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# Voltage Dependence of the Rheogenic $Na^+/K^+$ ATPase in the Membrane of Oocytes of *Xenopus Laevis*

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**Summary.** Electrophysiological experiments were performed to analyze the $Na^+/K^+$-ATPase in full-grown prophase-arrested oocytes of *Xenopus laevis*. If the $Na^+/K^+$-ATPase is inhibited by dihydroouabain (DHO), the resting potential of the membrane of $Na^+$-loaded oocytes may depolarize by nearly 50 mV. This hyperpolarizing contribution to the resting potential depends on the degree of activation of the $Na^+/K^+$-ATPase and varies with intracellular $Na^+$ activity ($a^i_{Na}$) and extracellular $K^+$ ($K^+_o$). It is concluded that variations of $a^i_{Na}$ among different oocytes are primarily responsible for the variations of resting potentials measured in oocytes of *X. laevis*. Under voltage-clamp conditions, the DHO-sensitive current also exhibits dependence on $a^i_{Na}$ that may be described by a Hill equation with a coefficient of 2. This current will be shown to be identical with the electrogenic current generated by the $3Na^+/2K^+$ pump. The voltage dependence of the pump current was investigated at saturating values of $a^i_{Na}$ (33 mmol/liter) and of $K^+_o$ (3 mmol/liter) in the range from −200 to +100 mV. The current was found to exhibit a characteristic maximum at about +20 mV. This is taken as evidence that in the physiological range at least two steps within the cycle of the pump are voltage dependent and are oppositely affected by the membrane potential.

**Key Words** $Na^+/K^+$ ATPase · electrogenic · sodium pump · current-voltage relation · oocyte · toad

## Introduction

The electrochemical gradients of $Na^+$ and $K^+$ across the membrane of cells are maintained by the $Na^+/K^+$-ATPase. Under physiological conditions $Na^+$ is pumped against its electrochemical gradient from the cytoplasm into the external medium and, correspondingly, $K^+$ is pumped into the opposite direction. It is generally assumed (Karlish & Yates, 1978) that the ATPase molecule can exist in a conformation $E_1$ that has high affinity binding sites for $Na^+$ directed to the cytoplasm if intracellular ATP is bound and in a conformation $E_2$ that has in its phosphorylated form high-affinity binding sites for $K^+$ directed to the external medium. During pump activity the ATPase cycles with a series of intermediates through these two conformations and transports more $Na^+$ ions than $K^+$ ions across the membrane (*see*, e.g., Garrahan & Glynn, 1967; Thomas, 1969). It is generally assumed that three $Na^+$ ions and two $K^+$ ions are sequentially transported per cycle and per molecule ATP that is split (Post & Jolly, 1957; DeWeer, 1985). As a consequence of the $Na^+/K^+$ imbalance of ion transport, activation of the $Na^+/K^+$ pump gives an "electrogenic" hyperpolarizing contribution to the membrane potential; the amount of this contribution depends on the current produced by the pump and on the passive conductances of the membrane.

To analyze the electrogenicity of the $Na^+/K^+$-ATPase electrophysiological experiments were performed on full-grown prophase-arrested oocytes of the South African clawed toad *Xenopus laevis*. These cells have a high density of molecules of the $Na^+/K^+$-ATPase in their plasma membrane (Richter, Jung & Passow, 1984), and the large size of the oocytes makes alterations of intracellular concentrations during an experiment unlikely. The current generated by the $Na^+/K^+$ pump can be directly monitored under voltage-clamp conditions, and this paper describes the voltage dependence of the pump current. A characteristic voltage dependence of the $Na^+/K^+$ ATPase in these cells will be demonstrated with a maximum of the pump activity at +20 mV. This result suggests models (Chapman, Johnson & Kootsey, 1983; DeWeer, 1984; Läuger & Apell, 1985; Reynolds, Johnson & Tanford, 1985) that have during one pump cycle at least two voltage-dependent steps that are oppositely affected by the membrane potential within the physiological range.

Parts of the presented results have been published in brief form previously (Lafaire & Schwarz, 1984, 1985). Current-voltage curves that may suggest a maximum have also been recently reported

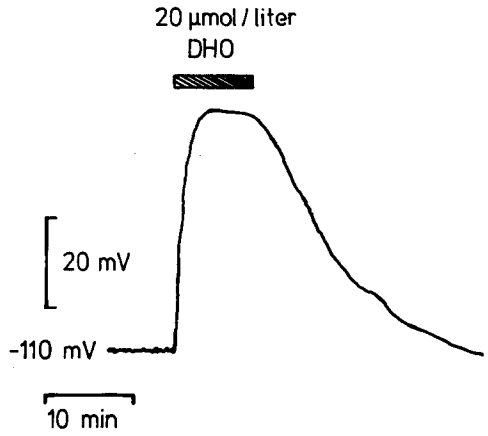


**Fig. 1.** Reversible depolarization of the membrane potential of $Na^+$-loaded oocytes by DHO in standard bath solution. (Exp. 15.06.83)

by Gadsby, Kimura and Noma (1985) for the $Na^+/K^+$ ATPase in cardiac muscle cells.

