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Thiamine Triphosphate Activates an Anion Channel of Large Unit Conductance in Neuroblastoma Cells

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Abstract. In neuroblastoma cells, the intracellular thiamine triphosphate (TTP) concentration was found to be about 0.5 μM, which is several times above the amount of cultured neurons or glial cells. In inside-out patches, addition of TTP (1 or 10 μM) to the bath activated an anion channel of large unit conductance (350–400 pS) in symmetrical 150 mM NaCl solution. The activation occurred after a delay of about 4 min and was not reversed when TTP was washed out. A possible explanation is that the channel has been irreversibly phosphorylated by TTP. The channel open probability (P_o) shows a bell-shaped behavior as a function of pipette potential (V_p). P_o is maximal for -25 mV $< V_p <$ 10 mV and steeply decreases outside this potential range. From reversal potentials, permeability ratios of $P_{Cl}/P_{Na} = 20$ and $P_{Cl}/P_{gluconate} = 3$ were estimated. ATP (5 mM) at the cytoplasmic side of the channel decreased the mean single channel conductance by about 50%, but thiamine derivatives did not affect unit conductance; 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (0.1 mM) increased the flickering of the channel between the open and closed state, finally leading to its closure. Addition of oxythiamine (1 mM), a thiamine antimetabolite, to the pipette filling solution potentiates the time-dependent inactivation of the channel at $V_p = -20$ mV but had the opposite effect at +30 mV. This finding corresponds to a shift of P_o towards more negative resting membrane potentials. These observations agree with our previous results showing a modulation of chloride permeability by thiamine derivatives in membrane vesicles from rat brain.

Key words: Thiamine triphosphate — Anion channel — Oxythiamine — Neuroblastoma — Patch clamp — HPLC

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Introduction

For more than two decades, thiamine triphosphate (TTP) has been suspected to play a role in membrane permeability (for review, *see*: Cooper & Pincus, 1979; Haas, 1988), but its molecular target was never identified. TTP exists at low concentrations (<1 μM) in most cell types (Bettendorff et al., 1991). The difficulty in studying the effects of TTP on membrane permeability stems from it probably acting only from the cytoplasmic side of the membrane.

We have previously studied a membrane-associated thiamine triphosphatase (TTPase) in the electric organ of *Electrophorus electricus* (Bettendorff et al., 1987, 1989; Bettendorff, Wins & Schoffeniels, 1988). This enzyme was activated by anions with a sequence of efficiency similar to that predicted by the lyotropic series, except for sulfate, which at millimolar concentrations, was strongly inhibitory. These results have recently been confirmed by studies on skeletal muscle (Matsuda et al., 1991). Furthermore, TTPase is irreversibly inhibited by 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS), a chloride channel blocker, and anions protect it against the effect of DIDS (Bettendorff et al., 1988). These results at least suggested the existence of an anion-binding regulatory site on the membrane-bound TTPase. Since then, we found substantial evidence that thiamine derivatives may play an important role in the regulation of anion permeability (Bettendorff et al., 1993*a*).

An investigation of $^{36}Cl^-$ uptake in membrane vesicles prepared from rat brain showed that extracellular TTP (≥1 mM) increased $^{36}Cl^-$ uptake by less than 10% (Bettendorff, Wins & Schoffeniels, 1990). We then tried to introduce TTP into the vesicles by preincubating them in the presence of the TTP precursors thiamine and thiamine diphosphate (TDP). Indeed, under these conditions, the TTP content and chloride uptake increased (Bettendorff et al., 1993*b*). On the other hand, oxythiamine, a thiamine antimetabolite, inhibits chloride uptake in these membrane vesicles (Bettendorff et al., 1993*a*). We presented evidence that the oxythiamine-sensitive $^{36}Cl^-$ uptake was not carrier-mediated and was increased by membrane depolarization, suggesting that a voltage-sensitive channel was involved. DIDS was also found to inhibit chloride uptake, and in the presence of DIDS, oxythiamine had no effect. We postulated that cytoplasmic TTP can activate chloride channels which can be suppressed by an action of oxythiamine from the extracellular side of the membrane.

