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Ultraviolet-Induced Alterations of the Sodium Inactivation in Myelinated Nerve Fibers*

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Summary. Ultraviolet radiation irreversibly reduces the sodium permeability in nerve membranes and, in addition, induces a change of the potential dependence of the kinetic parameters of sodium inactivation in the node of Ranvier. This second ultraviolet effect shifts the kinetic parameters of sodium inactivation $h_\infty(V)$, $\alpha_h(V)$, and $\beta_h(V)$ to more negative potentials (no changes of the slopes of the curves). The amount of the displacement ΔV along the potential axis is equal for the three parameters and depends on the ultraviolet dose. It is about $\Delta V = -10$ mV after an irradiation dose of 0.7 Ws/cm^2 at 280 nm. Both ultraviolet-induced effects depend on membrane potential and on the wavelength of the applied radiation. But while the potential shift is enhanced at more negative holding potentials, the ultraviolet blocking is diminished and *vice versa*. Further, the ultraviolet-induced potential shift is greater at 260 nm than at 280 nm, whereas a maximum sensitivity of ultraviolet blocking is found at 280 nm. Therefore, the two radiation effects are the result of two separate photoreactions. For explanation of the radiation-induced potential shift it is assumed that ultraviolet radiation decreases the density of negative charges at the inner surface of the nodal membrane. From this hypothesis a value for the inner surface potential ψ_i was derived. $-19\text{ mV} \leqq \psi_i \leqq -14\text{ mV}$.

Selective and irreversible reduction of the sodium current through the membrane of the node of Ranvier due to ultraviolet radiation (280 nm) has been reported previously (Fox, 1974*a*). The sodium current decreases exponentially with irradiation dose suggesting a first-order photochemical reaction (Fox & Stämpfli, 1971; Fox, 1974*a*). This decrease of current results from a radiation-induced reduction of the maximum sodium permeability (Fox, 1976*b*). Similar results were obtained from voltage-clamp experiments on lobster axons (Oxford & Pooler, 1975).

An additional effect was detected recently: a displacement of the sodium activation parameter $m_\infty(V)$ in the negative direction of the

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potential axis (Fox, 1976*b*). A similar shift was seen for the steady-state sodium inactivation parameter $h_\infty(V)$.

The objective of the present investigation was, therefore, to study the properties and the mechanism of this second photoreaction and to clarify whether the two ultraviolet radiation effects are independent of each other. A preliminary report has been published elsewhere (Schwarz, 1975*a*).

Materials and Methods

Measurement of Currents

The sodium currents of single nodes of Ranvier were studied under voltage-clamp conditions using the method described by Nonner (1969). Single motor and sensory fibers of the sciatic nerve of *Rana esculenta* were dissected according to the technique of Stämpfli (1969). In this paper, no distinction is made between motor and sensory fibers since no marked differences in their reaction to ultraviolet radiation were observed.

The Hodgkin-Huxley-Frankenhaeuser formalism describing the nodal sodium current is used throughout the present paper (*see*, e.g., Frankenhaeuser, 1960). Potentials are given as inside minus outside potential relative to resting membrane potential, thus $V=E-E_r$. Ionic currents were calibrated (*see*, e.g., Fox 1974*a*) as current densities for reasons of comparison with earlier work and measured on-line with a Honeywell DDP-516 computer as extensively described by Fox (1974*a*; 1976*a*): under real time conditions voltage pulses were set and the membrane currents were recorded and digitalized at intervals of 20 μsec. The maximum sodium inward current was evaluated on-line and stored on digital magnetic tape. The steady-state sodium inactivation curve $h_\infty(V)$ was measured as usual employing a sequence of voltage pulses consisting of a prepulse of variable amplitude (-50 to $+50$ mV) and fixed duration (50 msec) and of a fixed test pulse (60 mV; 2 msec). The time constant of sodium inactivation $\tau_h(V)$ was measured with the double pulse method (Frankenhaeuser, 1960) using a standard sequence of a prepulse of fixed amplitude (range -50 to $+50$ mV) and variable duration (0.5 to 50 msec) and of a fixed test pulse (60 mV; 2 msec). The time constant τ_h for each pulse amplitude was evaluated from the relative changes of I_{Na} with prepulse duration by a least-squares fit of an exponential to the experimental data. The rate constants α_h and β_h were calculated according to their definitions

$$h_\infty=\alpha_h/(\alpha_h+\beta_h); \qquad \tau_h=1/(\alpha_h+\beta_h).$$

The parameter α_h was preferred to τ_h for the detailed investigations for practical reasons and, particularly, because higher precision was obtainable (*see below*).

The holding potential V_H could be set to any desired value without changing the absolute voltage of the conditioning and test pulses. The standard condition was $V_H=0$ [approx. resting potential, i.e. $h_\infty(V=0)\approx 0.7$]. The base line of the current at holding potential was continuously recorded with an ink writer; after an initial period of stabilization of about 20 min, the drift of the base line usually did not exceed 1 mV during the entire experiment (1 to 2 hr), corresponding to less than a 3 mV change of holding potential (Fox, 1976*a*).

