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A DYNAMIC X-RAY DIFFRACTION STUDY OF ANESTHESIA ACTION

THICKENING OF THE MYELIN MEMBRANE BY *n*-PENTANE

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Summary

The structural changes induced in the myelin sheath by *n*-pentane nerve impulse blockage were studied by small-angle X-ray diffraction using a linear position-sensitive detector. The results show that the thickness of the myelin period lattice increases from 170 to 180 Å during *n*-pentane treatment.

Since the turn of this century the mechanism of action of anesthetics has been investigated a great deal [1—3]. However, the crucial question of how anesthetics act at a molecular level in an excitable membrane has not been answered yet.

One of the current theories of anesthesia was proposed by Seeman [2] who postulated that anesthetics and other nerve blocking drugs adsorb to hydrophobic regions of excitable membranes, thus, expanding the volume and area of the hydrophobic domains of membrane proteins and lipids, causing a blocking of the ionic channels responsables for action potentials. Recently, Haydon et al. [4] proposed a molecular mechanism for the anesthesia produced by *n*-alkanes. It states that during this process a thickening of the bimolecular lipid region of axonal membranes occurs which destabilises the ionic channels formed during electrical excitation.

In order to advance further the knowledge of the molecular mechanism of anesthesia it is desirable to analyze the phenomenon in excitable membranes with appropriate powerful physical techniques such as X-ray diffraction. Unfortunately, these experiments are not easy to realize since excitable membranes are not ordered. Nevertheless, it is possible to obtain structural

information related to this process by studying the effect of anesthetics on a favorable membrane system such as the nerve myelin sheath which is naturally ordered, and its structure has already been determined at high resolution by X-ray diffraction [5,6]. Besides that, myelin is also involved in the phenomenon of nerve conduction [7].

With the purpose to analyze the structural changes induced in myelin during *n*-alkane nerve impulse blockage, freshly dissected frog sciatic nerves (*Rana pipiens*) were desheathed, divided into two similar segments and mounted in a specimen holder with two compartments having separate perfusion systems. This set up facilitates collecting X-ray diffraction patterns from a nerve perfused with normal Frog Ringer saturated with *n*-pentane and its control perfused with normal Ringer's solution. During the perfusion, a series of X-ray diffraction patterns (34 Å spatial resolution) was alternatively recorded from both nerve pieces with a linear position-sensitive detector [8]. The X-ray pattern modifications of different nerve specimens exposed to *n*-pentane-containing Ringer's showed considerable time course variability depending on nerve dimensions and flow rate of perfusion. However, the control nerve, perfused at the same flow rate and with similar dimensions never showed any variation in their X-ray diffraction pattern, over the same period of time. The experiment reported here (Fig. 1, upper part) was particularly slow.

The experiment was initiated with the recording of a 15 min X-ray diffraction pattern from each segment of sciatic nerve perfused in normal Frog Ringer at 23°C. These patterns were found to be identical to each other and similar to those previously reported by other groups [5,6,9]. They show the first five reflections of a 170 Å one-dimensional unit cell characteristic of frog sciatic myelin. Immediately after recording these controls the perfusion medium of one of the nerves was changed to Ringer containing *n*-pentane and sequential X-ray diffraction patterns were recorded one after the other for periods of 15 min from each nerve segment.

During the first 12 h of the perfusion with the *n*-pentane-containing solution in the X-ray pattern were detected. After this time significant structural modifications in the *n*-pentane-treated nerve started without changes in the control nerve. At 18 h the lattice period in the *n*-pentane-treated segment increased to 172 Å and simultaneously a continuous variation in the relative intensity of the reflections was observed: the first, second, third and fifth orders were enhanced while the fourth order was diminished as compared to the pattern at 0 h (see Fig. 1). The repeat period of the treated nerve continued to increase, at 25 h it measured 180 Å. Simultaneously, a further modification of the relative intensities was observed: the odd orders continuously decreased whereas the even orders increased. The increase in the repeat distance was paralelled by a decrease in the diffracting power P (see Table I) which was calculated from the integrated intensity $I(h)$ of the reflections [10] The X-ray pattern at 25 h indicated there was about 50% as many ordered membrane pairs as in the pattern at 0 h (compare the spectra at 0 and 25 hours in Fig. 1). The control nerve which was perfused with Ringer's solution for 25 h exhibited an X-ray pattern identical to that recorded at 0 h, that is to say its 170 Å lattice did not change over this lapse of time.

