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A PERMEABILITY CHANGE OF MYELIN MEMBRANE VESICLES TOWARDS CATIONS IS INDUCED BY MgATP BUT NOT BY PHOSPHORYLATION OF MYELIN BASIC PROTEIN

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Summary

The existence of an endogenous protein kinase activity and protein phosphatase activity in myelin membrane from mammalian brain has now been well established. We found that under all conditions tested the myelin basic protein is almost the only substrate of the endogenous protein kinase in myelin of bovine brain. The protein kinase activity is stimulated by Ca^{2+} in the micromolar range. Optimal activity is reached at a free Ca^{2+} concentration of about 2 μM. Myelin membrane vesicles were prepared and then shown to be sealed by a light-scattering technique. After preloading with $^{45}Ca^{2+}$, $^{86}Rb^{+}$, or $^{22}Na^{+}$, the self-diffusion (passive outflux) of these ions from myelin membrane vesicles was measured. Ionophores induced a rapid, concentration-dependent outflux of 80—90% of the cations, indicating that only a small fraction of the trapped ions was membrane bound. There was no difference in the diffusion rates of the three cations whether phosphorylated (about 1 mol phosphate per myelin basic protein) or non-phosphorylated vesicles were tested. In contrast, a small but significant decrease in permeability for Rb^{+} and Na^{+} was measured, when the vesicles were pretreated with ATP and Mg^{2+}.

Introduction

It is now well established that myelin from the central and peripheral nervous system contains an endogenous, membrane-associated protein kinase,

Abbreviation: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid.

phosphorylating the myelin basic protein, a major protein of the myelin membrane. It has been demonstrated that the enzyme is cyclic AMP independent but could be regulated by Ca^{2+} [1—4]. The physiological significance of myelin phosphorylation is unknown and a number of functions have been proposed. Among these functions, a role in the compaction process of the myelin sheets or in the regulation of the membrane permeability for cations are the most favoured hypotheses [3—6].

Studies on membranes from a number of other mammalian sources reveal marked differences in the effect of membrane protein phosphorylation on permeability for cations. Whereas phosphorylation seems to decrease the permeability for Na^+ in the toad bladder epithelial cell membrane [7], there was no effect on Na^+ permeability in synaptosomes from rat brain [8] and in vesicles from human erythroyctes [9]. In contrast, an increase in Na^+ permeability with increasing phosphorylation was measured in avian erythrocyte membranes [10]. The permeability for Ca^{2+} is reported to be lowered by the phosphorylation of membrane proteins in rod outer segment discs from retina [11], in rat brain synaptosomes [8] and in human erythrocytes [9]. Most of the involved protein kinases are cyclic AMP dependent and it is not always clear if the measured effects on permeability are really due to phosphorylation and not to another (unknown) effect of the added cyclic AMP. In some studies the measurements of permeability were indirect and unphysiologically high concentrations of Ca^{2+} were used [8,9]. It appeared, therefore, of interest to study the effect of phosphorylation on permeability of the myelin membrane. The preparation and characterization of myelin membrane vesicles have recently been described [12]. The system is well defined in the sense that pure myelin membranes can be isolated by established methods and that the cyclic AMP-independent endogenous protein kinase phosphorylates almost exclusively one membrane protein [1].

In the present report we present further evidence that the myelin membrane vesicles are sealed. Direct flux measurements are performed with myelin membrane vesicles preloaded with radioactive cations. Ionophores are used to discriminate between bound and trapped ions. Our results indicate that there is no causal relationship between myelin membrane phosphorylation and permeability changes for Na^+, Rb^+ and Ca^{2+}. However, the treatment of myelin membrane vesicles with MgATP causes a small but significant decrease of the permeability for Rb^+ and Na^+.

Materials and Methods

Myelin isolation. Myelin was isolated by using the method of Norton [13] from bovine brain white matter. Bovine brains were obtained from a local slaughterhouse, frozen rapidly and kept at $-70°C$ until used. Protein estimations were performed according to the method of Lowry et al. [14], in the presence of 0.2% sodium dodecyl sulfate (SDS) and using bovine serum albumin as a standard.

Preparation of myelin membrane vesicles. The vesicles were prepared according to the method of Steck et al. [12]. Briefly, packed purified myelin was suspended in 5 vol. of 1 mM sodium phosphate buffer, pH 8.0, containing

2 mM dithiothreitol. For a sodium-free medium, sodium phosphate was replaced by Tris-HCl. After gentle homogenization, the membranes were diluted to 0.2 mg protein/ml final concentration with the same buffer and gently stirred at 4°C during 15 h. After centrifugation (25 000 × *g* for 30 min at 4°C), the pellet was resuspended in the incubation buffer by vortex mixing (10 mg protein/ml) and passed through a 26 gauge needle.

