

A QUANTITATIVE ANALYSIS OF COMBINED POTENTIAL AND CURRENT CLAMP EXPERIMENTS ON THE SINGLE MYELINATED NERVE FIBRE OF *RANA ESCULENTA*

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(Received April 16, 1981)

Abstract

Action potentials and membrane currents were recorded on the same myelinated nerve fibre of *R. esculenta*. During the running of the experiment all permeability variables were evaluated from clamp currents by computer on the basis of the Frankenhaeuser–Huxley equations. With the extracted data action potentials were computed for the same nerve fibre under investigation. This way the validity of the underlying equation system was tested in predicting membrane currents and action potentials for the single, individual nerve fibre. The standard data for *R. esculenta* correspond satisfactorily with those for *X. laevis*, in Ringer's solution, as well as in solutions with 3·1 nM tetrodotoxin in order to reduce the sodium currents, at 20°C. The same treatment revealed a discrepancy between the calculated action potential and the recorded one when an external solution with 10 mM tetraethylammonium chloride was applied, abolishing the potassium currents. The duration of the measured action potentials was about 2·5 times longer than that predicted, showing deficiencies in the system of equations with respect to the description of sodium permeability.

1. Introduction

In 1952 Hodgkin and Huxley described a quantitative analysis of potential clamp measurements on the unmyelinated axon of the squid, yielding a set of simultaneous differential equations in time with potential dependent rate constants. From these data action potentials were calculated, which showed good agreement with those measured. Later on, potential clamp experiments were performed on the myelinated nerve fibre of *Xenopus laevis* (Dodge and Frankenhaeuser, 1958) which resulted in an equivalent formal treatment for computing action potentials

*This work was supported by the Deutsche Forschungsgemeinschaft (Br 310/14).

on the nodal membrane (Frankenhaeuser and Huxley, 1964). For every complete potential clamp analysis, however, a set of experiments on different nerve fibres was required in order to separate the currents carried by the different ionic species from the net membrane currents. This separation was usually done by analysis of experiments with different extracellular ionic activities, or by analysis of experiments with selectively blocking substances. Thus all rate constants describing the membrane permeabilities, and all 'standard data' describing the rate constants, were mean values from a series of potential clamp experiments. As such, the action potentials calculated from these standard data represented averaged results only. Up to now there was no check of the agreement between the predictions of the potential clamp analysis and the current clamp measurements for one and the same single nerve fibre.

This paper describes the results of combined potential clamp and current clamp experiments on the single, individual nerve fibre of *Rana esculenta*, yielding complete data for every preparation—in contrast to standard data which are mean values. A preceding experimental separation of sodium and potassium currents was not a prerequisite. During the experiment, action potentials were computed from the extracted data; these were compared directly with measured action potentials in the same nerve fibre. By this procedure, based on the Frankenhaeuser–Huxley equations, both potential and current clamp experiments became of equal significance for the investigation of excitation mechanisms in nerve. The compatibility of potential and current clamp results was proven in a series of experiments in Ringer's solution at 20°C. Furthermore, experiments were performed in which the sodium and potassium permeabilities were reduced or blocked by tetrodotoxin or tetraethylammonium chloride. Thus the validity of the equations underlying the potential clamp analysis was examined for the individual nerve fibre, also under modified experimental conditions.

2. Experimental methods

Definitions, nomenclature and equations

In potential clamp experiments usually the membrane potential is given as axis cylinder potential minus outside potential, and is denoted E_m ; outward membrane current is consequently positive. In experiments on the node, deviations of membrane potential V_m from its resting value E_r are measured, thus $V_m = E_m - E_r$. In order to obtain a uniform nomenclature, all potentials are given as E_m . That potential at which $h_x = 0.7$ was defined as the resting potential (E_r) and was set as $E_r = -70$ mV (Huxley and Stämpfli, 1951). Breakdown stimulation at the end of the experiments verified this value roughly. The equations used for the potential clamp analysis and for the computation of action potentials are described in detail by Frankenhaeuser and Huxley (1964). In the following the same symbols are used, new symbols are explained in the text.

