl, 2.6 $CaCl_2$, 1.3 $MgCl_2$, 10 Na-HEPES, pH 7.4 or pH 8.2. (A) Representative recordings at −80 mV at the indicated pH. (B) Current-voltage plots for major conductance substate of the channel at pH 7.4 (open circles, $n = 39$ seals from $n = 5$ placentas) and at pH 8.2 (black triangles, $n = 15$, from $n = 5$ placentas). The conductance obtained as the slope for the linear regression curve for the data points was 213 ± 5.7 pS and 212 ± 6.4 pS at pH 7.4 and 8.2 respectively. (C) The classical bell-shaped curve was obtained at pH 7.4 condition (open circles, $n = 9$ seals from $n = 3$ placentas) and in pH 8.2 conditions (black triangles, $n = 9$ seals from $n = 3$ placentas).

we performed patch clamp experiments using a 500 mmol/L taurine solution at pH 8.2. In this NMDG-taurine solution the concentrations of negatively charged taurine was around 140 mmol/L. Fig. 2A,B show two I−V relationship curves obtained submitting the channel to voltage ramps while exposed to symmetrical chloride concentrations (NMDG-Cl) and taurine (NMDG-Tau) on the bath side, respectively. Under asymmetrical anion concentrations (148 mmol/L Cl^- pipette and 140 mmol/L taurine, 8 mmol/L Cl^- bath) in NMDGCl solution, the reversal potential shifted from 0 to −21.90 mV (Fig. 2B, arrow). Taurine to chloride permeability ratio was calculated from the shift in V_{rev} using the GHK equations (see Section 2) and gave a P_{Tau}/P_{Cl} value of 0.39 ± 0.08 ($n = 12$ from $n = 5$ separate placentas).

3.3. Single-channel taurine currents. Unitary conductance

In taurine symmetric conditions, single channel currents were smaller compared to amplitudes using equivalent Cl^- concentrations, but the pattern of the recording also presented different subconductance levels. Single-channel current traces, at the indicated holding potential, for chloride channels are shown in Fig. 3A. A linear current-potential relationship was obtained with reversal potential at 0 mV (Fig. 3B) for the dominant current level and the single-channel conductance was 62.4 ± 7.3 pS ($n = 19$, from $n = 6$ separate placentas). These conductances were observed in experiments performed with the bath and pipette solutions containing (in mmol/L): 140 NMDGTau, 2.6 $CaCl_2$, 1.3 $MgCl_2$, 10 Na-HEPES, and pH 8.2. The single channel taurine currents present an open probability similar to the typical voltage dependence for the placental Maxi-chloride channel in symmetric chloride solutions (Fig. 5) and it could be described by a bell-shaped curve. As shown by the voltage ramp in Fig. 3C the channel was normally open at potentials between −50 mV and +50 mV; only higher voltages, in either a positive or negative direction, induced channel closure.

3.4. DIDS blocking single-channel taurine currents

The Maxi-chloride channels from normal placental apical membrane are sensitive to the stilbene derivative 4,4′diisothio cyanostilbene-2,2′-disulphonic acid (DIDS) as previously reported in chloride symmetrical conditions [24,26]. The effect of DIDS on the single channel currents in taurine symmetric conditions was also investigated. Fig. 3D shows two I−V relationship curves obtained submitting the channel to voltage ramps when exposed to symmetrical taurine concentrations in control condition (0 mmol/L DIDS) and after the addition of 1 mmol/L DIDS.

The addition of DIDS reduced the total current in the patch to 30% with respect to the control currents (100%) at −80 mV holding potential while, at +80 mV there was a reduction to 50% of the total patch control currents ($n = 8$). These results are comparable to the results obtained for the Maxi chloride channel from normal placenta in chloride solutions [24,26].