Non-vesiculated myelin (control) was purified myelin, which was suspended immediately before use in the same assay medium as the vesicles.

Phosphorylation reaction, electrophoresis and autoradiography. Standard protein kinase assays were carried out at 22°C in a volume of 0.1 ml, containing 100 μg myelin proteins. Unless otherwise indicated, the medium contained 50 mM Tris-HCl, pH 7.4, 8 mM $MgCl_2$, 15 μM $CaCl_2$, 2 mM dithiothreitol, 10 μM [γ-^{32}P]ATP (specific radioactivity 500 cpm/pmol). The reaction was terminated usually after 2 min incubation by addition of 50 μl 'solubilizing buffer' containing 4% SDS, 10% 2-mercaptoethanol, 8% glycerol, 0.01% bromphenol blue in 50 mM Tris-HCl, pH 6.8, and then the solubilized membranes were heated at 65°C for 30 min.

SDS-polyacrylamide gel electrophoresis was performed on linear 7–15% acrylamide gradient slab gels, according to the method of Laemmli [15] with the modifications of Steck et al. [12]. The Coomassie blue-stained slabs were dried on a Whatman 3 mM chromatography paper under vacuum. For autoradiography the dried gels were placed in contact with Kodak X-ray film NS-T2 for 24 h. In some experiments, individual protein bands were cut from the dried slab, digested in 0.5 ml 50% Protosol (New England Nuclear) during 15 h at 37°C and after addition of 3 ml Aquasol (New England Nuclear) counted in a Beckman LS 350 liquid scintillation counter.

When total phosphate incorporation was measured, the phosphorylation reaction was terminated with 2 ml of ice-cold 20% (w/v) trichloroacetic acid containing 50 mM $Na_4P_2O_7$. The mixtures were pipetted onto Millipore filters (0.45 μM) and the precipitated membrane material washed three times with 15 ml of ice-cold trichloroacetic acid, 50 mM $Na_4P_2O_7$. After drying, the Millipore filters were counted in 10 ml Aquasol.

To determine the dependence of the protein kinase activity on low concentrations of Ca^{2+}, the enzyme assays were performed in 100 mM Tris-maleate buffer, pH 6.6, with different, well defined Ca^{2+} : EGTA ratios (20 mM EGTA final concentration) yielding the desired free Ca^{2+} concentrations (calculated according to the method of Portzehl et al. [16]).

Measurements of membrane vesicle permeability. Freshly prepared vesicles were phosphorylated as described for myelin with the following modifications: the reaction was performed in 1 ml containing 2 mg of membrane protein, 10 mM Tris-HCl, pH 7.4, 8 mM $MgCl_2$, 2 mM dithiothreitol, 5 mM ATP, during 1 h at 22°C. An aliquot of the mixture was phosphorylated with 5 mM [γ-^{32}P]ATP (specific radioactivity 5000 cpm/nmol) to measure the incorporated phosphate. Non-phosphorylating conditions were obtained either by omitting $MgCl_2$ and ATP in the incubation mixture (control vesicles), or by incubating heat-inactivated membrane vesicles (1 h at 45°C was sufficient for complete inactivation of the protein kinase [1]).

After phosphorylation, the membrane vesicles were rapidly cooled at 4°C

and washed three times with 14 ml of vesiculation buffer (1 mM phosphate or Tris-HCl, pH 8.0, 2 mM dithiothreitol) by repeated centrifugation (25 000 × *g*, 25 min, 4°C) resulting in a removal of more than 99.8% of the non-incorporated ATP.

The washed vesicles were resuspended in vesiculation buffer and incubated (loaded) with radioactive cations at 4°C during 16–20 h: for $^{45}Ca^{2+}$, 200 μM $^{45}CaCl_2$ (6—7 μCi $^{45}Ca^{2+}$) was added to 1.5 ml vesicle suspension containing 2 mg of proteins. For $^{86}Rb^+$, 50 μM $^{86}RbCl$ (5.5–9.0 μCi $^{86}Rb^+$, carrier free) was added to 0.5 ml vesicle suspension containing 1.7—1.9 mg of proteins. For $^{22}Na^+$, 50 μM $^{22}NaCl$ (4—4.5 μCi $^{22}Na^+$) was added to 0.25 ml vesicle suspension containing 1.6—2.0 mg of proteins. The loaded membrane vesicles were then resuspended in 14 ml vesiculation buffer (containing 500 μM EGTA when $^{45}Ca^{2+}$ flux was measured) and washed three times by repeated centrifugations (4°C) at 25 000 × *g* for 12 min.