Experimental setup

The experiments were performed on single myelinated motor nerve fibres of *R. esculenta* in the arrangement described in detail by Nonner (1969). The node under investigation was continuously superfused with Ringer's or test solutions. The neighbouring nodes were cut in isotonic KCl solution. A quick change between potential and current clamp was possible. The temperature of the solutions superfusing the node was adjusted to 20°C. Potential and current were converted to digital form; A/D conversion with 20 μ s sampling interval and 8-bit word length was used (Fig. 1). Before the next clamp pulse was started (repetition interval 2 s), the data were transferred to the computer and stored on magnetic tape or disk.

The membrane potential was adjusted so that $0.65 < h_{\infty} < 0.75$ and each potential step was preceded by a 50 ms prepulse of $E_m = E_r - 45$ mV. From this potential step leakage and capacity currents i_l and i_c were determined. Currents were assumed to be proportional to the potential drops across the internal resistance of one cut internode, and were converted to current densities following the procedure of Frankenhaeuser (1962a). The attenuation artifact for potential measurements was neglected in agreement with the findings of Nonner (1969).

Solutions

The Ringer's solution contained 110 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl_2 and 5 mM Tris (hydroxymethyl)-aminoethane-HCl buffered at pH = 7.3. The isotonic KCl solution (117 mM KCl) was adjusted to the same pH with 0.1 M

KOH. Test solutions with tetrodotoxin (TTX, Sankeyo, Tokyo) and tetraethylammonium chloride (TEA, Merck-Schuchardt) were prepared from stock solutions.

3. The potential clamp analysis

The leakage and capacity currents were subtracted from the measured membrane currents i_m^* ; thus

$$(1) \quad i_m^* - i_c - i_l = i_{Na} + i_K + i_p = i_m.$$

The steady-state values and time constants of the ionic permeabilities in the Frankenhaeuser-Huxley equations were fitted simultaneously to the corrected measured membrane currents i_m . The iterative algorithm (Marquardt, 1963) started from estimated values for the parameters involved, and stopped if no preset improvement in the agreement between measured and approximated curves was achieved by the following iteration step. Table 1 shows an example. For a potential step from $E_m = -115$ mV to -10 mV, the net membrane current was calculated from given steady-state values and time constants, which then had to be re-detected by iteration. For better demonstration of the approximation procedure, in this example equal initial values were chosen for all parameters. The p permeability variables could be neglected for potential steps $E_m > -40$ mV (see below). For faster convergence, the iterative approximation of only the nonlinear parameters (τ_m , τ_h , τ_n) was found to be sufficient. The steady-state values (m_{∞} , n_{∞}) could be determined in one step (Golub and Pereyra, 1973); h_{∞} was measured in the usual double pulse procedure. Thus, in every case, less than 10 iterations were required to reach the stopping criterion. To estimate the degree of deviation,

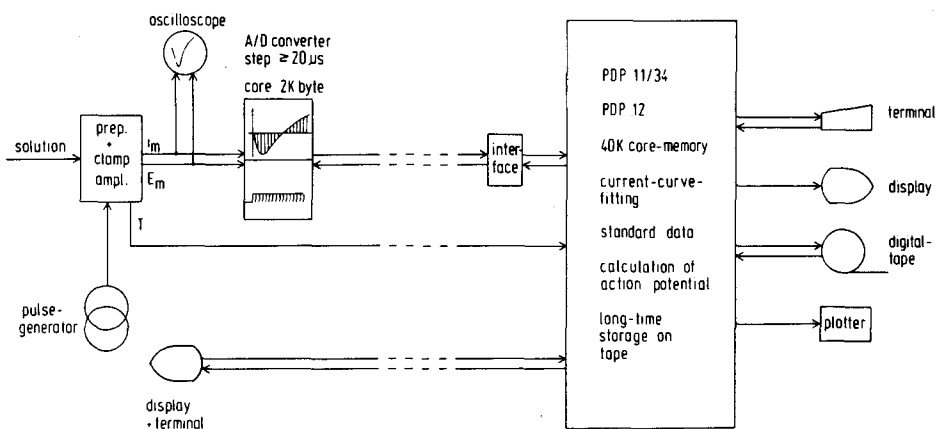


Fig. 1. Arrangement of potential clamp analysis. The node under investigation is placed in a perspex chamber with thermostatically controlled temperatures T , and is superfused with test solutions. Current i_m or potential E_m is kept constant in time, changes in the other variable are measured. After analog-digital conversion (A/D) of i_m and E_m the potential clamp currents are analysed by a 40 K computer. From the complete data action potentials are computed and compared with the current clamp measurements.