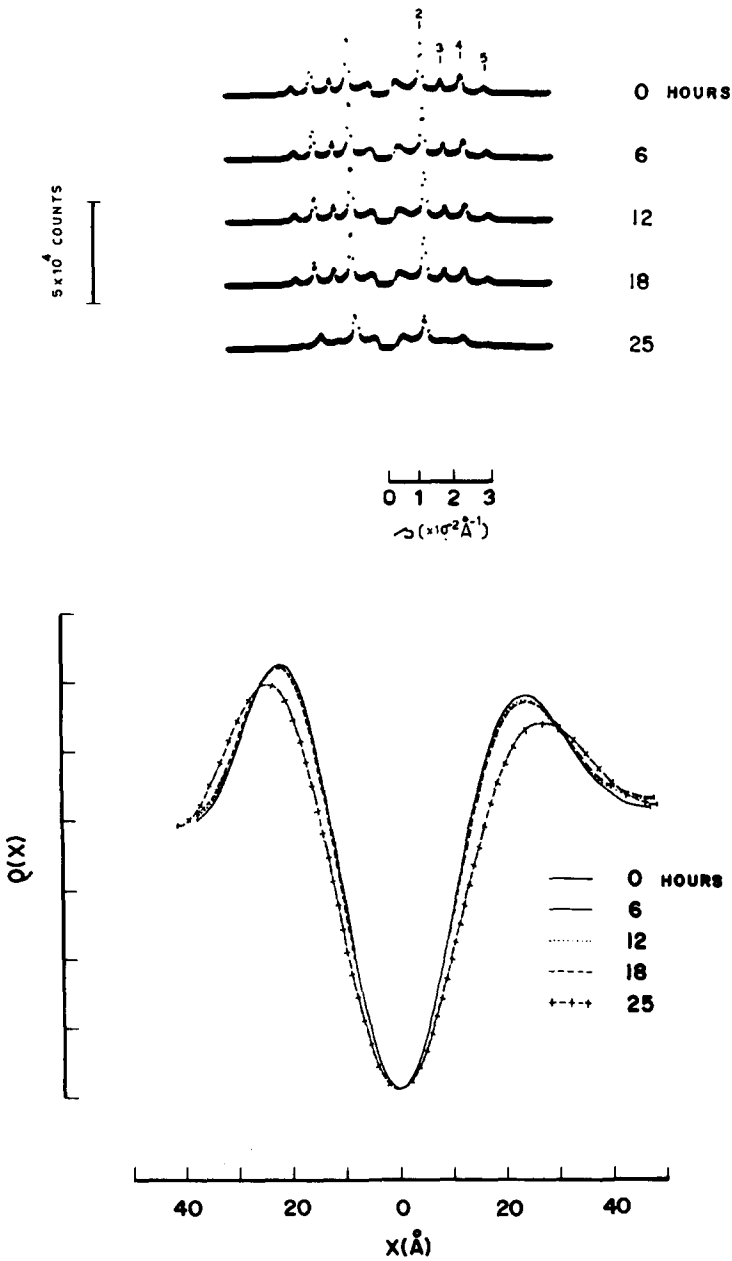

Fig. 1. Upper part. X-ray diffraction patterns from a frog sciatic nerve showing the kinetics of myelin structure modifications produced by perfusing the nerve with normal Ringer's solution saturated with *n*-pentane. The measurements were done for counting periods of 15 min using a position sensitive detector. Times indicated in the figure represent the onset of each data collection. Over the first 12 h of perfusion the spectra were very similar to each other showing Bragg reflections $h = 1$—5 from a 170 Å period lattice. The spectrum at 25 h shows an increase in the lattice dimension and decrease in the intensities of the reflections. $s = 2 \sin \theta / \lambda$ (2θ = diffraction angle, λ = wavelength (1.54 Å)). Lower part. Myelin bilayer profiles calculated from the patterns shown in the upper part of the figure with the phasing indicated in the text. The profiles have been normalized to constant diffracting power (see legend to Table I) and arbitrary matched at their low electron density centers.