In the present study, we measured the TTP content of cultured neuroblastoma cells and applied the patch-clamp technique to study the effects of thiamine derivatives on membrane currents. The experiments were focused on the question whether TTP can activate Cl^- currents at the cytoplasmic side, using the inside-out membrane configuration. The proposed inhibitory effect of oxythiamine was studied after its addition to the bath or to the pipette filling solution, since our previous findings suggested that, in contrast to TTP, oxythiamine acts from the extracellular side of the membrane. This electrophysiological approach provides a basis for studying a modulatory role of TTP at the single channel level of Cl^- currents.

Materials and Methods

Chemicals

TTP was from Wako Chemicals (Osaka, Japan). TDP, thiamine monophosphate (TMP), oxythiamine, ATP, DIDS and thiamine were from Sigma (St. Louis, MO).

Cell Culture

The experiments were performed on the mouse neuroblastoma clonal cell line Neuro-2a (a gift from Prof. G. Moonen, Laboratory of Human Physiology, University of Liège). The cells were grown in 100 mm petri dishes (Nunc, Roskilde, Denmark) with 10 ml Dulbecco's modified Eagle's medium (DMEM, GIBCO, Ghent, Belgium) supplemented with 10% fetal calf serum (GIBCO) and enriched with glucose (6 mg/ml) at 37°C in 5% CO_2.

Determination of Thiamine Derivatives in Cultured Cells

The procedure was essentially as described previously (Bettendorff et al., 1991). Briefly, the culture medium was carefully removed from dishes with confluent cells and replaced with saline (145 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM glucose and 10 mM HEPES-Tris, pH 7.4). This procedure was repeated twice to remove thiamine in the supernatant. After the last wash, the jet from the pipette was directed on the cells to detach them from the dish. The cells were collected in 10 ml of saline and centrifuged at 700 × *g* for 3 min; they were resuspended in 350 μl of saline. The cells were disrupted by addition of 70 μl trichloroacetic acid (60%) and incubated on ice for 15 min. Proteins were sedimented by centrifugation (5,000 × *g*, 15 min) and the supernatant extracted with 3 × 1.5 ml diethyl ether.

Thiamine derivatives were determined by HPLC (Bettendorff et al., 1991). Prior to injection on the column, they were oxidized to fluorescent thiochromes by addition of 50 μl alkaline ferricyanide solution (4.3 mM potassium ferricyanide solution in 15% NaOH) to 80 μl of sample.

The separation was achieved on a PRP-1 reversed-phase analytical column (Hamilton, Reno, NV) and the mobile phase was composed of sodium phosphate (50 mM), tetrabutylammonium hydrogen sulfate (25 mM) and 4% tetrahydrofuran adjusted at pH 9.0 with NaOH. The sample loop was 20 μl and the flow rate was 0.5 ml/min. The detector was a fluorescence spectrometer (LS-4, Perkin Elmer) with the wavelengths set at 365 nm for excitation and at 433 nm for emission.

Protein concentrations were determined by the method of Peterson, 1977.

Patch-Clamp Experiments

The cells were seeded on coverslips in petri dishes (30 mm, Nunc) and incubated for 1–3 days as described above. On the day of the experiment, the culture medium was replaced by a high-NaCl solution consisting of (in mM): 142 NaCl, 4 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 18 glucose and 20 HEPES at pH 7.4 (310 mOsm). The cells were used thereafter within 2–3 hr. Patch-clamp experiments were carried out as described (Hamill et al., 1981) with an EPC-7 patch amplifier (List Electronic, Darmstadt, FRG). The seal resistance was in the range of 10 to 80 GΩ. The pipette medium contained high-NaCl solution. After formation of an inside-out patch, the bath medium was replaced by high-KCl solution consisting of (in mM): 20 NaCl, 135 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 1 EGTA, (free Ca^{2+} about 1 μM), 10 glucose and 20 HEPES at pH 7.4 (310 mOsm). For determination of ion selectivity in the inside-out configuration the high-KCl solution in the bath was replaced by high-NaCl and NaCl was partially or entirely replaced by Na gluconate as indicated.