Table 1

Computer print-out of parameter approximation for a given clamp current

The membrane current is computed for a potential step from $E_m = -115$ mV to -10 mV using the time constants and steady-state values as given in the last line. The computer evaluation starts from arbitrary initial values. After a few iterations the squares of the total errors (last column) approach nearly zero, and the presumed values are re-detected.

τ_m	m_{∞}	τ_n	n_{∞}	τ_h	h_{∞}	Σe^2
0.9000	0.9000	0.9000	0.9000	0.9000	0.9000	85203.01175308
0.4777	0.7681	1.2711	1.5792	0.2897	0.2870	2618.64513843
0.0575	0.8460	0.5470	1.4080	0.2009	0.2136	16.27582593
0.0711	0.9549	0.7230	1.2124	0.1965	0.0998	4.832863533
0.0666	0.8964	1.3465	0.9759	0.2524	0.0191	0.25756796
0.0643	0.8769	1.6850	0.9665	0.2684	0.0032	0.00723645
0.0639	0.8731	1.7757	0.9693	0.2717	0.0001	0.00001156
0.063849	0.873454	1.780399	0.969806	0.271802	0.000016	Data used

the sum of squared errors was calculated for each curve and a χ^2 -test was performed.

From the extracted time constants and steady-state values the rate constants α and β could easily be determined, e.g. for the sodium activation m by

$$(2) \quad \alpha_m = \frac{m_\infty}{\tau_m}, \quad \beta_m = \frac{1 - m_\infty}{\tau_m}.$$

The rate constants as functions of membrane potential were approximated as usual by exponential functions, e.g. for α_m :

$$(3) \quad \alpha_m = A \cdot (E_m - \bar{B}) / (1 - \exp(-(E_m - \bar{B})/C)).$$

\bar{B} was used instead of B given by Frankenhaeuser and Huxley (1964) to avoid different designations for membrane potential (see above): $\bar{B} = B + E_r$. This procedure yielded a set of 24 constants describing the rate constants of all permeabilities. From these complete data action potentials, sub-threshold responses, afterpotentials and refractory periods could be calculated by a modified Heun-Runge-Kutta method as described in detail by Bromm and Frankenhaeuser (1968).

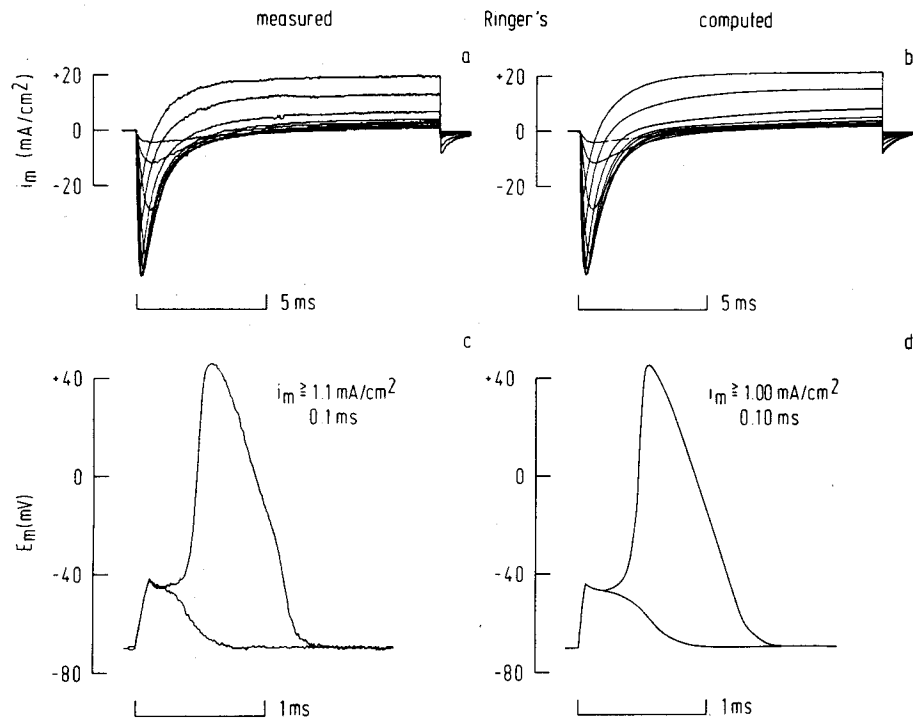


Fig. 2. Measured and computed membrane currents and action potentials of the individual nerve fibre: *a, b*; family of membrane currents i_m corrected for i_c and i_i . Potential steps to: $-33, -27, -23, -19, -15, -9, -6, +4, +22, +40$ (mV); *c, d*; action potentials and subthreshold responses. For stimulus parameters see inset. Computer plots for single motor nerve fibre in Ringer's solution, 20°C.

