EFFECT OF TWO BRAIN SPECIFIC PROTEINS (S100 and 14.3.2) ON CATION DIFFUSION ACROSS ARTIFICIAL LIPID MEMBRANES

P. CALISSANO* and A.D.BANGHAM

A.R.C. Institute of Animal Physiology

Babraham, Cambridge, England

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SUMMARY

The two brain specific proteins S100 and 14.3.2 interact with lipid membranes inducing an increase in their permeability to cations in physiological conditions of pH and ionic strength. Such effect is regulated by Ca^{++} and varies according to the membrane structure. Glucose permeability, in the same experimental conditions, is not affected by the two proteins.

S100 and 14.3.2 are two proteins unique to the nervous system. Although their specificity, amount, regional and cellular distribution in the nervous system has been throughly investigated (1,2) nothing is yet known about their function. Some indication on the possible function of the S100 has been recently suggested by the conformational change which follows its binding with Ca^{++} (3).

We report a possible interaction of these two proteins with lipid membranes by incubating them with liposomes sequestring monovalent salts, and following the diffusion out of cations as compared to control experiments, without protein. Liposomes are small spherules generally formed by two or more concentric lipid layers surrounding an aqueous phase,and

*On leave of absence from: Laboratorio di Biologia Cellulare del CNR, Roma
Present address: Laboratorio di Biologia Cellulare
Via Romagnosi 18A
00196 Roma, Italy

have been used in the recent years as a model of biological membranes to investigate the effect of several antibiotics, steroids, alcohols, and anaesthetics on ion permeability (4).

MATERIALS AND METHODS

Liposomes were prepared by adding a 1 ml solution of Tris-Cl buffer 5 mM containing KCl 60 mM, Rb 1 mM plus 20 μC of Rb^{86} to a mixture of PC 25%-PS** 75% previously evaporated to dryness (5).

The liposomes were sonicated to clearness under N_2 and allowed to stand overnight. The isotope outside the liposomes was removed by passage of the dispersion over a G50 Sephadex column equilibrated with an isotope-free salt solution. Proteins, dissolved in the same buffer, at the concentration indicated in the figures, were added in 0.5 ml portions to 0.5 ml of liposomes previously put into 8/32 Visinkg dialysis bags. The mixture in the dialysis bags was then allowed to incubate in tubes containing 10 ml of the same buffer-salt. The incubation was carried out at 37°C in a shaking bath with 120 cycles/min. At times intervals the bags were transferred to another set of 10 ml containing tubes, and the incubation continued. Radioactive counting was performed in a Packard scintillation liquid counter.

The S100 protein was purified according to Moore (6); it gave a single band in acrylamide gel electrophoresis in presence of EDTA (3) and it showed one single symmetrical peak when chromatographed on a Sephadex G100 column or on a DEAE Sephadex column. The 14.3.2 protein was a generous gift of Dr. B.W. Moore.

RESULTS AND DISCUSSION

As it can be seen in Fig. 1, the S100 and 14.3.2 incubated with liposomes at physiological conditions of pH and ionic strength induce a big increase in the leak of Rb^{86}. The effect of S100 is minimum in absence of Ca^{++} and 7-8 times higher than in control experiments in the presence

**The following abbreviations have been used:
PC=phosphatidylcholine; PS=phosphatidylserine; SA=stearylamine; C16-S= C 16 sulphate; SM=sphingomyelin; PA=phosphatidic acid.