To measure the ionic fluxes (self-diffusion), the pellet was resuspended in 5 ml of the same buffer (4°C), a first sample of 0.95 ml was taken (for zero time), then the vesicle suspension was rapidly warmed to 22°C and incubated in a shaking bath. Further 0.95 ml samples were collected at 20, 50, 100 and 200 min and immediately centrifuged (4°C) in conical 1.5 ml tubes at 25 000 × *g* for 15 min. The supernatants were carefully taken and transferred into counting vials. Supernatants and pellets were counted in the ^{32}P-channel of a Beckman LS 350 liquid scintillation counter with Aquasol as scintillation fluid. Whenever a ionophore was added, this was done after the zero-time sample had been taken.

The self-diffusion of the different cations is expressed as loss from the vesicle sediment (pellet) in percent, the zero-time value of the pellet being taken as 100%.

Rb^+ can be considered as a model for K^+. We used $^{86}Rb^+$ rather than $^{42}K^+$ because the half-life of the latter is inconveniently short. Many studies have documented that the two isotopes give identical results, for instance, with the (Na^+, K^+) pump [17] and in liposomal transport [18].

Materials. The ionophore A23187 was a gift from Lilly Laboratories. The radiochemicals were supplied by the Radiochemical Centre, Amersham. All the other chemicals used were reagent grade and purchased from Merck, Fluka or Sigma.

Results

Phosphorylation of myelin basic protein by the endogenous protein kinase

It has been shown that in myelin of the rat central nervous system, the endogenous protein kinase phosphorylates almost exclusively the myelin basic protein [1,2]. We found that this is also true for bovine myelin where more than 75% of the total ^{32}P radioactivity was associated with the pre-large and large myelin basic protein bands in SDS-polyacrylamide gels. With the exception of one unidentified faint band (molecular weight about 43 000), no further band could be seen on autoradiographs. In order to obtain a maximal phosphorylation of the myelin membrane, different phosphorylation conditions were tested. Adding 2 mM of the reducing agent, dithiothreitol,

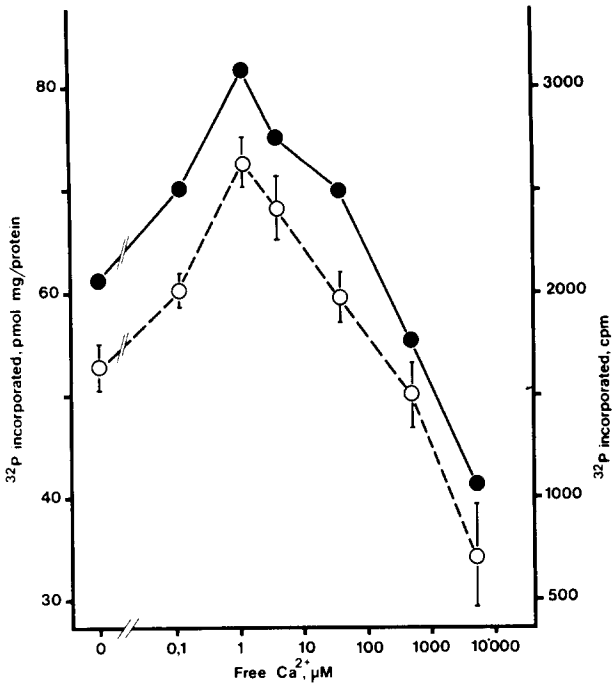

Fig. 1. Effect of Ca^{2+} on phosphorylation of myelin proteins. Free Ca^{2+} concentration was varied by use of Ca^{2+}/EGTA buffers (calculated according to the method of Portzehl et al. [16]). ○- - - - -○, phosphate incorporation in total myelin proteins (left-hand scale). The data shown are the means ±S.E. of four experiments. ●———●, phosphate incorporation in myelin basic protein (right-hand scale: cpm per cut band, corresponding to 25—30 µg of myelin basic protein).

improved the incorporation of phosphate by a factor of 1.5. The stimulation of the protein kinase activity by Ca^{2+} in the micromolar range is shown in Fig. 1. The lower curve shows the incorporation of ^{32}P in total myelin proteins expressed in pmol ^{32}P/mg protein (trichloroacetic acid precipitation technique), the upper curve the incorporation of ^{32}P in the major myelin basic protein band, excised from SDS-polyacrylamide gels (in cpm/25—30 µg myelin basic protein).