4. Results

Experiments in normal Ringer's solution

The validity of the Frankenhaeuser-Huxley equations for the individual nerve fibre was investigated in normal Ringer's solution at 20°C. Fig. 2, left side, demonstrates a typical experiment. In Fig. 2(a) the membrane currents, i_m , are presented for different potential steps. In Fig. 2(c) the current clamp experiment is given—current pulses near threshold strength elicited a subthreshold response and an action potential. The application of the evaluation method to the family of membrane currents yielded the rate constants and maximum permeabilities, which are collected in Fig. 3. From this experiment, standard initial values at $E_m = -70$ mV resulted in $m_0 = 0.0$, $h_0 = 0.67$, $n_0 = 0.016$, $p_0 = 0.005$. The curves in Fig. 3 (a), (b) and (c) are best least-squares fits to the measured α 's and β 's by the empirical analytic functions (see Methods, Equation (3)). The approximation of β_h was performed by the s-shaped (saturated) function (Frankenhaeuser, 1963), though the data points given here suggest an expression similar to α_m or α_n .

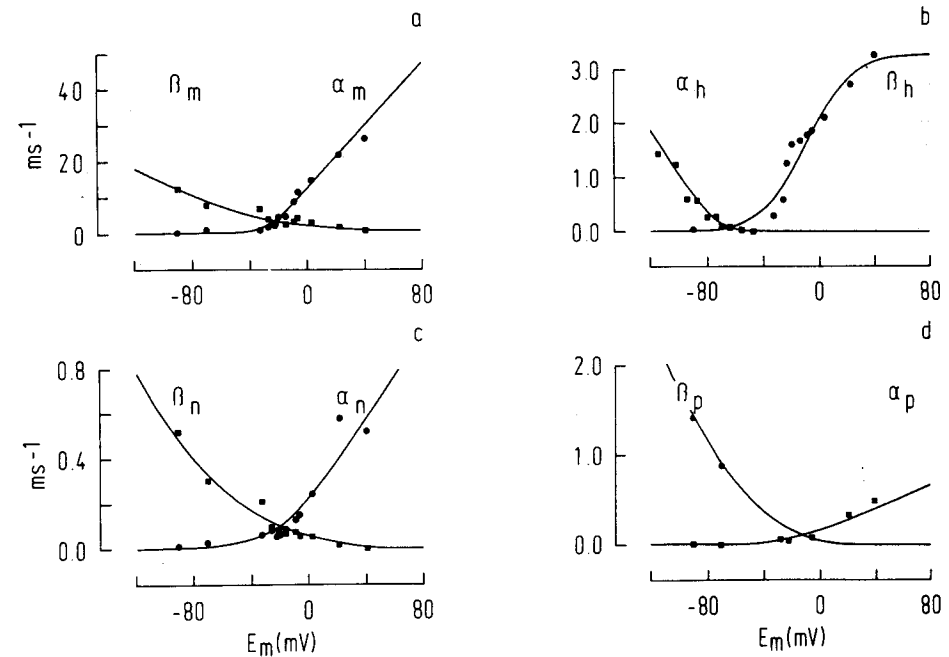


Fig. 3. Rate constants for m, h, n , and p of the individual nerve fibre. Points denote extracted values from the same potential clamp experiment as shown in Fig. 2, the curves representing best fits. Further data for this nerve fibre: $P_{Na} = 0.0135$ cm/s, $P'_K = 0.0008$ cm/s, $P_p = 0.0002$ cm/s, $g_l = 0.047$ S/cm²; $E_l = -69.66$ mV.