Irradiation

Monochromatic ultraviolet radiation of 280 and 260 nm wavelength was generated with a high pressure mercury lamp with a stabilized arc (Type St 75 (90 W); Quarzlampengesellschaft, Hanau), using interference filters of 9 nm bandwith (Type SSUV; Spec-

Table 1. Ionic composition (mM/l) of solutions

	Na^+	K^+	Ca^{++}	Cl^-	Buffer[c]	pH[d]
Ringer's[a]	110	2.5	1.8	119.3	5(T)	7.3
Internal solution[b]	13	105	–	118	5(P)	6.9

[a] +5 mM tetraethylammonium chloride for elimination of K^+-currents.
[b] In contact with the cut ends of the fiber.
[c] T=Tris(hydroxymethyl)aminomethane; P=$Na_2HPO_4+KH_2PO_4$(1:1).
[d] At 15 °C.

trum Systems, Waltham, Mass.). The radiation was focussed to the node of Ranvier with quartz optics and an aluminized toroidal mirror. The dose rate in the plane of the node as measured with a photodiode (Type PIN-10; United Detector Technology, Inc., Santa Monica, Calif.) was in the range of 2 mW/cm^2 at 280 nm, 3 mW/cm^2 at 260 nm.

Solutions

The solutions used are given in Table 1. Since only changes of the sodium current were studied, the potassium current was eliminated by adding 5 mM tetraethylammonium chloride to the Ringer's solution. The temperature was 13.5–15.5° C.

Calculations

The functions of sodium inactivation, $h_\infty(V)$ and $\beta_h(V)$, were approximated by the empirical function of Frankenhaeuser and Huxley (1964). Differing from these authors, in this paper the same representation was chosen also for α_h, thus

$$z(V)=A/(1+\exp[(V-V')/k]) \tag{1}$$

where $z(V)$ stands for $h_\infty(V)$, $\alpha_h(V)$, $\beta_h(V)$. For the potential range in question, $\alpha_h(V)$ is fitted by this equation as well as by the one given by Frankenhaeuser and Huxley (1964).

Two of the four voltage-dependent parameters of sodium inactivation, h_∞, τ_h, α_h, and β_h, are sufficient to describe the inactivation mechanism. $h_\infty(V)$ and $\alpha_h(V)$ were selected for detailed investigations, since they could be measured with the highest precision: Both curves show a quasi-linear central part with a steep slope in the range $0.3<h_\infty<0.7$ ($0\,\text{mV}<V<15\,\text{mV}$) and $0.4\,\text{msec}^{-1}<\alpha_h<0.9\,\text{msec}^{-1}$ ($-40\,\text{mV}<V<-20\,\text{mV}$). These slopes, $-1/(4k)$, are greater than the maximum slope of $\beta_h(V)$ and do not vary to a large extent for different fibers (*see* Table 2 in Results). Therefore, it is possible to calculate negative potential shifts from changes of $h_\infty(V=0)$ and $\alpha_h(V=-40\,\text{mV})$:

$$\begin{aligned}\Delta V_h&=4k_h\cdot\Delta h_\infty(V=0)\\ \Delta V_\alpha&=4k\cdot\Delta\alpha_h(V=-40\,\text{mV}).\end{aligned} \tag{2}$$

$h_\infty(V=0)$ can be determined by $h_\infty(V=0)=i^0_{Na}/i^P_{Na}$. The peak sodium currents i^0_{Na} and i^P_{Na} (as measured without and with a negative prepulse) have both been shown to follow an exponential decrease with irradiation dose $H\cdot t$ (Fox & Stämpfli, 1971; Fox, 1974*a*):

$$\begin{aligned}i^P_{Na}&=i^P_{Na}(t=0)\cdot\exp(-\gamma_{Na}Ht)\\ i^0_{Na}&=i^0_{Na}(t=0)\cdot\exp(-\gamma_0 Ht).\end{aligned} \tag{3}$$

Therefore, $h_\infty(V=0)$ is also expected to show an exponential decrease with irradiation dose and its rate constant γ_h is determined by $\gamma_h=\gamma_0-\gamma_{Na}$.

The rate constants of radiation-induced decrease γ_{Na}, γ_0, γ_h are a measure of the ultraviolet sensitivity of the nodal membrane: γ_h for radiation-induced reduction of $h_\infty(V=0)$, i.e. for potential shift. The radiation-induced change of $\alpha_h(V=-40\,\text{mV})$ can be described empirically with sufficient accuracy by

$$\alpha_h=\alpha_h(t=0)\cdot\exp(-\gamma_\alpha H t) \tag{4}$$

(*see* Fig. 3, and Schwarz, 1975*b*).

