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Changes of Ultraviolet Sensitivity of Voltage-Clamped Sodium Channels During their Potential-Induced Conductance Cycle

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Summary. The effects of ultraviolet (UV) radiation at various intermediate states of excitation of the frog nerve membrane were measured under voltage-clamp conditions. By synchronizing a UV flash source (flash duration: 10 µsec) with the stimulating voltage-clamp pulses it was possible to irradiate the membrane at any state of excitation. The resulting UV sensitivities γ^{Na} and γ^{h} of $\overline{g_{\mathrm{Na}}}$ and $h_\infty(V=0)$ were compared with those found at the end of a hyperpolarizing prepulse ($m=0$, $h=1$). The irradiation always induced a decrease of both parameters, $\overline{g_{\mathrm{Na}}}$ and $h_\infty(V=0)$, and the more "h-gates" there were in the closed position at the moment of irradiation, the more pronounced were the effects. The normalized sensitivity of both parameters can be described fairly well by linear relations of the form:

$$\gamma^x(m,h)/\gamma^x(0,1)=1+P_x(1-h)+Q_x m^2 h \qquad (x=\mathrm{Na}\text{ or }h).$$

The coefficients P and Q depend on wavelength. The results suggest a conformational change of the nodal membrane during excitation and an interdependence of m and h to some extent.

Key Words Ranvier node · ultraviolet irradiation · sodium conductance · sodium inactivation

Introduction

Ultraviolet radiation evokes specific and reproducible changes of the membrane of the node of Ranvier (Hutton-Rudolph, 1943; Booth, von Muralt & Stämpfli, 1950; von Muralt & Stämpfli, 1953; Fox, 1974). Experiments using the voltage-clamp technique have demonstrated that ultraviolet radiation essentially causes a specific damage of the sodium system. It was shown (Fox, 1974, 1976) that the maximum conductance $\overline{g_{\mathrm{Na}}}$ was irreversibly reduced. Since there was no indication of functional alterations of individual channels this UV effect was interpreted as a reduction of the number of operating sodium channels. The action spectrum of this "blocking" effect showed a maximum at 280 nm for the node of Ranvier (Fox, 1974). Oxford and Pooler (1974) reported a maximum at 290 nm for the lobster giant axon.

Further investigations by Schwarz (1975) and Schwarz and Fox (1977) have also demonstrated that the mechanism of inactivation can be influenced specifically by ultraviolet irradiation: The voltage dependence of the steady-state inactivation, $h_\infty=h_\infty(V)$, is shifted towards more negative potentials.

In the present work the ultraviolet sensitivity of the sodium channel at various states of excitation was investigated. For that purpose a UV flash source was synchronized with the stimulating pulses.

Materials and Methods

Irradiation

A high energy ultraviolet flash source was designed employing an Argon arc (PL1, Impulsphysik, Hamburg). Monochromatic ultraviolet radiation of 280 and 262 nm wavelength was generated using interference filters of 9 nm bandwith (Type SSUV, Spectrum Systems, Waltham, Mass.). The radiation was focussed to the node of Ranvier with quartz optics and an aluminized toroidal mirror. The dose in the plane of the node was measured in every experiment using a photodiode (Type PIN-10 CAL/UV, United Detector Technology, Inc., Santa Monica, Calif.) connected to an integrating amplifier. It was in the range of 1.8 mW sec/cm^2 per flash assuming homogeneous illumination. The flash had a duration of ca. 10 µsec and was released every second. Ultraviolet absorption could not be measured because of the small dimensions of the Ranvier node. Therefore relative changes of the irradiation effect were measured comparing the effects in various excitation states with a reference effect in every single experiment.

Measurement of Currents

Following the method of Stämpfli (1952, 1969) single motor fibers of the sciatic nerve of *Rana esculenta* were mounted in an acrylic chamber which was connected to a

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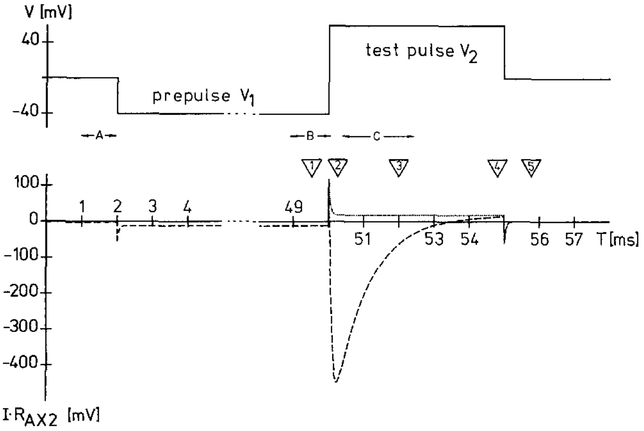

