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Dimethyl sulfoxide at high concentrations inhibits non-selective cation channels in human erythrocytes

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Abstract. Dimethyl sulfoxide (DMSO), a by-product of the pulping industry, is widely used in biological research, cryobiology and medicine. On cellular level DMSO was shown to suppress NMDA-AMPA channels activation, blocks Na^+ channel activation and attenuates Ca^{2+} influx (Lu and Mattson 2001). In the present study we explored the whole-cell patch-clamp to examine the acute effect of high concentrations of DMSO (0.1–2 mol/l) on cation channels activity in human erythrocytes. Acute application of DMSO (0.1–2 mol/l) dissolved in Cl^--containing saline buffer solution significantly inhibited cation conductance in human erythrocytes. Inhibition was concentration-dependent and had an exponential decay profile. DMSO (2 mol/l) induced cation inhibition in Cl^-- containing saline solutions of: 40.3 ± 3.9% for K^+, 35.4 ± 3.1% for Ca^{2+} and 47.4 ± 1.9% for $NMDG^+$. Substitution of Cl^- with $gluconate^-$ increased the inhibitory effect of DMSO on the Na^+ current. Inhibitory effect of DMSO was neither due to high permeability of erythrocytes to DMSO nor to an increased tonicity of the bath media since no effect was observed in 2 mol/l glycerol solution. In conclusion, we have shown that high concentrations of DMSO inhibit the non-selective cation channels in human erythrocytes and thus protect the cells against Na^+ and Ca^{2+} overload. Possible mechanisms of DMSO effect on cation conductance are discussed.

Key words: DMSO — Cation channels — Patch-clamp

Introduction

Dimethyl sulfoxide (DMSO), a by-product of the pulping industry, is extensively utilized as a solvent in biological research. It is also widely used as a preservative for bone marrow cells and organs (Boiko et all. 1982; Small and Goldstein 1982; Motta et al. 2010; Brockbank et al. 2011; Haack-Sørensen and Kastrup 2011). It shows radioprotective (Goddu et al. 1996) and antioxidant properties (Repine et al. 1981; Mobarok 1998; Dkhar and Sharma 2010), antitumor activity (Fahim et al. 2003), and inhibits necrosis of the cells (Camici et al. 2006; Iida et al. 2007). Medical doctors prescribe it for a variety of ailments including pain, inflammation, scleroderma, interstitial cystitis and arthritis, and elevated intracranial pressure (Goodnough et al. 1980; Karaca et al. 1991; McGee et al. 1991; Brien et al. 2008; Kim et al. 2011). For the variety of its positive effects it is considered to be the aspirin of our era. DMSO, alone or in combination with synergistic molecules, may help to neutralize pathological products harmful to the heart and brain in medical disorders involving head and spinal cord injury, memory dysfunction, and ischemic heart disease (Goodnough et al. 1980; Jacob and de la Torre 2009; Parisi et al. 2010).

DMSO demonstrates a strong inhibitory effect on platelet aggregation (Dujovny et al. 1983), presumably *via* the inhibition of prostaglandin platelet-aggregating arachidonic acid metabolites (Asmis et al. 2010). No post-thrombotic consequences for platelet disaggregation were reported in humans or animals following high dose of DMSO administration (Dujovny et al. 1983). The experiments on human erythrocytes also showed lower cell aggregation indexes in DMSO treated cells (Santos et al. 2002).

On cellular level DMSO was shown to suppress NMDA-AMPA channels activation, blocks Na^+ channel activation and attenuates Ca^{2+} influx (Lu and Mattson 2001).

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However, the reported effects of DMSO are controversial and not always positive. DMSO was shown to produce widespread apoptosis in developing central nervous system and can cause cardiotoxicity in mice (Hanslick et al. 2009). Nevertheless, the documented side-effects of DMSO administration are relatively mild and relate to high concentration used for the treatment (25–40% DMSO intravenously). Intravascular haemolysis due to high DMSO permeability in erythrocytes was reported in higher than 30% DMSO solutions (Karaca et al. 1991).

In erythrocytes the use of high dose of DMSO was shown to inhibit acetylcholinesterase (Jagota 1992), oxidize glutathione (Homer et al. 2005) and enhance hexose monophosphate shunt activity (Adler et al. 1991). Conflicting data on DMSO effect on Ca-ATPase activity show an inhibition (Rybczyńska and Chmiel 1982; Romero 1992; McConnell et al. 1999) as well as an activation (Benaim and de Meis 1990; Lehotsky et al. 1992) of Ca^{2+}-pump by high DMSO concentrations.

Since mature human erythrocyte are lacking intracellular Ca^{2+} stores (mitochondria and endoplasmic reticulum), a balance between the activities of Ca-ATPase and ion channels permeable to Ca^{2+} keeps intracellular free calcium concentration ($[Ca^{2+}]_i$) at low levels (less than 100 nmol/l; Tiffert et al. 2003). Ion channels which can rapidly increase intracellular Na^+ and Ca^{2+} concentrations play a key role in regulation of the cells survival. However, the data concerning the effect of DMSO on cation channels activity is missing and our study aims to provide the missing data.

