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ELECTROPHYSIOLOGICAL MODIFICATIONS INDUCED BY THE FLUORESCENT PROBE, PYRENE, ON *MYXICOLA* GIANT AXONS

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Summary

Current- and voltage-clamp experiments on *Myxicola* giant axons labelled with pyrene showed decreased Na^+ and K^+ conductances. The low-frequency membrane capacity and the gating charge transfer were slightly reduced. It may be inferred that pyrene is incorporated in some hydrophobic membrane domains close to the ionic channels

It has been postulated that membrane fluidity might play a role in nerve excitation [1,2]. Now, the fluorescent hydrophobic probe, pyrene, has proved to be a useful tool for monitoring membrane fluidity [3,4] and has allowed us to show, on stimulated and labelled nerve fibres, transient changes of pyrene monomer and excimer emissions [5]. Unlike pyrene, the fluorescent probes already used by others workers [6] to obtain fluorescent signals during nerve excitation are not directly relevant for the membrane fluidity changes. They were also found to be non-toxic and potential-dependent. Before the investigation of pyrene fluorescence signals during voltage-clamp experiments, it was important to carry out an electrophysiological analysis to decide if the probe induced some functional perturbations.

Giant axons from the annelid sea-worm, *Myxicola infundibulum*, were dissected and current- or voltage-clamped [7]. Modifications included an adjustable compensation for the series resistances. Gating charge transfer was measured from the asymmetry current yielded by the single-sweep procedure [8]. A separate set of impedance experiments, at resting membrane potential, has also been performed according to the method described for the squid giant axon [9]. As the effects of pyrene were particularly obvious with extensive labelling and since the latter required a long incubation (up to 2 h) with slight agitation at room temperature, the behaviour

of these labelled axons was compared to that displayed by a set of 'control axons' subjected to the same treatment, but in the normal physiological medium i.e., artificial sea-water. It must be stressed that these conditions were the standard procedure which had to be used for recording nerve extrinsic fluorescence either in the steady-state or during excitation. At each stage, the good condition of the fibres was tested by recording external diphasic action potentials. Labelling procedure using bovine serum albumin-pyrene complexes and fluorescence measurements were performed as already described for crab leg nerves [10]. The extent of labelling was assessed by the pyrene monomer to excimer emissions ratio (I_e/I_m) yielded from the fluorescence spectra of those labelled axons which had been subjected to the electrophysiological analysis.

Fig. 1 summarizes the results obtained under current-clamp conditions. The membrane potential responses to short depolarizing shocks are shown (Fig. 1a, b, c). The two upper oscilloscope records compare typical examples of action potentials for the most labelled axons (b) with the control (a). Note the reduced and wider spike, the increased threshold and the negative post-potential. In curve c, it is shown that the spike amplitude attained a maximum reduction (approx. 20%) as soon as I_e/I_m amounted to 0.15. On average, there was little or no alteration in resting membrane potential. The incubation of the axons with bovine serum albumin alone, at a concentration in artificial sea-water corresponding to the highest value used for the labelling solution (0.60 mg/ml), did not induce significant effects. The lower part of the figure (Fig. 1d) shows the membrane capacity, C_M, plotted as a function of frequency, the range of which was limited to 20 kHz because of larger relative errors at higher frequencies. Data are corrected for the series resistances [9], being estimated as 20 $\Omega \cdot cm^2$. On average, it appears that C_M decreased by 10% or the frequency-dependent part by 20%. This is only obvious for low frequencies as both curves tend to superimpose above 10 kHz.

Fig. 2a and b shows the voltage-clamped membrane currents at 0 mV, for a *Myxicola* giant axon in the control solution (a) and then for this same axon, 40 min after external exposure to 1 ml of the most concentrated solution of bovine serum albumin-pyrene in artificial sea-water (b). In both records, the temperature was first 8°C and then it was shifted to 18°C yielding the larger traces. The main effects of pyrene incorporation are clearly visible, i.e., reductions of Na^+ and K^+ conductances, especially marked at the higher temperature. The remaining conductances amounted to 75 and 50%, respectively, for g_{Na^+} and g_{K^+} at 18°C. The temperature coefficient (Q_{10}) for g_{Na^+}, the Na^+ conductance, fell from the control value of 1.5 in (a) to 1.25 in (b). This last figure is surprisingly close to Q_{10} of I_e/I_m (1.29 in the same temperature range) as measured from fluorescence experiments (unpublished results). Fig. 2 also indicates the lack of effect on the Na^+ conductance kinetics. Indeed, neither the time-to-peak nor the time course of the early current decay (and consequently, neither τ_m nor τ_h, the Na^+ activation and inactivation time constants, respectively) were significantly affected.

In order to see if the partial inhibition of the Na^+ conductance was due to a decreased gating charge transfer, we attempted measurements of