Corrections

a) Spontaneous changes (run-down). The sodium current decreases spontaneously with time of the experiment. This decrease can be described by an exponential function with a time constant of the order of 60–120 min (Fox & Duppel, 1975; Schwarz, 1975*b*). This effect is superimposed on the ultraviolet-induced decrease of the peak sodium current. It is corrected for by determination of the exact magnitude of the spontaneous current decrease during periods preceding and following the irradiation (Fox, 1974*a*).

b) Ultra-slow changes. The nodal peak sodium current measured at a fixed test voltage depends on the membrane potential even if the normal sodium inactivation is removed ($h_\infty=1$). This effect which is not included in the Hodgkin-Huxley formalism was investigated and quantitatively described by Fox (1976*a*):

$$i_{Na}^P(V_H, V)=u_\infty(V_H)\cdot i_{Na}^P(V) \tag{5}$$

with

$$u_\infty(V_H)=1/\left(1+\exp\frac{10.5-V_H}{-11.7}\right). \tag{6}$$

Since h_∞ and τ_h are determined from ratios of i_{Na}^P at fixed membrane potentials before and after irradiation, no correction is necessary for a radiation-induced shift of u_∞. Only for the evaluation of the rate constant of ultraviolet-induced sodium current decrease γ_{Na} [*see* Eq. (3)] a correction using Eqs. (5) and (6) (replace V_H by $V_H+\Delta V$) is necessary, which in our case never exceeded 10% of the uncorrected values.

c) Series resistance. With an uncompensated series resistance R_s the potential of the test pulse V_{test} selected for the determination of $h_\infty(V)$ and $\tau_h(V)$ or $\alpha_h(V)$ will be displaced by some amount ΔV. ΔV is entirely determined by the magnitude of the current during the test pulse and the fixed series resistance: $\Delta V=i_{Na}\cdot R_s$.

For the experimental set-up used for this study ΔV was estimated by Drouin and Neumcke (1974) on the base of extensive measurements of several factors contributing to the series resistance in the voltage-clamp system of Nonner (1969) including an analysis of the gap resistance of the node of Ranvier. The result was verified by measuring current-voltage relations of i_{Na} at different steady-state levels of sodium inactivation and using Ringer's solutions with low sodium concentration. According to this study the difference ΔV between the true voltage at the nodal membrane V_{true} and the test voltage V_{test} is:

$$\Delta V[\text{mV}]=V_{test}-V_{true}=R_s[\Omega\,\text{cm}^2]\cdot i_{Na}[\text{mA/cm}^2] \tag{7}$$

where $0.070\leqq R_s\leqq 0.084$ depending on the percentage cut from the neighboring internode of the node under investigation.

To determine the influence of the uncompensated series resistance on $h_\infty(V)$ it is necessary to solve numerically the full Hodkin-Huxley-Frankenhaeuser equations. However, a fairly good estimation can be obtained with the following assumptions: (a) for positive test pulses of sufficient amplitude ($V_{test} \geqq 60\,mV$) the ratio τ_m/τ_h will not vary considerably, if the membrane potential changes by a few mV; (b) the very small effect of the series resistance on the negative prepulse potentials can be neglected. Then:

$$i_{Na} = m_\infty^2\, h^{true}\, \overline{g_{Na}}(V_{true} - V_{Na}) \tag{8}$$

and

$$i_{Na}^{max} = m_{\infty,\,max}^2\, \overline{g_{Na}}(V_{true}^{max} - V_{Na}). \tag{9}$$

Dividing Eq. (8) by Eq. (9) and considering Eq. (7) we have

$$\frac{i_{Na}}{i_{Na}^{max}} = h_\infty^{obs} = \frac{m_\infty^2}{m_{\infty,\,max}^2}\left(\frac{V_{test} - i_{Na} R_s - V_{Na}}{V_{test} - i_{Na}^{max} R_s - V_{Na}}\right) h_\infty^{true}. \tag{10}$$

It appears that h_∞^{obs} does not depend directly on the maximum sodium conductance $\overline{g_{Na}}$. If V_{test} is in the range of $m_\infty = 1$, then for inward sodium currents ($i_{Na} < 0$) it is: $h^{obs} \geqq h^{true}$. This means that h_∞^{obs} is displaced from h_∞^{true} in the positive direction and with decreasing i_{Na} due to irradiation from there will be replaced towards h_∞^{true} (negative direction).

In our experiments ($V_{test} = 60\,mV$), m_∞ was slightly below 1 and in this case $m_\infty/m_{\infty,\,max} \leqq 1$ and hence $h_\infty^{obs} \leqq h_\infty^{true}$. Both effects balance each other, if m_∞ does not deviate too much from 1, as was the case in our experiments. Calculations of the actual shift of $h_\infty(V)$ by uncompensated series resistance even for extreme experimental situations, in fact, revealed shifts by not more than 1 mV (Schwarz, 1975*b*).

Results

Preceding Experiments

The standard parameters for the representations (Eq. 1) of the sodium inactivation parameters h_∞, α_h and β_h for the node of Ranvier from *Rana esculenta* are given in Table 2. To ensure that TEA necessary for K^+-current elimination does not influence the radiation effects, comparable irradiation experiments were performed in which the potassium current was either subtracted by TEA or by numerical calculation (Hille, 1967).