Human erythrocytes were shown to express several types of non-selective cation channels permeable for Ca^{2+}: i) the voltage-gated channel, coupled to an acetylcholine receptor (Bennekou et al. 1993, 2004; Kaestner et al. 1999) and ii) the voltage-independent cation channels activated by oxidation, osmotic shock, energy depletion (Huber et al. 2001) and prostaglandin PGE_2 (Lang et al. 2005).

The present study was performed to show the effect of high concentrations of DMSO, used in cryobiological and medical practice, on cation channels activity in human red blood cells.

Materials and Methods

Erythrocytes

Erythrocytes were drawn from healthy volunteers, who signed informed consent, or provided by the blood bank of the University of Tübingen from banked erythrocyte concentrates. Erythrocytes were washed twice (1200 × *g*, 5 min, 22°C) in 5 mmol/l glucose-containing NaCl Ringer bath solution (pH 7.4, see below).

Electrophysiology

Patch-clamp whole-cell recordings were performed at room temperature. The patch electrodes were made of borosilicate glass capillaries (150 TF-10, Clark Medical Instruments) using a horizontal DMZ puller (Zeitz, Martinsried, Germany). Pipettes with high resistance from 17 to 20 MOhm were connected *via* an Ag-AgCl wire to the headstage of EPC 9 patch-clamp amplifier (HEKA Elektronik Dr. Schulze GmbH, Germany). Data acquisition and data analysis were controlled by a computer equipped with an ITC 16 interface (InstruTech, HEKA Elektronik Dr. Schulze GmbH, Germany) and Pulse software (HEKA Elektronik Dr. Schulze GmbH, Germany). For current measurements, erythrocytes were held at a holding potential (V_h) of –10 mV and 200 ms pulses from –100 to +100 mV were applied with increments of +20 mV. The original current traces are depicted without filtering (acquisition frequency of 3 kHz). The currents were analyzed by averaging the current values measured between 90 and 190 ms of each square pulse (current-voltage relationship). The applied voltages refer to the cytoplasmic face of the membrane with respect to the extracellular space. The offset potentials between both electrodes were zeroed before sealing. The liquid junction potentials between bath and pipette solutions and between the bath solutions and the salt bridge (filled with NaCl bath solution) were calculated according to Barry and Lynch (1991). Data were corrected for liquid junction potentials. Recordings were obtained after reaching >10 GΩ seal in on-cell configuration.

The pipette solution consisted of (in mmol/l): 125 Na-gluconate, 10 NaCl, 1 $MgCl_2$, 1 MgATP, 1 EGTA, 10 HEPES/NaOH (pH 7.4). The NaCl Ringer bath solution contained (in mmol/l): 145 NaCl, 5 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 5 glucose, 10 HEPES/NaOH (pH 7.4). In the experiments where Cl^- was substituted with $gluconate^-$, the solution contained (in mmol/l): 150 Na-gluconate, 2 $MgCl_2$, 1 $CaCl_2$, 5 glucose, and 10 HEPES/NaOH (pH 7.4). The KCl, NaCl, NMDG-Cl, and $CaCl_2$ bath solutions contained (in mmol/l) either 150 KCl, 150 NaCl, 180 NMDG, or 100 $CaCl_2$, 10 HEPES (titrated with HCl, NMDG or CaOH to pH 7.4). DMSO/glycerol (0.1–2 mol/l), dissolved in Ringer or 150 mmol/l Na-gluconate bath solutions, were added acutely. Concentrations of DMSO (glycerol) were increased gradually starting with the lowest (0.1 mol/l) DMSO/glycerol concentration to avoid cell lysis due to osmotic shock in highly concentrated (1–2 mol/l) DMSO and glycerol solutions. The recordings were made after reaching a steady state (5–10 min incubation in DMSO/glycerol media).

Reagents were obtained from Sigma (Germany) and were of the highest grade.

Intracellular Ca^{2+}

Experiments were performed on erythrocytes loaded with 2 mol/l DMSO. Composition of the bath solution was following (in mmol/l): 145 NaCl, 5 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 5 glucose, 2 mol/l DMSO, 10 HEPES/NaOH (pH 7.4). Concentration of DMSO was gradually increased starting with 0.1 mol/l DMSO in the Ringer bath solution.

DMSO-treated erythrocytes (0.4 % Ht) were washed in Ringer solution containing 5 mmol/l $CaCl_2$ and 2 mol/l DMSO, then 2 μmol/l Fluo-3/AM was added (Calbiochem, Bad Soden, Germany). The cells were incubated at 37°C for 20 min., washed once and re-suspended in 5 mmol/l Ca^{2+}-containing Ringer (in the presence of 2 mol/l DMSO) and subsequently analyzed by flow cytometric analysis (FACS-Calibur from Becton Dickinson; Heidelberg, Germany) in fluorescence channel FL-1 with excitation wavelength of 488 nm and emission wavelength of 530 nm.