The determination of α_p and β_p as functions of membrane potential was rather ambiguous. Frankenhaeuser (1962*b*) measured the p variables in solutions of low and high external potassium concentrations. In Fig. 3 the data points for i_p were obtained in Ringer's solution by calculating the expected tail currents for i_{Na} and i_K , and subtracting these values from the measured tail currents. The curves plotted for α_p and β_p were taken from Frankenhaeuser's data. Within the accuracy of the measurements, the few points compiled by computer evaluation of the experiment in normal Ringer's solution agreed with Frankenhaeuser's predictions. Therefore, in the further analysis, the standard data of Frankenhaeuser and Huxley (1964) were adopted to describe the p permeability. For membrane potentials $E_m > -40$ mV, all the other rate constants could easily be determined from potential clamp steps and the accompanying membrane currents; thus most of the data points were obtained within this potential region. At more negative potentials, only a few points were determined as discussed below. The values at $E_m = -70$ mV and -90 mV were taken from preliminary tail experiments.

Applying the exponential equations (Equation (3)) underlying the approximation of the rate constants, yielded the complete data A , B , C for the individual fibre. In this way, during the experiment, it was possible to extract by the aid of the computer all information necessary to calculate membrane permeabilities and action potentials. For these computations the following values were assumed according to Frankenhaeuser and Huxley (1964): membrane capacitance $C_m = 2 \mu\text{F}/\text{cm}^2$, and axon internal concentrations $[\text{Na}^+]_i = 13.74$ mM and $[\text{K}^+]_i = 120$ mM. The calculated ionic currents are shown in Fig. 2(b). The congruence between computed and measured currents (cf. Fig. 2(a)) characterizes the quality of the evaluation method as the approximation of the measured clamp currents passed the χ^2 -test at the 5% level. In Fig. 2(d) the action potential is plotted as calculated from the complete data. The agreement between the measured action potential and that predicted from the potential clamp analysis establishes the validity of the Frankenhaeuser-Huxley equations even for the single myelinated nerve fibre, and also for *R. esculenta*.

Similar experiments were performed on a number of fibres and the complete set of data was obtained. Mean values and standard deviations from 6 experiments are summarized in Table 2 and are compared with those given by Frankenhaeuser and Huxley (1964). The term 'standard data' (Table 2) is used for averaged values, taken from a number of preparations, whereas the term 'complete data' describes the individual values extracted from one fibre. The standard deviations of single data were due to the variations of the different preparations. $\bar{B}\beta_m$ and $\bar{B}\alpha_h$ could not be determined from potential or current clamp measurements with sufficient accuracy to decide whether the individual values differed from the *X. laevis* values or not so the *X. laevis* values were adopted. Only a few of the complete data were of significance in computing action potentials and subthreshold responses. For example, a 20% increase of $\bar{B}\alpha_m$ elevated the threshold potential by 26%; of $A\beta_h$ reduced the duration (14%) and amplitude (3%) of the spike; and of $\bar{B}\beta_h$ prolonged the duration (12%). Nevertheless the approximation to each measured

Table 2

Standard data for *Rana esculenta* (*R. e.*)

Mean values and standard deviations for six motor nerve fibres at 20°C; $B = \bar{B} - E_r$. For comparison standard data of *Xenopus laevis* (*X. l.*) is given (Frankenhaeuser and Huxley, 1964).

	A		B		C	
	<i>R. e.</i>	<i>X. l.</i>	<i>R. e.</i>	<i>X. l.</i>	<i>R. e.</i>	<i>X. l.</i>
α_m	0.58 ± 0.21	0.36	36.3 ± 14.3	22.0	2.76 ± 1.23	3.0
β_m	0.28 ± 0.065	0.4	13.0*	13.0	24.75 ± 8.86	20.0
α_h	0.057 ± 0.035	0.1	-10.0*	-10.0	10.28 ± 5.2	6.0
β_h	3.72 ± 1.00	4.5	49.44 ± 6.4	45.0	17.4 ± 7.4	10.0
α_n	0.01 ± 0.003	0.02	39.4 ± 22.7	35.0	13.74 ± 6.5	10.0
β_n	0.015 ± 0.006	0.05	14.2 ± 9.5	10.0	16.2 ± 5.66	10.0

* Data adopted from *X. l.*

current in Ringer's solution at 20°C was within the 5% level, and each measured action potential corresponded to that predicted. The standard data for motor fibres of *R. esculenta* were of the same order of magnitude as those for *X. laevis*. They yielded slightly broader action potentials than for *X. laevis*, in agreement with the measurements of Schmidt and Stämpfli (1966).

