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Effect of K^+ accumulation removal and high extracellular osmolarity on K^+ current in insect axonal membrane

Maria Stankiewicz[1], Marcel Pelhate[2], Izabela Pancerzyńska[1], Andrzej Zienkiewicz[1] and Wojciech Kądziela[1]

[1]Institute of Biology and Environment Protection, N. Copernicus University, 9 Gagarin St., 87-100 Toruń, Poland;
[2]Laboratory of Neurophysiology, ERS CNRS 108, Angers University, Haute de Reculée St., 49-045 Angers, France

Abstract. The effect of a removal of K^+ accumulation on K^+ current in insect axonal membrane was observed. Experiments were performed on isolated giant axon of a cockroach using double oil gap technique. K^+ accumulation was reduced by: (1) an outward water flow induced by non-electrolytes (urea, glucose) added to extracellular saline and (2) by an increase of non-specific permeability of axonal glial layer obtained after the application of DMSO. The conclusions are: (1) osmolar effect depends on the type of molecule used for osmotic shock, (2) increase of outward K^+ current in conditions of high extraaxonal osmotic pressure is attributed to the decrease of K^+ accumulation and outward water flow, (3) removal of K^+ accumulation doesn't affect the kinetics and the time course of K^+ current, (4) experiments confirmed the presence of an inactivating component in the axonal outward K^+ current, (5) DMSO must be used cautiously as a solvent in electrophysiological experiments.

Key words: axonal membrane, K^+ accumulation, osmotic shock, K^+ current

INTRODUCTION

K^+ accumulation as a result of neuronal activity is a widely distributed phenomenon in the peripheral and central nervous systems. The increase of K^+ concentration in narrow intercellular space is an important factor in the regulation of neuronal excitability, intercellular communication and glial cell function (for reviews see: Orkand 1980, Syková 1983, Stankiewicz et al. 1994). However, the direct effect of K^+ accumulation on K^+ channels is not quite clear yet. Different physiological mechanisms for the clearance of accumulated K^+ ions have been proposed (review, Ritchie 1992). Excessive K^+ can be eliminated also by an enzymatic treatment destroying extracellular clefts but it may affect the studied active membrane (Stankiewicz et al. 1993). A decrease of K^+ accumulation has been also observed by the outward water flow induced by high extracellular osmotic pressure (Kukita 1988).

Cockroach giant axon is one of the main preparations for pharmacological study of insect ionic channels (Pelhate et al. 1990). However, definite characteristics of its K^+ conductance has not been done yet. The application of the patch clamp technique to study axonal single channel properties failed untill now because of the persistence of a very thin layer of Schwann cells. These glial cells create a periaxonal space where accumulation of K^+ can take place. It has been demonstrated that the presence of K^+ accumulation causes obvious difficulties in the description of the characteristics of the K^+ conductance in giant axon (Stankiewicz et al. 1993).

The aim of this study was (1) to observe the effect of high osmotic pressure in extracellular fluid on an outward K^+ current and K^+ accumulation in isolated giant axon; (2) to characterize the K^+ current in the absence of K^+ accumulation in this preparation.

METHODS

The experiments were performed on isolated giant axons dissected from abdominal nerve cords of adult male cockroaches, *Periplaneta americana.*

Electrophysiological recordings were made in voltage-clamp conditions, using the double oil-gap single fibre technique (Pichon and Boistel 1967). The method of axon isolation and the recording technique have been described previously in detail (Pelhate and Sattelle 1982). Experiments were made at room temperature (18-20°C). Current traces were corrected for non-specific leakage and capacitive currents. All recordings were obtained at holding potential (HP) = -70 mV, with protocols indicated in the results.

Isolated axon was superfused by physiological saline containing (in mmol/l): 210 NaCl; 3.1 KCl; 5.4 $CaCl_2$; 5.0 $MgCl_2$. The saline was buffered with 1 mmol/l Hepes and the pH was adjusted to 7.2 with NaOH. Tetrodotoxin (Sigma) was added at 10^{-7} mol/l to saline to block the Na^+ current.