Statistics

Data is expressed as arithmetic means ± SEM and the paired two-tailed t-test was employed as appropriate, $p < 05$ was considered statistically significant.

Results

In the first series of experiments we examined the effect of acute application of DMSO on cation channel activity of human erythrocytes in NaCl Ringer bath solution.

Prior to DMSO application, erythrocyte cation conductance (measured as inward current slope of the current-voltage (I/V) relationship) approached the value of 68 ± 2 pS (Fig. 1B). The conductance declined significantly reaching values of 66 ± 2 pS ($n = 13$; $p \leq 0.05$) following exposure to 0.1 mol/l DMSO; 56 ± 2 pS for 0.5 mol/l, 48 ± 2 pS for 1 mol/l and 42 ± 2 pS for 2 mol/l DMSO solutions respectively ($n = 13$; $p \leq 0.001$).

To check whether DMSO blocked cation (not Cl^-) conductance in the next series of experiments we dissolved DMSO in isotonic Na-gluconate bath solution. A combination of Na-gluconate bath and pipette solutions allowed us to characterize Na^+ influx. As it is shown in Fig. 2A and B, acute application of DMSO in Na-gluconate bath solution inhibited Na^+ conductance in human erythrocytes in concentration-dependent manner. Prior to DMSO application Na^+ conductance in isotonic Na-gluconate solution was higher (130 ± 29 pS) than that recorded in NaCl Ringer bath solution (Fig. 2B). The value decreased significantly in the presence of 2 mol/l DMSO and approached 95 ± 16 pS ($n = 7$; $p \leq 0.05$).

Fitting of the experimental data showed an exponential decay profile in both NaCl and Na-gluconate bath solutions

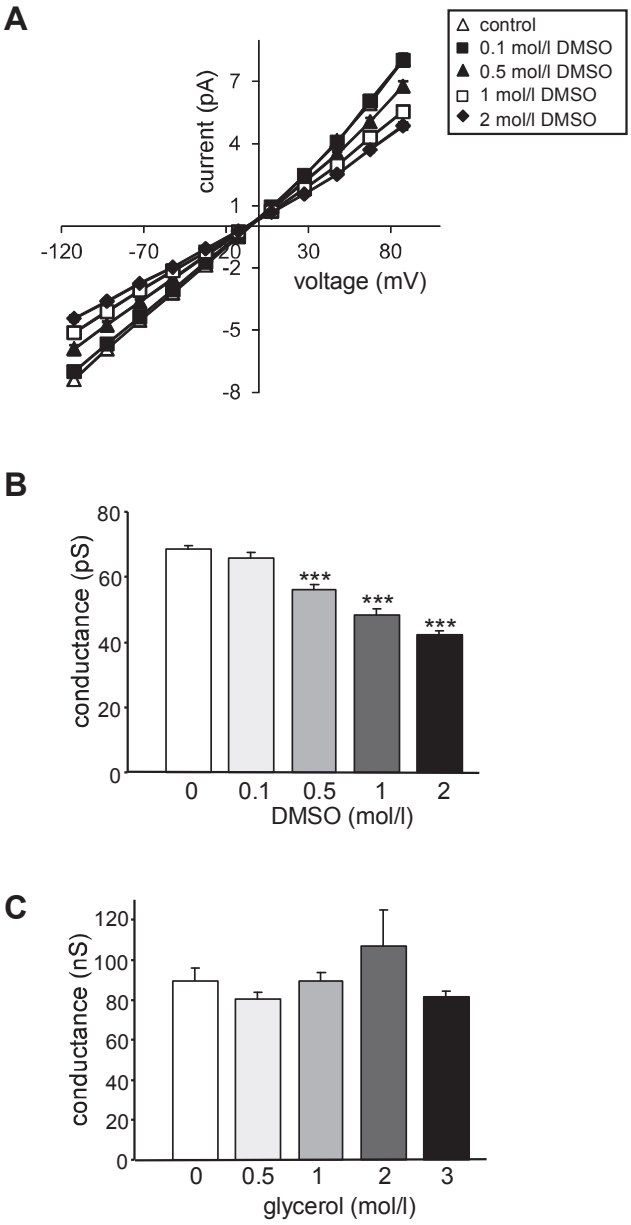

Figure 1. Acute application of DMSO dissolved in NaCl Ringer bath solution blocks cation conductance in human erythrocytes. **A**. Arithmetic means (±SEM, $n = 13$) of the current as a function of voltage (I/V relationship) recorded in NaCl Ringer bath solution prior to control and following acute application of 0.1 mol/l ; 0.5 mol/l ; 1 mol/l and 2 mol/l DMSO in NaCl Ringer bath solution. **B.** Arithmetic means (±SEM, $n = 13$) of the conductance (as calculated for the inward currents by linear regression) prior to control and following acute application of 0.1 mol/l; 0.5 mol/l; 1 mol/l and 2 mol/l DMSO in NaCl Ringer bath solution. *** indicates significant difference from control ($p \leq 0.001$; paired *t*-test). **C.** Arithmetic means (±SEM, $n = 3$) of the conductance (as calculated for the inward currents by linear regression) prior to control and following acute application of 0.5 mol/l; 1 mol/l; 2 mol/l and 3 mol/l glycerol in NaCl Ringer bath solution.