Fig. 1. Pulse program of clamp voltage for irradiation experiments and recordings of nodal membrane currents. ---- total current ($I_{Na}+I_L+I_C$); potassium current I_K being removed by TEA; .... leakage and capacitive currents (I_L+I_C). Period *A* indicates registration of the offset voltage (control of compensation of the whole measuring system); period *B* indicates the determination of the leak conductance and period *C* indicates registering of ionic currents. UV irradiation flashes of 10 µsec duration were triggered at various states of excitation marked by the open triangles containing a figure (1, ..., 5)

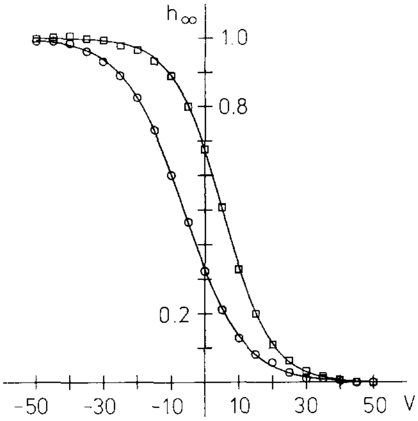

Fig. 2. $h_\infty(V)$-curves before and after 3 UV flash irradiations (first and last curve) taken from the experiment 9/78 shown in Fig. 6. The solid lines represent least-squares fits to the experimental data points using Eq. (2). The fitted parameter $V_{1/2}$ and k are: First curve (□): $V_{1/2}=3.8 \pm 0.081$ mV; $k=7.4 \pm 0.073$ mV. Last curve (○): $V_{1/2}=-6.4 \pm 0.102$ mV; $k=8.8 \pm 0.094$ mV

voltage-clamp arrangement described by Nonner (1969). The experiments were run on-line under the control of a computer [Honeywell DDP-516, DA-Converter (10 Bit), AD-Converter (12 Bit), 11 µsec sampling rate; for further details *see* Fox (1974)].

Figure 1 shows a typical voltage-pulse program. Using TEA for elimination of the potassium current the peak sodium inward current I^p_{Na} was determined on-line (corrected for the leakage current) and stored on digital magnetic tape. I^p_{Na} was registered with and without a prepulse $V_1=-40$ mV; $t_1=50$ msec. Thus, the inactivation parameter

$$h_\infty(V=0)=I^p_{Na}(V_1=0)/I^p_{Na}(V_1=-40\text{ mV}) \tag{1}$$

was easily determined.

Complete sodium inactivation curves $h_\infty(V)$ were measured by changing the prepulse amplitude V_1 from -50 to $+50$ mV; $t_1=50$ msec. These curves were analyzed off-line. They were approximated by the following function:

$$h_\infty(V)=\frac{1}{1+\exp\left(\frac{V-V_{1/2}}{k}\right)}. \tag{2}$$

(For example *see* Fig. 2.)

The accuracy of determination of $V_{1/2}$ and k is given on one hand by the standard deviation of the least-squares fit (*see* Waerden (1957) for method) of a single run (measuring the error of parameter determination) and on the other hand by the variation of the determined values of several successive runs (measuring the stability of the system). The standard deviation of the least-squares fit of single runs of h_∞-curves never exceeded 0.2 mV. The variation (±SD) of successive determinations of $V_{1/2}$ never exceeded 0.3 m.V. A test experiment is shown in Table 1.

Table 1. Test experiment (VN 1/78) on the accuracy of determination of the parameters of h_∞-curves

Time (sec)	$V_{1/2}$ (mV)	k (mV)
0	8.40±0.16	6.69±0.14
148	8.97±0.12	6.50±0.10
268	8.38±0.12	6.41±0.11
443	8.77±0.10	6.36±0.09
555	8.75±0.15	6.40±0.13
Mean±SD	8.65±0.26	6.47±0.13

Table 2. Ionic composition (mM/liter) of solutions

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

[a] T=Tris(hydroxymethyl)aminomethane; $P=Na_2HPO_4+KH_2PO_4$(1:1).