Different high osmolarity of extraaxonal fluid has been obtained by the application of non-electrolytes, namely urea and glucose, dissolved in normal physiological saline or in isotonic KCl saline. Solutions of 0.2; 0.5; 1; 1.5 mol/l of urea and 0.3; 0.5; 1 mol/l of glucose have been used.

In order to increase the non specific permeability of the layer surrounding the axon, a 1 % solution of DMSO (Dimethyl sulfoxide, Sigma) in physiological saline has been applied.

RESULTS

K^+ current in axonal membrane in control conditions

In control conditions, an increase of outward K^+ axonal current causes a rise of inward tail current observed when membrane potential is stepped back to the holding potential (HP) after applying a depolarizing pulse (Fig. 1a). The size of the inward tail current depends on the instantaneous reversal potential for K^+ (E_{rev}), which can be measured in experiments with test pulse followed by different postpulses (Fig. 1b). It has been demonstrated already (Stankiewicz et al. 1993) that in resting conditions E_{rev} in cockroach giant axon is equal to actual HP. Outward K^+ current evoked by 35 ms

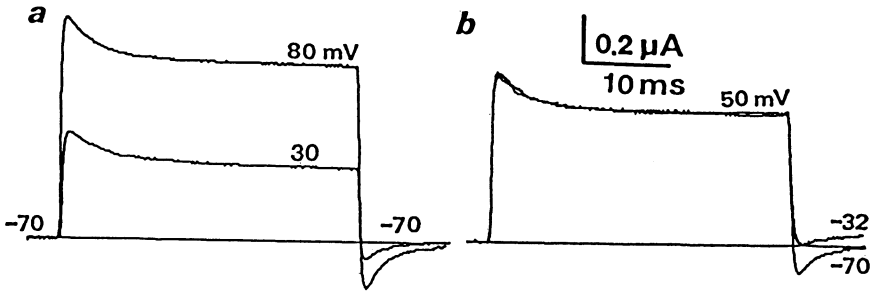

Fig. 1. a, dependence of an inward tail current on the size of outward K^+ current. K^+ currents were elicited from a holding potential (HP) of -70 mV by applying 35 ms pulses to +30 and +80 mV. b, measurement of a reversal potential (E_{rev}) for K^+ current. Tail current evoked on repolarization to different potentials indicated near each trace after 35 ms depolarization to +50 mV.

depolarizing pulses, +30 and +80 mV, changes E_{rev} from -70 mV to -37 and -25 mV, respectively. Assuming that axoplasmic potassium concentration $[K^+]$ remained constant (150 mmol/l), we may calculate that, although the axon was superfused with a saline containing 3.1 mmol/l KCl, outward current elicited by depolarizing pulse (+50 mV) can induce a shift in periaxonal $[K^+]$ from about 10 mmol/l to about 40 mmol/l (Fig. 1b).

Effect of urea

The effect of urea depended on its concentration. A solution of 0.2 mol/l was without effect whereas 0.5 mol/l induced, a progressive increase of outward current, concomitantly with the decrease of inward tail current (Fig. 2A a,b). After about 5 min a new equilibrium state was obtained and further changes in outward and tail current were not observed. One mol/l of urea had a similar effect but only at the begining. After this step (called step I - Fig. 2B a,b) the second one (II) appeared. The increase of outward current continued, but inward tail current became gradually larger reaching a value sometimes much higher than that in control (Fig. 2B c). After applying 1.5 mol/l of urea only the second (II) step was observed. The described increase of both currents usually continued to the death of the axon. Reversibility of effect of urea depended also on its concentration where for the first step (I) it was almost total (Fig. 2A c), and for the second step (II) it was usually only partial (not shown). Physiological saline applied after a long bath in 1 or 1.5 mol/l urea induced a large increase of leak current indicating the destruction of the axon.