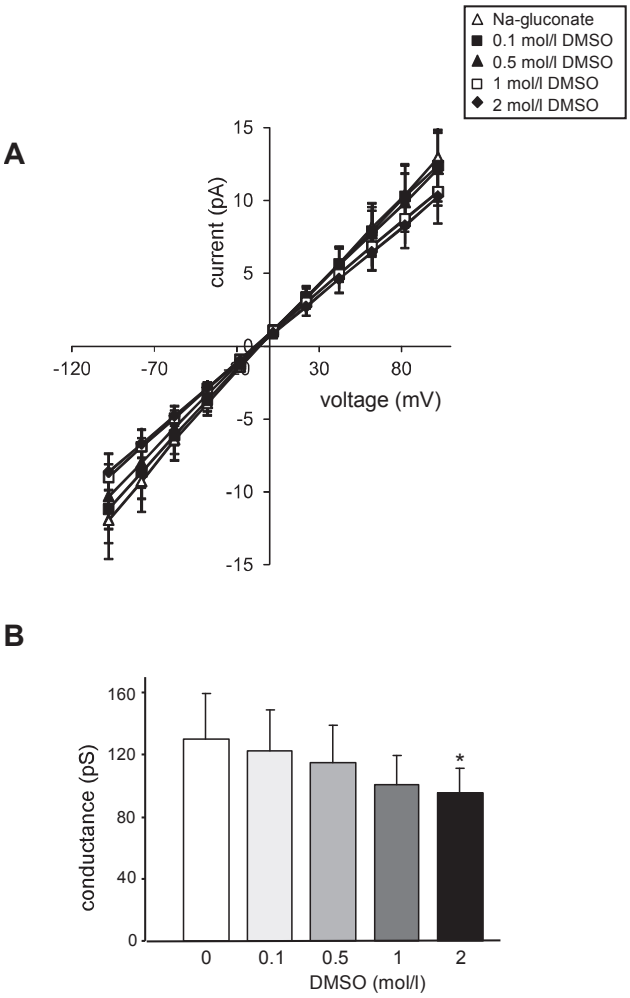

Figure 2. DMSO blocks Na^+ conductance in Cl^- free medium. **A**. Arithmetic means (±SEM, $n = 8$) of the current as a function of voltage (I/V relationship) recorded in Na-gluconate bath solution prior to control (△) and following acute application of 0.1 mol/l; 0.5 mol/l; 1 mol/l and 2 mol/l DMSO in Na-gluconate bath solution. **B.** Arithmetic means (±SEM, $n = 8$) of the conductance (as calculated for the inward currents by linear regression) prior to control (□) and following acute application of 0.1 mol/l; 0.5 mol/l; 1 mol/l and 2 mol/l DMSO in Na-gluconate bath solution. * significant difference from control ($p \leq 0.05$, paired t-test).

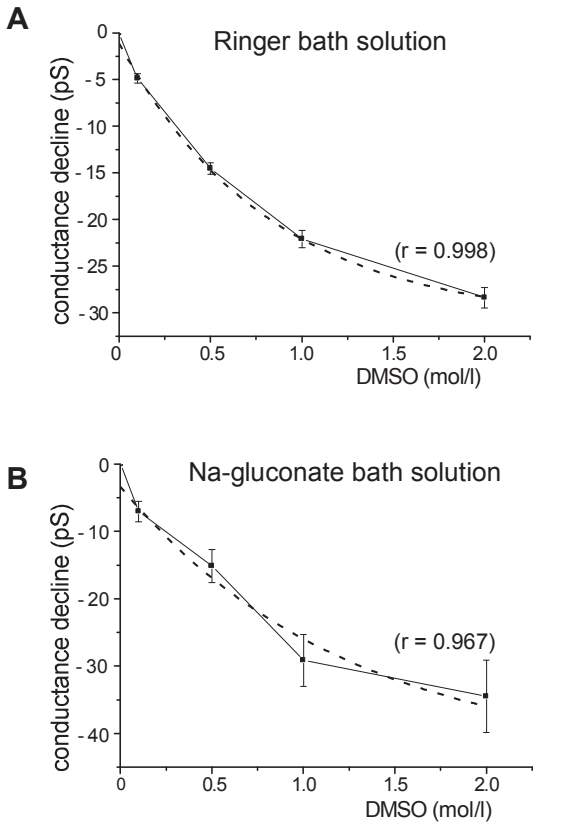

Figure 3. Exponential decay profile of the cation conductance decline in the presence of DMSO. **A.** Fitting of the experimental data for cation conductance inhibition by DMSO in NaCl Ringer bath solution (solid black line presents the original data while dotted black line depicts the exponential fitting). **B.** as in **A.** for 150 mmol/l Na-gluconate bath solution.

(correlation coefficient (r) is 0.998 and 0.967 for NaCl and Na-gluconate bath solutions, respectively) (Fig. 3).