Table 2. Representation of the sodium inactivation parameters by Eq. (1)

	A	V_h (mV)	k (mV)
h_∞	1	5.2 ± 0.5	6.9 ± 0.7
α_h	$1.05 \pm 0.02\ msec^{-1}$	-29.5 ± 0.4	13.1 ± 0.4
β_h	$0.44 \pm 0.10\ msec^{-1}$	36.8 ± 0.5	-16.5 ± 0.5

The parameters A, V_h, and k were determined by fitting Eq. (1) to experimental values of seven motor fibers.
Temperature 13.5–15.5 °C.

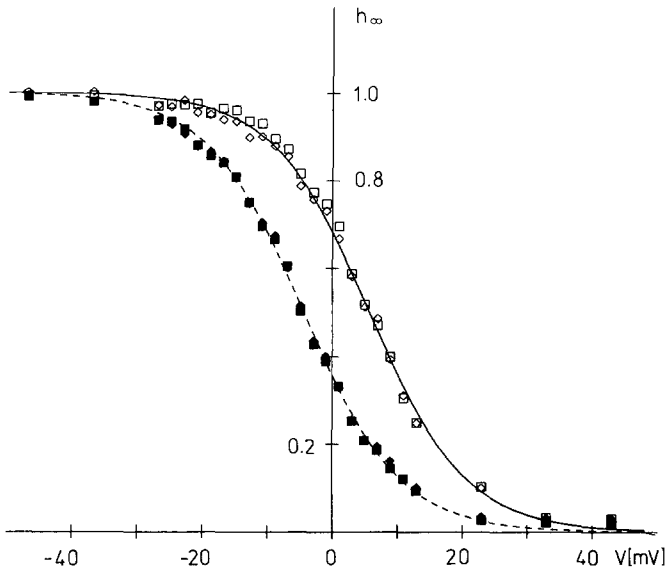

Fig. 1. Effect of ultraviolet radiation (280 nm) on the steady-state sodium inactivation curve. Open symbols before, closed symbols after irradiation of 6 min (dose: 0.72 Wsec/cm^2). Two curves with an interval of 2 min were measured before and after irradiation to exclude the occurrence of spontaneous shifts. Lines represent fits of Eq. (1) to the experimental points; resulting fitting parameters before irradiation: $A=1$, $k=7.25$ mV, $V'=+5.7$ mV; after irradiation: $A=1$, $k=7.25$ mV, $V'=-4.7$ mV. Temperature 15 °C

Both procedures led to the same radiation effects on the sodium peak current and the inactivation parameter $h_\infty(V=0)$.

Radiation-Induced Alterations of the Sodium Inactivation Parameters

Ultraviolet radiation of 280 nm wavelength induced a shift of the steady-state sodium inactivation curve $h_\infty(V)$ in the negative direction of the potential axis (Fig. 1); the amount of this translation depended on the ultraviolet dose *(see below)* and was by more than one order of magnitude greater than the spontaneous shift occurring without irradiation. There was no change in the central slope of $h_\infty(V)$ independent of ultraviolet dose, i.e. of voltage shift. This finding is in contrast to the observations of Oxford and Pooler (1975) in lobster axons after irradiation. The average central slope ($\pm$SEM) measured in nine different nerve fibers was (*see also* Table 2): $\frac{\partial h}{\partial V} \approx \frac{1}{4k_h} = 0.036 \pm 0.004\ \mathrm{mV}^{-1}$ before irradiation, and $\frac{\partial h}{\partial V}\frac{1}{4k_h} = 0.035 \pm 0.004\ \mathrm{mV}^{-1}$ after irradiation, independent of the amount of radiation-induced shift of $h_\infty(V)$.