[b] At 15 °C [c] TEA=tetraethylammoniumchloride for elimination of K^+-currents.

Determination of Ultraviolet Sensitivities of the Nodal Membrane

Figure 3 shows a typical recording. The numbers in the triangles refer to the state of excitation at which the flash was released (*see also* Fig. 1). Both sodium currents (with and without a negative prepulse) follow an exponential decrease with irradiation dose $H \cdot t$ ($H \cdot t$ = dose rate × irradiation time; Schwarz & Fox, 1977), and therefore $h_\infty(V=0)$ is also expected to show such a dose dependence:

$$I_{Na}^p(t)=I_{Na}^p(t=0)\exp(-\gamma^{Na}Ht) \qquad (h_\infty=1) \tag{3a}$$

$$h_\infty(V=0,t)=[h_\infty(V=0,t=0)-h_\infty(V=0,t=\infty)] \cdot \exp(-\gamma^h Ht)+h_\infty(V=0,t=\infty). \tag{3b}$$

The rate constants γ^{Na} and γ^h define the ultraviolet sensitivity of the corresponding membrane parameters. The constant $h_\infty(V=0, t=\infty)$ was determined to be of the order of 0.2 (Schwarz, 1975; Hof, 1979).

Since the radiation effect is always superimposed on a spontaneous effect (run-down) it was necessary to record radiation-free intervals before and after every irradiation period. With the aid of an off-line computer program simple functions were fitted to the time course of the peak sodium current (with and without prepulse): For the radiation-free intervals an exponential decrease was used; for the irradiation periods, the product of that function and another exponentially decreasing function (Eq. 3a or 3b). Thus, the pure irradiation effect could be derived immediately.

Using least-squares fitting of these functions to the measured values the maximum standard deviation of the fits observed in all experiments was 3.5×10^{-2} cm^2/W sec. This corresponds to a maximum relative error of 3.5% for γ^{Na} and of 13.5% for γ^h taking into account the lowest values of γ^{Na} or γ^h ever observed in these experiments.

Solutions and Temperature

The solutions are given in Table 2. The temperature was 15 °C stabilized automatically by a thermostat using Peltier elements and thermistor probes in a feedback arrangement.

Results

Effect of Flash Irradiation at Different States of Excitation

Figure 3 shows a typical registration. The condition of the membrane at the end of the hyperpolarizing prepulse was always used as a reference (state 1) because the parameters m and h are well defined in that state ($m=0$, $h=1$). The UV sensitivities γ^{Na} and γ^h of the states 2, 3, 4 and 5 were determined, as compared with the sensitivities of state 1.

Several experiments were evaluated for each state of excitation and this for the wavelengths 280 and 262 nm. The results are presented in Table 3 in terms of normalized sensitivities (mean ± SEM). A statistical analysis (t-test) was

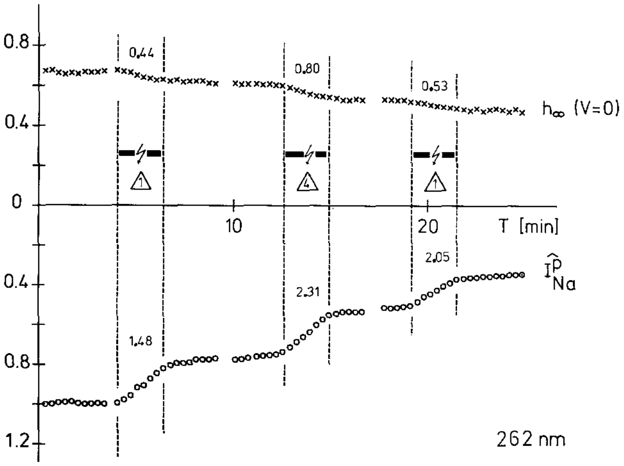

Fig. 3. Typical experiment for determination of the UV sensitivity (γ^{Na} and γ^h) of the nodal membrane to flash irradiation at different excitation states. (Here excitation state 4 was compared with the reference state 1.) During irradiation both I_{Na}^p and $h_\infty(V=0)$ are irreversibly reduced, more so in state 4 of excitation than in state 1. Normalized values of the sodium current are displayed: $\hat{I}_{Na}^p(t)=I_{Na}^p(t)/I_{Na}^p(t=0)$. The numbers within the graph represent the evaluated sensitivities γ^{Na} and γ^h (cm^2/W sec), respectively. Dose: 0.15 W sec/cm^2 per irradiation period

