Biochimica et Biophysica Acta, 649 (1981) 581–586
Elsevier/North-Holland Biomedical Press

BBA 79494

KINETICS OF NERVE IMPULSE BLOCKING BY PROTEIN CROSS-LINKING ALDEHYDES

APPARENT CRITICAL THERMAL POINTS

DORU-GEORG MĂRGINEANU [a,*], EVA KATONA [b] and JUNONA POPA [a]

[a] *Biophysics Laboratory, Faculty of Biology, University of Bucharest, Spl. Independentei 91-95, Bucharest 76201 and* [b] *Biophysics Laboratory, Medical Pharmaceutical Institute, Blv. Petru Groza 8, Bucharest 76241 (Romania)*

(Received June 30th, 1981)

Key words: Aldehyde; Action potential; Protein cross-linking; Amino group; Ionic channel; (Frog Sciatic nerve)

The effect of formaldehyde, crotonaldehyde, butyraldehyde, glutaraldehyde and cinnamaldehyde on the compound action potential of frog sciatic nerve was studied in the temperature domain 20–35°C at various aldehyde concentrations. All these reagents gradually decrease the amplitude of nerve action potential, up to the complete block, the order of effectiveness being: crotonaldehyde > cinnamaldehyde > butyraldehyde > formaldehyde > glutaraldehyde. The effect of cinnamaldehyde is almost completely reversible, while all others have irreversible action. The dependence of the blocking time on temperature and concentration is well expressed in all cases by the same empirical equation. This dependence points to the existence of critical temperatures, specific for each aldehyde, at which impulse blocking would be instantaneous, regardless of concentration. These temperatures (obtained by extrapolation) lie between 43°C (for crotonaldehyde) and 57.5°C (for butyraldehyde). The existence of free amino groups within ionic channels, as main sites of aldehyde attack, is inferred.

Introduction

Studies aimed at revealing the molecular bases of nerve membrane functioning almost exclusively deal with specific labeling, then extraction and characterization of membrane components acting as ionic channels (for review and discussion see Refs. 1 and 2). Though this approach is fully rewarding, in order to get a precise functional image of the axonal membrane at molecular level, it must be complemented by in situ chemical modification of the membrane and the correlation of chemical specificity of various reagents with their effects on nerve activity. As it is clear that the membrane components involved in impulse generation are chiefly of proteic nature [3,4], a wealth of data about the effects on nerve of various proteic reagents accumulated [5–8]. However, the effects of typical protein cross-linking reagents such as the aldehydes were checked only occassionally [9]. Recently, Horn et al. [10] studied the effect of glutaraldehyde and formaldehyde on membrane currents of squid axon.

Here we report a detailed investigation of the kinetics of impulse blocking in frog sciatic nerve by a representative selection of aldehydes: formaldehyde, crotonaldehyde, butyraldehyde, glutaraldehyde and cinnamaldehyde at various concentrations and temperatures. Though the nerve trunk as a whole seems a rather obsolete biological material as compared with isolated nerve fibres, we used it for several reasons: (i) the anatomical obstructions (connective material and sheaths) slow the drug effects so that they can be better monitored; (ii) the large number of axons ($>10^3$) form a true statistically significant population adequate for such neurochemical approaches; (iii) the existence of the above-mentioned study on single axons [10], though less estended, allows comparison. On the other hand, when dealing with whole nerves, only the amplitude of the compound action

* To whom correspondence should be addressed.

potential is a really significant parameter because of the additivity of contributions from single fibres, while the temporal parameters such as durations of various phases and conduction velocity are less informative. Recently, we published an account of the effects of formaldehyde, crotonaldehyde, butyraldehyde and glutaraldehyde on these latter parameters too [11].

The data obtained appear sufficiently detailed and regular to be accomodated within a proposed empirical equation, holding for all aldehydes in the concentration and temperature domains checked. The most distinctive feature is the finding of characteristic critical temperatures, specific for each aldehyde, at which nerve impulse blocking would be independent of concentration.

Material and Methods

The sciatic nerves were taken from large male specimens of *Rana temporaria*, weighing around 50 g. The length of the dissected nerves was about 50 mm and their ends were ligatured to prevent inactivation by sodium entrance from the bathing solution.