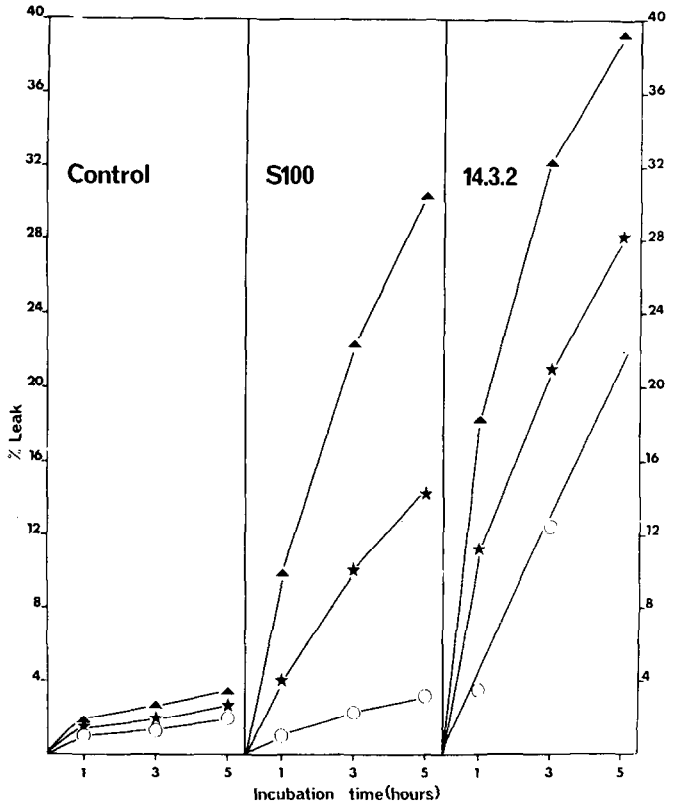

Fig. 1 - Leak from liposomes incubated at 37°C with o——o EDTA 0.1 mM, ●——● Mg^{++} 2mM or ▲——▲ Ca^{++} 2 mM. From left to right, Control, S100 0.4 mg/ml and 14.3.2 0.25 mg/ml. Each dyalisis bag contained 4.0 μM of phospholipid. Leak is expressed as % of the total counts captured by the liposomes.

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of this ion. Mg^{++} in the same experimental conditions has a similar, but smaller activating effect. An analogous, although quantitatively higher effect is induced by the 14.3.2 protein which increases the leak by a factor of 10-12 when incubated with Ca^{++} and 4-5 times in its absence. Again Mg^{++} had a minor activating effect.

We found that the increased diffusion depends both on the protein and on the Ca^{++} concentration. Experiments performed with increasing concentration of S100 from 50 μg to 1.0 mg/ml showed an increase in the effect reaching a plateau at 0.5-1.0 mg/ml; half maximum response was reached at a concentration of 50 μg/ml. Such a concentration, if expressed in terms of mole S100/mole of phospholipid represents a ratio of $1:2 \cdot 10^3$ or

approximately 2 protein molecules per liposome. The 14.3.2 protein was even more effective. Increasing concentrations of Ca^{++} from 0.05 mM to 2.0 mM in presence of constant amount of S100 (0.25mg/ml) and of phospholipid, showed an increased activation of the protein effect with a plateau at 0.5-1.0 mM Ca^{++}.

In order to see whether the action of these two proteins was similar for different kinds of phospholipids or whether they showed a certain degree of preference, the effect of S100 and 14.3.2 has been followed on liposomes prepared with constant amounts of PC (25%) but substituting the PS previously used with other negatively or positively charged lipids. Preliminary results indicate that both proteins, although to a different extent, have their optimum effect on liposomes containing PS at the concentrations used in the experiments shown in Figs. 1 and 2. The