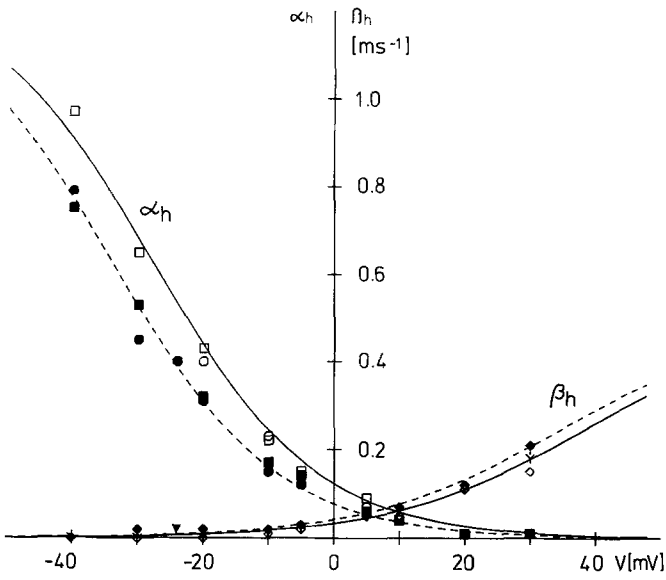

Fig. 2. Effect of ultraviolet radiation (280 nm) on the rate constants α_h and β_h of sodium inactivation. Open symbols before, closed symbols after irradiation of 3 min (dose 0.2 Wsec/cm^2). Two curves with an interval of 10 min were measured before and after irradiation to show that spontaneous changes may be neglected. Lines represent fits of Eq. (1) to the experimental points before and after irradiation. α_h: $A=1.25\,\text{msec}^{-1}$, $k=12.5\,\text{mV}$, $\Delta V'\approx 6\,\text{mV}$; β_h: $A=0.48\,\text{msec}^{-1}$, $k=-14.5\,\text{mV}$, $\Delta V'\approx 4\,\text{mV}$. Temperature 14 °C

Ultraviolet irradiation also caused a negative shift along the potential axis of the rate constants $\alpha_h(V)$ and $\beta_h(V)$ (Fig. 2). The solid and the broken lines represent least-squares fits of Eq. (1) to the experimental points before and after irradiation. The resulting fitting parameters revealed no change except for a translation of the curves to more negative potentials. There was no significant change of holding potential during the experiment which could account for the measured shifts. Such a shift would have been revealed by a change in the base line of the current at holding potential which was not observed (*see* Measurement of Currents).

Quantitative data for the radiation-induced shifts of the inactivation parameters in relation to the applied irradiation dose were obtained by measuring the rate constants of decrease of i_{Na}^P, $h_\infty(V=0)$ and $\alpha_h(V=-40\,\text{mV})$ as illustrated in Fig. 3; results are summarized in Table 3. The rate constants of radiation-induced decrease are the same for h_∞ and α_h within the limits of error, meaning that $\Delta V_\alpha=\Delta V_h$ for any certain ultraviolet dose. On the other hand, the rate constant of sodium current decrease differs from the two rate constants γ_h and γ_α by a factor of about 4.

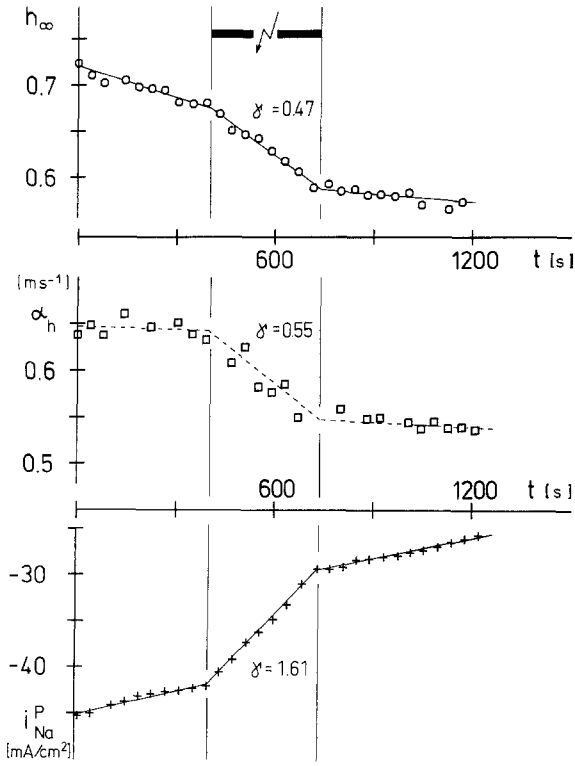

Fig. 3. Effect of ultraviolet radiation (280 nm) on i^P_{Na}, $\alpha_h(V=-40\,mV)$ and $h_\infty(V=0)$. The rate constants of radiation-induced decrease were determined according to Eqs. (3) taking into consideration the spontaneous changes. Units of γ: cm^2/Wsec. Temperature 14 °C

Table 3. Rate constants γ_α, γ_h, and γ_{Na} of radiation-induced decrease of $\alpha_h(V=-40\,mV)$, $h_\infty(V=0)$, and i^P_{Na}, respectively, according to Eqs. (3) and (4)

Exp. No.	γ_α	γ_h	γ_{Na}	γ_α/γ_h	$\gamma_{Na}/\gamma_\alpha$	γ_{Na}/γ_h
44	0.77	0.97	2.95	0.79	3.83	3.39
	0.72	0.78	2.99	0.92	4.12	3.83
72	0.56	0.47	1.61	1.19	2.88	3.43
	0.32	0.39	1.85	0.82	5.78	4.74
Mean ± SEM				0.93 ± 0.09	4.15 ± 0.60	3.85 ± 0.31

Units of γ: cm^2/Wsec; Temperature: 14 °C; Wavelength: 280 nm.

Modifications of the Effect of Ultraviolet Radiation on the Steady-State Sodium Inactivation

The following experiments were designed to establish that the ultraviolet-induced reduction of i^P_{Na} and potential shift are two separate radiation effects.