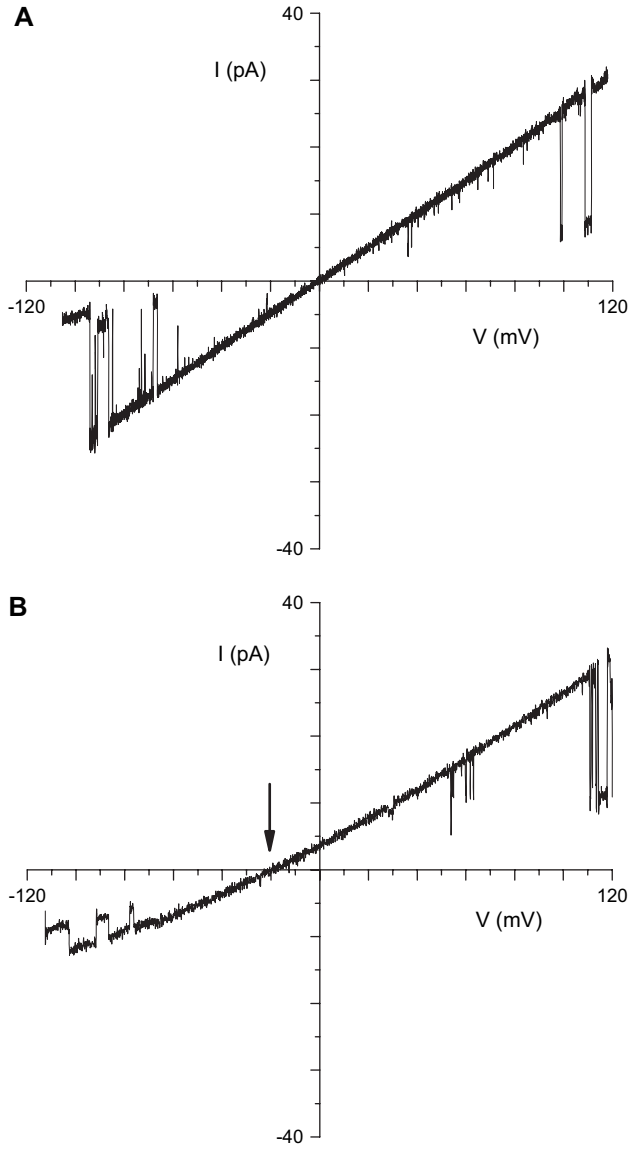

Fig. 2. Taurine permeability of the Maxi-chloride channel from human placenta. Current-voltage curves obtained from voltage ramps between −120 and 120 mV in excised patch recordings. (A) Maxi-chloride channel was exposed to a symmetrical chloride concentration (148 mmol/L). (B) 140 mM of chloride in bath solution was replaced by taurine (arrows indicate the reversal potential).

3.5. Relative aspartate and glutamate permeability of Maxi-chloride channels from human placental MVM

In order to determine the selectivity of this channel for other amino acids such as aspartate (Asp) and glutamate (Glu), 140 mmol/L bath chloride was replaced with Asp or Glu. The pipette solution contained 148 mmol/L Cl^-. When the same concentration of Cl^- was perfused in the bath solution, the current recorded in response to a ramp pulse showed an almost linear I–V relation with a reversal potential of 0 mV. The Asp or Glu- to- Chloride permeability ratio was calculated from changes in reversal potential brought about by anionic amino acids replacement using the equation derived from the GHK equation. The value for permeability ratio P_{Asp}/P_{Cl} was 0.42 ± 0.14 ($n = 7$ from $n = 2$ separate placentas) and the value for P_{Glu}/P_{Cl} was 0.42 ± 0.12 ($n = 8$ from $n = 2$ separate placentas). Figure 4 shows a typical recording of a voltage ramp pulse for the patches exposed to Asp (Fig. 4A) or Glu (Fig. 4B) in asymmetrical conditions, showing reversal potential displacement (arrow).

3.6. Conductance to aspartate and glutamate of Maxi-chloride channels from human placental MVM

The experiments were carried out in symmetrical solutions where Asp or Glu was the major permeate species.

Different subconductance levels were seen as described above for taurine, similar to the previous reports for the single chloride channel currents in symmetrical chloride solutions, however the dominant level conductance was smaller. The slope conductance was obtained from a linear current-potential relationship with reversal potential at 0 mV (Fig. 4C) either for the glutamate or aspartate. The values were 61.3 ± 3.9 ($n = 8$) and 74.7 ± 4.8 ($n = 7$) for glutamate and aspartate respectively. These conductances were observed in experiments performed with the bath and pipette solutions containing (in mmol/L): 140 NMDGGlu or NMDGAsp, 2.6 $CaCl_2$, 1.3 $MgCl_2$, 10 Na-HEPES, and pH 7.4.