To define cation selectivity of DMSO sensitive cation conductance we substituted the NaCl Ringer bath solution with isotonic buffered NMDG-Cl, $CaCl_2$ and KCl solutions. The I/V relationships for the cells in 180 mmol/l NMDG-Cl, 100 mmol/l $CaCl_2$ and 150 mmol/l KCl bath solutions with and without 2 mol/l DMSO are shown in Fig. 4A,B,C. As a result, DMSO inhibited inward (cation) currents irrespectively to positive charge carrier in extracellular bath solution (Fig. 4D). According to the patch-clamp data, the inhibitory effect of DMSO on cation conductance of human erythrocytes in Cl^- containing bath solutions was the following: 47.4 ± 1.9% for $NMDG^+$, 40.3 ± 3.9% for K^+, 35.4 ± 3.1% for Ca^{2+}, 15.4 ± 1.6% for Na^+, and 39.2 ± 1.1% for all the cations presented in the NaCl Ringer bath solution.

The blocking effect of 2 mol/l DMSO on Na^+ conductance depended on anion composition of the bath media and was reduced in NaCl bath solution in comparison with Na-gluconate (Fig. 4D). Activation of the non-selective cation channels stimulated by Cl^- removal in Na-gluconate bath solution was blunted by 2 mol/l DMSO (data are not shown).

To exclude the possibility that the blocking effect of high concentration of DMSO on non-selective cation channels in human erythrocytes may be due to high cellular osmolarity, we explored the effect of high concentrations of glycerol. Glycerol penetrates into the cells like DMSO. As shown in Fig. 1C, acute application of high concentrations of glycerol