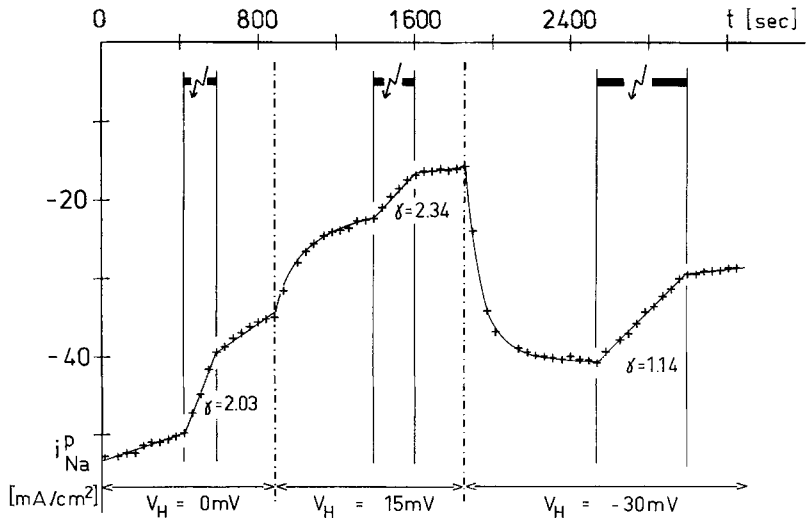

Fig. 4. Modification of ultraviolet blocking of sodium current by changes of holding potential V_H. Rate constants according to Eq. (3) in cm²/Wsec. The slow changes of i^p_{Na} due to variation of ultra-slow inactivation with V_H are extensively described by Fox (1976*a*) and by Neumcke, Fox, Drouin and Schwarz (1976). Temperature 13.5 °C. Wavelength 280 nm

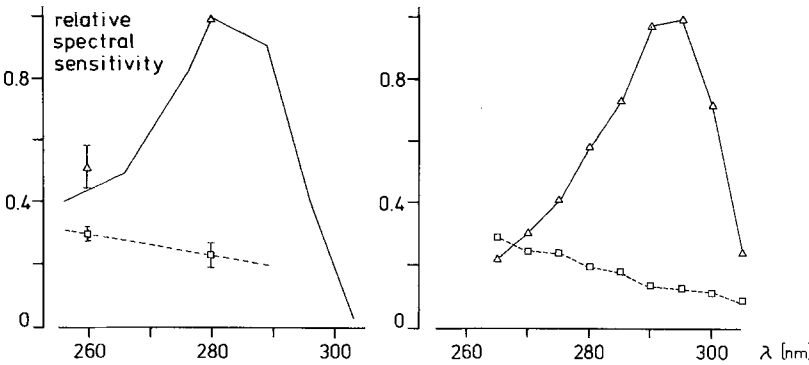

Fig. 5. *Left:* Rate constants γ_{Na} and γ_h of ultraviolet-induced decrease of i^p_{Na} (Δ) and of $h_\infty(V=0)$ (□) *vs.* wavelength. Mean values ± SEM relative to γ_{Na} at 280 nm. Solid line $=\gamma_{Na}/\gamma_{Na}$ ($\lambda=280$ nm) as measured by Fox (1974*a*). *Right:* Action spectra of ultraviolet radiation effects on embryonal chicken heart cells as measured by Nathan *et al.* (1976): △: blocking of beating activity; □: increase of beat rate prior to final block

Changes of the holding potential altered the sensitivity of peak sodium current against ultraviolet radiation. An example of a typical experiment is shown in Fig. 4. The results coincide with those reported by Fox (1974*b*) for negative holding potentials [at $V_H=-30$ mV (mean of six experiments ± SEM): $\gamma_{Na}/\gamma_{Na}(V_H=0)=0.69\pm0.09$]; on the other hand, an enhancement was observed for $V_H=+15$ mV [$\gamma_{Na}/\gamma_{Na}(V_H=0)$

$=1.33\pm0.09$]. Similar experiments were performed to investigate the ultraviolet sensitivity of radiation-induced potential shifts; while the ultraviolet sensitivity of i_{Na}^{P} increased with potential, the opposite was true for $h_\infty(V=0)$: $\gamma_h/\gamma_h(V=0)=1.32\pm0.18$ at $V_H=-30$ mV and 0.55 ± 0.15 at $V_H=+15$ mV.

Measurements of the spectral sensitivities of $h_\infty(V=0)$ and i_{Na}^{P} were performed at two different wavelengths (Fig. 5, left). The relative values found for i_{Na}^{P} at 280 and 260 nm were the same as those measured by Fox (1974*a*). The values measured for $h_\infty(V=0)$ differed from those of i_{Na}^{P}: while the peak sodium current showed a maximum sensitivity at 280 nm, the steady-state sodium inactivation was more sensitive at 260 nm.