3.7. Open probability (P_o) of the channels currents for amino acids

Experiments were carried out in symmetrical solutions where Tau, Asp or Glu was the major permeate species. The voltage dependence of the open channel probability of channels has been obtained as the ensemble-averaged current of consecutive current responses to a voltage ramp pulse applied in independent seals as described in Experimental Procedure. Fig. 5 shows the curve for P_o versus voltage for these results. The channel was normally open at potentials between −50 mV and +50 mV; only higher voltage steps, in either a positive or negative direction, induced channel closure either for Tau, Asp or Glu as permeate species. These results are in agreement with the characteristics of P_o reported previously for the placental Maxi-chloride channel from normal placenta and the open probability (P_o) versus voltage relationship for our normal contemporaneous experiments in symmetric chloride solutions, all of them could be described by a bell-shaped curve.

3.8. Comparative summary among the substitute anionic amino acids tested

The average results for all amino acids tested are shown in Fig. 6. The relative permeability (P_x/P_{Cl}) is shown in Fig. 6A, where the selectivity of the channel estimated from P_x/P_{Cl} gave the following sequence: Chloride > Taurine = Glutamate = Aspartate, to all of them the P_x/P_C values was over 0.3 and no significant difference was observed among these amino acids, demonstrating a high permeability of the Maxi-chloride channel for Asp, Glu and Tau.