To achieve a high incorporation of phosphate into the myelin membrane vesicles, a concentration of 5 mM ATP was used and the incubation time was increased to 1 h. Under these conditions, an incorporation of 9—13 µmol phosphate/mg protein could be obtained, corresponding to 0.7—1.1 mol phosphate per mol of myelin basic protein. There was no significant change in the incorporation pattern (as judged by visual comparison of the autoradiograms of SDS-polyacrylamide gels) whether high (5 mM) or low (10 µM) concentrations of ATP were used. There was also no difference in the amount and distribution of ^{32}P incorporation (based on autoradiograms and trichloroacetic acid precipitates) whether non-vesiculated or vesiculated myelin was phosphorylated (data not shown).

In self-diffusion experiments, a partial loss of the initially incorporated ^{32}P was noted during loading, washing and diffusion measurement. In a typical flux experiment, the loss between the phosphorylation step and the end of the

release measurement accounted for 30% of the total incorporated ^{32}P. This is most probably due to the endogenous phosphoprotein phosphatase which is present in myelin [5,19] and which is thought to be responsible for the relatively high turnover of phosphate in myelin basic protein in vivo and in vitro [1,6].

Characterization of the myelin membrane vesicles by light scattering

A prerequisite for permeability studies is to ensure that the vesicles are sealed. If so, the vesicles should obey van't Hoff's law and behave as osmometers. It has been found that for osmotic swelling and contraction (shrinking), the light absorbance of the particles varies nearly inversely with the volume [20], this is because the absorbance (i.e., the relative index of refraction) of the vesicles increases with osmotic contraction or inversely decreases with osmotic swelling. Therefore, osmotic contraction leads to an increase of the scattered light, resulting in an increase in absorbance. (This is true when the wavelength of the incident light and the size of the particles are comparable. For a detailed theoretical treatment of turbidity measurements, see Ref. 20).

In Fig. 2, the effect of NaCl, added in increasing amounts to vesiculated and non-vesiculated myelin suspensions, on the absorbance at 520 nm is plotted. These results suggest that the vesicles must be sealed, since salt addition causes a marked increase in absorbance, indicating an osmotic contraction of the vesicles. The slow decrease in the absorbance observed subsequently may reflect the reswelling caused by the diffusion of the ions (and thereby water) into the vesicles.

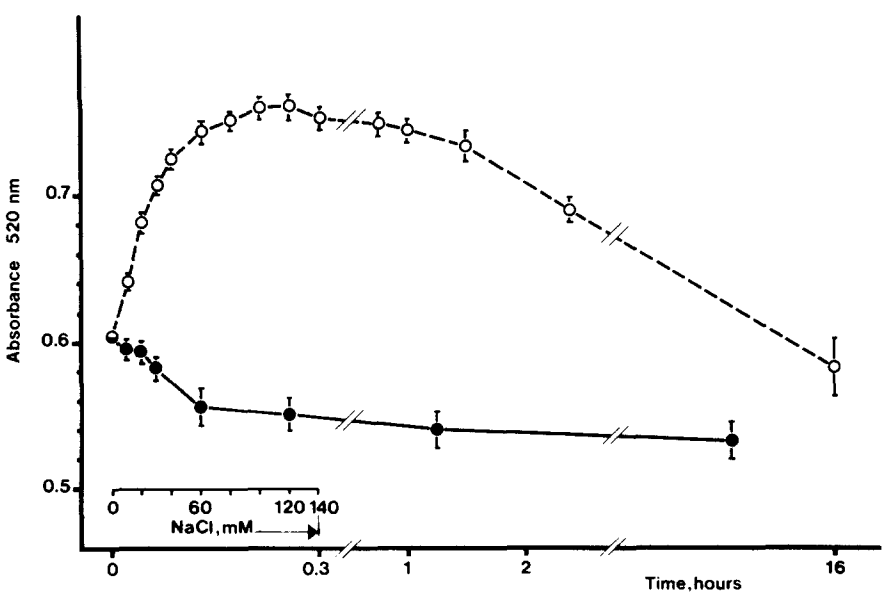

Fig. 2. Effect of increasing amounts of NaCl on the absorbance of vesiculated (○- - - - -○) and non-vesiculated (●———●) myelin preparations (means ±S.E. of five separate experiments).

Permeability studies

Permeability for Ca^{2+}. It has been shown [21] that the myelin membrane binds Ca^{2+}, having two binding sites, one of high affinity (with an association constant of $1.1 \cdot 10^3$ M^{-1}), the other of low affinity (20 M^{-1}). In agreement with these data, we found that non-vesiculated myelin binds 21 nmol Ca^{2+}/mg protein at 200 μM Ca^{2+}. After loading of myelin membrane vesicles with 200 μM Ca^{2+} and three washes in 1 mM phosphate buffer, pH 8, 14 nmol Ca^{2+}/mg myelin protein remain associated with the vesicles. This amount represents the sum of bound and trapped Ca^{2+}. To eliminate the external membrane-bound Ca^{2+}, 500 μM EGTA was added to the washing buffer, resulting in a 10-fold decrease of the vesicle associated Ca^{2+} level (1.2—1.6 nmol Ca^{2+}/mg protein).