Solutions. The nerves were initially immersed into Ringer solution whose composition, in mM, is: NaCl 110; KCl 2.5; $CaCl_2 \cdot 2\,H_2O$ 1.8; Tris-HCl 5. The aldehyde solutions were prepared, immediately before use, by diluting into Ringer the commercial glutaraldehyde (Merck); formaldehyde, butyraldehyde (Fluka) crotonaldehyde and cinnamaldehyde (Loba Chemie). Aldehyde concentrations are expressed in % (w/v) and not molar, because in the case of at least some aldehydes the polymerization state within aqueous solutions is uncertain [12]. The concentration domain for glutaraldehyde was 0.25–2.00%, for formaldehyde 0.10–0.50%, and for crotonaldehyde, butyraldehyde and cinnamaldehyde 0.01–0.10%. In each case the concentration domain was chosen in which the fall of action potential amplitude is clearly manifested but not too rapid. The pH of aldehyde-Ringer solutions ranged between 6.8 and 7.0.

Equipment and data collecting. The stimulation chamber has seven compartments formed by Plexiglass septa sealed with vaseline. In the first two from both ends there are the stimulating and recording electrodes (Pt wires), the propagation distance being 30 mm. Aldehyde solutions are introduced only in the compartments without electrodes. The whole chamber is thermostat-controlled and a Cu–Constantan thermocouple is placed in the central compartment.

The stimulating square pulses are delivered by a ST II-B No. 231 generator through a pulse separation unit No. 249 (Equipments Industriels, Paris). The stimuli were of 40 Hz frequency and 0.04 ms duration and their amplitude was taken (between 4 and 9 V) in order to obtain a full size nervogram, i.e., the maximal compound action potential. This is visualized on the screen of a Tektronix 564 B storage oscilloscope after preamplification by a R 106 A (Officine Galileo) amplifier.

The rough data essentially consist of the traces on the storage screen of the compound action potential elicited by stimulation at different time intervals following aldehyde application. Before any treatment, the height of the action potential recorded from our chamber was approx. 10 mV. The smallest readable value is around 1% of the maximal, this condition corresponding to the block of action potential. The recorded height of action potential at each moment after immersion in aldehyde was divided by the initial (control) value. The relative heights thus obtained in (at least) three experiments done under the same conditions were averaged. The standard deviation divided by the mean never exceeded 8% and is not represented on the graphs.

Results and Computations

All the aldehydes used gradually decrease the amplitude of nervogram in frog nerve, up to the complete block, the rate of this effect depending on aldehyde nature and concentration and on temperature. To illustrate the inhibitory effect of glutaraldehyde, in Fig. 1 the time evolution of action potential amplitude consecutive to nerve immersion in glutaraldehyde-Ringer is plotted, for the same aldehyde concentration at four temperatures.

The effect shows an identical curve for all the aldehydes we used, irrespective of temperature (T) and concentration (C), these parameters influencing only its rate. Accordingly, we can characterize the effect of each aldehyde at given C and T by means of temporal parameters such as the halving time of the

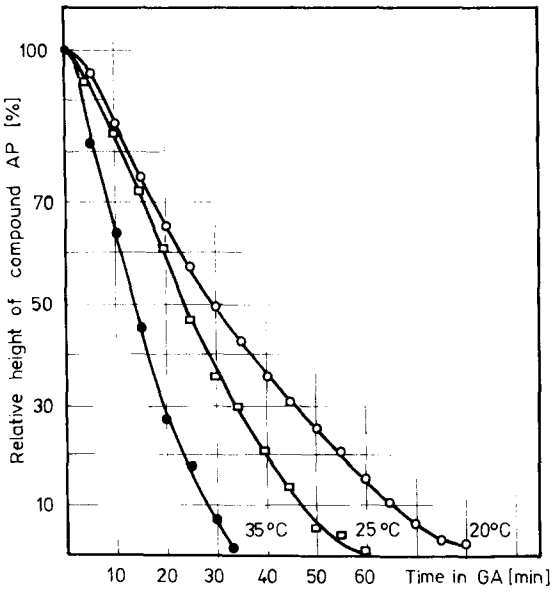

Fig. 1. The relative height of the compound action potential (AP) in frog sciatic nerve following exposure to glutaraldehyde (GA) 0.5% for various periods of time at the temperatures indicated.