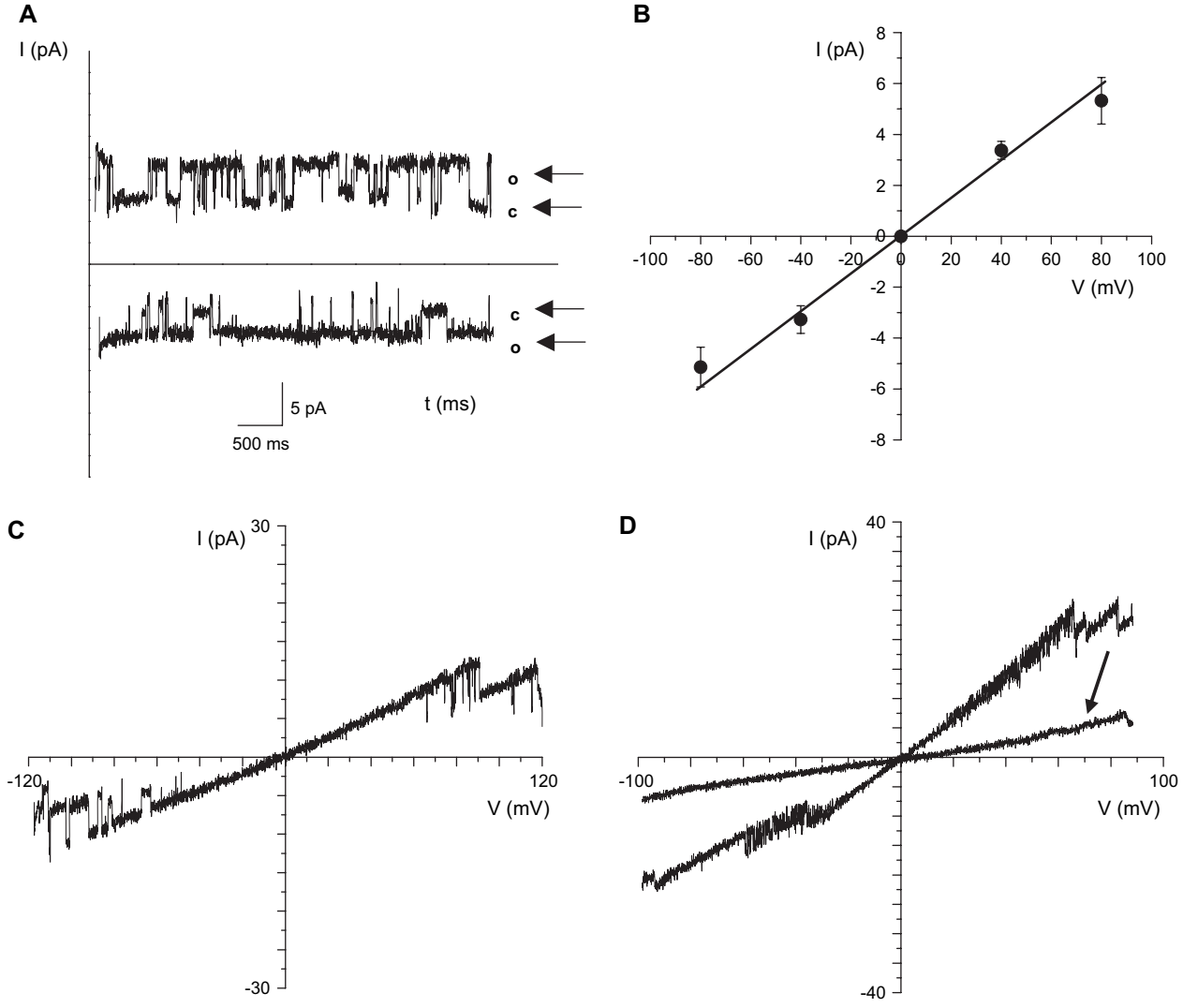

Fig. 3. Recordings of the Maxi-chloride channel from human placenta in taurine symmetrical conditions. Single-channel currents recorded in excised patch from MVM reconstituted into giant liposomes. Currents were recorded in bath and pipette solutions containing (in mmol/L): 140 NMDG-Tau, 2.6 $CaCl_2$, 1.3 $MgCl_2$, 10 Na-HEPES, pH 8.2. (A) Representative recordings at ±80 mV. (B) Current-voltage plots for major conductance substate of the channel ($n = 10$ from $n = 3$ placentas). The conductance obtained as the slope for the linear regression curve for the data points was 62.4 ± 7.3 pS. (C) Current-voltage curve obtained from a voltage ramp between −120 and 120 mV in excised patch recording. (D) Current-voltage curves obtained from a voltage ramp in absence of DIDS (control) and in presence of 1 mmol/L DIDS applied to the bath (arrow).

We also examined relative conductance in patches exposed to symmetrical substitute amino acids. Figure 6B shows the conductance ratio (G_x/G_{Cl}) determined by comparing symmetrical substitute anion solutions to Cl in the pipette and bath solutions. The amino acid conductance values were in the intermediate range for classical ion channels (over 60 pS). The relative conductances exhibited the following sequence: Chloride > Tau = Glu = Asp, where the conductance values were 1/4 of the chloride conductance. No significant difference was observed in the conductance among the different amino acids.

4. Discussion

The present study demonstrates that the Maxi-chloride channel from apical syncytiotrophoblast membrane is permeable to taurine and two other amino acids: aspartate and glutamate. The taurine single current has a lower conductance than the conductance of the channel in chloride symmetrical conditions, and also the taurine currents are sensitive to DIDS. The relative conductances in patches exposed to symmetrical substitute amino acids led to a slight increment in the conductance to aspartate with respect to taurine and glutamate, but no significative differences was observed among them. The range for conductances was 60–75 pS and the relative permeability of the amino acids to chloride ions was around 0.4, where the permeability reflects the ability of the ion to enter the channel pore and the conductance reflects the ability of the anion to traverse the entire length of the channel.

The taurine permeability ratio ($P_{Tau}/P_{Cl} = 0.39$) obtained in the present work can be compared with that reported by Banderali and Roy [5] for the outward rectifying chloride channel