Effect of glucose

Glucose applied in concentration 0.3 and 0.5 mol/l induced similar changes as those of 1 mol/l urea: an increase of outward current and decrease of inward tail current at the beginning (step I) and an increase of both currents later (step II), (not shown). Similar to 1.5 mol/l of urea after 1 mol/l of glucose only step II was observed. Independently of the glucose concentration the increase of outward and inward current continued to the death of the axon. Usually stronger depolarization of membrane augmented the described effect. The reversibility of the glucose effect was much smaller at corresponding concentration than in the presence of urea.

Characteristics of K^+ current in conditions of reduced K^+ accumulation in periaxonal space

Urea at concentration of 0.5 mol/l induced the most stable decrease of inward tail current indicating a lowering of $[K^+]$ in the immediate vicinity of the axonal membrane and this urea concentration has been chosen as the most convenient for detailed observation of K^+ current in conditions of reduced K^+ accumulation.

Table I shows examples of instantaneous equilibrium potential for K^+ ions, E_{K+} (determined as the instantaneous reversal potential of the tail current) at the end of two 35 ms depolarizing pulses before and after urea application. Moderately high extracellular osmolarity induced important shift in instantaneous E_{K+}. Periaxonal concentration of K^+ ($[K^+]_e$) at the end of the depolarizing pulses to +30

