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EFFECTS OF IONIC CONCENTRATION ON SODIUM PERMEABILITY PROPERTIES OF MYELINATED NERVE FIBRES OF *XENOPUS LAEVIS*

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SUMMARY

1. The nodal currents of single myelinated nerve fibres were recorded under potential clamp conditions, and the effect of [Ca], [Na] and [K] in the external solution on some of the Na permeability properties were analysed.

2. [Ca], [Na] and [K] all affected the position of the steady-state Na inactivation (h_∞) curve on the potential axis. The curve was displaced in positive direction by high ionic concentration.

3. The shift associated to different [Ca] was largest in low [Na] and [K]. Similarly the shift associated to different [Na] and [K] was largest in low [Ca].

4. The maximum peak sodium permeability (max. peak $-P_{Na}$) was affected by the [Ca], [Na] and [K]. It was greater in (i) low [Ca], (ii) high ([Na]+[K]) and (iii) high [Na]:[K] ratio.

5. The effect of [Ca] on peak $-P_{Na}$ was mainly a consequence of a change in $\bar{P}_{Na}$ (which is the value of P_{Na} if activation were complete, $m = 1$, and inactivation fully removed, $h = 1$).

INTRODUCTION

The ionic currents associated with the nervous action potential are known to be dependent on the ionic permeability properties of the nerve membrane and the electrochemical driving force of the permeating ions. By use of an electrical feed-back system, the potential clamp technique makes it possible to record the ionic currents associated with changes of membrane potential in rectangular steps. Thereby the dependence on potential and time of the specific ionic permeabilities has been revealed (e.g. Hodgkin & Huxley, 1952*b*; Frankenhaeuser & Huxley, 1964; Cole, 1968). Positive potential steps of certain amplitudes elicit an initial transient increase in Na permeability and a delayed sustained increase

in K permeability. The potential level preceding the step affects the size of the Na permeability. This behaviour has been empirically described by a potential and time dependent activation and inactivation of the specific ionic permeabilities. The steady-state values of these parameters are continuous functions of potential, asymptotically approaching a level of fully turned off at large negative potentials and turned on at positive potentials.

In a potential clamp analysis on squid axons Frankenhaeuser & Hodgkin (1957) found that the position of the curves describing the relation between Na activation, inactivation as well as K activation and membrane potential is affected by the external [Ca]. When [Ca] is increased e-fold, the Na activation curve is shifted +9 mV along the potential axis. The other parameters mentioned are similarly affected, and it was found that changes in [Mg] have a qualitatively similar effect.

These effects are, however, not specific for Ca and Mg; changes in uni-univalent salt concentration cause a qualitatively similar effect: Chandler, Hodgkin & Meves (1965) thus found that a change in ionic strength of an internal perfusate in squid axons affects the relation between Na inactivation and membrane potential. A reduction from 300 to 24 mM of the internal ionic concentration shifts the inactivation curve about +30 mV along the potential axis. The threshold for excitation increases similarly. Mozhayeva & Naumov (1970) showed on myelinated nerve fibre that a change in external [NaCl] affects the potential at which the K permeability is turned on. The size of this effect depends on pH and [Ca]. The shift is about −7 mV for a 2·5-fold reduction of external ionic concentration at ordinary pH and [Ca]. In a potential clamp investigation on the myelinated nerve fibre of the frog (*Xenopus laevis*) it was found that the uni-univalent salt concentration affects the potential dependence of both Na and K permeability curves (Brismar, 1973). A decrease in salt concentration is associated with a shift of the permeability curves along the potential axis in negative direction. This effect is enhanced by low [Ca] and diminished by high [Ca].

The aim of the present investigation was to find out whether the relation between Na inactivation and membrane potential in the nodal membrane is affected by the external uni-univalent salt concentration. Potential clamp measurements showed that the Na inactivation curve was shifted along the potential axis depending on the ionic concentration. The curve was displaced in negative direction associated with a decreased concentration. This effect was larger when [Ca] was low. The dependence of the peak Na permeability at large positive potential steps on the ionic concentration was also analysed.