TABLE I

CHANGES INDUCED IN MYELIN BILAYERS THICKNESS d_1, UNIT CELL DIMENSIONS d, STRUCTURE FACTORS $F(h)$ AND RELATIVE DIFFRACTING POWER $P(t)/P(0)$ BY *n*-PENTANE ANESTHESIA

The structure factors $F(h)$, $F = |hI(h)|^{1/2}$, $h = 1-5$, have been calculated from the integrated intensity $I(h)$ measured of reflections from X-ray diffraction patterns shown in Fig. 1 (upper part). The values were normalized by the $P(0)/P(t)$ ratios (inverse of the relative diffraction power).

$$P(t) = (\Sigma_h I(h))/d^2$$

The thickness of the single membrane unit (d_1) is measured as indicated in the text. The parameters shown in the table at time 0 h correspond to X-ray diffraction patterns from both nerve segments at 0 h (see text) and that recorded from the control nerve after 25 h perfusion with normal Ringer's solution since the three spectra were identical to each other.

Time (h)	Structure factors					$P(t)/P(0)$	d (Å)	d_1 (Å)
	$F(1)$	$F(2)$	$F(3)$	$F(4)$	$F(5)$			
0	0.12	1.41	0.80	1.32	0.72	1.00	170	45.9
6	0.12	1.42	0.80	1.31	0.72	0.99	170	46.1
12	0.13	1.44	0.80	1.27	0.74	0.98	170	46.2
18	0.14	1.45	0.82	1.26	0.75	0.87	172	46.5
25	0.09	1.65	0.47	1.41	0.51	0.46	180	51.7

The electron density profiles $\rho(x)$ shown in Fig. 1 (lower part) have been determined from the X-ray diffraction patterns of *n*-pentane treated nerve shown in Fig. 1 (upper part). The corresponding structure factors $F(h)$ and lattice dimensions (d) are indicated in Table I. Since none of the 5 reflections fell down to zero during the experiment, it was plausible to assume that their phases remained unchanged. Therefore, the profiles were all calculated with the same sequence of signs (—, +, +, —, —). This combination has been previously established by other workers [5,6,9,11]. Variations in the membrane thickness d_1 were determined from each electron density profile by measuring the distance between the two high electron density peaks of one of the bilayers. These values are also indicated in Table I.

During the first 12 h after perfusing the nerve with alkane containing solution the thickness of the myelin single membrane increased by less than 1 Å (from 45.9 to 46.2 Å) without changes in the lattice. After prolonged perfusion (25 h) with the same solution it increases up to 51 Å suggesting that *n*-pentane has been incorporated into the hydrocarbon region of the lipid bilayer.

The preliminary experiments reported here demonstrate that the X-ray diffraction patterns sequentially recorded from a frog sciatic nerve perfused with Ringer's solution saturated with *n*-pentane show progressive changes which have been interpreted as due to a significant increase in the unit membrane thickness. However, the thickening effect of pentane on myelin is too slow (more than 12 h) as compared with the impulse conduction blockage effect on the excitable membrane (less than 1 h) [4]. This in a way is not surprising since it has to be expected that the effects on *n*-pentane on nerve conduction have to be very much faster than on myelin structure since the nodes of Ranvier (where the action potential generates) are much more accesible to the external solutions than myelin.

Summarizing, it has been shown that myelin, a membrane system asso-

ciated with the excitable membrane increases its thickness when exposed to n-pentane-containing solution (as suggested by the thickness-tension hypothesis of Haydon et al. [4]). However, a direct relationship between the observed structural changes and the nerve impulse blocking action of *n*-pentane can not be unambiguously infered from the present results.

References

1 Mullins, L.J. (1971) in Handbook of Neurochemistry (Lajtha, A., ed.), Vol. 6, pp. 395—421, Plenum, New York
2 Seeman, P. (1972) Pharmacol. Rev. 24, 583—655
3 Fink, B.R. (1975) Progress in Anesthesiology, Vol. 1, Raven Press, New York
4 Haydon, D.A., Hendry, B.M., Levinson, S.R. and Requena, J. (1977) Nature 268, 356—358
5 Caspar, D.L.D. and Kirschner, D.A. (1971) Nat. New Biol. 231, 46—52
6 Worthington, C.R. and King, G.I. (1971) Nature 234, 143—145
7 Stampfli, R. (1954) Physiol. Rev. 34, 101—112
8 Gabriel, A. and Dupont, Y. (1972) Rev. Sci. Inst. 43, 1600—1602
9 Blaurock, A.E. (1971) J. Mol. Biol. 56, 35—52
10 Kirschner, D.A. and Caspar, D.L.D. (1975) Proc. Natl. Acad. Sci. U.S. 72, 3513—3517
11 Moody, M.F. (1963) Science 142, 1173—1174