Data were digitized (VR10, Instrutech, Elmont, NY), stored on tape and analyzed using patch-clamp software of Instrutech on a Mega Atari St4. The indicated potentials are the pipette potentials (V_p) referred to a bath potential of 0 mV. Positive current deflections correspond to a cation flux from the pipette to the cytoplasmic side and vice-versa. Experiments were performed at 20–22°C. Data are given as mean ± SD; *n* denotes the number of independent experiments.

Table 1. Thiamine derivatives in tumor-derived cell lines

Experimental condition	TTP	TDP	TMP	Thiamine	*n*
		(pmol/mg)			
Neuroblastoma	2.1 ± 0.2	220 ± 50	9.7 ± 14	66 ± 10	4
	(0.7)	(74)	(3.2)	(22)	
PC-12	4.8 ± 0.8	320 ± 50	27 ± 6	168 ± 48	6
	(0.9)	(61.4)	(5.2)	(32.4)	
Glioma C6	0.4 ± 0.1	354 ± 48	17 ± 8	24 ± 3	3
	(0.1)	(90)	(4.3)	(6.1)	

Thiamine derivatives were determined as described in Materials and Methods. The results are expressed in mean ± SD. The percentage of each thiamine derivative compared to total thiamine is indicated in parentheses.

ABBREVIATIONS

DIDS, 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid; TMP, thiamine monophosphate; TDP, thiamine diphosphate; TTP, thiamine triphosphate; TTPase, thiamine triphosphatase.

Results

The measured content of thiamine derivatives for neuroblastoma, PC-12 and glioma cells is given in Table 1. In neuroblastoma cells, TTP amounts to about 2.1 pmol/mg or 0.7% of total thiamine. Using an intracellular volume of 4.5 μl/mg of protein which was determined from the difference between the ^3H_2O and ^{14}C-inulin spaces (L. Bettendorff, *unpublished results*); the intracellular TTP concentration was about 0.5 μM. This value is three times higher than in cultured cerebellar neurons. Cell counting revealed that the total number of TTP molecules per cell is $0.4 \times 10^6 \pm 0.2 \times 10^6$ ($n = 4$). Although the total amount of thiamine derivatives is more or less the same in neuroblastoma, PC-12 or glioma cells, the latter contain much less TTP than neuronal cells. This is in agreement with the results obtained from primary cultures of nervous cells: astrocytes contain six times less TTP than neurons, though the total thiamine content is comparable (Bettendorff et al., 1991).

Patch-clamp experiments were performed with the neuroblastoma cell line. To assess the effect of TTP on ion channels, the inside-out patch configuration was used. After formation of an inside-out patch, the high-NaCl solution of the bath was replaced by the high-KCl solution. As it is known that different populations of Cl channels can be activated in cell-free inside-out membranes by application of potential gradients preferentially by depolarization (Schwarze & Kolb, 1984; Li et al., 1989), a definite protocol of voltage pulses was applied. V_p was changed continuously between −60 to 60 mV in steps of 10 to 20 mV with a step length being 20 sec. Under these conditions, an ion channel of large unit conductance (*see below*) was observed in 25 (41%) patches out of a total of 61. In those patches where this channel type was not observed within the first 8 min, the perfusion medium was replaced by a high-KCl solution containing either TTP (1 or 10 μM) or no additional compound, and a constant potential of +20 mV was applied. Table 2 shows that under control conditions, the channel became activated in 2 patches out of 27. The addition of TTP activated this channel type in about 40% of the experiments after an average delay of 4 min (*see also* Fig. 1), while it was not activated by addititon of TDP. It is a characteristic fingerprint that in general the current pattern of this channel type does not appear in an all-or-none step (*compare* Nelson, Tang & Palmer, 1984; Kolb, Brown & Murer, 1985; Kolb & Ubl, 1987). As Fig. 1 shows, no channel activity is observed prior to the addition of TTP (trace *A*). About 100 sec after addition, a transient and fluctuating increase of the patch current is observed. About 10 to 20 sec after the first occurrence of increased cur-