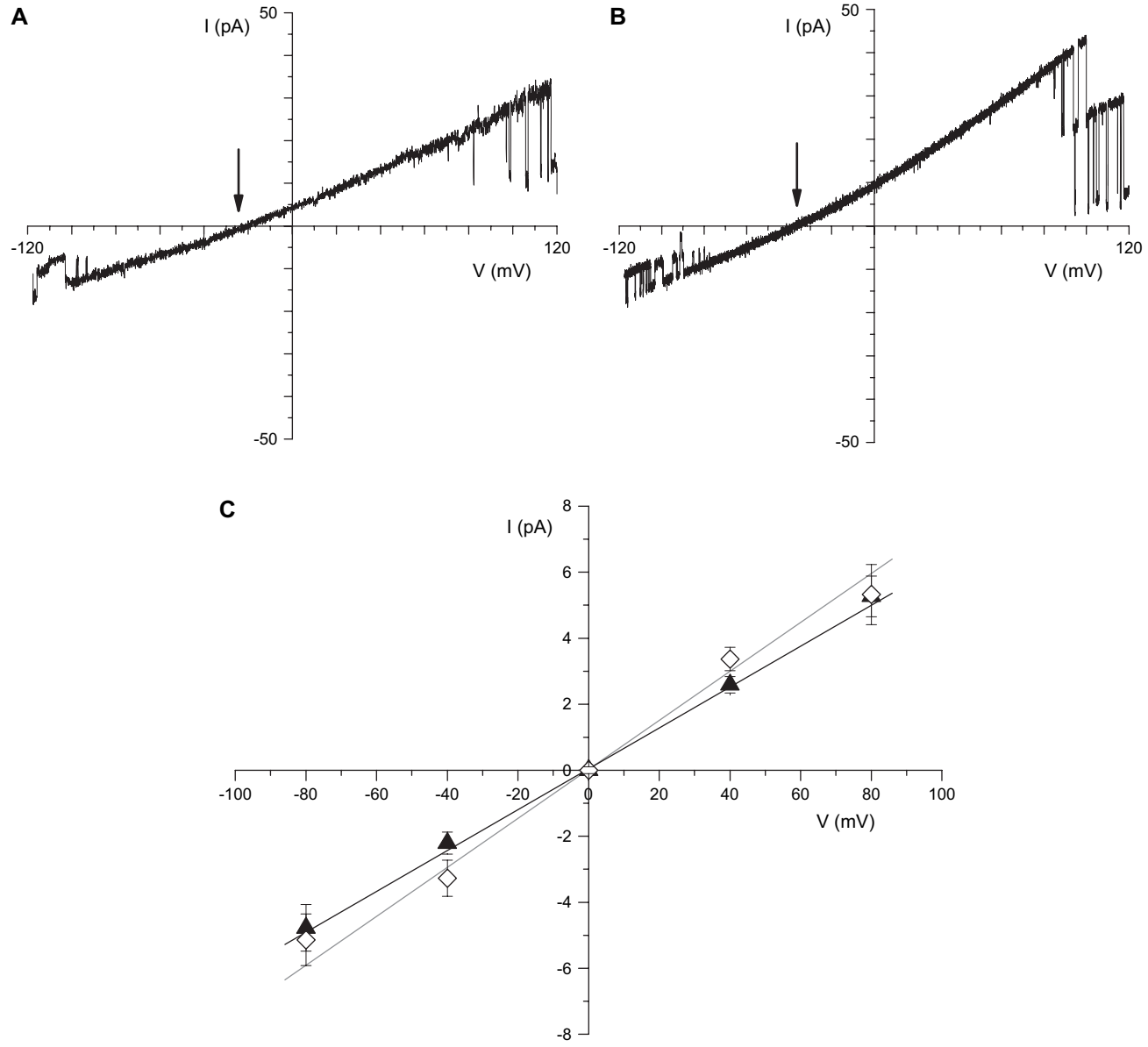

Fig. 4. Glutamate and aspartate permeabilities of the Maxi-chloride channel from human placenta. Maxi-chloride channel was exposed to asymmetrical conditions containing (in mmol/L): 140 NMDGCl, 2.6 $CaCl_2$, 1.3 $MgCl_2$, 10 Na-HEPES, pH 7.4 in pipette and 140 NMDG-Glu or Asp, 2.6 $CaCl_2$, 1.3 $MgCl_2$, 10 Na-HEPES, pH 7.4 in bath solution. Current-voltage curves obtained from voltage ramps between −120 and 120 mV in excised patch recordings. (A and B) Arrows indicate the reversal potential when part of chloride in the bath solution was replaced by 140 NMDG-Glu or 140 NMDG-Asp respectively. (C) Current-voltage (I/V) curves. Black line (black triangle) represents the channel exposed to symmetrical conditions 140 mmol/L NMDG-Glu, the slope conductance was 61.3 ± 3.9 pS ($n = 8$ from $n = 2$ placentas). Grey line (open squares) represents the channel exposed to symmetrical conditions 140 mmol/L NMDG-Asp pH 7.4, the slope conductance was 74.7 ± 4.8 pS ($n = 7$ from $n = 2$ placentas).

in MDCK cells. These authors obtained two values with different methods: 0.75 and 0.43. However the value reported here is higher than the value reported by Boese et al. [7] for the VSOAC in rat IMCD cells, where the relative permeability P_{Tau}/P_{Cl} was 0.15−0.2.