Table 3. The relative UV sensitivity in various states of excitation (including a statistical analysis (t-test) for difference between UV sensitivities at excitation states x and 1)

Wave-length (nm)	State of excitation x	$\frac{\gamma_x^{Na}}{\gamma_1^{Na}}$	SEM	Statist. signif.	Number of exp.
262	2	0.82	0.04	**	6
	3	1.23	0.07	n.s.	2
	4	1.39	0.07	***	6
	5	1.31	0.04	***	5
280	2	0.87	0.05	*	10
	3	1.15	0.06	n.s.	3
	4	1.20	0.06	**	8
	5	1.15	0.04	**	4

Wave-length (nm)	State of excitation x	$\frac{\gamma_x^h}{\gamma_1^h}$	SEM	Statist. signif.	Number of exp.
262	2	0.73	0.21	n.s.	5
	3	1.91	0.05	*	2
	4	2.08	0.28	**	5
	5	2.02	0.10	**	4
280	2	0.98	0.10	n.s.	6
	3	1.58	0.08	*	3
	4	1.68	0.11	***	7
	5	1.56	0.15	*	4

* $p<0.025$; ** $p<0.01$; *** $p<0.001$; n.s.: $p>0.05$.

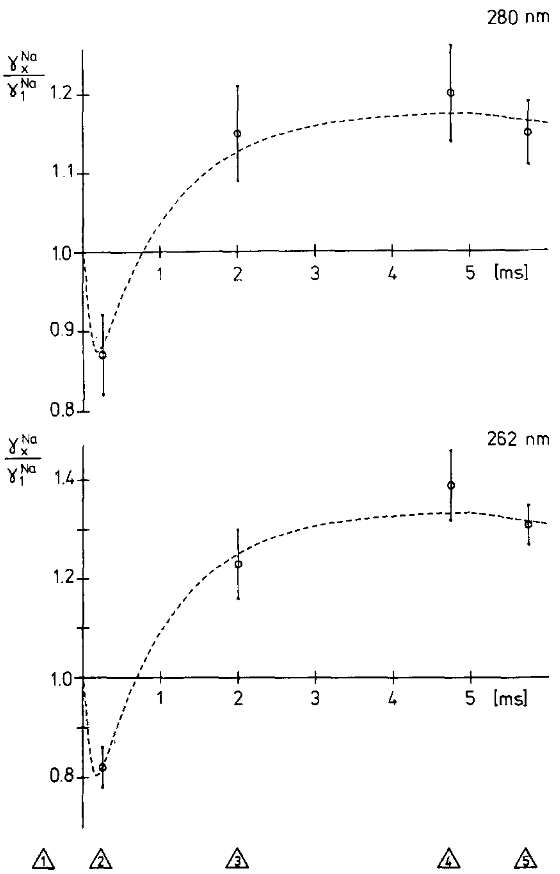

Fig. 4. UV radiation effects on I^p_{Na} at different excitation states (*see also* Fig. 1 for experimental design). For the single states their UV sensitivity at 280 and 262 nm relative to those at the reference states ±SEM are depicted; the absolute values of sensitivity of I^p_{Na} to UV flashes are: $\gamma_1^{\mathrm{Na}}(280\ \mathrm{nm})=1.83\pm0.18\ \mathrm{cm}^2/\mathrm{W\,sec}$; $\gamma_1^{\mathrm{Na}}(262\ \mathrm{nm})=1.18\pm0.19\ \mathrm{cm}^2/\mathrm{W\,sec}$. Dashed lines represent a least-squares fit to Eq. (4). 280 nm: $P_{\mathrm{Na}}=0.18$, $Q_{\mathrm{Na}}=-0.24$; 262 nm: $P_{\mathrm{Na}}=0.35$, $Q_{\mathrm{Na}}=-0.39$. *Note:* Eq. (4) is a function of $m(t, V)$ and $h(t, V)$ and therefore shows discontinuities in slope as m and h do if V is discontinuous (voltage step)

performed to evaluate whether or not the resulting UV sensitivities at various excitation states did differ from the reference state.