Table 2. Effect of thiamine derivatives on the appearance of an anion channel of large unit conductance

Experimental condition	%	*n*	*t* (min ± SD)
Control	7.4	27	6 ± 3
TTP (10 μM)	38	8	4 ± 3
TTP (1 μM)	41	17	4 ± 2
TDP (10 μM)	0	8	

Inside-out patches were used and the perfusion medium was replaced by high-KCl solution. A voltage protocol was applied for 8 min (*see text*). If the patch remained blank, the perfusion medium was either replaced by an identical solution (control) or by a high KCl solution containing TTP or TDP at the concentrations indicated. A test potential of 20 mV was applied for a further 8 min to identify channel activation. (Chi-square test: $P = 0.012$).

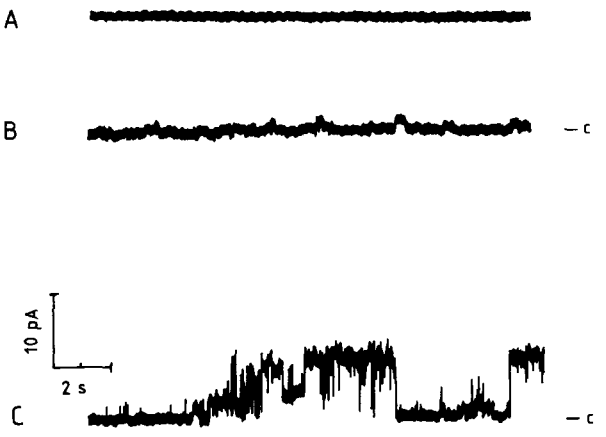

Fig. 1. Single channel current fluctuations in an inside-out patch of a neuroblastoma cell at a pipette potential of +20 mV. The pipette filling solution contained high-NaCl saline and the bath solution high-KCl. (*A*) No channel activity was observed several (>8) min after excision. (*B*) 100 sec after addition of TTP (1 μM) a 50 pS channel appeared which (*C*) finally grew into a 400 pS channel. (*c:* current level of channel closed state).

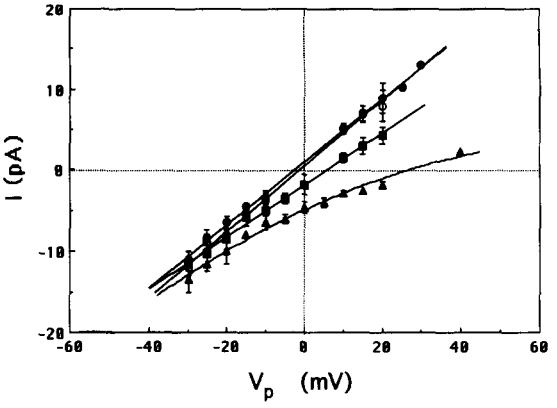

Fig. 2. Single channel currents as a function of pipette potential ($I - V_p$ relationship) in inside-out patches. The pipette filling solution was high-NaCl. The bath contained 142 mM KCl (●), 142 mM NaCl (○), 79 mM NaCl + 71 mM Na gluconate (■), 142 mM Na gluconate (▲).

rent fluctuations, the current starts to switch between well-defined current levels. The resulting regular current pattern can be observed thereafter and, in addition, current transitions to conducting substates can be observed. The latter observation will not be considered further.

Note that addition of TTP to an already active patch did not significantly alter channel behavior, though a definite statement is difficult to make as TTP seems to act only with some delay.