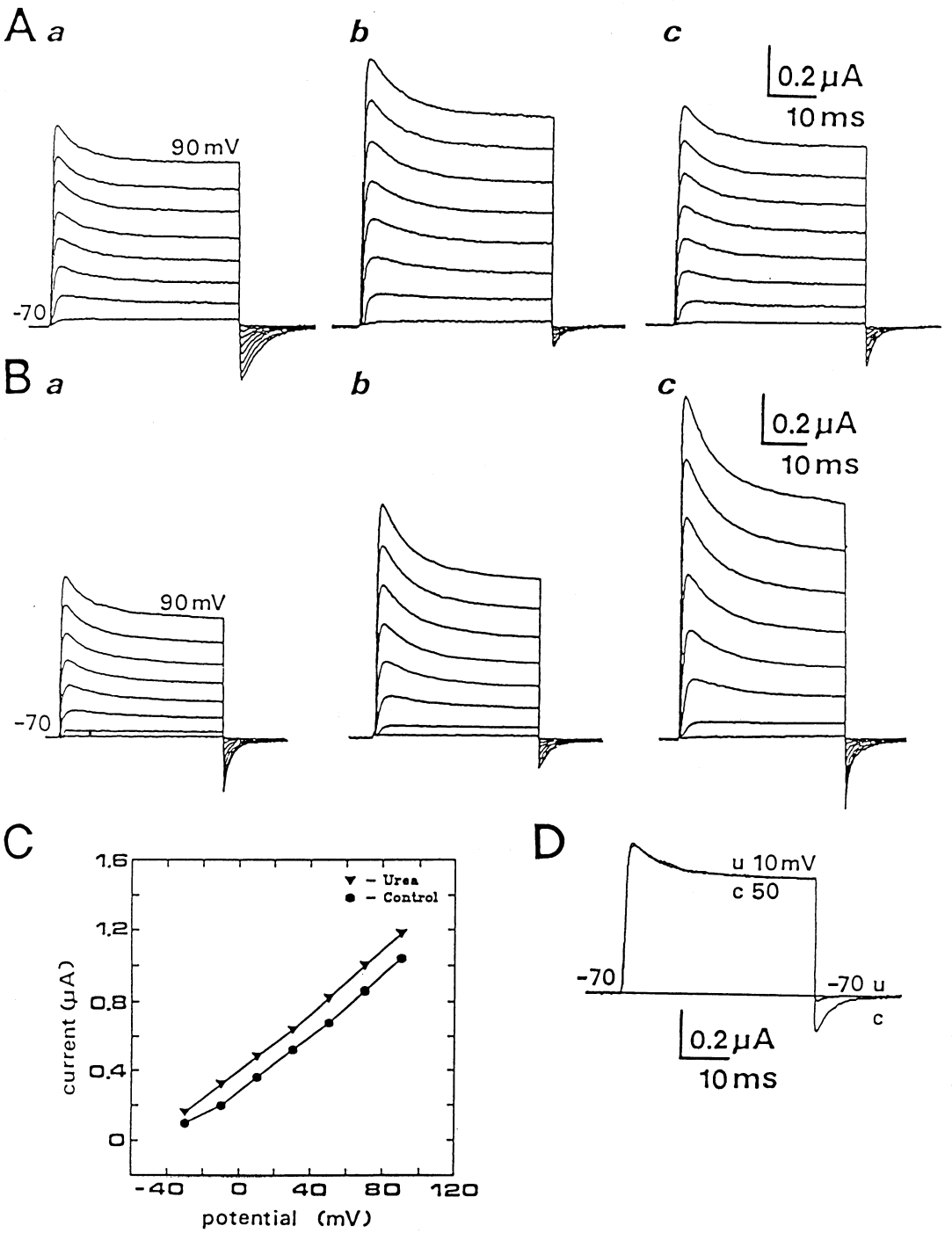

Fig. 2. Effect of urea on K^+ current. A, effect of 0.5 mol/l of urea. Control (a), family after urea application (b), reversibility of urea effect in physiological saline (c). B, effect of 1 mol/l of urea. Control (a), families after urea application: first step (I) of the effect (b), second step (II) of the effect (c). K^+ currents were elicited from HP of -70 mV by applying 35 ms depolarizing pulses from -50 to +90 mV in 20 mV steps. C, voltage dependence of outward K^+ current before and after 0.5 mol/l of urea. D, time course comparison of similarly sized outward K^+ currents before (c) and after (u) urea application. K^+ currents were elicited from HP of -70 mV by applying 35 ms pulses to +50 mV before and to +10 mV after urea application.

TABLE I

Values of a reversal potential for K^+ current (E_{rev}) after 35 ms depolarizing pulses to +30 and +80 mV obtained in experiments with test pulse and different postpulses protocol in control conditions and after 0.5 mol/l of urea application

No. of experiment	Membrane potential	Erev control	Erev urea
	(mV)	(mV)	(mV)
1		-35	-55
2	30	-40	-60
3		-35	-50
1		-20	-45
2	80	-30	-55
3		-25	-35

and +70 mV, calculated on the basis of E_{K+} values, changed after applying of urea from 35 mmol/l to 16 mmol/l and from 55 mmol/l to 25 mmol/l, respectively. The aim of experiments with very short depolarizing test pulses and different postpulses was a verification of resting E_{K+}. No current was observed only when potential returned to the holding potential (not shown). It indicates no important change in E_{K+} and in $[K^+]_e$ in resting conditions after application of urea.

A decrease of K^+ accumulation after 0.5 mol/l urea has been observed concomitantly with the increase of outward current (by about 30 ± 6 %, n=10, P<0.01). Reduced K^+ accumulation and shift in E_{K+} to more negative values effects in an increase of driving force (Em - E_{K+}, Em actual membrane potential) for K^+ ions flowing through channels opened by depolarization. Is it the only cause for the observed increase of outward K^+ current? To answer this question, experiments with isotonic KCl saline have been performed. Application of 0.5 mol/l urea under such conditions induced a decrease of inward K^+ current (Fig. 3a) and an increase of outward K^+ current (Fig.3b) as compared to control. In isotonic KCl saline, changes in $[K^+]_e$ induced by outward current can be ignored. In this manner it has been demonstrated that a decrease of $[K^+]_e$ by high extracellular osmolarity is not the only factor responsible for the increase of outward current.