## Materials and Methods

Females of the South African clawed toad *Xenopus laevis* were anaesthetized with 2 g/liter *m*-aminobenzoeacid-ethylester-methan-sulfonat (MS222, Sandoz Ltd., Basle, Switzerland), and parts of the ovary were removed. Full-grown prophase-arrested oocytes of stages V and VI (Dumont, 1972) were selected after removal of the enveloping tissue by treatment of parts of the ovary with collagenase (1.5 U/ml bath solution). The cells were stored in standard bath medium (*see below*) at room temperature and used for experiments within four days after dissection. Before an experiment the oocytes were usually preincubated in $K^+$-free solution for two to ten hours to elevate the activity of intracellular $Na^+$.

The standard bath solution was a modified Barth's medium (Barth & Barth, 1959) with (in mmol/liter) 88 NaCl, 0.41 $CaCl_2$, 0.33 $Ca(NO_3)_2$, 0.82 $MgSO_4$, 2.4 $NaHCO_3$, 3 KCl; the pH was buffered to 7.6 by 5 mmol/liter N-2-hydroxylethylpiperazine-N'-ethansulfonic acid, and 20 mg/liter of penicillin and streptomycin were added. In the solutions with other $K^+$ concentrations the KCl content was changed but the concentrations of all the other substances remained unaltered. In the $Na^+$-free solution all sodium salts were isosmotically replaced by tetramethylammoniumchloride.

In order to achieve complete block of the activity of the $Na^+/K^+$-ATPase 10 µmol/liter dihydroouabain (DHO) were usually added to the standard bath solution. We convinced ourselves that higher concentrations of DHO (20 µmol/liter) (*see also* Fig. 11) and other cardiotonic steroids (ouabain (up to 100 µmol/liter) or 2 µmol/liter *k*-strophanthidine) produce the same maximum inhibition of the membrane current (*compare also* Fig. 11).

The oocytes were potential-clamped by conventional two-microelectrode technique. Potential-recording and current-delivering electrodes were filled with 1 and 3 mol/liter KCl, respectively, and they were usually beveled to give a final resistance of 20–30 and 10–15 MΩ, respectively. For evaluation of the current-voltage dependencies rectangular voltage pulses of varying amplitude and 500 msec duration were applied every 5 sec from a holding potential that was set to the respective resting potential of the oocyte. Repetitive depolarizations or hyperpolarizations at this frequency lead to the same steady-state current and did not alter the resting potential. Membrane currents were measured through a low-pass filter of 100 Hz, and steady-state currents were determined during the last 100 msec of the test pulse.

The intracellular $Na^+$ activity $a_{Na}^i$ was monitored with $Na^+$-selective microelectrodes by using liquid ion exchanger ($Na^+$ cocktail ETH227, Fluka AG, Buchs, Switzerland); the electrodes were calibrated according to the method described by Dagostini and Lee (1982); for a tenfold change in the $Na^+$ activity the electrode potential varied by 57.1 ± 0.8 mV (±SEM; $n$ = 15).

## Results

Specific inhibitors of the $Na^+/K^+$ ATPase are the cardiotonic steroids. In our experiments we usually used the ouabain derivatives dihydroouabain (DHO) or *k*-strophanthidine that block the $Na^+/K^+$-ATPase in oocytes of *X. laevis* reversibly, in contrast to ouabain (*see also* Dascal, Landau & Lass, 1984; Richter et al., 1984).

### Effects of Cardiotonic Steroids on the Resting Potential

The activation of the $Na^+/K^+$ pump may considerably contribute to the membrane potential in the oocytes of *X. laevis*. This is demonstrated in Fig. 1; application of 10 µmol/liter DHO may depolarize the resting potential by more than 40 mV. This depolarization is fully reversible if the bath solution is again replaced by the solution without DHO. The time course of the potential changes reflects the kinetics of inhibition and recovery and is not limited by the speed of exchange of the bath solution. This was confirmed by control experiments, which demonstrate much faster effects after exchanging bath solutions of different $K_o^+$ concentrations (*not documented*).

Normal pump activity requires the presence of external $K^+$ ($K_o^+$) (Harris & Maizels, 1951). Correspondingly, removal of $K_o^+$ abolishes the DHO-dependent depolarization in the oocytes (*see* Table). In addition, the DHO-dependent depolarization depends on $K_o$; at concentrations exceeding 3 mmol/liter the DHO-dependent depolarization already saturates (Table). Since DHO behaves as a competitive inhibitor with $K_o^+$ for binding to the $Na^+/K^+$-ATPase, in experiments with $K_o^+$ concentrations exceeding 3 mmol/liter, pump activity was always inhibited by the noncompetitively binding *k*-strophanthidine.

At constant $K_o^+$ the DHO-dependent depolarizations vary among the individual oocytes even of the same donor from −40 mV to potentials less neg-

**Table.** Effect of external $K^+$ on the membrane potential $E_R$ (with the $Na^+/K^+$ pump stimulated by $K_o^+$-free preincubation), $E_M$ (after inhibition of the pump by 2 $\mu$mol/liter *k*-strophanthidine), $\pm$SEM($n$), and of the steroid-dependent polarization $\Delta E$

| $K_o^+$ (mmol/liter) | $E_R$ (mV) | $E_M$ (mV) | $\Delta E$ (mV) |
|---|---|---|---|
| 0 | −71.2 ± 5.5(10) | — | 0 |
| 1 | −84.7 ± 7.5( 7) | −73.8 ± 1.3( 5) | 10.9 |
| 3 | −104.8 ± 2.2(53) | −64.2 ± 2.0(38) | 40.6 |
| 7 | −96.0 ± 8.9( 7) | −52.3 ± 6.1( 6) | 43.7 |
| 20 | −70.7 ± 8.8(10) | −37.5 ± 2.0(11) | 35.0 |
| 40 | −77.4 ± 14.8( 6) | −24.7 ± 3.3( 6) | 41.5 |