The ion selectivity of the single channel was analyzed by measuring single channel, current-voltage ($I - V_p$) relationships in the inside-out configuration for different ionic compositions of the bath. Figure 2 shows that for high-NaCl as pipette filling and high-KCl as bath, the current reverses at a potential (V_{rev}) close to 0 mV and a single channel conductance of about 370 pS can be estimated. Substitution of high-KCl by high-NaCl has no significant effect on V_{rev}; yet, partial or complete replacement of chloride by gluconate shifted V_{rev} to more positive pipette potentials. The corresponding results are summarized in Table 3. The observed shifts of V_{rev} could be described by the Goldman-Hodgkin-Katz (GHK) relationship for an anion-selective channel. Adjusted parameters resulting from a GHK-fit are $P_{Cl} : P_{gluconate} : P_{Na} = 1 : 0.35 : 0.05$.

Figure 3 shows the open probability (P_o) after a jump from 0 mV to the corresponding test potential V_p. P_o was calculated from the time spent in the highest conducting state during a recording time of 0.5–1.0 min. It shows a bell-shaped behavior and decreases steeply outside the potential range of −25 to +10 mV.

Table 3. Single channel conductance (γ) and V_{rev} of TTP elicited current fluctuations at different bath solutions in the inside-out configuration

Bath	γ(pS)	V_{rev}	n
High-KCl	368 ± 61	−1.3 ± 2.9	6
High-NaCl	382 ± 59	−1.7 ± 2.5	3
Na (Cl + Gluconate)	306 ± 67	7.1 ± 2.0	3
Na Gluconate	185 ± 24	26.9 ± 3.5	5

The pipette medium contained high-NaCl saline (150 mM Cl^-). The perfusion was composed of either high-KCl (150 mM Cl^-), high-NaCl (150 mM Cl^-), isoNa (Cl + Gluconate) (79 mM Cl^- and 71 mM gluconate) or isoNa Gluconate (8 mM Cl^- and 142 mM gluconate).

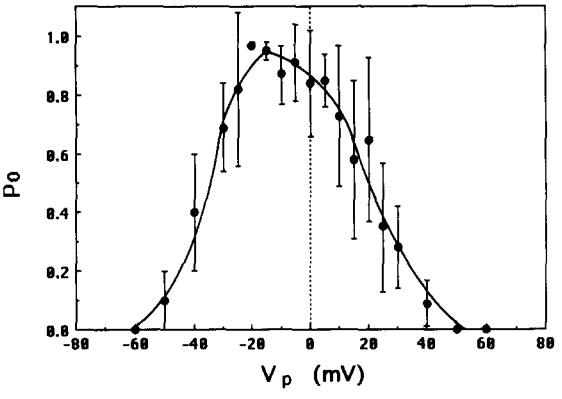

Fig. 3. Voltage dependence of the single channel open probability (P_o) in inside-out patches. P_o was calculated as the mean time the channel spent in its fully open state over a total recording time of 0.5–1 min after application of the test potential (V_p). The holding potential was set to 0 mV. Each point gives the mean ± SD for 3–8 patches. The pipette filling solution was high-NaCl and the bath solution (cytoplasmic side) high-KCl.

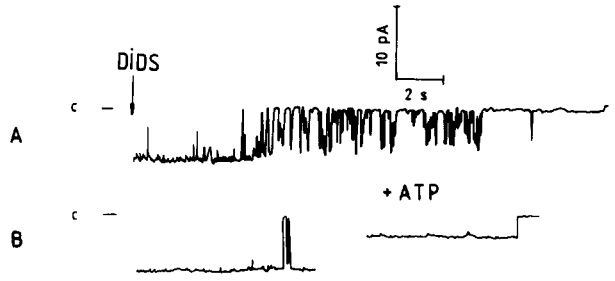

Fig. 4. (*A*) Effect of DIDS (0.1 mM) on the single channel current pattern in inside-out patches. The arrow indicates addition of DIDS. (*B*) Single channel current before and after addition of 5 mM ATP to the bath solution. The pipette potential was −20 mV. Solutions were as in Fig. 3.