An increase of outward current after applying urea is reflected in the shift to more negative values of its voltage-dependence, compared to control, Fig. 2C. No change in the time constant of outward current activation has been induced by urea. The time constant of inward tail current decline was not significantly smaller in high extracellular osmolarity. The time course of similar sized outward current was often the same before and after urea (Fig. 2D). The presence of "droop" (decrease of outward current amplitude during the first 5 ms of the depolarizing pulse) has been observed with high K^+ accumulation (Fig. 2D, c) as well as with a reduced one (Fig. 2D, u).

Effect of DMSO

DMSO is known to increase non-specific permeability of membranes. It was assumed that elevated permeability of layer surrounding the axon will facilitate a movement of water and K^+ ions in different compartments around the axon.

Figure 4 illustrates the effect of 1% DMSO. The decrease of outward K^+ current observed after

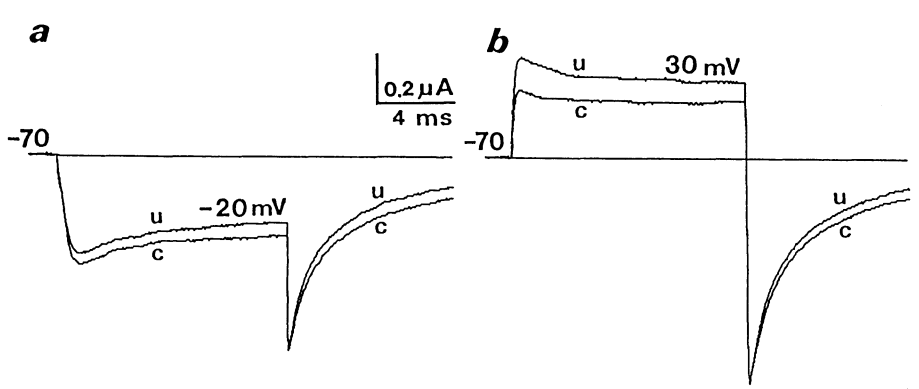

Fig. 3. Effect of 0.5 mol/l of urea in isotonic KCl saline on K^+ current. Effect on inward K^+ current (a) and on outward K^+ current (b). c, control; u, after urea application.