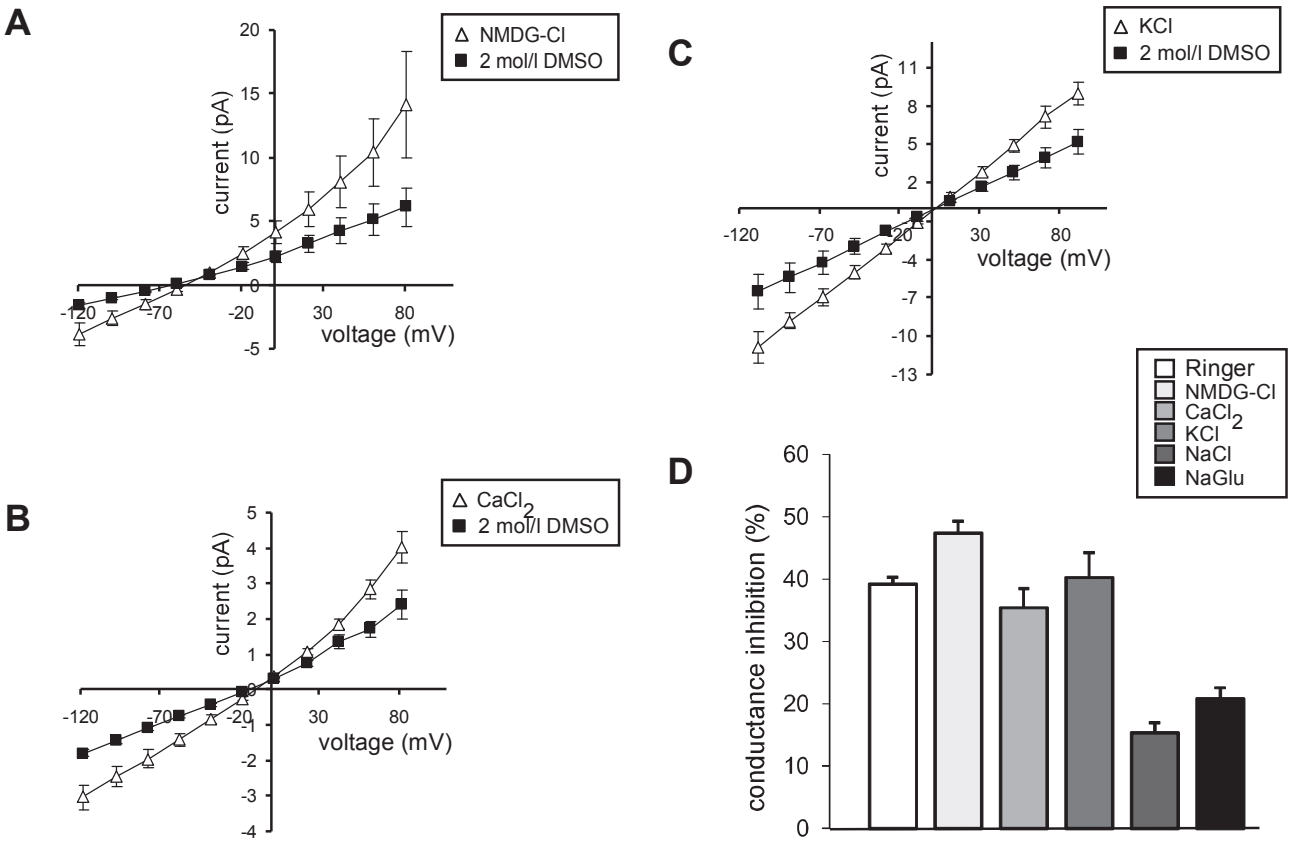

Figure 4. Non-selective inhibitory effect of 2 mol/l DMSO on cation conductance in human erythrocytes. **A**. Arithmetic means (±SEM, $n = 6$) of the current as a function of voltage (I/V relationship) recorded in 180 mmol/l NMDG-Cl solution prior to control (△) and following acute application of 2 mol/l DMSO in 180 mmol/l NMDG-Cl solution. **B.** Arithmetic means (±SEM, $n = 4$) of the current as a function of voltage (I/V relationship) recorded in 100 mmol/l $CaCl_2$ solution prior to control (△) and following acute application of 2 mol/l DMSO in 100 mmol/l $CaCl_2$ solution. **C.** Arithmetic means (±SEM, $n = 3$) of the current as a function of voltage (I/V relationship) recorded in 150 mmol/l KCl solution prior to control (△) and following acute application of 2 mol/l DMSO in 180 mmol/l KCl solution. **D.** Percent of cation conductance inhibition (±SEM, $n = 3 \div 13$) calculated by linear regression for the difference in inward currents at the beginning of experiment and after acute application of 2 mol/l DMSO dissolved in the NaCl Ringer, 180 mmol/l NMDG-Cl, 100 mmol/l $CaCl_2$, 150 mmol/l KCl, 150 mmol/l NaCl and 150 mmol/l Na-gluconate bath solutions.

had no inhibitory effect on cation conductance in human erythrocytes.

To check whether the blocking effect of DMSO on cation channels activity may be due to its oxidative properties, the whole-cell patch-clamp experiments were performed with a strong oxidant (1 mmol/l H_2O_2). When applied acutely, 1 mmol/l H_2O_2 decreased cation conductance of human erythrocytes following the first 2–5 minutes. Further incubation with the oxidant resulted in a significant increase in cation conductance of human erythrocytes (Fig. 5A,B).

We further examined the effect of high DMSO on $[Ca^{2+}]_i$. The experiments performed with fluorescent Ca^{2+} dye Fluo-3 showed significantly reduced $[Ca^{2+}]_i$ in DMSO treated cells in comparison with control cells (Fig. 6). The effect, however, could relate to quenching of Fluo-3 fluorescence by high DMSO concentration.

Discussion

The present patch-clamp study on human erythrocytes revealed a gradual concentration-dependent decline in cation conductance induced by DMSO. Inhibitory effect of high concentrations of DMSO was observed for all tested monovalent (Na^+, K^+ and $NMDG^+$) as well as for divalent (Ca^{2+}) cations suggesting that DMSO affects the non-selective cation channel activity. The observed effect was not due to a decline in lipid cation conductance since Na^+ conductance measured in our patch-clamp experiments was 10^4 times

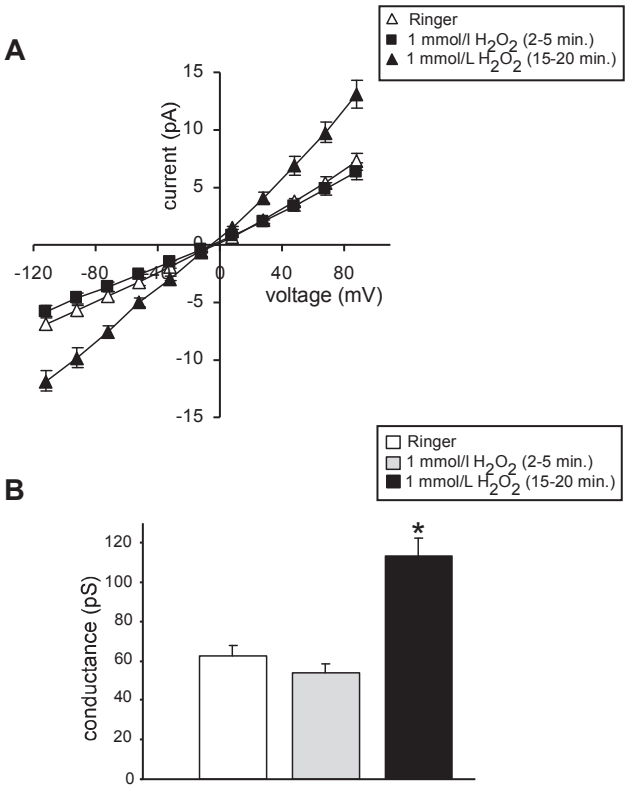

Figure 5. Time-dependent effect of 1 mmol/l H_2O_2 on cation conductance in human erythrocytes. **A**. Arithmetic means (±SEM, $n = 4$) of the current as a function of voltage (I/V relationship) recorded in the NaCl Ringer bath solution prior to control (△), following 2–5 min and after 15–20 min of acute application of 1 mmol/l H_2O_2 in NaCl Ringer bath solution. **B.** Arithmetic means (±SEM, $n = 4$) of the conductance (as calculated for the inward currents by linear regression) prior to control (□), following 2–5 min and after 15–20 min of acute application of 1 mmol/l H_2O_2 in NaCl Ringer bath solution. * significant difference from control ($p \leq 0.05$; paired t-test).