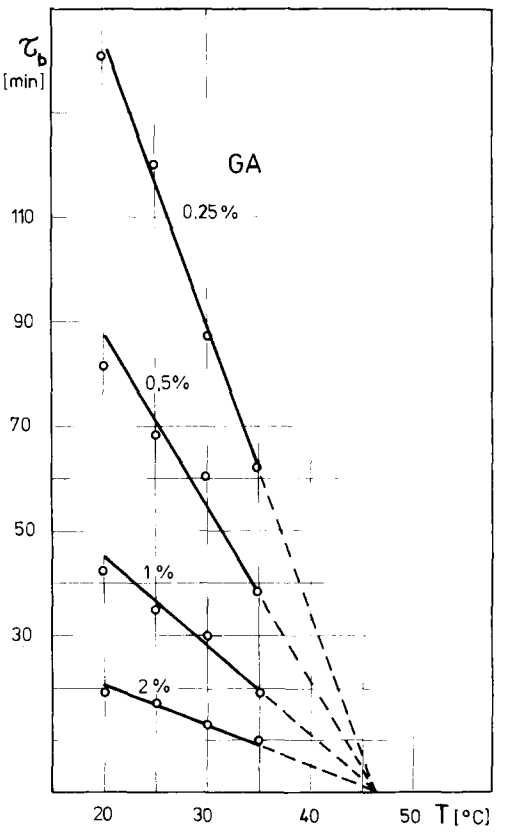

Fig. 2. The blocking time of nerve impulse (τ_b) as a function of temperature, at four glutaraldehyde (GA) concentrations given in % near each solid line.

action potential amplitude, or the blocking time (τ_b) in which the amplitude falls below the sensitivity of the equipment. Both descriptions convey essentially the same information and, in order to avoid redundant data, we shall further refer to only τ_b which directly appears from experiments and bears relevant physiological significance.

The blocking time in glutaraldehyde-Ringer solution is plotted in Fig. 2 as function of temperature. The linear dependence of τ_b on T at each aldehyde concentration is clearly apparent. If the solid lines which fit the experimental points are extrapolated, they intercept the abscissa at the same point. This appears as a critical point (T_c) characterizing the effect of glutaraldehyde on frog sciatic nerve. This quite unexpected regularity holds in the case of the other aldehydes too.

The data in Fig. 3 show that the blocking times in formaldehyde-, butyraldehyde-, and crotonaldehyde-Ringer solutions also linearly depend on T and there are such T_c values specific for each aldehyde.

The reduction in action potential amplitude up to the complete block is irreversible in all these cases, even at the smallest concentration. Only in the case of cinnamaldehyde, nerve excitability is restored almost completely upon reimmersion in normal Ringer so that the effect of a second treatment can be studied (Fig. 4).

The second blocking time is much shorter than the first one (about half of it) at the same T and C, but (when plotted together) they all reveal the same T_c, as clearly appears from Fig. 5.

The sigmoid dependence of the action potential amplitude on the immersion time (t) in aldehyde solutions, as represented in Fig. 1, is fairly described by the equation:

$$y = 1 - [1 - \exp(-t/\tau)]^n \qquad (1)$$

where y is the normalized (relative) AP amplitude and both parameters τ and n should a priori be con-

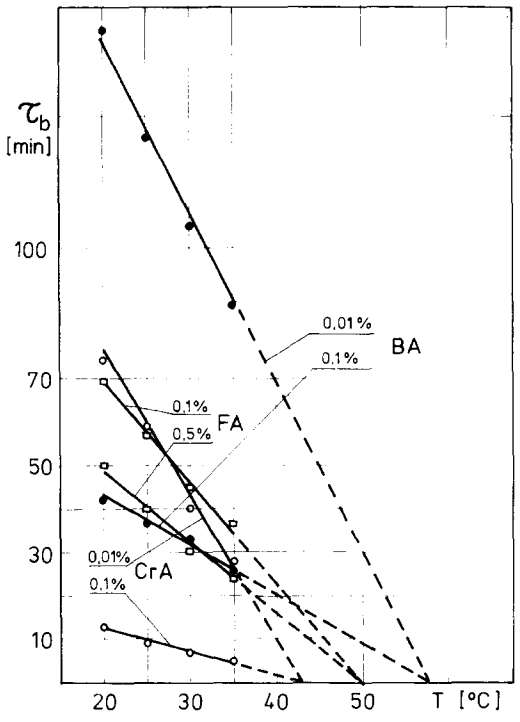

Fig. 3. The blocking time of nerve impulse (τ_b) as a function of temperature, at two concentrations of formaldehyde (FA, □), crotonaldehyde (CrA, ○) and butyraldehyde (BA, •).

sidered as depending on aldehyde type and on C and T.