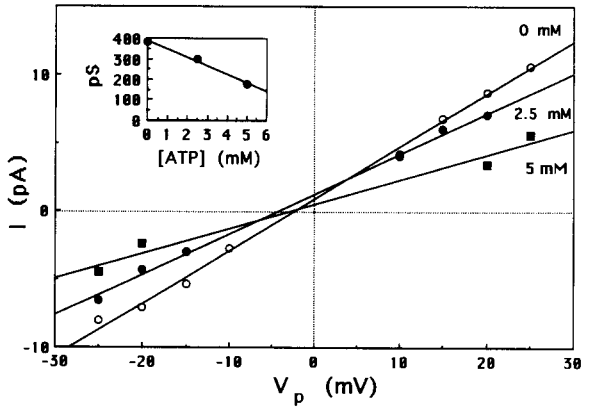

Fig. 5. Effect of ATP on the single channel conductance of the maxi chloride channel. Solutions are as in Fig. 3, except that ATP (0, 2.5 or 5 mM) was present in the bath. Each point is the mean of 2–3 patches. The inset represents the mean slope conductance as a function of ATP concentration.

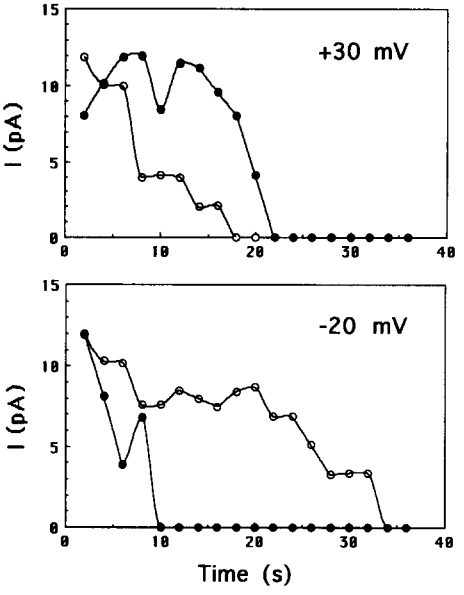

Fig. 6. Voltage-dependent relaxation of the single channel current as a function of time at two test potentials in the absence (○) or the presence (●) of 1 mM oxythiamine in the pipette filling solution. The pipette potential was stepped from 0 to either +30 or −20 mV as indicated. Each point represents the ensemble average of voltage-jump current-relaxations of five different recordings. High-NaCl was used for the bath and pipette filling.

After addition of DIDS (0.1 mM), a known chloride channel inhibitor, to the perfusion medium, the channel entered a state of rapid flickering reducing the mean open time (Fig. 4, *A*). The single channel conductance was not affected. The effect of DIDS was irreversible in agreement with the observations of Pahapill and Schlichter (1992).

For comparison with TTP, we tested the effect of ATP, another triphosphate compound, on channel activity. At a concentration of 10 μM at the cytoplasmic side, no effect of ATP was observed. At higher concentrations (5 mM), it reduced the single channel conductance by about 50% (Fig. 4, *B*); yet, Soejima and Kokubun (1988) could not find any effect of ATP on maxi Cl channels in vascular smooth muscle cells. Figure 5 shows the $I - V_p$ relationship in the absence and presence of 2.5 and 5 mM ATP in the bath solution.

We had previously shown that oxythiamine (1–5 mM), when applied on the external side of the vesicles, reduced $^{36}Cl^-$ uptake (Bettendorff et al., 1993*a*). In inside-out membrane experiments, the presence of thiamine antimetabolites like oxythiamine, pyrithiamine (another thiamine antimetabolite) or thiamine itself in the pipette solution and/or in the bath had no significant influence on the single channel conductance. But if oxythiamine (5 mM) was present in the pipette medium before formation of inside-out patches, the probability of this anion channel appearing seemed to be reduced: no channel activity was observed in seven patches. Addition of a lower concentration of oxythiamine (1 mM) produced a shift in the voltage dependence of P_o which is indicated by the corresponding behavior of the channel inactivation current in Fig. 6. After a voltage jump from a holding potential of 0 mV to a test potential of +30 mV, the presence of oxythiamine slowed down the time-dependent inactivation, but for a test potential of −20 mV inactivation was accelerated. Thiamine at 1 mM did not affect the voltage gate (*not shown*).