Experiments with TTX

To further test the applicability of the Frankenhaeuser-Huxley equations to the individual nerve fibre, the membrane permeabilities were altered by substances with known effects. Fig. 4(a) shows the membrane currents under 3.1 nM tetrodotoxin (TTX), a specific blocker of the Na permeability constant \bar{P}_{Na} (Hille, 1968). The maximum inward Na currents were reversibly reduced to 52% of the control in Ringer's solution and the steady-state K currents, as well as the tail currents were unchanged. In the current clamp experiment (Fig. 4(c)) the spike amplitude (E_{AP}) was reduced to 90%, the maximum rate of rise (dE_{AP}/dt) to 54%, and the threshold potential (E_{th}) was increased by 9%, compared with the control.

Again the rate constants were extracted, and the membrane currents, computed from the complete data (Fig. 4(b)), demonstrated the sufficient approximation (χ^2 -test at 5% level). The calculated action potential (Fig. 4(d)) agreed as well with the measured one. The complete analysis revealed that TTX blocked solely the maximum sodium permeability \bar{P}_{Na} without any effect on the rate constants of all ionic permeabilities. In particular i_p was not influenced by TTX (in agreement with the findings of Dubois and Bergman (1975)). Mean results of six experiments under 3.1 nM TTX showed a reduction of \bar{P}_{Na} to $57.2 \pm 5.4\%$, of E_{AP} to $91.9 \pm 2.5\%$, of dE_{AP}/dt to $62.7 \pm 5.7\%$, and an increase of E_{th} by $5.8 \pm 3.5\%$.

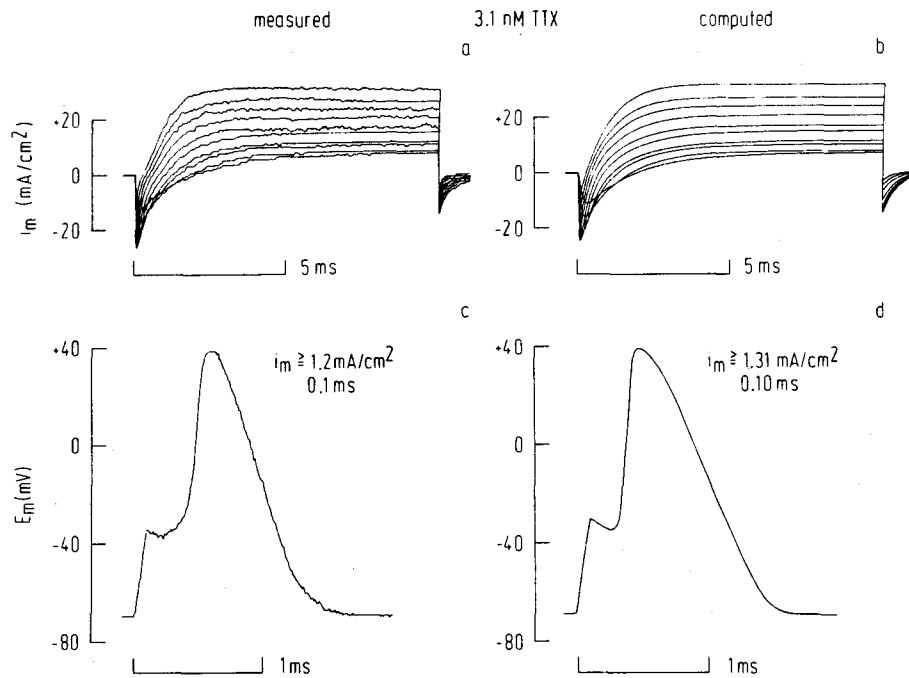


Fig. 4. Measured and computed membrane currents and action potentials of the individual nerve fibre under 3.1 nM TTX. TTX reduces solely the maximum sodium permeability P_{Na} . Measurements (a, c) and predictions (b, d) are in good agreement. Membrane currents i_m corrected for i_e and i_i ; stimulus parameters see inset. Computer plots for single motor nerve fibre, 20°C.

In all experiments the current clamp results could be predicted by the potential clamp analysis.