METHODS

Large myelinated nerve fibres from the sciatic nerve of the frog (*Xenopus laevis*) were isolated. A single fibre was mounted in a Perspex chamber consisting of four pools connected through salt bridges and calomel electrodes to a two-amplifier feed-back system. The technique was similar to the one developed and described by Dodge & Frankenhaeuser (1958). Modifications have since been made of the amplifier set-up, now exchanged to operational amplifiers (Brismar & Frankenhaeuser, 1972).

The purpose of the method is to measure the nodal membrane currents during step changes of the membrane potential.

The experiments were carried out at a temperature between 2 and 6° C. Low temperature provided experimental conditions where the petroleum jelly seals kept their shape for a long time and provided an increased resolution of the initial phase of the ionic current.

An analysis was made of the effect of changes in the salt composition of the external solution on (*a*) the inactivation of the Na permeability mechanism and (*b*) the peak of the Na permeability at large positive potential steps. The solutions used are listed in Table 1.

TABLE 1. Composition of solutions (mM)

	$[CaCl_2]$	[NaCl]	[KCl]	$[NaHCO_3]$	[Tris buffer]	[sucrose]
Solutions with	0·27	25·0	0	2·5	0	180
25·0 mM-NaCl	0·27	25·0	30·0	2·5	0	120
	0·27	25·0	90·0	2·5	0	0
	0·27	25·0	180	2·5	0	0
	2·0	25·0	0	2·5	0	180
	2·0	25·0	30·0	2·5	0	120
	2·0	25·0	90·0	2·5	0	0
	2·0	25·0	180	2·5	0	0
	14·8	25·0	0	2·5	0	140
	14·8	25·0	70·0	2·5	0	0
Solutions with	0·27	25·0	90·0	0	2·5	0
[NaCl] + [KCl]	0·27	115	0	0	2·5	0
= 115 mM	2·0	25·0	90·0	0	2·5	0
	2·0	115	0	0	2·5	0

pH 7·6–7·8

The peak Na permeability-potential curve was obtained in the usual manner from a family of current records associated with potential steps of different amplitudes. The membrane was polarized to $E = -120$ mV between test pulses, in order to remove inactivation fully. The peak value of the Na permeability was determined from measurements of the peak initial current at positive potential steps by use of the constant field equation (Goldman, 1943; Hodgkin & Katz, 1949). The limited Na-specificity of the channel for the initial current ($P_{Na}:P_K$ ratio about 20:1, Frankenhaeuser & Moore, 1963) was accounted for by measurements of the equilibrium potential of the initial current and the assumption of unaltered internal concentrations of Na and K during the run of the experiment. This was essential for the calculation of P_{Na} in solutions with low [Na] and high [K]. At

large positive potentials Na permeability reaches a maximum level, the maximal peak Na permeability (max. peak-P_{Na}).

The analysis of the Na inactivation was performed according to the method described by Hodgkin & Huxley (1952*a*). The membrane was polarized to $E = -120$ mV in order to remove inactivation fully. Double pulses were applied so that conditioning polarizations of 100 ms duration and various amplitudes preceded a test step. The potential (E) during the test step was between -20 and 0 mV, in which potential region the Na current is maximal. The amplitude of the Na current (measured as the peak inward current minus the instantaneous current at the beginning of the test pulse) in units of its maximum value was plotted against the membrane potential during the conditioning polarization. This plot represented the steady-state Na inactivation curve.

Nomenclature. Membrane potential (E) is given as inside potential minus outside potential. The expressions 'hyperpolarization' and 'depolarization' were avoided and substituted with 'negative' and 'positive potential steps' respectively.