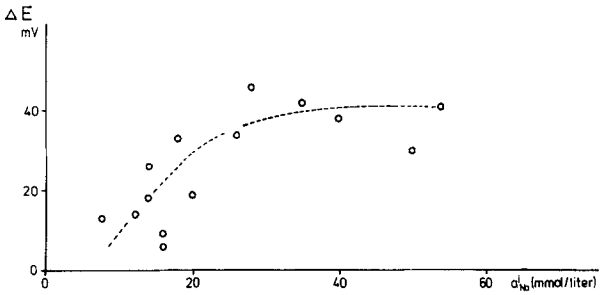


**Fig. 2.** Dependence of the DHO-sensitive polarization of the membrane on intracellular $Na^+$ activity. The broken line is drawn by eye

ative than −100 mV. This is due to the fact that the intracellular $Na^+$ activity $a^i_{Na}$ of the individual oocytes considerably varied (7–20 mmol/liter) if the cells were not preincubated in $K_o^+$-free solution. Figure 2 demonstrates that variations of the DHO-dependent depolarization are systematically related to variations in $a^i_{Na}$. To reduce the scatter of $a^i_{Na}$ the experiments were usually performed on oocytes whose intracellular sodium activity had been increased to an average value of 32.7 mmol/liter by preincubation in $K_o^+$-free solution; this is about twice the average value of untreated cells (*see* Lafaire & Schwarz, 1985). Under these conditions the DHO-dependent polarizations have an average of about 40 mV in the standard bath solution (*see* Table).

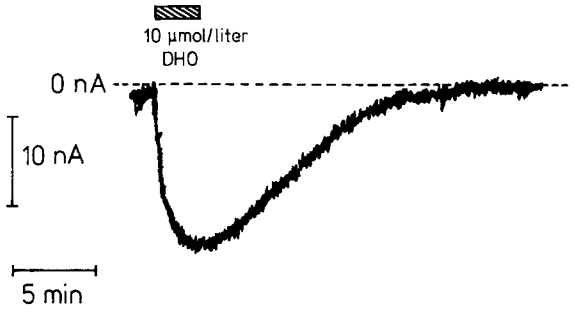


**Fig. 3.** Reversible inhibition of a component of the membrane current by DHO in standard bath solution. While recording the membrane current, the membrane potential was clamped to −50 mV; zero current refers to this holding potential. Before and during application of DHO, the voltage clamp was briefly turned off to determine the respective membrane potentials which were −110 and −65 mV, respectively. (Exp. 28.06.85)

## The Voltage Dependence of the Membrane Current Sensitive to Cariotonic Steroids

The membrane of the oocytes contains voltage-dependent channels for ions (Baud, Kada & Marcher, 1982; Miledi, 1982; Kusano, Miledi & Stinnakre, 1982; Barish, 1983; Peres & Bernardini, 1983; Dascal et al., 1984; Miledi & Parker, 1984). The DHO-dependent depolarization, therefore, possibly may not represent a direct measure for the activity of the $Na^+/K^+$ pump. For this reason, membrane currents were measured under conditions of clamped membrane potentials. Application of DHO results in changes of the current (*see* Fig. 3) that represents a direct measure of pump activity provided neither passive membrane conductances nor the concentrations of $Na^+$, $K^+$, ATP, ADP and $P_i$ alter during the application of DHO.

The primary object of this investigation is the voltage dependence of the pump activity. We, therefore, analyzed under voltage-clamp conditions the contribution of the membrane current that can be blocked by DHO. For this purpose the membrane of the oocyte was clamped to its resting potential (zero net current) and test pulses were applied first under control conditions (i.e., in the absence of DHO), then in the presence of DHO, and again under control conditions. Experiments were only analyzed if the current-voltage curves before and after the exposure to DHO were nearly identical. Figure 4 shows that currents reach steady state at the end of the test pulses and that DHO reduces these steady-state currents; the outward current produced by large depolarizing pulses are primarily affected. This indicates voltage dependence of the DHO-sensitive current that becomes more obvious if the steady-state currents are plotted versus the test potentials. An example of such current-voltage curves without and with DHO is shown in Fig. 5. The voltage dependence of the DHO-sensitive current is represented by the difference between these two curves (Fig. 6). Characteristic for the voltage dependence of this difference current is the maximum at about +20 mV. In some

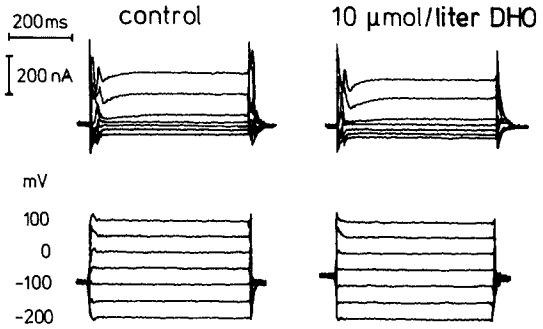


**Fig. 4.** Time dependence of the membrane current during voltage-clamp pulses of different amplitudes without and with DHO. Top: membrane current; bottom: membrane potential during clamp pulses. The holding potential was set to the respective resting potential. (Exp. 09.06.85)

