Effects of Dimethylsulfoxide (DMSO) and Potassium Chloride on the Resting Potential of the Isolated Frog Nerve

DMSO appears to be effective in altering the configuration of proteins[1]. This effect is reversible and seems to be related to the size of the DMSO molecule and the capacity to substitute for, or bind water[2,3]. Another explanation of the DMSO effects is to assume reversible interactions of DMSO with SH groups of enzymes[4]. These properties may be discussed as a mode of action of DMSO in living systems. The effects of DMSO on nervous tissue were studied with regard to nerve conduction[5] and blockade[6].

In this paper we tried to elucidate 1. the direct influence of DMSO on the resting potential, 2. the indirect influence of DMSO on potassium chloride mediated depolarizations and, 3. the possibility of seasonal differences in the action of DMSO and potassium chloride.

Method and material. We used the sucrose gap method[7]. Sciatic nerves of winter and summer frogs (*Rana temporaria*) were prepared for a distance of about 4 cm; nerve sheaths and perineural epithelium[8] were removed. From October to March the frogs were defined as winter-, from April to September as summer frogs.

Non-polarisable calomel electrodes connected to a cathode follower with a DC amplifier, an oscilloscope and pen recorder were used to record the alterations of the resting potential.

Results. 15% DMSO Ringer solution depolarizes reversibly the resting potential of frog nerves. Compared with the depolarizing action of a 20 mmol/l potassium chloride Ringer solution as standard, there were fundamental differences in the depth and velocity of the depolarization between these 2 depolarizing substances (Figure 1).

Composition of the Ringer-, sucrose- and test solutions

The pH value of the solutions was adjusted between 7.3 and 7.5

Ringer solution

NaCl	110.50 mmol	
KCl	2.50 mmol	
$NaHCO_3$	2.40 mmol	/l aqua bidest
$CaCl_2$	1.05 mmol	

Isotonic sucrose solution

$C_{12}H_{22}O_{11}$ 73 g/l aqua bidest

15% resp. 30% DMSO Ringer solution

150 or 300 ml of 97% DMSO were filled up with aqua dest. to 1 l. To this mixture the electrolytes corresponding to 1 l Ringer solution were added.

20 mmol/l resp. 60 mmol/l KCl Ringer solution

The sodium chloride contents was lowered while that of potassium chloride was raised.

Furthermore there was an obvious concentration-effect-relationship in the action of DMSO- and potassium Ringer solution. There was no seasonal difference in the depolarizing effect of DMSO but in that of potassium chloride Ringer solution[9].

In Figure 2 is shown that the DMSO effects on the resting potential of nerves from summer and winter frogs are equal, while those of potassium chloride are significantly different.

In a series of experiments summarized in Figure 3, we found that there is a longer lasting effect of DMSO which outlasts the acute DMSO mediated depolarization and which could be demonstrated by subsequent potassium chloride depolarizations. Successive combination of DMSO first and potassium after it shows that the final restitution in the membrane for the following KCl mediated depolarizations is DMSO and time-dependent. It takes about 40 min to abolish completely the persistent DMSO effect.

In contrast to the successive combination, we also tried a simultaneous one. Depolarization caused by KCl Ringer shows a narrow deviation of the resting potential; depolarization caused by the mixture DMSO and KCl, i.e. simultaneously applied, is characterized by a narrow peak followed by a plateau of lower value. The duration of the plateau depends on the perfusion time of the mixture. When the nerve is perfused with the mixture after DMSO alone has been applied, the depolarization peak of the mixture is cut off. Application of 20 mmol/l potassium chloride caused a standard depolarization of normal height.

To prove or refute the hypothesis of the DMSO/SH group interaction, we tried to block the SH groups of nervous enzymes with SH group blockers e.g. *p*-chlormercuri-benzoeacidic sodium. Though we used a relative high concentration (10^{-3} *M*), which produces a hyperpolarization of the resting potential, the DMSO depolarization was not influenced.

Discussion. Attempts to elucidate the mode of action of DMSO are as numerous as the physico-chemical and biological qualities of the DMSO-molecule. It has broad

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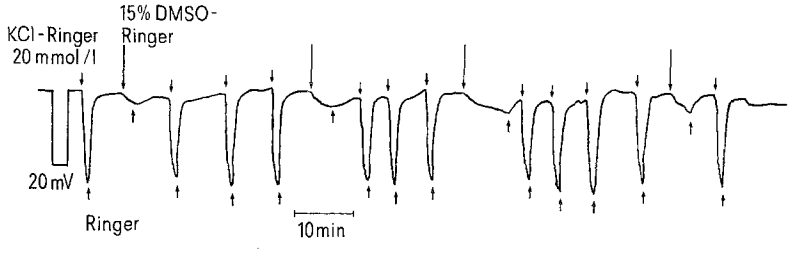

Fig. 1. Depolarization evoked by applying 20 mmol/l KCl Ringer respectively 15% DMSO Ringer solution; repolarization is performed by perfusion with Ringer solution. ↓, 15% DMSO Ringer solution; ↓, KCl Ringer solution 20 mmol/l; ↑, Ringer solution.

solvent characteristics and can permeate innocuously through protein barriers[10]. DMSO is able to penetrate regions on the protein subunits interfaces more readily than other bulkier solvents. The other explanation of the DMSO effects is to assume reversible interactions of DMSO with the SH groups of enzymes e.g. of the membrane ATPase[4].