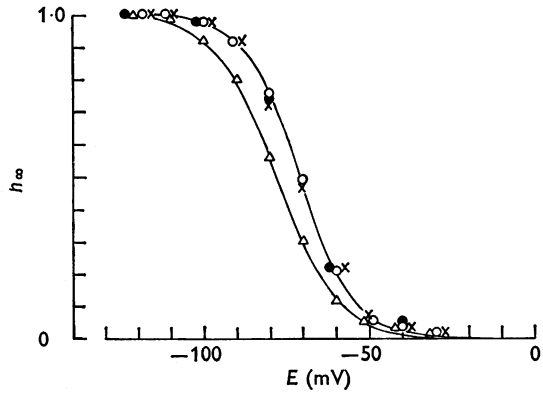

Fig. 1. Relation between steady-state Na inactivation (h_∞) and membrane potential (E) in different external solutions. Abscissa: membrane potential during conditioning pulse. Ordinate: Na current associated with a test pulse ($E = -10$ mV) following a 100 ms conditioning pulse, expressed in units of maximum current. The curves were drawn according to the empirical equation (Hodgkin & Huxley, 1952*a*)

$$h_\infty = \frac{1}{1+\exp[(E-E_h)/k]},$$

with $E_h = -71$ mV, $k = 8$ mV (right curve) and $E_h = -78$ mV, $k = 9$ mV (left curve). Temp. *ca.* 4° C.

×, 115 mM-NaCl; ○, 25·0 mM-NaCl + 90·0 mM-KCl; △, 25·0 mM-NaCl + 180 mM sucrose; ●, 25·0 mM-NaCl + 90·0 mM-KCl.

RESULTS

Na inactivation

Potential clamp experiments were performed on single myelinated nerve fibres. The Na permeability during a test step was plotted against the potential preceding the test step. This plot represents the Na

inactivation-potential curve. The effect on this relation of different uni-univalent salt concentrations and [Ca]:s in the external solution was analysed. A solution containing 25·0 mM-NaCl, 90·0 mM-KCl, 2·5 mM-$NaHCO_3$ and 2·0 mM-$CaCl_2$ was used as reference, and was applied after each test solution.

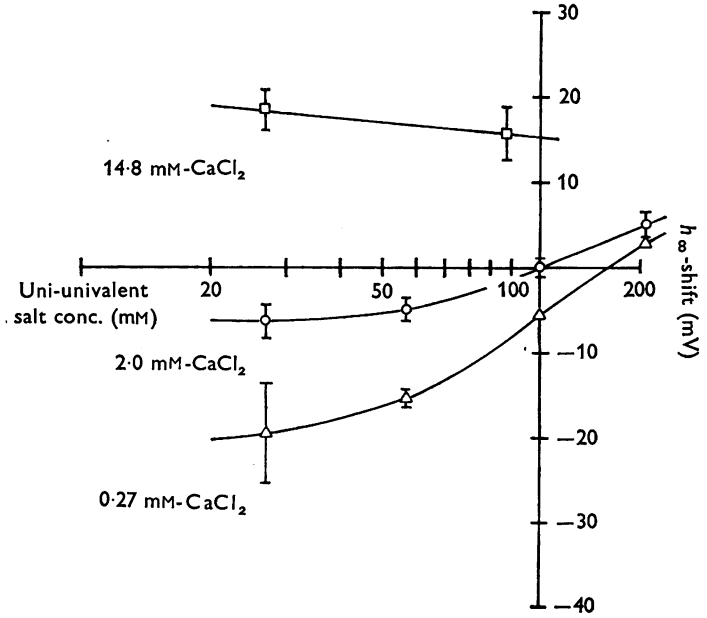

Fig. 2. Shift of steady-state sodium inactivation curve (h_∞) *vs.* concentration (logarithmic scale) of uni-univalent salts. Shift given as E_h in the test solution minus E_h in the reference solution, where E_h is the potential at which $h_\infty = 0{\cdot}5$. Measurements made as indicated with three different [Ca]. Temp. *ca.* 4° C. Smooth curves were drawn to fit the mean values.

