Section: Neurobiology and Cybernetics

Passive permeability of bovine rod outer segment disc membranes to various substances.

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Under hypotonic conditions, the plasma membrane of a rod outer segment lyses and the osmotic intact discs swell to spherical vesicles. Due to this, swollen discs can be floated to an osmotically inert Ficoll/water interface (Smith et al., Exp. Eye Res. 20 (1975) 211). Upon variation of the Ficoll concentration, approximately equal amounts of floating discs were obtained for 2.5% to 5% Ficoll, whereas below 2.5% Ficoll the amount of floating discs decreased, reaching zero at ca. 1.5% Ficoll.

Due to disc shrinking, the amount of floating discs decreases upon increasing the osmolarity with an osmotically active substance. Osmotically inactive substances, on the other hand, have no effect on the flotation behavior of the discs. Thus, one can adopt this centrifugation technique to probe the passive permeability properties of osmotically intact discs.

The critical osmolarity, where only half of the maximally floating discs are floated, was found to be 8-10 mOsmol/l for osmotically fully active substances like e.g. Hepes. This value is shifted to greater osmolarities for substances which are not fully osmotically active. Tris and imidazole are examples for buffers which were found to be not fully osmotically active. This observation is probally due to the fact that these two buffers are electrically neutral in the deprotonated form and, therefore, can permeate the membrane as neutral molecules. An example for an osmotically inactive substance is ethanol which was found to be osmotically inactive up to 150 mOsmol/l, i.e. permeated the disc membrane readily (Fig. 1).

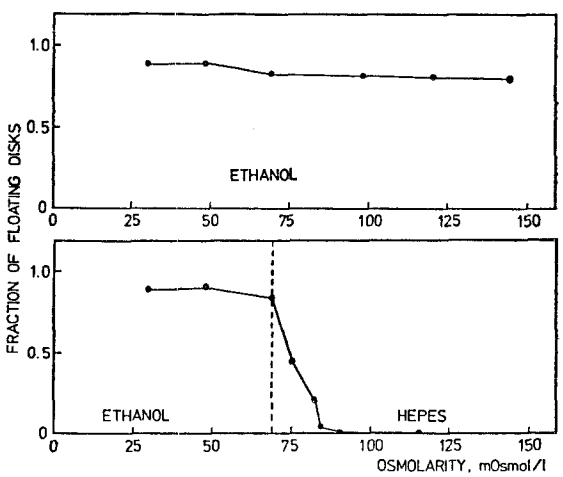

Fig. 1:
Dependence of the fraction of floating discs from the osmolarity of the suspending medium:
ethanol is osmotically inactive, Hepes is osmotically active.

No differences in the passive permeability properties were, thus far, detected between bleached and unbleached discs.

In summary, the disc membrane has a definite permeabilty to small electrically neutral organic molecules, whereas ions and zwitterions cannot permeate the disc membrane and, therefore, are osmotically fully active.

Supported by Deutsche Forschungsgemeinschaft (SFB 160)

Time course of dark adaptation in the Limulus ventral nerve photoreceptor - measured as constant response amplitude curve -, and its dependence upon extracellular calcium

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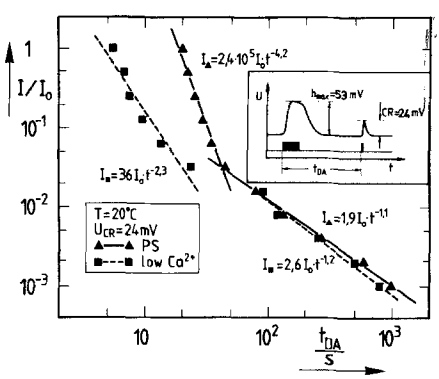
The time course of dark adaptation of Limulus ventral nerve photoreceptor following light adaptation by a bright 1 or 5 s illumination ($4{,}4 \cdot 10^{16}$ photons $\cdot$ cm^{-2} $\cdot$ s^{-1} at 543 nm), was measured by determining the intensity I_c of a 300 µs light flash, necessary to evoke a criterion response amplitude of the receptor potential.