However, the computer fitting of the experimental points with Eqn. 1 revealed that (within approximation limits corresponding to our experimental

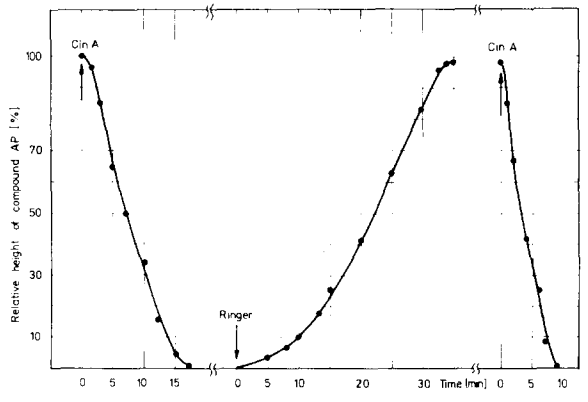

Fig. 4. The time course of the reversible effect of cinnamaldehyde (CinA) 0.1% on the relative height of the compound action potential (AP) in frog sciatic nerve at 20°C.

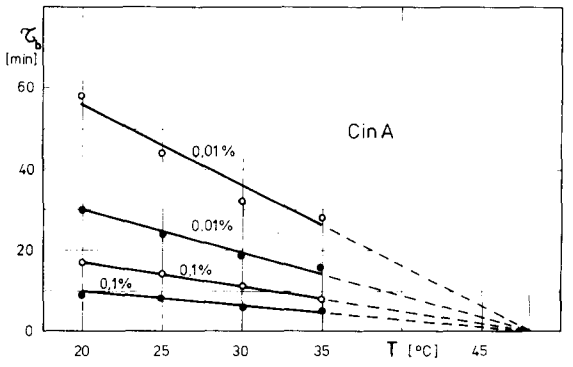

Fig. 5. The blocking time of nerve impulse (τ_b) as a function of temperature at two cinnamaldehyde concentrations at a first treatment (○) and a second one (•) following recovery.

method) the parameter n does not vary except with the type of aldehyde. If now for a given aldehyde n is set constant, irrespective of C and T, one easily obtains that the blocking time, for which $y = 0.01$, equals the parameter τ appearing in Eqn. 1 multiplied by a constant:

$$\tau_b = [-\ln(1 - 0.99^{1/n})] \cdot \tau \qquad (2)$$

This equation shows that τ will depend on C and T exactly as τ_b, experimentally obtained.

The data plotted in the Figs. 2, 3 and 5 show that the dependence of τ_b on T has in all cases the form:

$$\tau_b = K(C) \cdot (T_c - T)$$

where $K(C)$ stands for the concentration-dependent factor. Always τ_b decreases at higher C, so that a

TABLE I

EMPIRICAL PARAMETERS DESCRIBING IMPULSE BLOCKING BY ALDEHYDES IN FROG SCIATIC NERVE

Both n and α are pure (dimensionless) numbers. FA, formaldehyde; CrA, crotonaldehyde; BA, butyraldehyde; GA, glutaraldehyde; CinA, cinnamaldehyde.

	FA	CrA	BA	GA	CinA
T_c (°C)	49.5	43.0	57.5	46.5	48.0
n	2.98	1.32	1.38	1.92	1.84
α	0.23	0.78	0.53	0.95	0.54

complete empirical equation giving the dependence of τ_b on C and T can be formed as:

$$\tau_b = (A/C^{\alpha}) \cdot (T_c - T) \quad (3)$$

Here A and α, as well as T_c, depend only on the type of aldehyde. Table I lists the values of T_c, n and α.

Discussion

The reduction in action potential amplitude by aldehydes is a gross consequence of the block of ionic channels, mainly sodium channels, in full agreement with data on single axons [10]. The exact chemistry of protein cross-linking by aldehydes is not clear even in simpler in vitro cases [13] so that a fortiori it is even less in such complex situations as in situ channels of nerve fibres. However, several conclusions on the molecular aspects of nerve impulse blocking by aldehydes can be inferred from the data above.

The main sites of aldehyde attack on proteins (at neutral or slightly acid pH as in our experiments) are the free amino groups [14], so that one can infer that the ionic channels, particularly their 'selectivity filters' [2], actually bear such free groups.

