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Investigation of the Relation between Structure and Function in Myelinated Nerve Fibres with the Aid of Ultraviolet Radiation*

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Abstract. Ultraviolet radiation induces two photochemical alterations relevant to excitability in the nodal membranes: A selective blocking of the sodium permeability and a potential translation of the voltage dependent kinetic parameters of sodium inactivation *and* activation along the potential axis in the negative direction. The underlying processes are two different photoreactions, since 1) the action spectrum of the blocking effect shows a marked peak near 280 nm and rapidly decreasing sensitivity towards higher and lower wavelengths, while the action spectrum of the potential shift increases with lower wavelengths; 2) the blocking effect is enhanced by a more positive holding potential, while the potential shift is decreased; 3) the potential shift can be prevented intraaxonal application of l-cysteine or 2-mercaptoethanol, but the blocking effect is not affected.

Key words: Membrane structure and function — Excitability — Myelinated nerve fibre — Sodium channel — Ultraviolet radiation.

Ultraviolet radiation has been shown to selectively block the sodium channels in nerve membranes [1, 6]. The blocking effect appears as a one hit event eliminating irreversibly sodium channels as a whole, since neither the ionic selectivity nor the kinetics of the sodium permeability are changed by the blocking reaction, which follows an exponential dose effect curve of a first order process [3]. The blocking photoreaction does not depend on temperature [1]. It probably takes place in the outer leaflet of the nodal membrane, since Ca^{++} and H^+ binding to unspecific fixed charges of the outer membrane surface sensitize ultraviolet blocking. This sensitizing effect is not due to changes of the intrinsic electric field caused by Ca^{++} or H^+ [2].

A second effect of ultraviolet radiation relevant to excitability was observed by Schwarz [7]. He demonstrated that the potential dependent kinetic parameters

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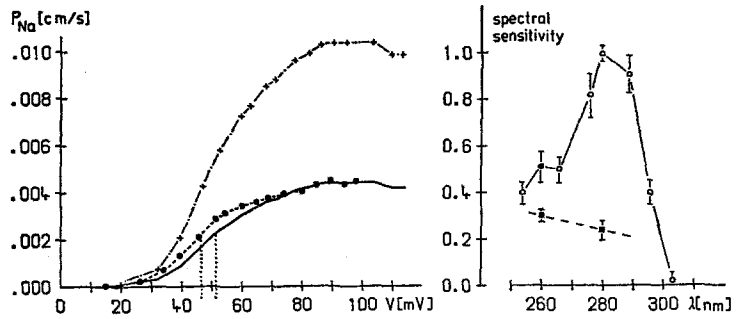

Fig. 1. Twofold action of ultraviolet radiation on the sodium channels of myelinated nerve fibres. Left: Reduction and leftshift of the sodium permeability vs. potential curve. + before, ● after an ultraviolet dose of 600 mWs/cm². The solid curve represents the values before irradiation times 0.42. Potential shift $\Delta V_m = -5.0$ mV. $\lambda = 280$ nm, T = 14° C, motor fibre. Data corrected for series resistance. — Right: Action spectra of the blocking (○) and of the potential shifting (□) photoreactions. Open symbols: 31 experiments on 17 different nerve fibres, closed symbols: 5 experiments by Schwarz. Motor fibres. $T = 13-16°$ C

of the sodium inactivation are translated along the potential axis in the negative direction simultaneously with the blocking photoreaction. The objective of the present study, therefore, was to investigate whether the radiation-induced potential shift is an effect restricted to the sodium inactivation mechanism only and whether the two photoreactions are independent of each other.

The methods used were identical to those already described [1]. In short, isolated nodes of Ranvier of the sciatic nerve from *Rana esculenta* were exposed to monochromatic ultraviolet radiation (bandwidth ± 3 nm). The ionic membrane currents were measured under voltage clamp conditions on-line using a Honeywell DDP-516 computer. Potentials are given as deviations from the resting potential.

The twofold effect of ultraviolet radiation is illustrated in Fig. 1 (left). Due to the applied ultraviolet dose of 600 mWs/cm² the sodium permeability — as defined by Frankenhaeuser [4] — is reduced to about one half (blocking photoreaction). Simultaneously, the sodium permeability vs. potential curve is shifted by − 5 mV (potential shifting photoreaction).

The potential translation of the kinetic parameters of the sodium inactivation (as determined by the decrease of the steady state inactivation parameter h_∞ (V = 0) measured at resting potential) is of the same amount as for the sodium activation: The ratio of the potential shifts, ΔV_m and ΔV_h, of the activation and inactivation parameters was determined in 4 experiments applying different doses of ultraviolet radiation. The mean ± S.D. was found to be:

$$\overline{\Delta V_m/\Delta V_h} = 1.09 \pm 0.09.$$

The two photoreactions are independent of each other as evidenced by the finding that the action spectra of the two effects are different (Fig. 1, right). While the blocking of the sodium permeability most effectively occurs at 280 nm and is reduced at higher and lower wavelengths, the potential shift is enhanced at lower wavelengths.

Supporting evidence for the view of two separate photoreactions is derived from results previously reported: 1. The ultraviolet sensitivity depends on the membrane holding potential. But, while the blocking effect is decreased by a more negative holding potential [2], the radiation-induced potential shift is increased in this case [7]. 2. Intra-axonal application of l-cysteine or of 2-mercaptoethanol prevents the ultraviolet-induced potential shift, but the blocking effect is not affected by the action of these SH-group containing compounds [5].

This latter result provides some information on a probable localization of the potential shifting photoreaction at the inner leaflet of the nerve membrane, while the blocking photoreaction is likely to occur at the outer leaflet (see above). The finding that the potential shifts both of the activation- and of the inactivation parameters are the same supports the hypothesis that the potential shift might be caused by a photochemical alteration of the inner surface charges of the nodal membrane.

In conclusion, the highly selective action of ultraviolet radiation on the sodium system of nerve membranes [1, 6] and the separability of different actions at probably separate localizations reveal this physical agent as a promising tool for future investigations of structure-function relationships in the mechanism of excitation.

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