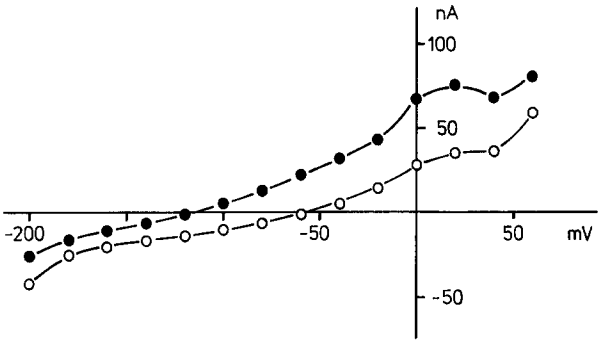


**Fig. 5.** Dependence of the steady-state membrane current on the amplitude of the test pulses. Filled symbol in standard bath solution, open symbols during application of 10 µmol/liter DHO. The lines are drawn by eye. Same experiment as in Fig. 1

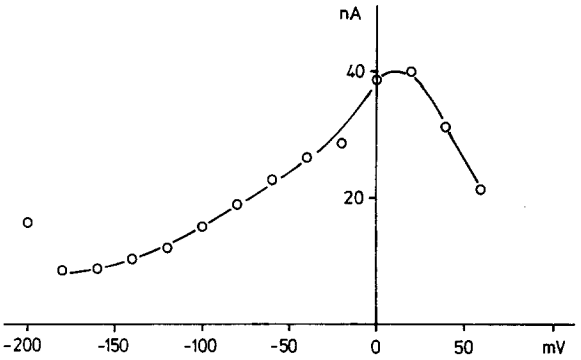


**Fig. 6.** Voltage dependence of the difference current (filled minus open symbols of Fig. 5) representing the component of the membrane current sensitive to DHO. The line is drawn by eye.

experiments a secondary increase of the difference current was found at potentials above +80 mV (*see* Lafaire & Schwarz, 1985), and in a few cases a reversal of the DHO-sensitive current was detected at potentials more negative than −150 mV (*see* Fig. 10).

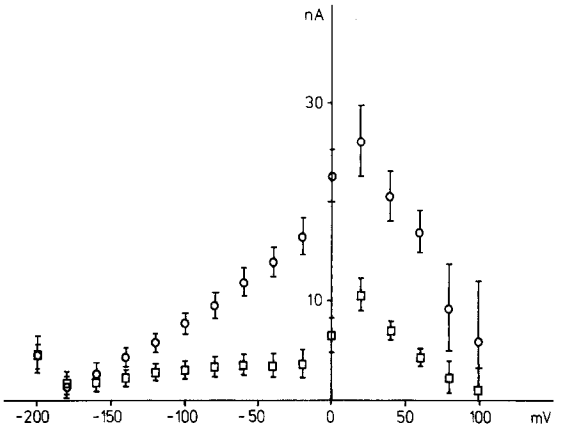


**Fig. 7.** Current-voltage dependence of the DHO-sensitive component of the membrane current. Circles: averaged data (±) from 15 oocytes with elevated $a^i_{Na}$ by $K^+_o$-free preincubation; squares: averaged data (±) from six oocytes without preincubation

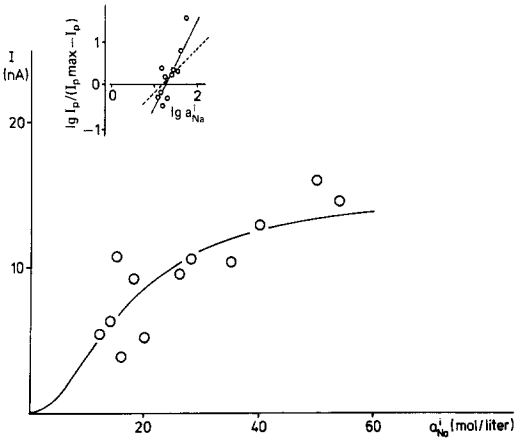


**Fig. 8.** Dependence of the DHO-sensitive current on intracellular $Na^+$ activity. The solid line represents a fit of Eq. (1) to the data points with $I_{pmax}$ kept fixed at 15 nA (the fitted parameters are $n = 1.90 \pm 0.07$ and $K_m = 225 \pm 47$). The inset shows the Hill presentation of the data; the solid line corresponds to $n = 2$, the dotted line to $n = 1$

## The Current Sensitive to Cardiotonic Steroids Depends on $a^i_{Na}$

Above we have demonstrated that $a^i_{Na}$ stimulates the DHO-dependent depolarization (*see* Fig. 2). Elevation of $a^i_{Na}$ by $K^+_o$-free incubation also enhances the DHO-sensitive current over the entire voltage range. This is demonstrated in Fig. 7 that shows current-voltage dependencies (determined as for Fig. 6) for oocytes whose $a^i_{Na}$ had been raised by preincubation in $K^+_o$-free solution, and for untreated oocytes. DHO-sensitive currents averaged over the investigated potential range are plotted versus the different values of $a^i_{Na}$ in Fig. 8. Though the scatter