In Fig. 3 the time course of the $^{45}Ca^{2+}$ self-diffusion is shown for vesicles which had been washed three times in the presence of 500 μM EGTA. The addition of the Ca^{2+} ionophore, A23187, induces a rapid concentration-dependent release of Ca^{2+} from the vesicles. If the washes were carried out in the

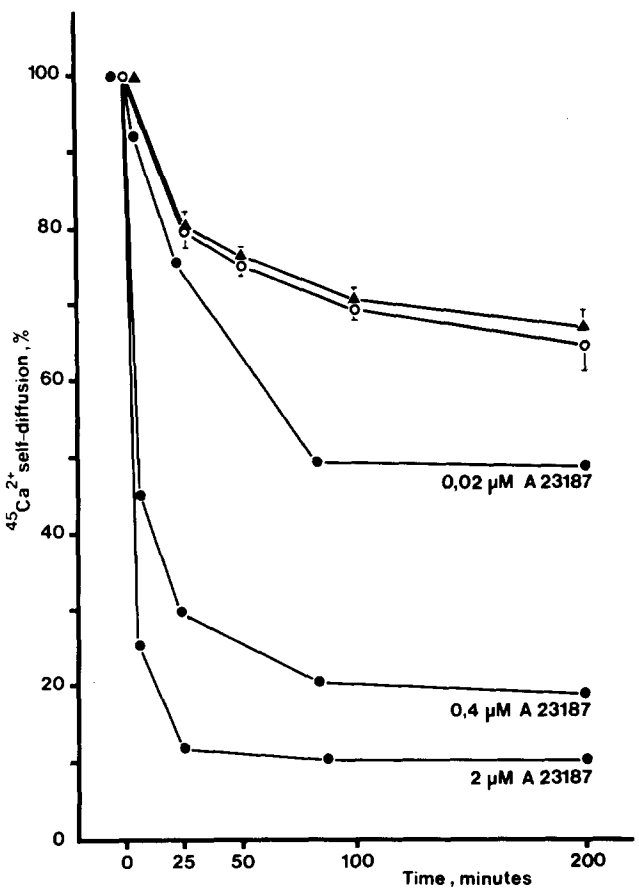

Fig. 3. $^{45}Ca^{2+}$ self-diffusion (outflux) from myelin membrane vesicles. ○———○, phosphorylated vesicles. ▲———▲, control vesicles. (Means ±S.E. of five separate experiments). ●———●, vesicles in the presence of different concentrations of the ionophore A23187.

absence of EGTA the ionophore had no effect on the Ca^{2+} release (data not shown). From these findings, we conclude that in EGTA-washed vesicles we were indeed measuring ionic fluxes through the vesicle membrane and that tightly bound Ca^{2+} accounted only for about 10% of the vesicle-associated Ca^{2+} (see release curve in the presence of 2 μM A23187). As this residual vesicle-bound Ca^{2+} pool remained constant over a large period of time (25–200 min) we assumed that it had no effect on Ca^{2+} diffusion.

A high phosphorylation state of the myelin basic protein (about 1 mol phosphate/mol myelin basic protein) had no significant effect on the Ca^{2+} outflux from the vesicles (Fig. 3). There was also no effect on the binding of Ca^{2+}, measured in the absence of EGTA (14 nmol Ca^{2+}/mg protein).

Permeability for Rb^+. As shown in Fig. 4, the ionophore gramicidin D which is specific for monovalent cations causes a rapid outflux from $^{86}Rb^+$-preloaded vesicles. From this ionophore-induced outflux it can be estimated that about 80% of the vesicle-associated Rb^+ is trapped in the vesicles.