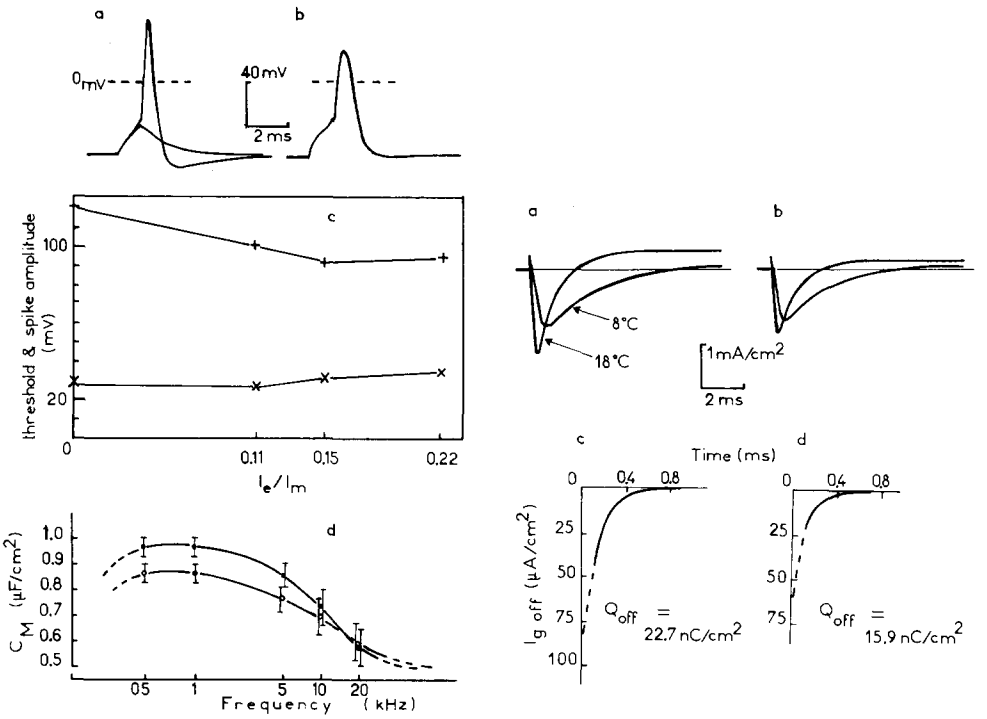

Fig. 1. *Myxicola* giant axons under current-clamp conditions. Room temperature 18—20° C. After incubation with bovine serum albumin-pyrene complexes, the labelled fibres were washed twice for 30 min in K^+-free artificial sea-water. (a) Resting potential level and space-clamp action potential from a 'control' axon; the just sub-threshold response is also shown. (b) The same parameters for an extensively labelled axon (I_e/I_m = 0.22). Graph c summarizes the mean behaviour found as result of three increasing pyrene labellings. The action potential amplitude (+) and the threshold (X) are shown. Graph d is a plot of membrane capacity, C_M, as a function of frequency. The peak-to-peak amplitude of the sinusoidal oscillations of membrane potential was settled at 4—6 mV. Only the frequency-dependent part of C_M (i.e., above 0.5 μF/cm²) is shown. Data are corrected for the series resistances, R_S = 20 Ω•cm², estimated from the high-frequency limit of resistance: (●) averaged from three raw axons as a control and (○) averaged from the same number of pyrene labelled axons (I_e/I_m approx. 0.20). Vertical bars show the standard error of the mean.

Fig. 2.(a,b) Membrane currents for a depolarizing pulse to 0 mV (*Myxicola* giant axon) at 8 and 18° C: (a) control in K^+-free artificial sea-water and (b) 40 min after exposure to the most concentrated bovine serum albumin-pyrene solution ($5 \cdot 10^{-5}$ M in artificial sea-water). Records corrected for leak which was unaffected. Holding potential V_H=—80 mV; compensation for R_S (10 Ω•cm²). (c,d) Off-asymmetry currents, respectively, for a control and a pyrene-labelled axon, both bathed in K^+-free artificial sea-water + 3 μM tetrodotoxin + 10 mM 4-amino-pyridine. Holding potential V_H=—100 mV and amplitude of voltage steps = ±100 mV. The charge transfer, Q_{off} (return of gating charges to their original position), has been calculated as the area under the trace extrapolated to zero time (end of pulses): temperature 8° C.

asymmetry or displacement current by a single-sweep subtraction of currents yielded by a pair of opposite voltage pulses. Because the off-current trace was found to be less noisy and somewhat more stable than the on-current, we chose to calculate the charge transfer, Q_{off}, assuming for this pulse width (1 ms) that $Q_{off}=Q_{on}$. Fig. 2c and d shows that the ratio, Q_{off}(labelled)/Q_{off}(control), was approx. 0.65. There is good agreement between this figure and the impedance results. As a matter of fact, if one is interested by the relative change of the capacity component, $C_{M(LF-HF)}$, responsible for the low-frequency (LF) dispersion and hypothetically specific

of the proteinous channels, it was found (Fig. 1d) that $C_{M(LF-HF)}$-(labelled)/$C_{M(LF-HF)}$(control) = 0.75—0.8.

On the whole, there seems to be no doubt that pyrene exerts some irreversible constraints on membrane conductances and on the gating charges, possibly by immobilization of a fraction of them (up to 30%). These effects resemble those displayed by some local anaesthetics like procaïne [11]. The lipid bilayer in which the 'voltage-sensors' of excitable channels have been assumed to protrude [2] seems to be the main candidate for pyrene inclusion, according to its solubility characteristics, although one cannot exclude a direct incorporation into some channels' hydrophobic sites. Further experiments are in progress to decide the ultimate mechanism (block of a fraction of the channels' population or reduction of single channel conductance) and to eventually correlate these electrophysiological effects with structural perturbations. It may already be inferred that pyrene incorporation does not significantly affect membrane thickness, since the high-frequency limit of membrane capacity appeared unchanged. These results differ from those obtained with squid giant axons and with solvent-free phosphatidylcholine-cholesterol treated with *n*-alkanes [12,13]. As regards to the search for specific fluorescence signals and the involvement of membrane fluidity in the excitation process, as still postulated recently [14,15], the above reported sensitivity of excitable channels towards pyrene may be considered as positive information although it stresses some limitations, particularly for the concentration range, in the use of the 'probe methods'.

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