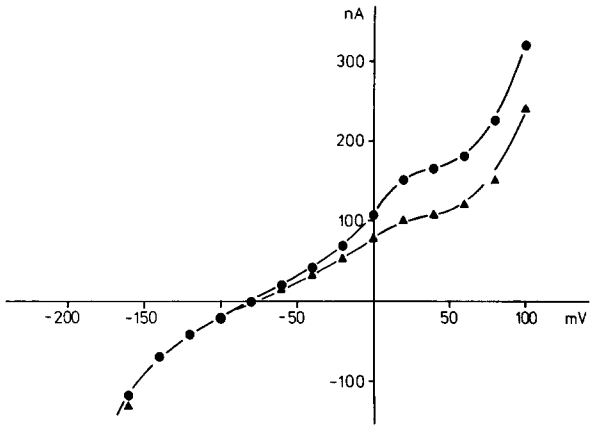


**Fig. 9.** Voltage dependence of membrane current in standard bath solution (circles) and during application of 20 mmol/liter TEACl (triangles). (Exp. 14.01.85)

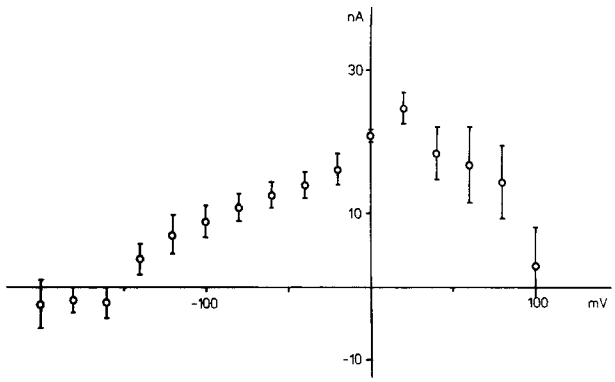


**Fig. 10.** Current-voltage dependence of the DHO-sensitive component of the membrane current in the presence of $TEA^+$. Data are average values ± SEM

of the data is considerable, we tried to fit the expression

$$I_p = \frac{I_{p\max} \cdot (a^i_{Na})^n}{K_m + (a^i_{Na})^n} \quad (1)$$

to the data with a fixed $I_{p\max}$ value. If $K_m$ and $n$ are fitted simultaneously, a reasonable approximation is obtained with a value of about 2 for $n$ (Fig. 8). A fit by simple Michaelis-Menten kinetics (n = 1) is also possible but gives less good approximation (*see* Hill presentation in inset of Fig. 8).

## The Current Sensitive to Cardiotonic Steroids Does Not Depend on Passive Conductances of the Membrane

If the $Na^+/K^+$ pump is inhibited, the resting potential of the membrane of oocytes of *X. laevis* is primarily governed by the $K^+$ permeability (*compare* Table, column with *k*-strophantidine). Therefore, possible relations between the activity of the $Na^+/K^+$ pump and the passive $K^+$ permeability were tested. Under the experimental condition of 3 mmol/liter external $K^+$, the oocytes exhibit an outwardly rectified $K^+$ current (R. Grygorczyk & W. Schwarz, *unpublished*). Figure 9 demonstrates that addition of 20 mmol/liter tetraethylammonium ($TEA^+$) to the bath solution considerably reduces the outward current most likely carried by the $K^+$. When this component of membrane current is inhibited by $TEA^+$, the DHO-sensitive current still exhibits its original voltage dependence (Fig. 10) with a maximum at about +20 mV; in addition, the data indicate that reversal of the DHO-sensitive current is occasionally observed at extremely negative potentials.

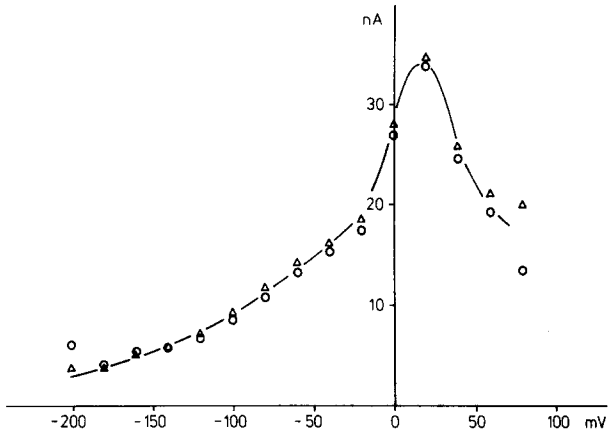


**Fig. 11.** Current-voltage dependence of the DHO-sensitive component of the membrane current. Circles: data obtained from measurements with 10 μmol/liter DHO; triangles: from measurements with 20 μmol/liter DHO. The solid line is drawn by eye. (Exp. 07.05.83)

To demonstrate that 10 μmol/liter DHO are sufficient for complete inhibition of the pump and that higher concentrations do not affect passive ion movements, current-voltage relations were determined at 10 or 20 μmol/liter DHO in the bath solution. At these concentrations DHO leads to nearly identical current-voltage curves; Fig. 11 demonstrates that the respective DHO-sensitive currents are also nearly identical. A similar result is obtained if 2 μmol/liter *k*-strophantidine are used.