Experiments with TEA

K channels are reversibly blocked by tetraethylammonium chloride (TEA) (Tasaki and Hagiwara, 1957). An almost total block of the K permeability is achieved with concentrations larger than 5 mM, when applied to the node of Ranvier (Hille, 1967). Fig. 5(a) shows the measured membrane currents, i_m , when the node was superfused with 10 mM TEA. The K currents are totally blocked, the Na inward currents seem not to be affected and the tail currents are abolished. The effect of TEA on the p permeability had to be expected, if the p currents were assumed to be caused by external K accumulation after long lasting potential steps $E_m > E_r$ (Dubois and Bergman, 1975; Ochs and Bromm, 1976). The current

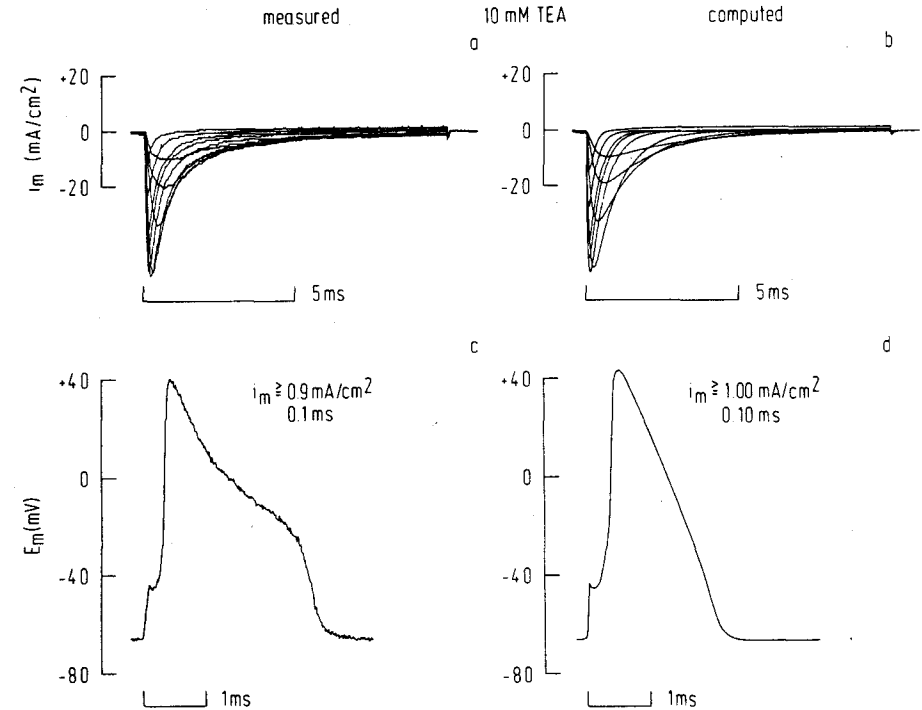


Fig. 5. Measured and computed membrane currents and action potentials of the individual nerve fibre under TEA. 10 mM TEA blocks P_K totally. The current approximation is not as precise as in Ringer's or under TTX. Note the slower decays of the measured Na currents (a), compared with the approximations, (b). The measured action potential (c) has a plateau phase and is much longer than that computed from the complete data of the same fibre (d). Membrane currents i_m corrected for i_e and i_i ; stimulus parameters, see inset. Computer plots for single motor nerve fibre, 20°C.

clamp experiment (Fig. 5(c)) demonstrates the typical shape of the action potential under supramaximal doses of TEA. The duration, as measured between maximum rate of rise and fall of the action potential, was prolonged by a factor of 3.1 compared with the control, and a predominant plateau phase was induced (Schmidt and Stämpfli, 1966). The resting potential was reduced by +4 mV, the threshold potential E_{th} was somewhat lower and the peak amplitude E_{AP} , referred to $E_r = -70$ mV, was unchanged.

However, the attempt to approximate the rate constants under TEA on the basis of the equations led to difficulties. The evaluation of the rate constants caused deviations, which exceeded the 5% level, though the stopping criterion was reached; i.e., the Frankenhaeuser-Huxley assumptions did not describe the membrane currents under TEA with the same precision as in normal Ringer's solution. The computed membrane currents in Fig. 5(b) show smaller peak

values, and the decays caused by Na inactivation develop faster, reaching the zero level earlier than the measured currents (Fig. 5(a)). After extracting the complete data, action potentials were computed as shown in Fig. 5(d). Experiments on six fibres supported the results. The action potential computed from the currents in TEA differed markedly from the measured ones. A plateau phase was not induced and the computed action potential was prolonged by a factor of 1.2 ± 0.1 , compared with the controls in Ringer's solution, whereas the measured action potential duration was increased by a factor of 3.2 ± 0.2 .