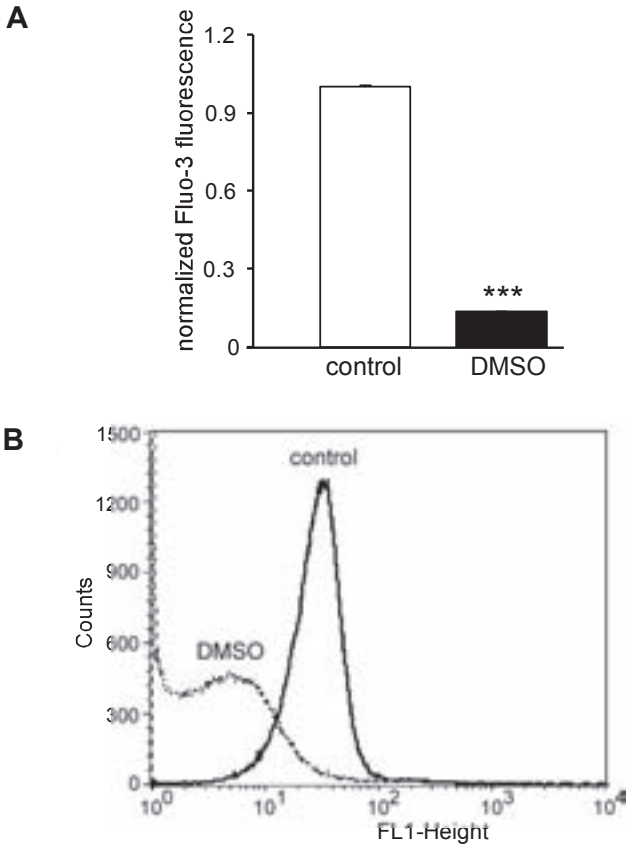

Figure 6. Effect of short-term DMS treatment cytosolic free Ca^{2+} concentration in human erythrocytes measured by Fluo-3. **A.** Arithmetic means ±SEM of normalized Fluo-3 fluorescence of control cells in Ringer solution in the absence ($n = 3$) and after short-term incubation with 2 mol/l DMSO ($n = 3$). *** significant difference from control ($p \leq 0.001$; t-test). **B.** Histogram of Fluo-3 fluorescence in a representative experiment of human erythrocytes in isotonic Ringer (solid line), and hypertonic Ringer bath solution containing 2 mol/l DMSO added (dotted line).

higher than that reported for Na^+ conductance (0.1 mol/l NaCl, pH 7.4) of pure lipid bilayers (Cala et al. 1982).

The highest inhibition by 2 mol/l DMSO was observed for the largest monovalent ($NMDG^+$) among all tested monovalent cations which ruled out lipid bilayer involvement in the DMSO effect.

The cation channels were originally active in our experiments. The calculated value of cation conductance for human erythrocytes in NaCl Ringer bath solution (68 ± 2 pS) was in a good agreement with the data of Desai (Desai et al. 2000), who reported less than 100 pS whole-cell conductance for untreated erythrocytes. Interestingly, the value for leak cation current in erythrocyte measured by radiotracers is much lower (Richter et al. 1997) than that measured by whole-cell patch-clamp. The discrepancy could be due to perturbation induced by whole-cell formation in the cell membrane that results in activation of mechanosensitive non-selective cation channels. Mechanosensitive cation channels were found in erythrocyte progenitor cells (K562 cells; Staruschenko and Vedernikova 2002) as well as in mature erythrocyte (Cordero and Romero 2002). Another possible explanation for the difference in cation currents is the use of loop diuretics (furosemide, bumetamide) for Na^+, K^+, Cl^--cotransporter inhibition in radiotracer measurements. The most recent data (Kucherenko et al., 2012) have shown a blocking effect of furosemide on non-selective cation channel activity in human erythrocytes. Noteworthy, a chemical blocker HOE642 inhibited (up to 40%) cation leak current in human erythrocytes in isotonic NaCl bath solution in radiotracer experiments (Bernhardt et al. 2007).

It suggests that cation channels could be not only activated by toxins or chemical treatment (Tosteson et al. 1991; Qadri et al. 2011) but they have basal activity in untreated human erythrocytes.

DMSO at clinical doses was shown to be a Na^+ channel blocker and to suppress, in a reversible manner, opening of ionotropic receptor N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor channels, which are known to be activated by glutamate during oxidative or metabolic stress (Karaca et al. 1991; Lu et al. 2001).