Since the activity of the $Na^+/K^+$ pump can also be blocked by removal of external $K^+$, we compared current-voltage curves obtained with 3 mmol/liter $K^+_o$ and 10 μmol/liter DHO with the current-voltage curves obtained in $K^+_o$-free solution but without DHO (Fig. 12). In the voltage range where the DHO-sensitive current exhibits its characteristic voltage dependence with the maximum at +20

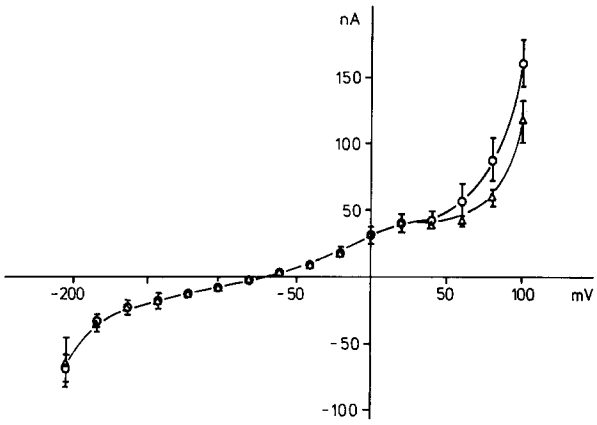


**Fig. 12.** Voltage dependence of membrane current in standard bath solution with 10 μmol/liter DHO (circles), and in $K_o^+$-free solution (triangles). Data are average values (± SEM) from six oocytes

mV, the two curves are nearly identical. The curves only deviate above +40 mV; this is possibly due to inhibition of the outwardly rectified $K^+$ currents by zero external $K^+$ as known from other preparations (*see*, e.g., Hille & Schwarz, 1978).

## Discussion

The presented data demonstrate that the $Na^+/K^+$ ATPase in the membrane of full-grown, prophase-arrested oocytes of *X. laevis* may considerably contribute to the resting potential. The large variations of the membrane potentials among different oocytes are partly due to seasonal variations of passive ion permeabilities (A.V.L., *unpublished*; also compare the values reported previously by Kado, Marcher and Ozon (1981), Kusano et al. (1982), Barish (1983), and Dascal et al. (1984)), but the results demonstrate that large hyperpolarizing contributions may also originate from different degrees of activation of the electrogenic $Na^+/K^+$-ATPase. In the following we will demonstrate that the current component sensitive to the cardiotonic steroids represents the current generated by the $Na^+/K^+$ ATPase. Therefore, the voltage dependence of the pump current will be considered in terms of voltage-dependent variations of the pump activity.

### The Current Sensitive to Cardiotonic Steroids is Produced by the $Na^+/K^+$ ATPase

The steroid-dependent current can only be considered to represent the pump activity (1) if the substrate concentrations do not alter during an experiment, and (2) if other membrane permeabilities are not altered by inhibition of the pump. These points will be discussed in the following:

1) The maximum electrogenic contribution to the membrane potential produced by the $Na^+/K^+$ pump can be estimated (*see* Mullins & Noda, 1963; Heinz, 1981) under the assumption of no net movements of ions across the membrane:

$$\Delta E_{pump} = \frac{RT}{F} \ln \frac{(a_K^i + \frac{2}{3}(p_{Na}a_{Na}^i + p_{Cl}a_{Cl}^o)) \cdot (a_K^o + p_{Na}a_{Na}^o + p_{Cl}a_{Cl}^i)}{(a_K^o + \frac{2}{3}(p_{Na}a_{Na}^o + p_{Cl}a_{Cl}^i)) \cdot (a_K^i + p_{Na}a_{Na}^i + p_{Cl}a_{Cl}^o)}$$

with $p_{Na} = P_{Na}/P_K$ and $p_{Cl} = P_{Cl}/P_K$ being the permeability ratios, and the $a$'s being the respective ion activities. For the calculation we used $p_{Na} = 0.02$ and $p_{Cl} = 0.11$; if the $Na^+/K^+$-ATPase is inhibited these permeability ratios yield resting potentials nearly identical to the measured values of the Table. Taking for the external solution an activity coefficient of 0.75, for the internal activities of $K^+$ and $Cl^-$ the values reported by Dascal et al. (1984) of $a_K^i$ = 88 mmol/liter, $a_{Cl}^i$ = 33.2 mmol/liter and of $Na^+$ the value given by Lafaire and Schwarz (1985) $a_{Na}^i$ = 32.7 mmol/liter, an electrogenic contribution $\Delta E_{pump}$ of 5.8 mV is obtained. The measured DHO-induced depolarizations were usually considerably larger than the calculated value of the pump-induced depolarization. This could be due to the pump operating under nonsteady-state conditions; but alterations of membrane permeabilities could also be responsible when the pump is inhibited by DHO (*see below*).

A $Na^+/K^+$ pump working under conditions where the intracellular $Na^+$ activity changes seem unlikely for two reasons; first, even during long-lasting experiments no alterations of $a_{Na}^i$ could be detected by ion-selective microelectrodes and second, the turnover rate of the $Na^+/K^+$ ATPase in the oocytes is extremely low under the experimental conditions (i.e., at a temperature of 20°C and at the resting potential); a value of 5 $sec^{-1}$ has been estimated from the number of ouabain binding sites and the rate of ouabain-sensitive $^{86}Rb^+$ uptake in the oocytes of *X. laevis* (Richter et al., 1984). This is by more than one order of magnitude lower than the maximum turnover rate of the $Na^+/K^+$ ATPase (*see* Cantley, 1981).