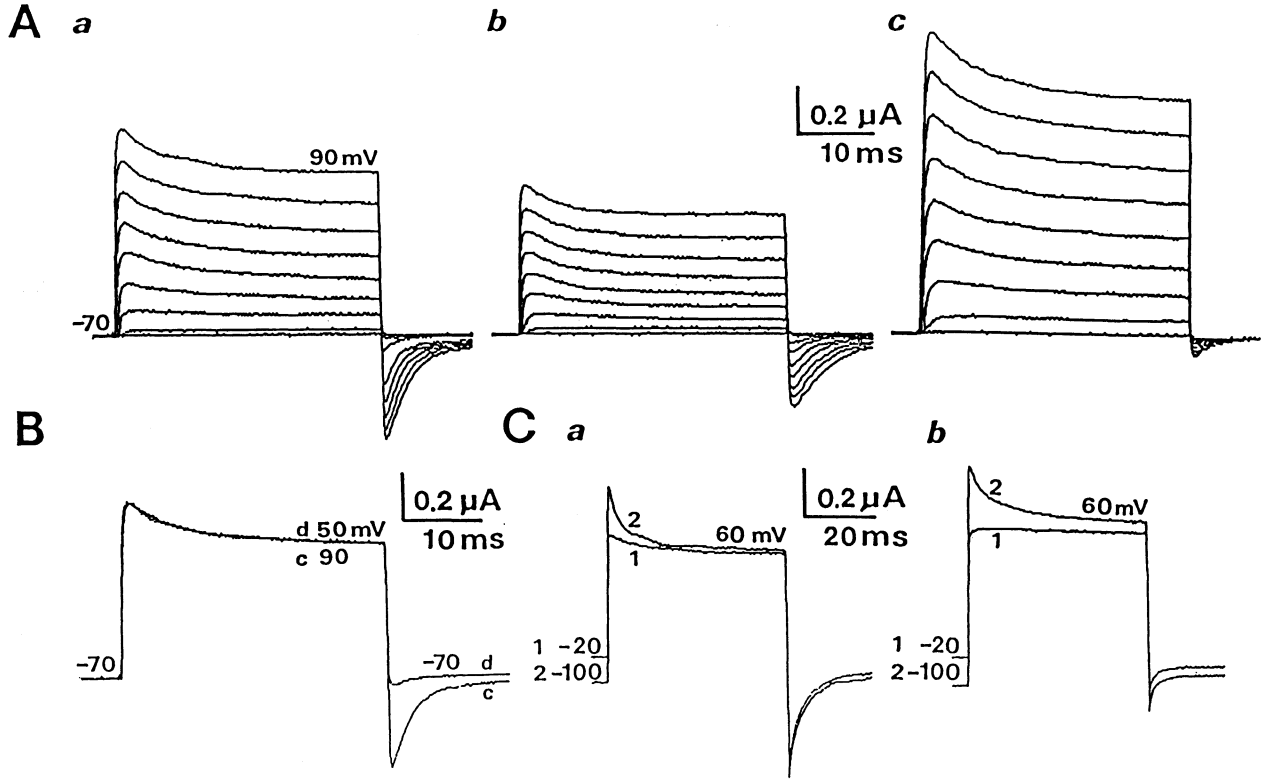

Fig. 4. Effect of DMSO on K^+ current. A, effect of 1% DMSO. Control (a), family after DMSO application (b), after wash in physiological saline (c). B, time course comparison of similarly sized outward K^+ currents before (c) and after (d) DMSO application. K^+ currents were elicited from HP of -70 mV by applying 35 ms pulses to +90 mV before and to +50 mV after DMSO. C, time course comparison of outward K^+ current elicited by 50 ms test pulse to +60 mV following prepulses to -20 (1) and to -100 (2) mV before (a) and after (b) DMSO application.

DMSO application is similar to that obtained after, for example, ethanol (Fig. 4A a,b). However, after a wash with physiological saline, an increase of outward K^+ current and a decrease of inward tail current, when compared to the control has been observed (Fig. 4A a,c). A forty percent increase of outward current has been observed together with a shift of E_{K+} from -20 to -55 mV after depolarizing pulse to +30 mV and from 0 to -45 mV after pulse to +80 mV. In spite of the almost total elimination of additional K^+ accumulation, induced by outward current, no change in the time course of this current has been obtained (Fig. 4B).

Experimental protocols using conditioning prehyperpolarizing and predepolarizing pulses can be used to isolate this transient K^+ current component (Fig.4C). In conditions of reduced K^+ accumulation the presence of the transient component is more evident. Moreover, strong predepolarization is also able to inactivate part of normally noninactivating current (Fig. 4C b).

DISCUSSION

The neuronal environment is an important factor decisive upon function of neuronal membrane. The glial cells create extracellular space for neurones and form the blood-brain barrier (Schofield and Treherne 1984). In axon, Schwann cells play a similar role. The elevation of osmolarity of the fluid superfusing the axon induced outward water flow between different compartments: axon, periaxonal space, Schwann cells, mesaxon. Comparing the effect of urea and glucose, it is clear that intensity of outward water flow depends on the type of molecule. Glucose as a larger and much less diffusive

molecule through the membranes induced a similar effect at lower concentration as urea, molecule that diffuses rapidly across the membranes (Macey 1984). An irreversible effect obtained with a higher concentration of non-electrolytes (1 mol/l glucose and 1.5 mol/l urea) concurs with the observation that the blood-brain barrier can be disrupted by a short exposure to 3mol/l urea (Treherne et al. 1973).