At our present state of knowledge we join the widely held hypothesis that DMSO does not act as an osmotic active agent or with SH group because there was no alteration of DMSO induced depolarizations by SH group blockers, but as an ideal physico-chemical solvent[1, 11, 12]. The experimental results of osmotic DMSO effects are contradictionary and the existence of experimental data which classify DMSO solutions at one time as hypertonic and at the other as hypotonic are numerous.

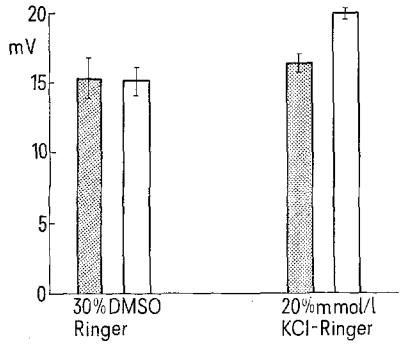

Fig. 2. Comparison between the resting potential depolarizing effects of 30% DMSO Ringer solution and 20 mmol/l KCl Ringer solution from winter and summer nerves .The black bars represent the values for summer nerves, size of sample 11 resp. 17; the white bars those for winter nerves, size of sample 22 resp. 69 (probality of error $< 0.1\%$).

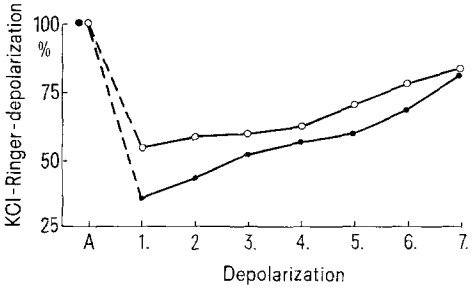

Fig. 3. Comparison between the depolarizations (sample mean of 6 experiments) evoked by 20 mmol/l KCl Ringer with winter (represented by the filled circles) and by 60 mmol/l KCl Ringer with summer nerves (represented by the open circles) after pretreatment with DMSO. A) The depolarization mediated by potassium chloride Ringer solution before DMSO Ringer was applied is arbitrarily plotted as 100% (standard depolarization). The first KCl mediated depolarization after DMSO application reaches about 55% in summer nerves and in winter nerves only 35% of the standard depolarization. There is a permanent restitution in the height of the depolarization amplitude with the following KCl depolarizations.

A comparison of the van't Hoff i-values shows that DMSO contributes very little to the osmotic pressure of extracellular aqueous solutions. Hemolysis is prevented when 0.9% NaCl or isotonic concentrations of other compounds are added to DMSO solutions[13].

25% DMSO produced blocking in an isolated nerve and it has been suggested that this might be an osmotic effect[6]. Whether DMSO solutions are hypo- or hypertonic, the isoosmolarity of the solutions used in measurements of the resting potential of nerves is not important since 50% hypertonic solutions did not alter the resting potential[14].

Summarizing we suggest that DMSO influences the conductance and the diffusion coefficient of the membrane and possibly the structure of water. It seems likely that alterations of these membrane qualities may be responsible for variations in the carrier mechanism of the passive ion transport.

The apparent seasonal differences in the sensitivity of the resting potential, i.e. the nervous membrane towards potassium chloride ions, is a well known phenomenon but has not as yet been explained satisfactorily[15–17].

There are similar results of the depolarizing effects of nicotine on frog's skeletal muscle. This effect is more marked from October to March than in spring and summer[17].

In our experiments we saw the same temporal limitation for the sensitivity towards potassium chloride mediated depolarizations of the resting potential in frog's peripheral nerves (Figure 2). Furthermore there is a parallelism between seasonal changes in enzyme activity and KCl depolarizations which might explain the seasonal differences in KCl depolarizations of the resting potential. The specific activity of the toad's cardiac muscle membrane ATPase during the summer months was repeatedly found to be high when compared to that of similar preparations isolated during the winter months[16].

At present we cannot say if this parallelism of our results with those of the literature, in which the differences of tissue must also be considered, is casual or causal.

Zusammenfassung. Da DMSO das Ruhepotential depolarisiert, verglichen wir seine Wirkung mit der von Kaliumchlorid. Infolge verschieden schneller Depolarisationen und einer jahreszeitlich abhängigen Ruhepotentialänderung durch Kaliumchlorid schliessen wir auf unterschiedliche Wirkungsmechanismen beider Substanzen. DMSO hat ausser einer Kurzzeitwirkung einen länger persistierenden reversiblen Effekt auf die Nervenmembran, der durch nachfolgende kurzwirksame Kaliumchloriddepolarisationen nachgewiesen werden kann.

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