Discussion

Interpretation of the Observed Voltage Shifts

Our observation of ultraviolet-induced voltage shifts could, in principle, be caused by uncompensated series resistance, since ultraviolet radiation also decreases the sodium permeability and hence the membrane current. However, as shown in Materials and Methods, only a shift of $h_\infty(V)$ of about 1 mV is expected under our experimental conditions. This theoretical estimate from the data is supported by the following observations:

1. In experiments designed to show the independence of the ultraviolet blocking effect of blocking by tetrodotoxin (TTX) (Fox, 1974*a*), $h_\infty(V=0)$ was also currently measured *(unpublished data)*. A change of $h_\infty(V=0)$ was never observed indicating that a shift of $h_\infty(V)$ of less than 1 or 2 mV occurred due to the reduction of P_{Na} by TTX ($V_{test}\geqq60$ mV!). On the other hand, $h_\infty(V=0)$ was lowered after ultraviolet irradiation, whether TTX was present or not. Likewise, no indication for a considerable shift of $h_\infty(V)$ was obtained in experiments with low TTX concentrations (no complete block of sodium channels) designed to determine a possible interaction of thiamine and TTX (Fox & Duppel, 1974).
2. The rate of ultraviolet-induced shift of $h_\infty(V)$, if due to series resistance, should be increased with more negative holding potential, since then the ionic currents are larger. However, most of our experiments were designed so that usually a reference irradiation at $V_H=0$ preceded the irradiation at negative or positive holding potentials. Under these circumstances the sodium current at negative holding

potential was smaller than at zero potential (due to the irreversible ultraviolet blocking of P_{Na}; *see* Fig. 4); nevertheless, a greater rate of shift of $h_\infty(V)$ was observed in contradiction to the hypothesis.

3. The different ratios of ultraviolet sensitivity of the blocking effect and of the voltage shift at 280 and at 260 nm (Fig. 5, left) indicate that per blocked sodium channel (i.e. per unit loss of i_{Na}) the rate of voltage shift at 260 nm is about 3 times higher than at 280 nm. This would contradict an explanation of the voltage shift as an effect of series resistance.
4. Experiments with SH-reagents have shown that it is possible to prevent the ultraviolet-induced shift of $h_\infty(V)$, even though P_{Na} is reduced by the irradiation at the same time (Hof, Schwarz & Fox[1], *see also below*).

Conclusively, it appears that most likely the observed shifts of $h_\infty(V)$, $\alpha_h(V)$ and $\beta_h(V)$ are due to a direct action of UV radiation.

Hypothetical Explanation of the Photo-Effect

To explain our results we assume that the sodium inactivation depends on the distribution of charged groups or particles within the nerve membrane. By applying Boltzmann's law, the potential dependence of h_∞ can be described by:

$$h_\infty(V) = 1/[1 + \exp(z^*(V - V')/kT)] \tag{6}$$

where z^* is the effective charge of the group or particle, meaning the actual charge times the fraction of electric field acting upon it. Then, in principle, two explanations of the radiation-induced potential shift would be possible: a change of z^* or a change of the electric field.

The experiments showed (*see* Fig. 1) that there is no change in the central slope of $h_\infty(V)$ implying no change of k_h. Comparing Eqs. (6) and (1) it is obvious that z^* is unaltered, too. The resting potential, on the other hand, is not affected by ultraviolet radiation (Fox, 1974*a*) nor is the holding potential. Therefore it seems obvious to suggest an alteration of the membrane surface charges by ultraviolet radiation. This suggestion is

[1] Hof, D., Schwarz, W., Fox, J.M. Modification of ultraviolet radiation effects on the membrane of myelinated nerve fibres by sulfhydryl compounds. *Pfluegers Arch. (submitted for publication).*

supported by the observation that ultraviolet radiation displaces $m_\infty(V)$ along the potential axis by almost the same amount as $h_\infty(V)$ (Fox, 1976*b*). Hof *et al.*[1] demonstrated that SH-compounds applied from the inside of the nerve fiber prevent the radiation-induced potential shift. Further, experiments of Fox and Duppel (1975) indicated that intracellularly applied thiamine polyphosphates diminished the spontaneous decrease of $h_\infty(V=0)$. Therefore, it may be hypothesized that ultraviolet radiation reduces the density of negative surface charges at the inner surface of the nodal membrane by a first-order photochemical reaction (exponential dose effect curve).

Following this hypothesis the internal surface potential can be estimated by extrapolating the observed potential shifts to infinite time of irradiation assuming that all surface charges can be destroyed in this way. From the shift of $\alpha_h(V)$ an upper limit of $-14\,\text{mV}$ was taken; from $h_\infty(V)$ a lower limit of $-19\,\text{mV}$:

$$-19\,\text{mV} \leqq \psi_i \leqq -14\,\text{mV}.$$

This value is close to the one determined by Chandler, Hodgkin and Meves (1965) in the squid giant axon. A mean surface potential of $-16.5\,\text{mV}$ corresponds at 15 °C to a density of surface charges: $\sigma_i \approx -1\,e_0/(3.5\,\text{nm})^2$ which was determined using the method described by Gilbert and Ehrenstein (1969). The distance of elementary charges at the inner surface of the nodal membrane seems to be greater by a factor of about two than the distance at the outer surface as determined by Drouin and Neumcke (1974).