2) A more likely explanation for the large hyperpolarizing contribution to the membrane potential by stimulation of the pump could be that voltage-dependent permeabilities amplify the effect of the $Na^+/K^+$ pump. Such interaction with the voltage-dependent channels in the oocytes have been avoided by performing voltage-clamp experiments. But even under voltage-clamp conditions activation

or inhibition of the $Na^+/K^+$ pump may alter passive ion permeabilities. High pump rates may particularly result in $K^+$ depletion at the external membrane surface that would affect passive movements of $K^+$ ions. Application of $TEA^+$ obviously blocks passive $K^+$ currents (Fig. 9) but still gives the characteristic voltage dependence of the DHO-sensitive current. In addition, the low turnover rate of the $Na^+/K^+$ ATPase in the oocytes (*see above*) does not support the view of $K^+$ depletion; even assuming a pump rate an order of magnitude higher (corresponding to a pump current of nearly 100 nA) could produce changes of the $K^+$ concentration at the external membrane surface not exceeding a few $\mu$mol/liter.

Another possibility that the DHO-dependent current might not only originate from the current generated by the $Na^+/K^+$ pump could be due to direct alterations of passive ion permeabilities by DHO. But the experiments with different cardiotonic steroids, with different concentrations of these inhibitors as well as with the inhibition by $K_o^+$-free solution always demonstrate the same voltage-dependence of the difference current. This agrees with findings of Deslauriers, Ruiz-Ceretti, Schanne and Payet (1982) that ouabain does not alter the passive electrical properties.

In conclusion, these results indicate that the DHO-sensitive current can be considered as the current generated by the $Na^+/K^+$ pump.

## The Voltage Dependence of the Current Generated by the $Na^+/K^+$ ATPase

In the past, much effort has been put on the question whether the pump activity depends in the physiological range on the membrane potential; but so far different laboratories came to different conclusions based on different observations. Results were reported that the pump activity does not depend on membrane potential (*see* e.g. Hodgkin & Keynes, 1955; Nakajima & Takahashi, 1966; Sjodin, 1982) or that less negative membrane potentials enhance pump activity (*see*, e.g. Thomas, 1982). DeWeer and Rakowsky (1984) report a negative slope in the current-voltage dependence of the reversed operating $Na^+/K^+$ pump. In addition, strong and non-monotonic voltage dependence of the current generated by the $Na^+/K^+$-ATPase in the oocytes has been described in this paper (*see also* Lafaire & Schwarz, 1984, 1985), and the data for heart cells by Gadsby et al. (1985) may also suggest a maximum in the current-voltage curve. These latter results were obtained from experiments on cells and under conditions where a large potential range could be investigated and where alterations of intracellular ion activities could be excluded, in case of the oocytes due to the large cell volume and in case of the heart cells due to the application of the patch-clamp technique with the whole-cell configuration. In addition, particularly in the experiments on oocytes an extremely large potential range could be investigated. The discrepancies with resect to the different voltage dependences of the $Na^+/K^+$ ATPase may be due to species differences or to differences in substrate concentrations. But the restricted potential ranges that have usually been investigated could also account for the different reported observations. Depending on the location of such a limited voltage range, different results will be obtained (*compare* Figs. 6, 7, 10 and 11); at hyperpolarizing potentials and around zero mV nearly no voltage dependence will be detected, but at depolarizing potentials one will expect stimulation and at positive potentials inhibition of pump activity.

Different models have recently been discussed in terms of a simplified reaction scheme based on the Post-Albers sequence (Chapman et al., 1983; DeWeer, 1984; Läuger & Apell, 1985; Reynolds et al., 1985). These authors demonstrated that one can expect within the physiological range changes of pump activity if the membrane potential is altered. Our results with a maximum pump current at +20 mV suggest that more than one voltage-dependent step can be rate-limiting during the pump cycle. One possibility would be the transfer of $Na^+$ and $K^+$ across the membrane (steps 3 and 5 as assumed by Chapman et al. (inset Fig. 13*A*)). With values for the concentrations of the substrates that are characteristic for the oocytes we can simulate for this reaction scheme current-voltage curves (Fig. 13*A*) qualitatively similar to the measured curves; the current generated by the pump exhibits a maximum at about +20 mV with steep voltage dependence on both sides of the maximum and a similar dependence on $a_{Na}^i$. Putting the voltage dependence on other steps (including the binding of $Na^+$ and $K^+$) with only slight modifications of the values of the rate constants can yield similar results. Another possible explanation for the observed voltage-dependence would be a voltage-dependent gate in series with the cycle of the $Na^+/K^+$-ATPase (DeWeer, 1984). So far, the only conclusion we can draw is that at least *two* voltage-dependent steps are involved during one cycle of the pump activity. For more detailed conclusions experiments are necessary that investigate the dependence of the electrogenic current under different substrate conditions.