Electrophysiological studies revealed several channels expressed in human erythrocytes: the Ca^{2+}-activated K^+ channels with the molecular identity of SK4 (KCNN4) (Hoffman et al. 2003), voltage-gated (Bennekou et al. 1993, 2004; Kaestner et al. 1999), and voltage-independent (Huber et al. 2001) non-selective cation channels. The voltage-independent cation channels were shown to be partially related to TRPC3/6/7 channels (Foller et al. 2008). Recent data demonstrated the existence of NMDA receptors in rat (Makhro et al. 2010) and AMPA receptors in human (Föller et al. 2009) erythrocytes. In our recent study (Kucherenko and Lang 2010) we have shown that an AMPA receptor antagonist NASRM (1-naphthyl acetyl spermine) was effective not only in blocking cation currents induced by Cl^- removal but also significantly blunted the basal cation conductance in Cl^--containing bath solution. Non-selective blockage by high concentrations of DMSO and its ability to inhibit cation conductance in Cl^- containing bath media observed in the present study suggest a possible involvement of AMPA receptor non-selective cation channels. Higher inhibition by DMSO of Na^+ conductance in isotonic Na-gluconate bath solution in comparison with NaCl bath solution (20.8 ± 1.7% *versus* 15.4 ± 1.6%) points out that DMSO blocks Cl^- removal stimulated as well as basal Na^+ conductance and thus might affect the activity of different types of cation channels in human erythrocytes.

Our present data showing the blocking effect of DMSO on cation conductance seemed to be in conflict with the data of (Santos et al. 2002) which demonstrated that K^+, Na^+ and Ca^{2+} plasma concentrations are elevated in DMSO-treated cells. The contradiction can be due to the fact that DMSO suppresses Na^+-K^+-ATPase as well as Ca^{2+}-Mg^{2+}-ATPase activities (Rybczyńska and Chmiel 1982; McConnell et al. 1999). Our fluorescent calcium measurements showed significantly lowered intracellular calcium levels in erythrocytes treated with high concentration of DMSO in comparison with native cells. High concentrations of DMSO that permeates into the cells, however, might quench Fluo-3 fluorescence resulting in apparent decline of measured intracellular free calcium levels. Conversely, our direct patch-clamp experiments clearly showed that DMSO affects the activity of non-selective cation channels in human erythrocytes suppressing Ca^{2+} and Na^+ influxes.

The precise mechanism of DMSO effect on the activity of the non-selective cation channels is unknown since DMSO was shown to have diverse effects on different cell structures. Previous studies have shown that DMSO is a powerful inhibitor of platelet aggregation (Repine et al. 1981; Dujovny et al. 1983), a reaction that might involve the inhibition of prostaglandin platelet-aggregating arachidonic acid metabolites by DMSO (Panganamala et al. 1976). Erythrocytes also participate in a clot formation. Prostaglangin PGE_2 which is produced by human erythrocytes was shown to play a key role in the non-selective cation channels activation increasing intracellular free calcium (Lang et al. 2005).

Interestingly, while showing a scavenging effect on free radicals formation, DMSO at the same time demonstrates oxidative properties, being a mild oxidant. A number of cation channels (TRPC3/6 as well) were shown to be activated upon oxidative stress (Poteser et al. 2006; Hecquet and Malik 2009; Graham et al. 2010; Miller and Zhang 2011). Our whole-cell patch-clamp experiments with a strong oxidant 1 mmol/l H_2O_2 also demonstrated enhanced cation conductance in human erythrocytes. The effect was time-dependent and at the beginning (2–5 min) of oxidation a decline in cation conductance was observed followed by a gradual increase. In the absence of H_2O_2, the cells treated with DMSO showed just a declined cation conductance. It is known that oxidative effect of DMSO is mostly due to methionine oxidation to methionine sulfoxide. Moreover, SH groups of proteins are also oxidized but this reaction proceeds after a lag period of 2 hours (Shecher 1986). Participation of SH-groups in the cation channels regulation in human erythrocytes was shown by Duranton (Duranton et al. 2002). Since acute application of a strong oxidant (H_2O_2) also resulted in a short-term inhibition of the cation conductance in our experiments, it is possible to speculate that the effect was due to methionine oxidation. Thus methionine residues of cation channel-forming membrane proteins might participate in regulation of cation permeability and their oxidation results in suppression of the basal activity of the channels.

Another mechanism by which DMSO might affect the activity of the cation channels in human erythrocyte is connected with the structural properties of the DMSO molecule. DMSO is a strongly polar substance with high dipole moment (3.96 D). Perturbations caused by dipole-dipole interactions of DMSO with amino acid residues of proteins forming the channel might influence conductivity of the channels. For example, by way of a concrete calculation, a simple model system like gramicidin channel shows a strong dependence of the conductance obtained on the location and orientation of dipole rings at the channel (Sancho and Martinez 1991).

In conclusion, we have shown that cytotoxicity of high concentrations of DMSO on human erythrocyte does not relate to the activation of cation channels permeable to Na^+ and Ca^{2+}. High concentrations of DMSO exert inhibitory effect on the non-selective cation channels activity in human erythrocytes. Reduced activity of the cation channels upon the treatment with high concentration of DMSO protects the cells against excessive Na^+ and Ca^{2+} download; preventing accelerated cell death and lowing cell aggregation.

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