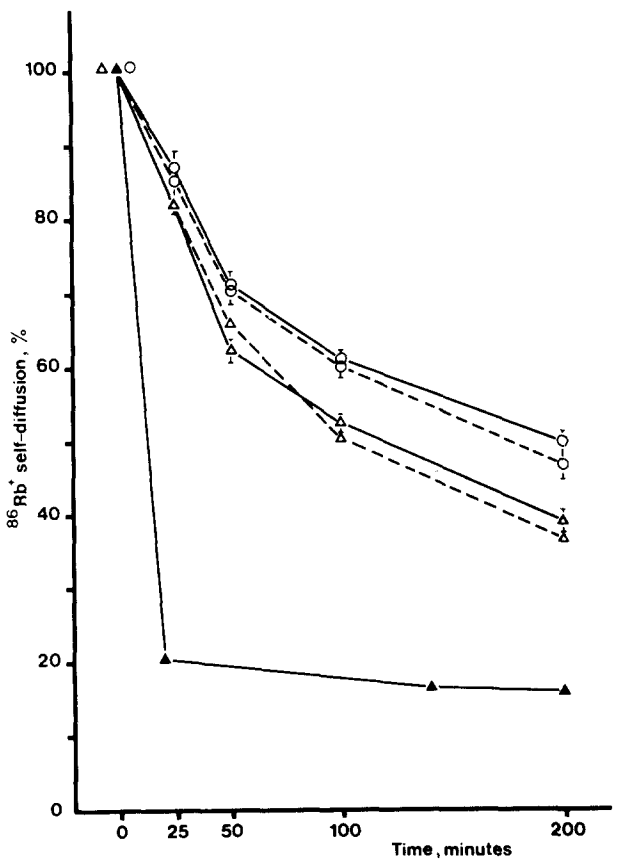

Fig. 4. $^{86}Rb^+$ self-diffusion (outflux) from myelin membrane vesicles. ○———○, phosphorylated vesicles. △———△, control vesicles. ○- - - - -○, heat-inactivated vesicles, treated with MgATP. △- - - - -△, heat-inactivated vesicles. ▲———▲, vesicles in the presence of 0.5 μM gramicidin D. (Means ±S.E. of three separate experiments). (Open circles, with MgATP treatment; open triangles, without MgATP treatment).

For permeability studies as a function of the phosphorylation state of the vesicle proteins, the following results were obtained (Fig. 4): a slight but significant decrease in $^{86}Rb^+$ outflux is caused by the incubation of the vesicles in the presence of 5 mM ATP and 8 mM Mg^{2+} as compared with vesicles without MgATP treatment. The Student's *t*-test gives $P < 0.02$ for the values at 50, 100 and 200 min. This permeability change could be observed whether the phosphorylation of the vesicle proteins (1 mol phosphate/mol myelin basic protein incorporated) had occurred or not (in heat-inactivated vesicles, less than 0.02 mol phosphate/mol myelin basic protein incorporated). Therefore, it must be concluded that the phosphorylation per se is not decreasing the Rb^+ release, but that the treatment of the vesicles with ATP and Mg^{2+} is sufficient to produce this effect.

The phosphorylation did not influence the loading of the vesicles with $^{86}Rb^+$ (about 0.35 nmol Rb^+/mg protein being vesicle associated after three washes, for a loading concentration of 50 μM Rb^+ and 3.6 mg protein/ml).

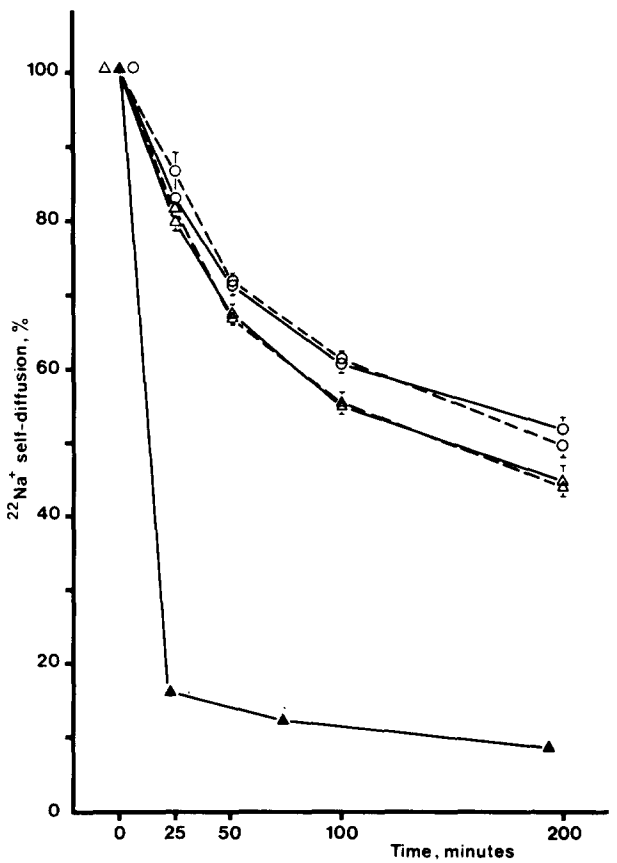

Fig. 5. $^{22}Na^+$ self-diffusion (outflux) from myelin membrane vesicles. ○———○, phosphorylated vesicles. △———△, control vesicles. ○- - - - -○, heat-inactivated vesicles, treated with MgATP. △- - - - -△, heat-inactivated vesicles. ▲———▲, vesicles in the presence of 0.5 μM gramicidin (means ±S.E. of two or five separate experiments).