An attempt was made to describe the dynamics of the sensitivity changes in terms of the dynamics of the gating parameters $m(V, t)$ and $h(V, t)$ (Figs. 4 and 5). Apparently it was not possible to approximate the course of the sensitivity changes in terms of either $m(V, t)$ or $h(V, t)$ alone. On the other hand the time course of the sodium current ($\sim m^2 h$) alone did not satisfactorily describe the data: $I_{\mathrm{Na}}=0$ at states 1, 4 and 5 (Fig. 1) whereas the sensitivities at states 4 and 5 differ significantly from that of state 1. Therefore the next simplest approach was a linear relationship of the following form:

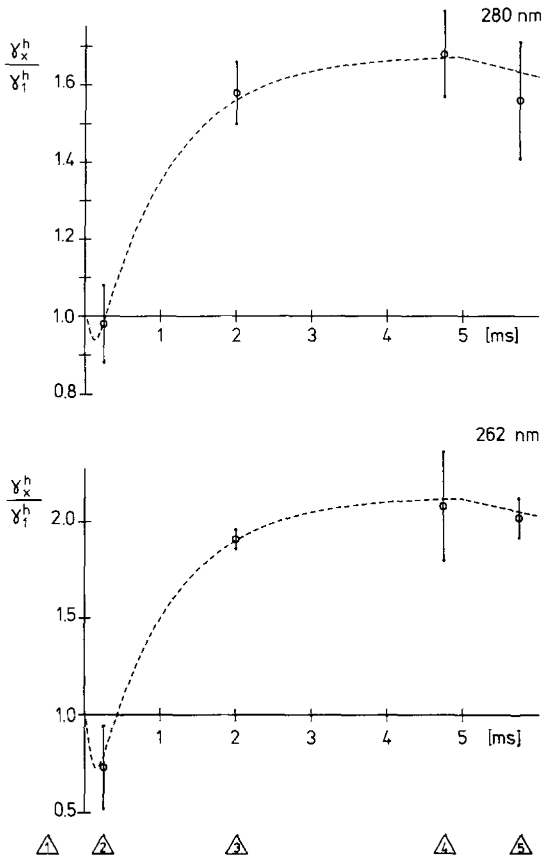

Fig. 5. UV radiation effects on $h_\infty(V=0)$ at different excitation states (wavelength 280 and 262 nm). (For details *see* Fig. 4.) Absolute values of sensitivity of $h_\infty(V=0)$ to UV flashes are: $\gamma_1^h(280\ \mathrm{nm})=0.45\pm0.07\ \mathrm{cm}^2/\mathrm{W\,sec}$; $\gamma_1^h(262\ \mathrm{nm})=0.26\pm0.04\ \mathrm{cm}^2/\mathrm{W\,sec}$. Dashed lines represent a least-squares fit to Eq. (4). 280 nm: $P_h=0.69$, $Q_h=-0.25$; 262 nm: $P_h=1.15$, $Q_h=-0.70$

$$\gamma^x(m,h)/\gamma^x(0,1)=1+P_x(1-h)+Q_x m^2 h \qquad (4)$$

($x=$Na or h).

Taking standard parameters for $m(V, t)$ and $h(V, t)$ (average values determined in our laboratory) at irradiation moments 1, 2, 3, 4, and 5, this equation satisfactorily fitted the data (*see* dashed lines in Figs. 4 and 5). This means that the sensitivity changes depend on changes on m *and* h as well.

The relative changes of γ^{Na} and γ^h during the excitation cycle are quite similar for both wavelengths. The absolute sensitivities at 280 nm are almost twice as high as at 262 nm.

Influence of UV Flash Irradiation on the Voltage-Dependence of the Steady-State Inactivation

In the case of continuous irradiation a shift of the curve $h_\infty(V)$ along the voltage axis was