Urea as a small molecule is able to diffuse into periaxonal space and probably even into axon. When it is applied in moderate concentration (0.5 mol/l) the molecules which crossed the Schwann barrier are able to balance the extrabarrier quantity of urea. The movement of water is limited and a new equilibrium state is obtained, characterized by larger outward K^+ current and smaller tail current indicating reduced K^+ accumulation as compared to control. At higher concentrations of urea (1, 1.5 mol/l) the increase of outward current in the second step was so large that it caused an increase of tail current, indicating the elevation of $[K^+]_e$.

The increase of outward current induced by high osmolarity of saline can have two causes. In normal conditions the accumulation of K^+ induces a shift of E_{K+} to less negative values. As a result of the reduction of K^+ accumulation, (I step of the effect after non-electrolyte application) E_{K+} does not much change during the development of K^+ current and the driving forces for K+ ions under depolarization are larger compared to control. The second cause can be outward water flow, as it has been described for squid axon (Kukita and Yamagishi 1983). Water flowing by opened K^+ channels amplifies the stream of K^+ ions causing outward current increase which is especially large in the presence of glucose and higher concentrations of urea. In experiments with isotonic KCl saline and 0.5 mol/l of urea, the decrease of inward current and the increase of outward current indicate that the water flow facilitates an ionic flow through the potassium channels in the same direction and suppresses the ionic flow in the opposite direction. The influence of K^+ accumulation can be ignored in these experiments. The percentage change of outward current was similar to that obtained in a normal saline with 0.5 mol/l of urea. This indicates that water flow is mainly responsible for the increase of outward current observed after 0.5 mol/l of urea. However, the experiments with DMSO showed that decrease of K^+ accumulation can play a dominant role in the increase of outward current. Elevated permeability of Schwann cells after treatment with DMSO facilitating a movement of water and ions causes a decrease of K^+ accumulation. However, an opening of additional K^+ channels induced by DMSO can not be excluded.

Almost total elimination of K^+ accumulation was without effect on K^+ current kinetics. Moreover, also high osmolarity of the extracellular fluid didn't affect the kinetics of K^+ current. Urea is known to interfere with the structure of various proteins (Kellis et al. 1988) however, urea as well as DMSO neither change the kinetics nor the voltage dependence of outward current. The observed shift in voltage dependence of outward current resulted only from an increase of current size at each membrane potential.

DMSO is often used as a solvent for different substances tested on biological membranes. Its long term effect on K^+ current and K^+ accumulation must be taken into account at least in preparations where K^+ accumulation is observed.

In the previous paper (Stankiewicz et al. 1993) it has been demonstrated that the presence of "droop" can not only be due to the K^+ accumulation in periaxonal space and the decrease of driving forces during depolarizing pulse, but also it can indicate the existence of a transient, inactivating component of outward current. The "droop" in outward current time course has been conserved in spite of the important reduction of K^+ accumulation by urea and by DMSO indicating rather the presence of the transient component of outward current.

The conclusion is that high osmolarity of extracellular fluid is able to change not only the volume of neurones (Syková and Chvátal 1993) but also their microenvironment and the amplitude of ionic currents. The osmolar effect depends on the size, ability to diffuse by membrane and concentration of the molecule, inducing the osmotic shock. The in-

fluence of K^+ accumulation on the outward K^+ current amplitude remains still not quite clear and further studies are necessary. However, excessive K^+ in periaxonal space does not affect the kinetics of axonal K^+ current. The next important conclusion from this study is that presence of two components of outward axonal K^+ current is more conclusive than it was before (Stankiewicz et al. 1993). In spite of the almost total elimination of K^+ accumulation, the "droop" persisted indicating the existence of a transient, inactivating, A type, current in axonal membrane. However, to confirm this, putch-clamp studies are necessary. Nevertheless, the elevated osmolarity of the extracellular environment can be useful in more precisely characterizing the ionic channels in preparation where accumulation of K^+ is observed.

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