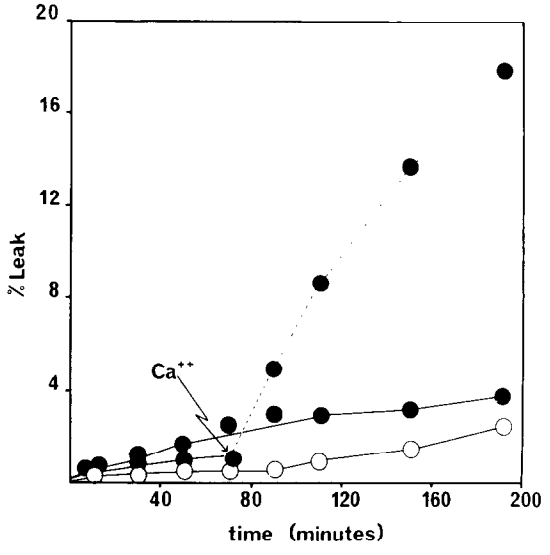

Fig. 2 - Effect of Ca^{++} added to a mixture of liposomes plus S100. Liposomes made of PC25%-PS 75% were incubated at 37°C in duplicate sets with o———o EDTA 0.1 mM or •———• EDTA 0.1 mM plus S100 0.5 mg/ml. After 70' of incubation, to two bags of control and 2 bags containing the protein Ca^{++} was added to reach a final concentration of 2 mM; they were then transferred to tubes containing 2 mM of Ca^{++} instead of 0.1 mM EDTA. The remaining set of control and experimental bags were further incubated in 0.1 mM EDTA until the end of the experiment. The arrow indicate the incubation time when Ca^{++} was added and the dotted line refers to the % leak from bags containing the S100 subtracted from the leak of the analogous control experiment.

apparent order of effectiveness for the S100 being PS > SA > C16S > SM > PA > PC and for the 14.3.2 being PS > C16S > SA > PC > PA > SM.

The experiments described above have been performed by adding the protein to the liposomes already contaning EDTA or Ca^{++}. It seemed of interest to see whether Ca^{++}, added to liposomes preincubated with the protein, exhibited the same "activating" ability. Fig. 2 shows that this is indeed the case and seems to suggest that (a) the S100 and Ca^{++} bind in a reversible way to the lipid membrane and (b) that they act in a synergistic rather than independent manner.

The substantial change in the permeability properties of the liposomes described above must be caused by some change in the negatively charged lipid barrier which is normally almost impermeable to cations. In order to investigate whether the increased leak to Rb^{+} was a non-specific effect involving any kind of solute, liposomes made of three different mixtures of phospholipids (PC alone, PC 90%-PS 10% and PC 25%-PS 75%) were prepared as before but also containing labelled glucose, 5 mM. Leak from the liposomes of Rb^{86} and glucose C^{14} was followed in presence of both the S100 and 14.3.2 with and without Ca^{++} 2 mM. In these conditions we found that the Rb diffusion was affected to a different extent by the two proteins being slightly higher than the controls with liposomes made with PC alone and 7-12 times higher with liposomes containing 25% PC and 75% of PS. However, the glucose exchange was unaffected being identical to the controls in all three preparations. Similar results were obtained with positively charged liposomes made of PC 25%-SA 75%; the two proteins showed a stimulation of the Rb leak (although lower than that shown with PS) but with no effect on glucose diffusion. Several other proteins with various isoelectric points between 1.0 and 9.0 were also tested for their possible effect on ion leakage in the same experimental conditions described above. Among these, RNAase, Pronase and the synthetic poli-glutamic acid (m.w. 140.000) had no effect; stimulation of 2-4 times was found with Pepsin and BSA which could, to some extent, be activated by Ca^{++}. Finally, of two haemoproteins investigated, one (cytochrome C) had a very small effect while the other (beef haemoglobin) showed a 20-25 times increased leak but was not regulated by Ca^{++}. Further experiments

are necessary in order to test whether the effect induced by such proteins shows the ions selectivity and Ca^{++} regulation exhibited by the two brain proteins.

The data presented show that the S100 and the 14.3.2 interact with lipid membranes inducing a change in their permeability. The finding that simple univalents cations but not glucose permeability is affected, seems to indicate that it is not due to a simple breakdown of the lipid barrier. The mechanism by which S100 induces a big increase of the cation diffusion in the presence of Ca^{++} may lie in the conformational change that the ion induces (3). The exposure to the solvent of several aminoacids, some of them with hydrophobic side chains, which follows tha binding of Ca^{++} to the protein, may act as a trigger for a diffusion process by facilitating the binding of the protein and of its penetration into the liposome and/or, by changing the affinity and the number of binding sites for cations.

Although further experiments are necessary to elucidate the mechanism of action of such proteins, these artificial lipid membranes seem to provide a useful model of interaction between lipids and proteins, as well as to give some suggestion on the role the S100 and 14.3.2 might play in the nervous tissue.

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