Fig. 1 shows the effect of different ionic composition on the Na inactivation, beginning with measurements in ordinary Ringer solution containing 115 mM-NaCl. A substitution of about 80 % of the NaCl with KCl drastically decreased the inward current associated with the test pulse ($E = -10$ mV), but did not affect the relation between steady-state Na inactivation and membrane potential. When, instead, an equal amount of NaCl was replaced by sucrose, then the inactivation curve was shifted to more negative values along the potential axis. In this experiment all solutions contained 2·0 mM-$CaCl_2$. The maximum slope of the inactivation curve was negligibly affected; k in the empirical equation (see legend for Fig. 1) describing this relation was 8 mV (right-hand curve) and 9 mV (left hand curve) while E_h, which describes the position of the

curve on the potential axis, changed from −71 mV (right-hand curve) to −78 mV (left-hand curve). Thus the shift was −7 mV in this case. The effects were well reversible.

Collected measurements of the changes of E_h with different concentrations of uni-univalent salts and of Ca are shown in Fig. 2. The major findings from these measurements are: (1) the inactivation curve was located at more positive potential values at high [Ca] compared to the location at low [Ca]; this dependence of E_h on the [Ca] was somewhat smaller than the [Ca] dependence of the curve relating turn on of Na permeability to potential; (2) a qualitatively similar change in E_h was associated with changes in uni-univalent salt concentrations; (3) the effect on the inactivation curve of the uni-univalent salt concentration was larger in solutions with low [Ca] compared to high; the effect of [Ca] was similarly greater at low uni-univalent salt concentrations; in very high [Ca] (14·8 mM) E_h was nearly independent of the uni-univalent salt concentration.

Peak Na permeability

The effect of the ionic concentration of the external solution on the Na permeability of the nodal membrane was investigated. From measurements of the membrane currents associated with rectangular pulse steps of various amplitudes the peak value of the Na permeability (peak $-P_{Na}$) was calculated, and peak $-P_{Na}$ to potential curves were plotted. It was found that the peak $-P_{Na}$ to potential relation was affected by the [Ca], by the [Na]:[K] ratio of solutions, and by the total ionic concentration. The dependence of the peak $-P_{Na}$ curve on the [Na]:[K] ratio and on the [Ca] is shown in Fig. 3. It was found that the maximum value of the peak $-P_{Na}$ at large potential steps was smaller in a solution with 25·0 mM-NaCl + 90·0 mM-KCl compared to a solution with 115 mM-NaCl. Maximum peak $-P_{Na}$ was further larger in 0·27 mM-$CaCl_2$ compared to 2·0 mM-$CaCl_2$. This was more pronounced in solutions with low [Na] and high [K]. The position of the peak $-P_{Na}$ curve on the potential axis was affected by the [Ca]. This [Ca]-dependence was more pronounced in solutions with a high [Na]:[K] ratio. The [Na]:[K] ratio did, however, not affect the position of the peak $-P_{Na}$ curve at 2·0 mM-Ca. Measurements of the position of the peak $-P_{Na}$ curve can be described by the potential at which peak $-P_{Na}$ is half the peak $-P_{Na}$ at large potential steps. Collected data of the effect of [Na]:[K] ratio and [Ca] are given in Table 2*A*.

It was further found that the peak $-P_{Na}$ to potential relation was dependent on the total uni-univalent salt concentration. Solutions containing 25·0 mM-NaCl and different [KCl] were tried. The [Ca] was 2·0 mM or 0·27 mM. A decrease in uni-univalent salt concentration was associated

with a decrease in max. peak $-P_{Na}$ at large positive potential steps. A decrease in [Ca] was associated with an increase in P_{Na} independently of uni-univalent salt concentration (Table 2*B*).

