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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## 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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trolled elution was developped for the second time range. From data in this time domaine the blocking effect of tetrodotoxin was found to occur at $K_{app} \leq 5 \times 10^{-8}$ M. Simple theoretical models are used to estimate the influence of molecular transport parameters on experimental data from efflux assays.

## Spectroscopical studies of the ASTACUS visual pigment

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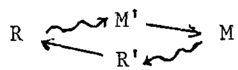
The rhabdoms of a single dark adapted (20 h) crayfish retina were isolated by density gradient centrifugation. The quantity of isolated and purified rhabdoms, which was entirely free from screening pigments, was sufficient to allow measurements of the extinction spectra of the membrane-bound chromoprotein obtained from a single retina.

Extinction spectra of the membrane-bound chromoprotein show that at 0° C and pH 7.0 the system is stable and photoreversible for several days. The maximum of the extinction spectrum of ASTACUS rhodopsin (0° C, pH 7) is $\lambda_{max}$ = 530 nm. Rhodopsin (R) is isomerized nearly quantitatively to metarhodopsin (M) ($\lambda_{max}$ = 500 nm) by irradiation at $\lambda$ = 630-640 nm.

Kinetic studies of the conversion from R to M and vice versa were performed by flash photolysis. The resolution of flash-induced transmission changes of the rhabdom suspension allowed single shot experiments (pulse length of the exciting light 6 ns). From our measurements we postulated the following temporary reaction scheme:




Both reaction pathways, $R \rightarrow M$ and $M \rightarrow R$, consist of a photoreaction and at least one dark reaction; additional dark reactions exist presumably in the time range < 10 µs. The kinetic data for both dark reactions are investigated in the temperature range from 0° to 20° C. Increasing the temperature from 0° to 20° C the half times for the dark reactions decrease from 3.9 ms to 0.24 ms in the case of $M' \rightarrow M$ and from 16 ms to 1 ms in the case of $R' \rightarrow R$.

The activation energies were calculated from the slope of the Arrhenius plot and seem to be the same for both reactions, $\Delta E_{M' \rightarrow M} = \Delta E_{R' \rightarrow R}$ = 22.5 kcal $mol^{-1}$.

Flash-spectroscopic measurements in the wavelength range from 430 to 650 nm show that the difference spectra of M-R' and M-R have the same maximum at 495 nm and the same minimum at 575 nm. This suggests that the absorption maxima of R and R' are similar, but differ in the molar extinction coefficient.

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