Discussion

Most cell types contain significant amounts of TTP in the cytoplasm (Bettendorff et al., 1991). In the present paper, we found comparable concentrations

of the thiamine derivatives in neuroblastoma, PC-12 and glioma cells. Total thiamine content is about 300 pmol/mg of protein *vs.* 63 pmol/mg for neurons in primary culture (Bettendorff et al., 1991). TTP content is 2.1 pmol/mg of protein, which is three times as much as in cultured neurons prepared from rat cerebellum. Glioma cells contain much less TTP than cells from neuronal origin. A similar observation has been made with primary cultures of astrocytes compared to neurons (for comparison, *see* Bettendorff et al., 1991). This would suggest a particular role of TTP in neuronal function.

To study the proposed action of TTP on Cl^- permeability (Bettendorf et al., 1990, 1993*a*), the patch-clamp experiments were focused on the action of TTP in the inside-out membrane configuration. As the dominant Cl-selective channel in the cell-free configuration, we observed a well-known anion channel of large unit conductance—a maxi Cl channel of 300 to 400 pS—which has been described in various mammalian cell types: rat myotubes (Blatz & Magleby, 1983; Schwarze & Kolb, 1984); macrophages (Schwarze & Kolb, 1984; Kolb & Ubl, 1987); Schwann cells (Gray, Bevan & Ritchie, 1984); epithelial cells (Nelson et al., 1984; Kolb et al., 1985; Schneider et al., 1985; Krouse, Schneider & Gage, 1986); sarcoplasmic reticulum (Hals, Stein & Palade, 1989); smooth muscle cells (Soejima & Kokubun, 1988) and human T lymphocytes (Schlichter et al., 1990; Pahapill & Schlichter, 1992). In neuroblastoma cells, this channel was first described by Bolotina et al. (1987) in excised patches, and Falke and Misler (1989) showed that a hypotonic shock activates a large conductance anion channel in cell-attached preparations of neuroblastoma cells. This channel population can be identified by its remarkable single channel properties which were confirmed in this study. The channel stays in the closed configuration at cell-attached membrane patches and can be activated by potential gradients in the inside-out configuration. P_o shows a bell-shaped behavior as a function of voltage (Fig. 3; *compare* Blatz & Magleby, 1983; Gray et al., 1984; Schwarze & Kolb, 1984; Hals et al., 1989; Schlichter et al., 1990). The anion selectivity is low. Our single channel data are in good agreement with those previously reported. Schlichter et al. (1990) report a relative permeability for Cl^- to gluconate of 0.29 for gluconate and less than 0.1 for Na^+ in human T lymphocytes. In Schwann cells $P_{Na}/P_{Cl} = 0.2$ (Gray et al., 1984) and 0.25–0.17 in myotubes and macrophages (Schwarze & Kolb, 1984). The observed irreversible effect of DIDS, increased rate of transitions between the fully open and closed state, agrees with the observations of Pahapill and Schlichter (1992). But until now, the mechanisms, most probably biochemical pathways, which are involved in the primary activation of this channel type are unknown.

Our results show that addition of TTP to the cytoplasmic side of the membrane of neuroblastoma cells increased the probability of appearance of a large anion channel. In 59% of all membrane patches, no channel activity was observed within 8 min after excision. TTP at physiological concentrations (1 μM) induced the activation of the channel in about 40% of the patches after a mean delay of 4 min after addition. Removal of this compound did not lead to channel closure, whereas TDP did not induce channel activity and ATP at concentrations >1 mM proved to be inhibitory. These observations suggest that more than a simple binding of TTP to the channel is involved in creating these phenomena, and an appealing possibility would be a phosphorylation leading to the activation of this channel.