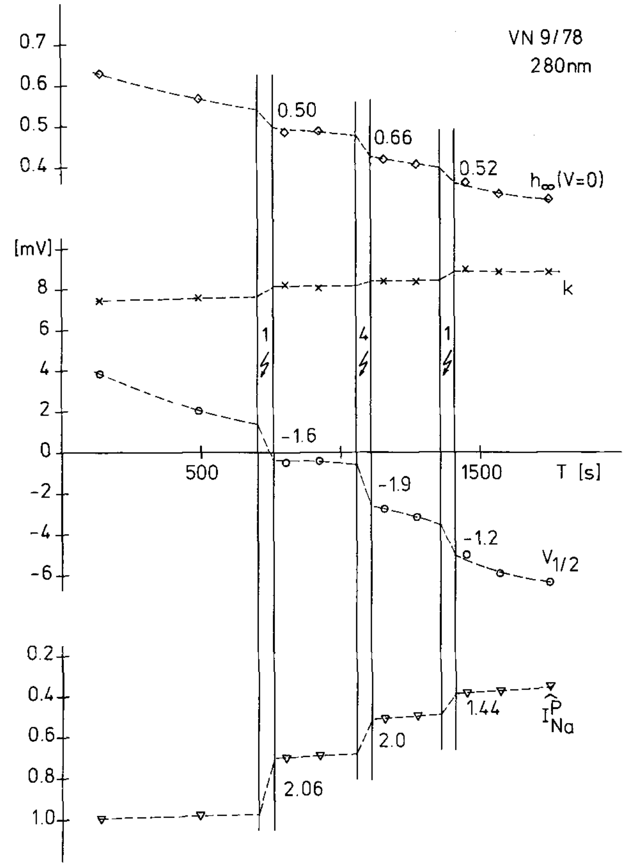

Fig. 6. Typical experiment to determine the UV radiation effect (high power flashes) on the sodium inactivation $h_\infty(V)$. Single $h_\infty(V)$-curves were registered and I^p_{Na} was measured before and after each irradiation and the parameters $h_\infty(V=0)$, k, and $V_{1/2}$ were determined (*see also* Fig. 2). Normalized values of the sodium current are displayed: $\bar{I}^p_{Na}(t)=I^p_{Na}(t)/I^p_{Na}(t=0)$. The numbers within the graph represent the evaluated sensitivities γ^{Na} and γ^h (cm^2/W sec), respectively. Dose: 0.15 W sec/cm^2 per irradiation period

observed (Schwarz & Fox, 1977). This finding was examined anew using discontinuous flash irradiation. Before and after every irradiation period several complete curves $h_\infty(V)$ were measured and function (2) fitted to them. The parameters $h_\infty(V=0)$, $V_{1/2}, k$ and, in addition, the time course of the peak sodium current are presented in Fig. 6.

For I^p_{Na} and $h_\infty(V=0)$ the rate constants γ^{Na} and γ^h were determined from these data as described above. The radiation-induced changes of the parameters $V_{1/2}$ and k are given in mV corrected for the spontaneous effect.

For quantitation 24 fibers were irradiated at excitation states 1 and 4 which produced the most pronounced differences (Fig. 7). By every irradiation $h_\infty(V=0)$ and $V_{1/2}$ were reduced corresponding to a shift of the curve to the left. In addition, a small increase of k was observed indicating a slight slope change of the curve $h_\infty(V)$. These results are given in Fig. 7.

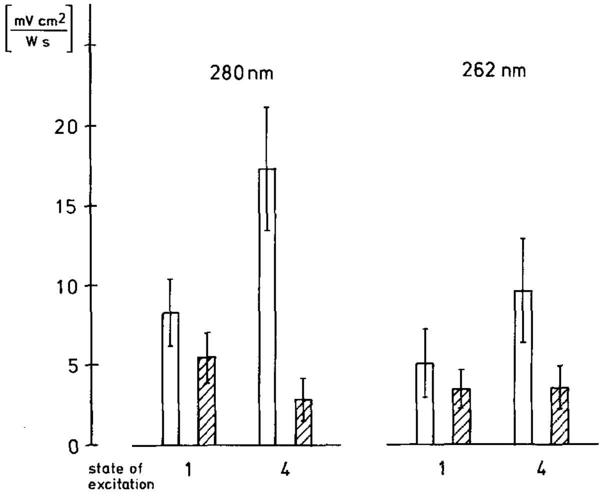

Fig. 7. Effect per unit dose (D) of UV flash irradiation of 280 and 262 nm on the parameters $V_{1/2}$ and k of the steady-state inactivation curve as determined from experiments as shown in Fig. 6. The effects at state 1 and state 4 of excitation are compared. Open bars: $-V_{1/2}/D$; hatched bars: k/D

Discussion

The Blocking of Sodium Channels

The reduction of the peak sodium current by ultraviolet irradiation is equivalent to a reduction of the maximum sodium conductance $\overline{g_{Na}}$. This parameter can be interpreted as the product of channel number and single-channel conductance. Several findings support the assumption that UV irradiation diminishes the number of sodium channels without altering the conductance of the remaining channels: No alterations of the selectivity could be detected after irradiation (no change of the sodium reversal potential) and the kinetics of the sodium system remained unchanged (Fox & Stämpfli, 1971; Fox, 1974). In addition, with continuous irradiation the sodium conductivity decreases exponentially with dose. Therefore first-order kinetics ("one-hit-events") can be assumed (Fox & Stämpfli, 1971). The total irradiation energy released during a flash of 10 µsec duration was in the order of the irradiation dose during one second of continuous irradiation in the experiments of Schwarz and Fox (1977). The blocking effect obtained in both cases is of the same magnitude, though the radiation intensity (dose rate) during the flashes was at least 5 orders of magnitude higher. This finding supports the view of a first-order kinetics, since the radiation effect is apparently independent of dose rate.