Equations of the form of (1) are common for describing the survival probability as function of dose in cell populations exposed to radiations, in which each cell is killed when the incident particles hit n 'sublethal targets'. Similarly, as the relative amplitude of the compound action potential is proportional to the fraction of active fibres in the 'population' forming the nerve, the parameter n appearing here could be viewed as the mean number of 'targets' which must be 'hit' by aldehyde in order to block impulse propagation in each fibre. Though this might be a useful analogy, the real nature of 'aldehyde targets' in the nerve still remains rather obscure. Anyhow, from Table I it should be noted that the largest n corresponds to formaldehyde, which is the simplest aldehyde molecule, and that butyraldehyde and crotonaldehyde, which do not differ but by a double bond, have very close n values.

Phenomenologically T_c is the temperature at which nerve impulse blocking by a given aldehyde would be instantaneous, regardless of concentration, and it is evidence of how pronounced is the effect of temperature. T_c values are specific for each aldehyde, thus probably characterizing the chemical changes induced in the ionic channels. It is noteworthy that all T_c's lie in the domain where thermal denaturation of proteins occurs, this being suggestive of the nature of molecular changes produced by aldehydes.

Because τ_b^{-1} is the rate of impulse blocking and $\tau_b^{-1} \sim C^{\alpha}$, the parameter α expresses the apparent stoichiometry of aldehyde interaction with its targets in the nerve; it also indicates how steep is the effect of concentration. Only in the case of glutaraldehyde does α approach unity.

From the data in Figs. 2, 3 and 5 one can establish the order of effectiveness of aldehydes, showing how fast is impulse blocking at the same T and C. This order is: crotonaldehyde > cinnamaldehyde > butyraldehyde > formaldehyde > glutaraldehyde. As no obvious correlation with the molecular weights of aldehydes appears, this order does not reflect mere differences in diffusion rate, but the chemical blocking potency. Notice that, rather intriguingly, glutaraldehyde has the least blocking potency though, strictly speaking, it is the only true cross-linking reagent among the aldehydes we used, having two lateral carbonyl groups. Also, it should be mentioned that the only aldehyde (in the group investigated) whose blocking effect on nerve impulse is reversible is that having a benzene ring, the cinnamic aldehyde.

If confirmed in the case of other nerves too, and completed for an even larger group of aldehydes, these observations would seem able to substantiate theoretical chemical developments on aldehyde – ionic channel molecular interactions.

Acknowledgement

Thanks are due to Professor V. Vasilescu for facilities in his department, to Dr. Eva Turcu for a gift of chemicals and to Mr. Mircea Marinescu for help with computations.

References

1 Hucho, F. and Schiebler, W. (1977) Mol. Cell. Biochem. 18, 151–172
2 Hille, B. (1978) Biophys. J. 22, 283–294
3 Nachmansohn, D. (1978) In Neuronal Information Transfer (Karlin, A., Tennyson, V.M. and Vogel, H.J., eds.) Academic Press, New York
4 Schoffeniels, E. and Dandrifosse, G. (1980) Proc. Natl. Acad. Sci. USA 77, 812–816

5 Keana, J.F.W. and Stämpfli, R. (1974) Biochim. Biophys. Acta 373, 18–33
6 Marquis, J.K. and Mautner, H.G. (1974) J. Membrane Biol. 15, 249–260
7 Shrager, P. (1975) Ann. N.Y. Acad. Sci. 264, 293–303
8 Baumgold, J., Matsumoto, G. and Tasaki, I. (1978) J. Neurochem. 30, 91–100
9 Shrager, P.G., Strickholm, A. and Macey, R.I. (1969) J. Cell. Physiol. 74, 91–100
10 Horn, R., Brodwick, M.S. and Eaton, D.C. (1980) Am. J. Physiol. 238, C127–C132
11 Mărgineanu, D.-G., Katona, E. and Popa, J. (1981) Arch. Int. Physiol. Biochim. 89, 159–165
12 Richards, P.M. and Knowles, J.R. (1968) J. Mol. Biol. 37, 231–233
13 Peters, K. and Rochards, P.M. (1977) Annu. Rev. Biochem. 46, 523–551
14 Ziegler, K., Schmitz, I. and Zahn, H. (1977) Adv. Exp. Med. Biol. 86A, 345–354