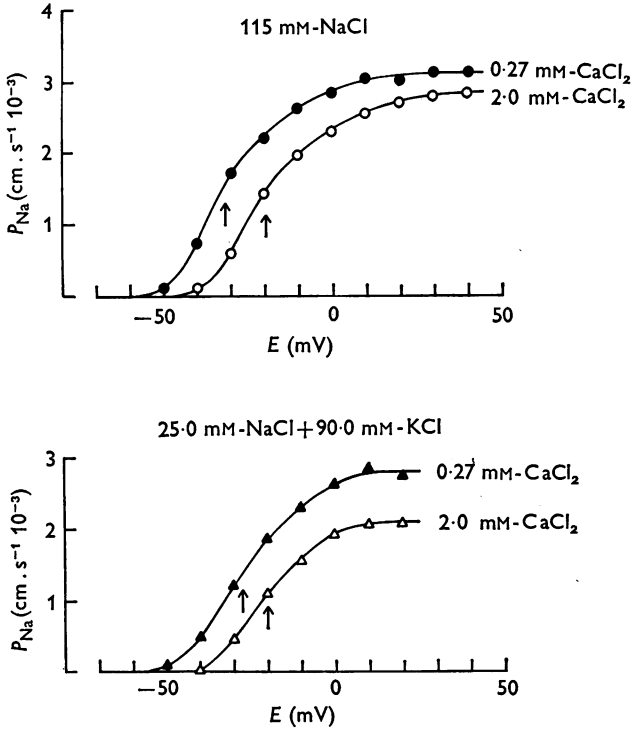

Fig. 3. Peak Na permeability (P_{Na}) plotted against membrane potential (E) in different external solutions. The membrane was polarized to $E = -120$ mV between test pulses. The arrows indicate the membrane potential at which peak sodium permeability is half maximum. Temp. *ca.* 4° C.

It is concluded from these experiments and the results given in Table 2 (*A* and *B*) that max. peak $-P_{Na}$ was greater in (1) low [Ca], (2) high ([Na]+[K]) and (3) high [Na]:[K] ratio. The effect of [Ca] on max. peak $-P_{Na}$ might depend on a change in $\bar{P}_{Na}$ (which is the value of P_{Na} if activation were complete, $m = 1$, and inactivation fully removed, $h = 1$) or on a changed ratio between the time constants related to activation (τ_m) and inactivation (τ_h) of the Na permeability mechanism. In order to distinguish between these two possibilities some additional experiments were performed. The time constants τ_m and τ_h at large positive steps were measured (see Frankenhaeuser, 1960). τ_m was found to be reduced but τ_h was negligibly affected by a reduction in [Ca] from 2·0 to 0·27 mM.

A decrease in the τ_m/τ_h ratio would cause an increase in the peak value of P_{Na} at constant $\bar{P}_{Na}$. However, since τ_h was the same in 2·0 and 0·27 mM-Ca, a measurement of I_{Na} at large steps and a time when $m = 1·0$ give a measure of changes in $\bar{P}_{Na}$. From such measurements it was found that the increase in peak $-P_{Na}$ was mainly a consequence of an increase in $\bar{P}_{Na}$ accompanied to a reduction in [Ca].

TABLE 2. Max. peak $-P_{Na}$ in different [Ca], [Na] and [K] measured in seven experiments

				Max. peak $-P_{Na}$ (cm.s^{-1}.10^{-3})				
	[Ca]	[Na] (mM)	[K]	(1)*	(2)	(3)	(4)	Mean
A. Expts. with	0·27	115	0	3·39	3·07	2·57	2·32	2·84
[Na]+[K] = 115 mM	2·0	115	0	3·17	2·82	2·38	1·94	2·58
	0·27	25·0	90·0	2·73	2·31	2·48	2·04	2·39
	2·0	25·0	90·0	2·12	1·78	2·02	1·96	1·97
				(5)	(6)	(7)		
B. Expts. with	0·27	27·5	0	—	2·23	1·78		2·01
[Na] = 27·5 mM	2·0	27·5	0	1·83	1·97	1·56		1·79
	0·27	27·5	30·0	—	2·60	1·98		2·29
	2·0	27·5	30·0	2·33	2·07	1·58		1·99
	0·27	27·5	90·0	—	2·87	2·01		2·44
	2·0	27·5	90·0	2·41	2·43	1·80		2·21
	0·27	27·5	180	—	—	2·05		2·05
	2·0	27·5	180	—	2·50	1·76		2·13

Temp. *ca.* 4° C.