The radiation-induced potential shift apparently is caused by a photoreaction separate from the one blocking the sodium permeability. This can be derived from the finding that negative holding potentials increase the sensitivity of the node of Ranvier with regard to the potential shift, but decreases the blocking effect and *vice versa* for positive holding potentials. Further, the different action spectra indicate two separate photoreactions. Additional evidence may be derived from the results of Hof *et al.*[1], who demonstrated that the radiation-induced potential shift is prevented by intracellularly applied SH-compounds, whereas the blocking effect is not.

The results of the present investigation are very close to data reported by Nathan, Pooler and De Haan (1976). These authors observed in embryonic chicken heart cells a blocking of the beating activity and an increase of the beat rate (prior to final blocking) after ultraviolet irra-

diation. The two effects followed different action spectra (Fig. 5, right), quite similar to those observed in the node of Ranvier. The blocking effect was attributed by Nathan and his co-workers to ultraviolet blocking of the sodium conductance, while the increase of the beat rate was shown to be caused by depolarization. Nathan *et al.* (1976) point out the divergences between neural preparations which do not produce significant membrane depolarization (Fox, 1974*a*; Oxford & Pooler, 1975) and embryonic heart muscle cells. The striking similarities, however, suggest that possibly part of the effects might have the same underlying mechanism.

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References

Chandler, W.K., Hodkin, A.L., Meves, H. 1965. The effect of changing the internal solution on sodium inactivation and related phenomena in giant axons. *J. Physiol. (London)* **180**:821

Drouin, H., Neumcke, B. 1975. Specific and unspecific charges at the sodium channels of the nerve membrane. *Pfluegers Arch.* **351**:207

Fox, J.M. 1974*a*. Selective blocking of the nodal sodium channels by ultraviolet radiation. I. Phenomenology of the radiation effect. *Pfluegers Arch.* **351**:287

Fox, J.M. 1974*b*. Selective blocking of the nodal sodium channels by ultraviolet radiation. II. The interaction of Ca^{++}, H^{+}, and membrane potential. *Pfluegers Arch.* **351**:303

Fox, J.M. 1976*a*. Ultra-slow inactivation of the ionic currents through the membrane of myelinated nerve. *Biochim. Biophys. Acta* **426**:232

Fox, J.M. 1976*b*. Investigation of the relation between structure and function in myelinated nerve fibres with aid of ultraviolet radiation. *Biophys. Struct. Mechanism* **2**:1

Fox, J.M., Duppel, W. 1975. The action of thiamine and its di- and triphosphates on the slow exponential decline of the ionic currents in the node of Ranvier. *Brain Res.* **89**:287

Fox, J.M., Stämpfli, R. 1971. Modification of ionic membrane currents of Ranvier nodes by UV-radiation under voltage clamp conditions. *Experientia* **27**:1289

Frankenhaeuser, B. 1960. Quantitative description of sodium currents in myelinated nerve fibres of *Xenopus laevis*. *J. Physiol. (London)* **151**:491

Frankenhaeuser, B., Huxley, F.A. 1964. The action potential in the myelinated nerve fibres of *Xenopus laevis* as computed on the basis of voltage clamp data. *J. Physiol. (London)* **171**:302

Gilbert, D.L., Ehrenstein, G. 1969. Effect of divalent cations on potassium conductance of squid axons: Determination of surface charge. *Biophys. J.* **9**:447

Hille, B. 1967. The selective inhibition of delayed potassium currents in nerve by tetraethylammonium ions. *J. Gen. Physiol.* **50**:1287

Nathan, R.D., Pooler, J.P., DeHaan, R.L. 1976. Ultraviolet-induced alternations of beat rate and electrical properties of embryonic chick heart cell aggregates. *J. Gen. Physiol.* **67**:27

Neumcke, B., Fox, J.M., Drouin, H., Schwarz, W. 1976. Kinetics of slow variation of peak sodium current in the membrane of myelinated nerve following changes of holding potential or extracellular pH. *Biochim. Biophys. Acta* **426**:245

Nonner, W. 1969. A new voltage clamp method for Ranvier nodes. *Pfluegers Arch.* **309**:176

Oxford, G.S., Pooler, J.P. 1975. Ultraviolet photoalteration of ion channels in voltage-clamped lobster giant axons. *J. Membrane Biol.* **20**:13

Schwarz, W. 1975*a*. The effect of ultraviolet radiation on the sodium inactivation of myelinated nerve fibres. *Pfluegers Arch.* **355**:R68

Schwarz, W. 1975*b*. Einfluss ultravioletter Strahlung auf die Inaktivierung des Na-Systems myelinierter Nervenfasern. Ph.D. Thesis, University of Saar, Saarbrücken, Faculty of Science

Stämpfli, R. 1969. Laboratory Techniques in Membrane Biophysics. H. Passow and R. Stämpfli, editors. Springer Verlag, Berlin-Heidelberg-New York