Permeability for Na^+. For the $^{22}Na^+$ self-diffusion, essentially the same results as for $^{86}Rb^+$ were obtained (Fig. 5), although small quantitative differences could be observed. After incubation (loading) with 50 μM Na^+ at 7.2 mg protein/ml and after three washes, about 0.20 nmol Na^+/mg protein was associated with the vesicles. On one hand, this lower loading (as compared with Rb^+) is probably partly due to the different loading conditions. On the other hand, corresponding to this lower loading, a slightly slower release rate was measured for Na^+ (Fig. 5).

The lowering in the Na^+ self-diffusion, which is again caused by the incubation of the vesicles in the presence of ATP and Mg^{2+} (whether resulting in a phosphate incorporation or not) is small but significant. ($P < 0.01$, Student's t-test, for the values at 100 and 200 min).

Discussion

The passive role of myelin as an insulator of the axon has been questioned recently. The occurrence of enzymes such as carbonic anhydrase [22,23], protein kinase [1,2] and phosphoprotein phosphatase [5,19], which are active in vivo, supports the view that myelin could act in some way facilitating neuronal function. One possibility is that myelin could regulate the ionic environment of the axon, or act as a sink for ions. These speculations are based on electrophysiological and ultrastructural evidence suggesting the existence of ionic channels in the paranodal region of myelin [24], on the presence of potassium channels in the internodal axonal membrane [25] and on the presence of calcium binding sites [21] and possibly calcium channels in myelin [26]. By analogy to other systems, it has been proposed that the phosphorylation of the myelin basic protein may be involved in the regulation of the membrane permeability for cations [3,4,12]. Our results cannot support this hypothesis. We found no evidence that the phosphorylation of the myelin basic protein by the endogenous protein kinase does regulate the self-diffusion of $^{45}Ca^{2+}$, $^{22}Na^+$ or $^{86}Rb^+$. No difference in the outflux (release) of the three tested cations from myelin membrane vesicles could be measured when phosphorylated and non-phosphorylated (heat-inactivated) vesicle preparations were compared. Thus, the physiological significance of the phosphorylation-dephosphorylation system in myelin remains unknown at the present time.

In order to differentiate between the bound and free state of cations, two different types of ionophores were used. These substances mediate rapid transport of ions through membranes [27]. Ionophores can be divided in two groups by their mode of action [28]: ionophores of one group form lipid-soluble ion-ionophore complexes and are designed as cage carriers. Ionophores of the other group form channels through the membrane, creating artificial pores. In the present study, we used the Ca^{2+}-specific ionophore A23187 which is a cage carrier and gramicidin D, a channel-type ionophore, specific for monovalent cations. Both ionophores, although very different in chemical nature and mode of action, induced rapid release of ions (A23187 of $^{45}Ca^{2+}$, gramicidin D of $^{86}Rb^+$ and $^{22}Na^+$) from preloaded myelin membrane vesicles. Therefore, it can be assumed that after loading, these vesicles contain a freely diffusable pool of trapped ions. These findings, together with the

fact that they behave as osmometers (as shown by the light-scattering technique), strongly suggest that the myelin membrane vesicles are sealed. The amount of ions which remains associated with the vesicles after ionophore-induced outflux can be assigned to another, distinct pool of non-diffusable ions. It is reasonable to assume that most of the ions of this non-diffusable pool are bound to the myelin membrane. About 10% of total Ca^{2+}, 15% of total Na^+ and 20% of total Rb^+ can be assigned to this pool and therefore are thought to be membrane bound. As this bound pool decreases only very slowly during diffusion measurement, it can be assumed that binding does not affect the diffusion measurement.