Since the electrogenic contribution depends on the activity of the $Na^+/K^+$-ATPase one has to expect that the membrane potential conversely affects

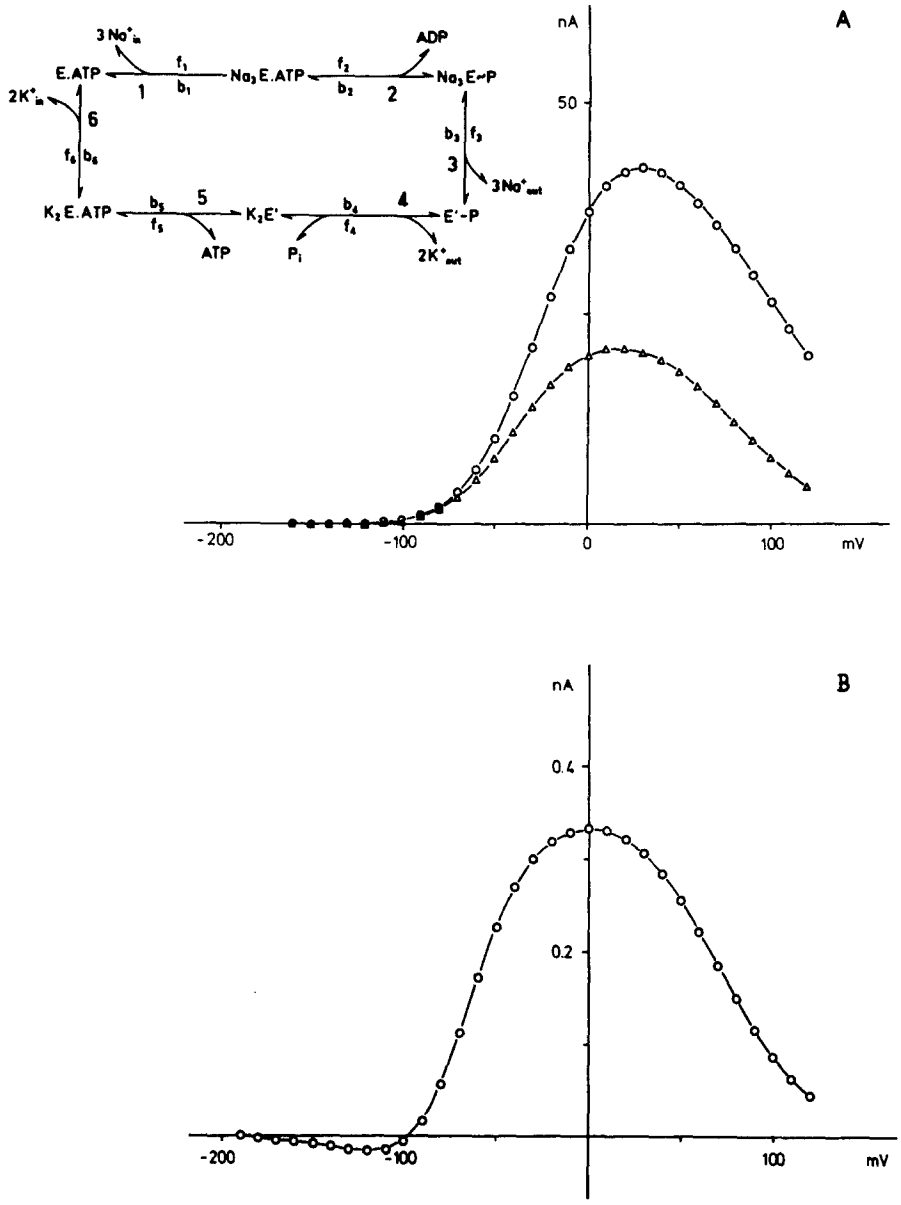


**Fig. 13.** Voltage dependence of the current generated by the $3Na^+/2K^+$ ATPase using the simplified reaction scheme (inset) and the same rate coefficients as given by Chapman et al. (1983). For the voltage-dependent steps it is assumed that half of the potential drop across the membrane acts on the transfer of two positive charges from inside to outside in step 3, and of one positive charge from outside to inside in step 5; this partial charge compensation during the translocation of the $3Na^+$ and $2K^+$ is necessary to account for the steepness of the current-voltage curve. The substrate activities were as follows: 5 mol/liter ATP, 0.06 mol/liter ADP, 8 mol/liter $P_i$, 3 mmol/liter $K_o^+$, 68 mmol/liter $Na_o^+$, 88 mmol/liter $K_i^+$ and for $Na_i^+$ 33 mmol/liter (*A*, circles), 16 mmol/liter (*A*, triangles), and 1 mmol/liter (*B*). For the number of pump sites per oocyte a value of $10^{10}$ (Richter et al., 1984) was used

the pump activity; particularly strong hyperpolarization should inhibit pump activity, and for thermodynamic reasons reversal of the pump can be predicted at potentials more negative than about −200 mV. Under normal conditions a reversal of the pump can hardly be detected because of the shallow voltage dependence at the extremely negative potentials. In addition, for the parameters used in the simulation a reversal of the pump can be expected at about −190 mV and at these strong hyperpolarizations the procedure of subtracting membrane current with inhibited $Na^+/K^+$-ATPase from the total current is not very accurate. Reduction of $a_{Na}^i$ would shift the reversal potential to less negative potentials and increase the steepness of the current-voltage curve near the reversal potential (*see* Fig. 13*B*). Though the size of the currents generated by the pump is also reduced, in a few experiments on oocytes that have been preincubated in $Na_o^+$-free solution (*not shown*) application of DHO occasionally produced slight hyperpolarization instead of the depolarization always observed in oocytes with elevated $a_{Na}^i$. This might indicate that in the cells with reduced $a_{Na}^i$ the pump may operate at the resting potential in opposite direction. Reversal of a $3Na^+/2K^+$ pump under artificial conditions ($K_o^+$-, $Na_i^+$-, and $ATP_i$-free) has been demonstrated in squid axons (DeWeer & Rakowski, 1984) by detection of voltage-dependent inward current sensitive to cardiotonic steroids.

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