The glutamate and aspartate permeation through the Maxi-chloride channel demonstrated that there are no significant differences between the relative permeability of these two amino acids. The values of the permeability ratios for aspartate and glutamate (0.42) are higher than those obtained previously for anion channels in MDCK cells [5] and in rat hippocampal neurons [36], where the range of the values was 0.13−0.19.

These results provide strong evidence that the placental Maxi-chloride channel is permeable to organic anions like taurine, glutamate and aspartate and it would mean that this channel plays a role in phenomena such as volume regulation and other biological processes.

As commented in the Introduction, in human placental explants [14] and several types of cells, on the basis of pharmacological inhibition, cell-swelling stimulates the release of taurine via a pathway that could be a chloride channel [1−4]. Even though in some reports two separate pathways were also proposed for taurine fluxes and chloride ions [8−11], various reports propose a common pathway for both chloride and

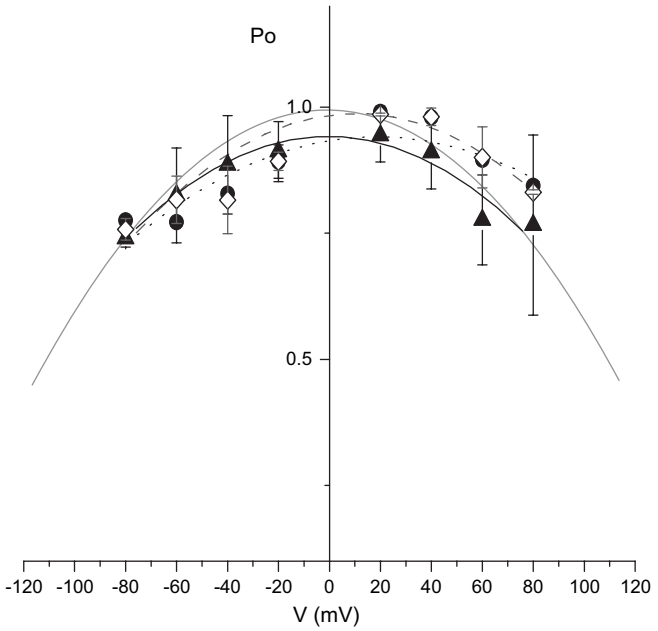

Fig. 5. Comparative open probability of the Maxi-chloride channel from human placenta in taurine, glutamate and aspartate solutions. The classical bell-shaped curve was obtained with taurine (black circles, dot line), glutamate (black triangles, black line) and aspartate (open diamond, dashed line) and for the historical experiments in chloride solution (grey line). Significant differences were not observed.

amino acids during the regulatory volume decrease (RVD). This hypothesis has been supported by electrophysiological experiments which have demonstrated that volume activated Cl^- channels in some cells are permeable to taurine, aspartate and glutamate [1,5–7,37,38]. In the present work, we show that taurine, glutamate and aspartate are able to permeate across the Maxi-chloride channel from MVM placental syncytiotrophoblast giving convincing evidence for a shared pathway for both chloride and anionic amino acids.

Additionally, there is evidence that suggests that the Maxi-chloride channel, activated by cell swelling or patch excision from non-swollen cells, is regulated by the cytoskeleton and it is involved in volume regulation processes in other cells [2,28], including serving as a pathway for swelling-induced ATP release [27].

In addition to the above observations, our present data from excised patches suggests, that the Maxi-chloride channel from syncytiotrophoblast could be the conductance pathway involved in placental volume regulation and underlies the volume activated taurine efflux sensitive to the classical chloride channel blockers describe by Shennan [14] for term human placenta. Our results are in agreement with previous evidence that imply a DIDS-sensitive anion conductance for volume-activated taurine release from human placental tissue, and also propose a common pathway for amino acid and anion effluxes.