As a response to a depolarizing voltage step, the subunits responsible for the opening and closing of the sodium channel pass through certain conformations in which they exhibit different sensitivities to UV radiation. A sodium channel can be destroyed less easily if its "*h*-gate" is open. If, at the same time, all subunits of the "*m*-gate" are open, a further decrease in sensitivity is observed. This holds for both wavelengths (280 and 262 nm).

Fox, Neumcke, Nonner and Stämpfli (1976) have found that the displacement current correlated with the sodium current reacts on UV irradiation half as sensitive as the maximum sodium conductance $\overline{g_{Na}}$ while the kinetics of the displacement current remains unchanged. The simplest explanation for this finding is (if one assumes the existence of physical "*m*-" and "*h*-particles"): A sodium channel ceases to work if one of its two *m*-units is hit. The blocking of sodium channels thus might be explained by the destruction of *m*-gates. From there it would follow that a certain interaction takes place between the structures of activation and inactivation, e.g. that the state of the *h*-gate influences the probability to destroy the *m*-gate by UV radiation. This conclusion is supported by recent measurements of displacement currents by Nonner (1980) indicating a functional coupling of *m* and *h* in contrast to the hypothesis of Hodgkin and Huxley.

The wavelength dependence of the blocking effect due to discontinuous (flash) or continuous irradiation at excitation state 1 is very similar: the sensitivity at 262 nm is 0.64 of that at 280 nm.

Effect of UV Flash Irradiation on the Sodium Inactivation

As was concluded by Schwarz and Fox (1977) two different mechanisms of UV radiation appear to be responsible for the "blocking" effect on $\overline{g_{Na}}$ and for the effect on the inactivation, respectively. The blocking effect appears to be the same as well under discontinuous as under continuous irradiation.

However, three major differences were observed comparing the effects of discontinuous (flash) and continuous UV irradiation on the sodium inactivation of the nodal membrane:

1. A slope change occurs in addition to the radiation-induced shift of $h_\infty(V)$ (*see* Fig. 2) which does not occur under continuous irradiation (Schwarz & Fox, 1977). (Strictly spoken, the parameter $h_\infty(V=0)$ represents not only parallel shifts of $h_\infty(V)$. Deriving Eq. (2) it can be shown, however, that the relative UV sensitivities γ_x^h/γ_1^h practically depend only on $V_{1/2}$.)
2. Discontinuous (flash) irradiation of 280 nm produces a more pronounced shift of $h_\infty(V)$ than 262 nm (*see* Fig. 5; legend). The opposite is true for continuous irradiation (Schwarz & Fox, 1977).
3. Discontinuous (flash) irradiation produces a more pronounced shift of $h_\infty(V)$ at state 4 of excitation than at state 1 (*see* Fig. 7). State 4 corresponds with a depolarization of the nodal membrane. Schwarz and Fox (1977), on the other hand, reported a decrease of the UV-induced shift due to a depolarizing holding potential.

The explanation for these discrepancies may be obtained from the extreme differences in irradiation dose rate and from the differences in membrane polarization changes.

Though the average doses applied are of the same order of magnitude in both the continuous and the discontinuous irradiation experiments, the latter include dose rates being higher by 5 orders of magnitude. For the blocking effect a "one-hit-event" kinetics was made probable (Fox & Stämpfli, 1971) and no differences were found for continuous and discontinuous irradiation (*see above*). However, no such first-order kinetics was established for the UV effects on the sodium inactivation. The discrepancies observed with respect to slope change and wavelength dependence of the UV-induced shift lead to the hypothesis that more than one reaction step is involved in the radiation effect, thus explaining the dose rate dependence.

Differences in the radiation effect due to short time and long-term polarization are not unexpected, since changes of the holding potential are followed by, e.g., changes in ultraslow inactivation (Fox, 1976), while depolarization of msec during the excitation cycle does not involve ultraslow inactivation.

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