Indeed, it has been suggested previously that the overall functional state of this channel might be modulated by unknown factors like phosphorylation (Blatz & Magleby, 1983; Schwarze & Kolb, 1984; Pahapill & Schlichter, 1992). The role of TTP, thus, could be to turn the channel on without interfering with the voltage-dependent gating mechanism. Those channels which we observed immediately after excision of the patch could have been previously phosphorylated by endogenous TTP. At 5 mM oxythiamine in the pipette-filling solution, no channel activity was observed. This could mean that at these concentrations the channel is completely inhibited or its open probability is shifted close to zero for all voltages. Further experiments are necessary to elucidate the reaction pathway which finally leads to channel activation.

These results are in good agreement with the effects of thiamine derivatives we observed on chloride uptake in rat brain vesicles (Bettendorff et al., 1993*a*, *b*): (i) a positive correlation between TTP content and chloride permeability; (ii) inhibition of a diffusion-driven chloride uptake by oxythiamine from the extravesicular side and increase of the inhibition by depolarization; (iii) inhibition of chloride uptake by DIDS; (iv) the channels implicated do not saturate at concentrations of chloride <0.1 M, which is indeed the case for the chloride channel of large unit conductance (K_m = 77–120 mM; Hals et al., 1989; Schlichter et al., 1990). The channel has no specificity for a particular cell type just as TTP is an ubiquitous compound, though it seems more abundant in skeletal muscles (Egi et al., 1986), electric organ (Eder & Dunant, 1980; Bettendorff et al., 1987) and neurons than in other tissues.

The maxi Cl channel has been shown to exist in many cell types, though its relative abundance is hard to determine in the absence of known high-

affinity ligands. Several authors have estimated the density of these channels from their abundance in patches of known area. Thus, Hals et al. (1989) calculated a channel density of 1 channel per 6.97 μm^2 in sarcoplasmic reticulum, Gray et al. (1984) 1 channel for 10–100 μm^2 in Schwann cells and Woll et al. (1987) calculated a density of about 10 channels/μm^2 in amphibian skeletal muscle. If we take into account the percentage of channels activated by TTP, we observe the maxi channel in about 65% of the patches. Considering further that in a few patches two or three channels were simultaneously active and that the mean patch area was 1–4 μm^2, we can estimate a channel density of 0.25–1 channel/μm^2. This value is larger than estimated for Schwann cells but smaller than in skeletal muscle cells. This relation could fit with the corresponding TTP content which is lowest in glial cells and highest in skeletal muscle. The abundance of Na channels, for instance, is reported to be about 25 channels/μm^2 in neuroblastoma cells (Catterall, 1977), one or two orders of magnitude higher than for maxi Cl channels. The high single channel conductance would be compensated by a low abundance. By activation of this channel population, the total membrane conductance would not increase to a larger extent than by activation of another channel population such as Na channels, as outlined by other authors (Gray et al., 1984; Pahapill & Schlichter, 1992). Activation of this Cl channel population would result in a clamping of the membrane potential at resting values, and in nervous cells it could cause a facilitation of hyperpolarization after the action potential and control the Cl gradient (Bureau et al., 1992). This view would be compatible with the effects of thiamine antimetabolites on nerve conduction: depolarization of the resting membrane and prolongation of the action potential sometimes causing repetitive firing (Goldberg & Cooper, 1975). Some thiamine antimetabolites could indeed be specific inhibitors of the maxi Cl channels and constitute important tools for the study of this channel.

For the first time we have shown an effect of TTP on ion channels at physiological concentrations. Fox and Duppel (1975) reported that TTP prevents the exponential decline of ionic current at the node of Ranvier, but high concentrations of 1 mM were necessary, suggesting that this could be caused due to a nonspecific effect by stabilization of surface charges. TTP, for instance, could present a new parameter involved in the regulation of ion channels in parallel to GTP-binding proteins.

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