* Numbers in parentheses indicate individual fibres.

DISCUSSION

The present investigation shows that the steady-state Na inactivation was affected by the ionic composition of the external solution. The effect was very similar to the effect of Ca concentration and uni-univalent salt concentration on the turn on of the Na and K permeability (Brismar, 1973). A decrease in uni-univalent salt concentration was associated with a displacement of the inactivation curve in negative direction along the potential axis. This displacement was larger at low [Ca]:*s* compared to high. It was thus found that the potential dependent permeability parameters were affected by changes in the ionic composition in a manner similar to a change in membrane potential. There were, however, quantitative differences between the effects of salt concentration on Na inactivation as compared to the effects on Na permeability and K permeability. In agreement with earlier investigations (Frankenhaeuser, 1957; Frankenhaeuser & Hodgkin, 1957 and Hille, 1968) it was found that an e-fold

change in [Ca] shifted the inactivation curve less than the permeability curves. The inactivation curve was correspondingly less affected by changes in uni-univalent salt concentration than the permeability curves. The finding that changes in uni-univalent salt concentration as well as Ca concentration shift steady-state Na inactivation curve and the direction of these shifts are qualitatively consistent with the existence of fixed negative charges at the external surface of the nodal membrane. As will be seen below a quantitative agreement was not obtained. According to the description of Chandler *et al.* (1965) such charges would set up a surface potential in size dependent on the ionic concentration. The surface potential would add to the membrane potential and affect the potential dependent permeability parameters in the same way as a change in membrane potential caused by passing current through the membrane.

Calculations of the change in surface potential as a function of the ionic concentration were carried out on the basis of different assumed densities of negative charges. An assumed charge density of $-5{\cdot}5\ \mu C.cm^{-2}$ gave satisfactory agreement between prediction and experimental measurements of the effect on the turn on of the Na permeability and K permeability (Brismar, 1973). The effect on the Na inactivation was smaller and it was impossible to obtain a satisfactory agreement on the basis of different assumed charge densities.

In these calculations it was assumed that the ions in the external solution have free access to the fixed charges and that an electroneutralization takes place in the form of electrical screening only (according to Gouy–Chapman), i.e. without binding in a chemical sense. Further the approximation was made that the electric field created by the fixed charges simply adds to that provided by the membrane potential (Chandler *et al.* 1965). It is, however, likely that the electric field created by the fixed charges at the external surface is greater close to this surface than deeper within the membrane. If the structures responsible for the Na inactivation mechanism are separate from those connected to the activation of the Na permeability, and located closer to the internal membrane surface, then the effect of the external ionic concentration in fact would be less on the inactivation mechanism. This idea is supported by the finding that internal perfusion of squid axons with pronase selectively destroys the inactivation of the Na system (Armstrong, Bezanilla & Rojas, 1973).

Another part of the investigation was concerned with the effect of ionic concentration on the Na permeability at large positive potential steps. The Na permeability has earlier been described as function of membrane potential and time, but independent of the external Na concentration (Dodge & Frankenhaeuser, 1959). The present investigation

shows that large changes in the [Na]:[K] ratio at ordinary ionic strength, as well as changes in the ionic strength, do affect P_{Na}, measured as maximum peak Na permeability. A decrease in [Ca] was found to be associated with an increase in max. peak $- P_{Na}$. This finding is in agreement with Vogel's (1974) recent report that an increase in [Ca] from 2·0 to 10·0 mM reduces P_{Na} by about 20 %. A measurement of the time constants for Na activation and inactivation revealed that the effect was essentially dependent on an increase in $\bar{P}_{Na}$.

It is thus concluded that the concentration of the current carrying cations, Na and K, not only affect the size of the ionic current as predicted by the constant field equation: the permeability properties of the nodal membrane are affected as well.

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