The time course of dark adaptation shows two phases with time constants in the range of 5 - 9 s and 300 - 500 s, respectively (15° C). The first phase is much more influenced by changed extracellular Ca^{2+}-concentration.



In the figure, I_c is plotted versus the dark adaptation time (t_{DA}) in a double logarithmic plot. The time course of the two phases of dark adaptation can be described by the function

$$I_c = a \cdot I_o \cdot t_{DA}^{-b},$$

fitted separately for the two phases.

First phase: The exponent b characterizing the slope is decreased from 3,5 ($s_{\bar{x}} = 0{,}4$) to 1,7 ($s_{\bar{x}} = 0{,}5$) when the external Ca^{2+}-concentration is lowered from 10 mmol/l to 250 µmol/l; b is increased to 4,7 ($s_{\bar{x}} = 0{,}5$) when the extracellular Ca^{2+}-concentration is raised to 40 mmol/l.

The factor a characterizing the sensitivity for $t_{DA} = 1$ s varies greatly from experiment to experiment; a is decreased-corresponding to a greater sensitivity by lowering and strongly increased by raising external Ca^{2+}-concentration.

Second phase: The exponent b is about 1,0 ($s_{\bar{x}} = 0{,}1$'; b and a are not much changed when the extracellular Ca^{2+}-concentration is varied.

The two phases of dark adaptation reflect the behaviour of the two components C_1 and C_2 of the receptor potential (1), which differ in sensitivity to light adaptation and in time dependence of dark adaptation. Under the conditions described here C_1 determines the height of the receptor potential in the first phase of dark adaptation whereas C_2 is responsible for the response amplitude in the second phase.

Interpretation: There are two processes governing the dark adaptation; one determining the first, fast phase, is strongly Ca^{2+}-dependent - probably controlled by the level of the intracellular Ca^{2+}-concentration-, the second one represents another slower recovery process which is much less Ca^{2+}-dependent.

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J. Comp. Physiol. 141, 303-310

Supported by Deutsche Forschungsgemeinschaft (SFB 160)

Interaction of specific neurotoxins with voltage-sensitive Na-channels in membrane vesicles

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The excitable face of the electroplaques in the electric organ of Electrophorus electricus propagates an action potential. The voltage-sensitive Na-channels in this membrane resemble pharmacologically those in nerve and striated muscle. To study their molecular and functional properties, Na-channel rich membrane fragments are prepared from the homogenized electric organ.

Standard preparation procedures including density gradient centrifugation yield fractions with specific tetrodotoxin binding up to 5 pMol/mg protein. After carrier-free column electrophoresis membranes with specific binding up to 30 pMol /mg are obtained, which corresponds to a tetrodotoxin-receptor density of ca $80/\mu m^2$. A major part of the membrane fragments forms vesicles (electron microscopy), and at least 50% of them are inside-out (affinity chromatography on bound lectins / asymmetric binding of neurotoxins).

For binding studies, tetrodotoxin is chemically tritiated to high specific activity (with M.Rack, R.Stämpfli). It binds with a thermodynamic equilibrium constant $K_D=(20\pm10)nM$ to its receptor on these membrane fragments. The experimentally determined kinetic constants for a bimolecular binding equilibrium are $k_f \geq 3\times10^5\ M^{-1}s^{-1}$ and $k_b=2.5\times10^{-2}s^{-1}$.

To analyze the transport function of the channels, the efflux of ^{22}Na from tetrodotoxin-receptor-rich membrane vesicles is monitored by filtration techniques. A modulation of the efflux by specific neurotoxins was observed only after having transiently established asymmetric Na- and K-ion concentrations inside and outside the vesicles. To improve the time resolution of these filtration assays, a technique of con-

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2) Grünhagen,H.H., Dahl,G., Reiter,P., (1981), Biochim. Biophys. Acta 642, 267
3) Grünhagen,H.H., (1980), Anal. Biochem. 109, 18