An interesting question is whether taurine moves via the swelling activated pathway in the anionic or in the zwitterionic form, because taurine is almost completely zwitterionic at physiological pH. Probably the large amount of taurine

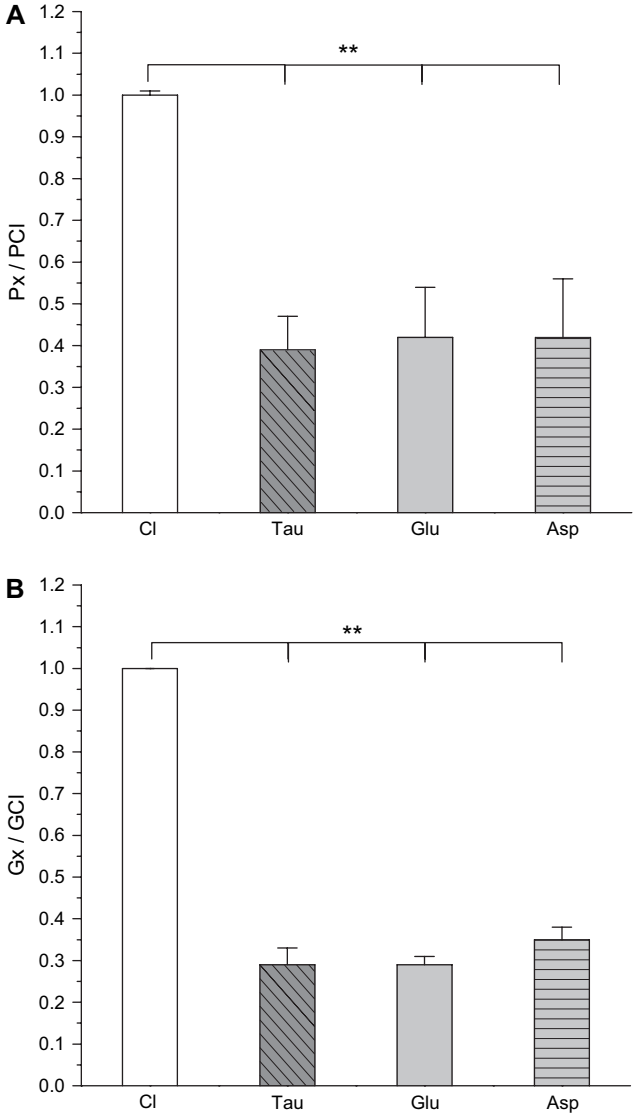

Fig. 6. Relative permeability and conductance of the Maxi-chloride channel from human placenta to amino acids. (A) Relative permeability of different amino acids respect to chloride. The shift in Vrev was obtained from experiments such as those shown in Figures 2B and 4A,4B. These values were used to calculate relative permeabilities from the GHK equations (see Section 2). (B) Relative conductance of different amino acids respect to chloride. It was determined by measured the conductance with substitute amino acid and dividing this value by the conductance with symmetrical chloride solution. The data show comparable relative permeability and conductance for the different amino acids with respect to chloride, with no significant differences between amino acids tested (**p-value <0.01 relative to value chloride conditions, one way ANOVA).

released during RVD (50% of all solutes) is transported as an electroneutral zwitterion and not as an anion. Recently some reports give information about the permeation of non-charged solutes across ion channels, specifically for taurine. Guizouarn et al. [39], gave the first experimental evidence that under physiological conditions, taurine is released as a zwitterion via the DIDS sensitive channel.

In summary, we propose that for the placenta, a specific molecular element in the mechanism termed RVD is represented by the Maxi-chloride channel from placental apical membrane which is

sensitive to DIDS and is also permeable to organic osmolytes such as taurine. RVD occurs the following of extracellular osmolarity and the consequent cell swelling induced potassium, chloride and taurine efflux from term placental tissue explants. The resulting exit of KCl and taurine drives water efflux and therefore restores the original cell volume. Because taurine in placenta is accumulated at high concentrations through active transporters, the opening of Maxi-chloride channels could be responsible for a significant proportion of the taurine efflux. We believe that this evidence support an important role for chloride channels in taurine mediated placental volume regulation.

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