5. Discussion

This paper describes a computer supported evaluation of potential and current clamp experiments on the individual nerve fibre. Complete data were extracted for all ionic permeabilities from which action potentials were computed. Thus the validity of the Hodgkin-Huxley-Frankenhaeuser formalism was established for the single myelinated nerve fibre. The main result was that the equations were sufficient to describe both potential and current clamp results for the individual nerve fibre of *R. esculenta* in normal Ringer's solution, at 20°C. This was still true, if the maximum Na permeability was reduced, as shown in experiments with TTX. Inconsistencies arose when the K permeability was blocked by TEA. In contrast to previous investigations (e.g. Dodge, 1963; Schoepfle and Johns, 1970; Khodorov, 1974) the computations given here are based on individual experimental data, not on computer simulations.

The evaluation method involved a least-squares fit procedure (Marquardt, 1963) which approximated the predicted membrane currents to the measured by iterative variation of the coefficients of the differential equations. Up to now, in publications concerning computer supported potential clamp analysis, the ionic currents were evaluated separately, for example by blocking the other membrane permeabilities (e.g. Adelman *et al.*, 1973; Campbell and Hille, 1976) or at least by assuming different time dependencies for the single currents (e.g. Moore, 1971). The method described here did not require any preceding separation of single current components. It was immediately applicable for the total net membrane current, and of course also for quite different mathematical models. For instance, the approximation of the A , \bar{B} , C 's in the analytic expressions for the rate constants were made by the same formal treatment. The main principle in applying such methods was to insert precautions to achieve unique results. In the eight-dimensional space with m_∞ , h_∞ , n_∞ , p_∞ , τ_m , τ_h , τ_n , τ_p , there were many relative slopes suggesting solutions. Values of h_∞ , p_∞ and τ_p were determined separately. It was further shown that by means of the algorithm of Golub and Pereyra (1973) the iterative procedure could be restricted to a three-dimensional subspace, spanned by the parameters τ_m , τ_h , τ_n . Thus a fast convergence was obtained, and the evaluation became easily manageable in practice.

The equations led to a set of at least 24 constants describing time and potential dependence of membrane permeability. Following a suggestion of Frankenhaeuser

(personal communication), this data set for the individual fibre was denoted 'complete data', whereas the mean values taken from a series of experiments were called 'standard data' in the sense of averaged, 'typical' data. The standard data from experiments at 20°C for *R. esculenta* agreed approximately with those given for *X. laevis*. Some of the complete data showed large deviations from their mean values; these were due to variations in clamp currents and action potentials from experiment to experiment. In all experiments, however, the measured action potentials agreed with those predicted from the potential clamp analysis for the same fibre. For clamp potentials $E_m > -50$ mV the steady state values and time constants, and thus the complete data, were readily available from the clamp analysis. The rate constants in this potential range were of greatest importance for computing action potentials.

On the other side, for $E_m < -50$ mV the permeability variables could only be determined from tail experiments, with less accuracy (Hodgkin and Huxley, 1952; Frankenhaeuser, 1960), except for the Na-inactivation. Also, the current clamp experiment (by eliciting action potentials from potentials $E_m < -70$ mV) did not give much more information. We guess that for the determination of the rate constants in this potential region, as well as for an accurate measurement of the p parameters, tail experiments in different extracellular ionic concentrations have to be performed, as usually done. To check the validity of the Hodgkin-Huxley-Frankenhaeuser model for the individual nerve fibre by comparing measured and predicted action potentials, the rate constants in the corresponding potential region were readily available.

Limits of the validity of the underlying equations were found, when the potassium currents were blocked by TEA. In spite of the reduction of parameters, the measured currents could never be approximated within the preset level of the χ^2 -test. Measured and computed action potentials did not agree when the K currents were blocked by 10 mM TEA. The computed action potentials were too short compared to those measured, in agreement with the computations of Frankenhaeuser and Huxley (1964) by setting $P_K = 0$. Better agreement seemed to be obtained when a further slow Na-inactivation process was involved in the equation system (cf. Chiu, 1977; Ochs *et al.*, 1981). However, it is not the aim of this paper to modify the Frankenhaeuser-Huxley equations in order to achieve better agreement with the experimental results, but to show their validity and limitations in predicting action potentials for the individual nerve fibre.

Acknowledgement

We thank Prof. B. Frankenhaeuser for many helpful suggestions and Prof. B. Khodorov for reading a first draft of the manuscript.

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