The incubation of the myelin membrane vesicles in the presence of ATP and Mg^{2+} results in a small but significant decrease in the $^{86}Rb^+$ and $^{22}Na^+$ self-diffusion. These permeability changes were measured whether or not an incorporation of phosphate in myelin basic protein had occurred. As the myelin membrane vesicles were washed extensively after the phosphorylation step (three times before and three times after loading), only traces of ATP and Mg^{2+} remained associated with the vesicles during diffusion measurements. There was no significant change in the trapping rate, or the gramicidin-induced outflux in MgATP-treated vesicles as compared with non-treated vesicles (data not shown). Therefore, we conclude that the differences in outflux are not due to a direct interaction (binding) of ATP and Mg^{2+} with $^{86}Rb^+$ and $^{22}Na^+$. One could imagine several explanations for an effect of MgATP on changing the permeability properties of the membrane, for instance, through a direct structural effect on the membrane, or by an action on an energy-dependent enzyme system other than the protein kinase system studied. One could also consider whether the treatment with MgATP modifies the lamellar structure (mostly unilamellar vesicles as shown by Steck et al. [12]), or the aggregation state of the vesicles, inducing a change of the ionic outfluxes. If this were the case, one would, however, expect that MgATP would cause a change in permeability for all tested ions and not only for Na^+ and Rb^+. Sheetz and Singer [29] demonstrated a marked structural change in erythrocyte ghosts induced by MgATP treatment, resulting in a shape change of the vesicles. These authors found a correlation between the spectrin phosphorylation state and the shape of the erythroycte ghosts and concluded that the shape of erythrocyte ghosts could be controlled by endogenous protein kinase and phosphatase activities [30]. In contrast, Anderson and Tyler [31] have recently shown that phosphorylation of spectrin does not control red cell shape, i.e., they found no causal relationship between spectrin phosphate levels and either red cell shape or spectrin binding to the membrane. It is possible that MgATP induces a structural effect on the myelin membrane (resulting in a permeability change for Na^+ and Rb^+) by a related mechanism as it does induce a shape change in erythrocyte ghosts. At the present time, it must be emphasized that there is no direct evidence for this hypothesis and further investigations are needed to clarify this question.

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References

1 Steck, A.J. and Appel, S.H. (1974) J. Biol. Chem. 249, 5416—5420
2 Miyamoto, E. (1976) J. Neurochem. 26, 573—577
3 Petrali, E.H., Thiessen, B.J. and Sulakhe, P.V. (1980) Int. J. Biochem. 11, 21—36
4 Sulakhe, P.V., Petrali, E.H., Thiessen, B.J. and Davis, E.R. (1980) Biochem. J. 186, 469—473
5 Miyamoto, E. and Kakiuchi, S. (1975) Biochim. Biophys. Acta 384, 458—465
6 Matthieu, J.M. and Kuffer, A.D. (1978) in Myelination and Demyelination (Palo, J., ed.), pp. 159—170, Plenum Publishing Corp., New York
7 De Lorenzo, R.J., Walton, K.G., Curran, P.F. and Greengard, P. (1973) Proc. Natl. Acad. Sci. U.S.A. 70, 880—884
8 Weller, M. and Morgan, I.G. (1977) Biochim. Biophys. Acta 465, 527—534
9 Weller, M. (1978) Mol. Cell. Biochem. 20, 95—102
10 Rudolph, S.A. and Greengard, P. (1974) J. Biol. Chem. 249, 5684—5687
11 Weller, M., Virmaux, N. and Mandel, P. (1975) Nature 256, 68—70
12 Steck, A.J., Siegrist, P., Zahler, P., Herschkowitz, N.N. and Schaefer, R. (1978) Biochim. Biophys. Acta 509, 397—409
13 Norton, W.T. (1971) Adv. Exp. Med. Biol. 13, 327—337
14 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
15 Laemmli, U.K. (1970) Nature 227, 680—685
16 Portzehl, H., Caldwell, P.C. and Rüegg, J.C. (1964) Biochim. Biophys. Acta 79, 581—591
17 Schwartz, A., Lindenmayer, G.E. and Allen, J.C. (1975) Pharmacol. Rev. 27, 3—134
18 Hilden, S. and Hokin, L.E. (1975) J. Biol. Chem. 250, 6296—6303
19 McNamara, J.O. and Appel, S.H. (1977) J. Neurochem. 29, 27—35
20 Koch, A.L. (1961) Biochim. Biophys. Acta 51, 429—441
21 Hemminki, K. (1974) Biochim. Biophys. Acta 363, 202—210
22 Yandrasitz, J.R., Ernst, S.A. and Salganicoff, L. (1976) J. Neurochem. 27, 707—715
23 Cammer, W., Bieler, L., Fredman, T. and Norton, W.T. (1977) Brain Res. 138, 17—28
24 Livingstone, R.B., Pfenninger, K., Moor, H. and Akert, K. (1973) Brain Res. 58, 1—24
25 Chiu, S.Y. and Ritchie, J.M. (1980) Nature 284, 170—171
26 Schlaepfer, W.W. (1977) Nature 265, 734—736
27 Pressman, B.C. (1976) Annu. Rev. Biochem. 45, 501—530
28 Ovchinnikov, Y.A. (1979) Eur. J. Biochem. 94, 321—336
29 Sheetz, M.P. and Singer, S.J. (1977) J. Cell Biol. 73, 638—646
30 Birchmeier, W. and Singer, S.J. (1977) J. Cell Biol. 73, 647—659
31 Anderson, J.M. and Tyler, J.M. (1980) J. Biol. Chem. 255, 1259—1265