J. Physiol. (1987), **383**, *pp.* 251–267
With 6 *text-figures*
Printed in Great Britain

PROPERTIES OF AN ELECTROGENIC SODIUM–POTASSIUM PUMP IN ISOLATED CANINE PURKINJE MYOCYTES

BY I. S. COHEN, N. B. DATYNER, G. A. GINTANT, N. K. MULRINE AND P. PENNEFATHER*

*From the Department of Physiology and Biophysics, Health Sciences Center, S.U.N.Y., Stony Brook, NY 11794, U.S.A. and the * Faculty of Pharmacy, University of Toronto, Canada*

(*Received 29 January 1986*)

SUMMARY

1. Purkinje myocytes were isolated from canine Purkinje strands by collagenase exposure and gentle trituration. The myocytes were studied by a switched single-micro-electrode voltage-clamp technique at 37 °C in Tyrode solution containing 8 mM-K^+ and 2 mM-Ca^{2+}.

2. The dose–response relation for the cardiotonic steroid dihydroouabain (DHO) was obtained by measuring the change in membrane current caused by application of concentrations of 1–100 μM. The K_D obtained in fourteen experiments was $3{\cdot}7 \pm 1{\cdot}1$ μM (mean ± S.E. of mean).

3. We employed 100 μM-DHO (a concentration more than 25-fold greater than the K_D) to estimate the resting pump current (I_p) in the isolated myocytes. A value of $0{\cdot}27 \pm 0{\cdot}02$ μA μF^{-1} (mean ± S.E. of mean, $n = 32$) was obtained.

4. Myocytes were also exposed to K^+-free solution for a period of 200 s. On return to K^+-containing Tyrode solution there was a slowly decaying outward current. The time constant of decay of this pump current transient was 87 ± 8 s (mean ± S.E. of mean, $n = 8$). The integral beneath this transient was used to obtain a second estimate of the resting pump current. In four preparations where exposures in DHO and in K^+-free solutions were employed the ratio $I_{p,\,DHO}/I_{p,\,K\text{-free}}$ was $1{\cdot}76 \pm 0{\cdot}15$ (mean ± S.E. of mean).

5. From the magnitude of resting pump current, in the presence of total pump blockade the Na^+ activity should rise at a rate of 1·3 mM min^{-1}.

6. Reducing $[K^+]_o$ from 8 to 1 mM reduced I_p by more than 40 % initially. I_p then slowly increased over the next 30 min. These results suggest that the steady-state inward background current is not greatly altered by changes in $[K^+]_o$, and that $[Na^+]_i$ rises to a new level. The changes in I_p obtained at early times following reduction of $[K^+]_o$ to 1 or 0·5 mM ($t < 1{\cdot}75$ min) were used to estimate the K_m for external K^+; a value of 0·8 mM was obtained.

7. The results suggest that the properties of the Na^+–K^+ pump in isolated canine Purkinje myocytes are similar to those in canine Purkinje strands. This argues against major distortions of measured pump properties in the canine Purkinje strand and for the physiological state of the Na^+–K^+ pump in the isolated Purkinje myocyte.

INTRODUCTION

The exchange across cell membranes of intracellular Na^+ for extracellular K^+ that is mediated by the Na^+ and potassium adenosine triphosphatase (Na^+– K^+ATP-ase) is asymmetric and thus electrogenic (Thomas, 1972). In heart cells, most evidence points to a coupling ratio of 3 Na^+ to 2 K^+ transported for every ATP molecule hydrolysed. Furthermore, this coupling ratio remains constant over a wide range of $[Na^+]_i$ and $[K^+]_o$ (see Gadsby, 1984). As a result, the 'pump current' provides a direct measure of Na^+ pump activity under a range of conditions. The pump current can be identified by its sensitivity to cardiotonic steroids. Conversely, since cardiotonic steroids are full antagonists (see Forbush, 1983), the inward current resulting from the blockade of a standing pump current can be used to quantify their interaction with the Na^+–K^+-ATPase.

The kinetic properties of the Na^+–K^+ pump in cardiac muscle have been studied by exposing Purkinje fibres to nominally K^+-free solution. This exposure results in an elevation of $[Na^+]_i$ (Ellis, 1977; Eisner, Lederer & Vaughan-Jones, 1981*b*). On return to K^+-containing Tyrode solution there is an increase in electrogenic Na^+–K^+ exchange, resulting in a slowly decaying membrane hyperpolarization or outward current (Gadsby & Cranefield, 1979; Gadsby, 1980; Eisner & Lederer, 1980). The time constant of the decay of a^i_{Na} to base-line levels is well correlated with the decay of this outward current (Eisner *et al.* 1981*b*; Glitsch & Pusch, 1980). Furthermore, the outward current transient is eliminated by application of cardiotonic steroids, providing further evidence that this current is generated by increased activity of the Na^+–K^+ pump (Gadsby & Cranefield, 1979; Eisner & Lederer, 1980).

Steady-state properties of the Na^+ pump in cardiac muscle and its binding of cardiotonic steroids have also been examined recently. Eisner *et al.* (1981*b*) estimated a resting pump current of 0·15 μA cm^{-2} from the rate of increase of a^i_{Na} in K^+-free Tyrode solution. Ellis (1977) used the rate of increase of a^i_{Na} on blockade of the pump by the cardiotonic stereoid dihydroouabain (DHO) to obtain a dissociation constant (K_D) of about 6 μM. In contrast to these values obtained in sheep Purkinje strands, Daut & Rudel (1982) measured the change in membrane current on application of DHO in guinea-pig ventricle and obtained a K_D of 46 μM, and a resting pump current of 0·8 μA cm^{-2}.

Although these studies suggest significant differences in the resting pump current and cardiotonic steroid sensitivity of Purkinje strands and ventricular muscle, some caution must be exercised. Different protocols were used in the two preparations and both preparations (ventricular trabeculae and Purkinje strands) are multicellular with narrow intercellular spaces. In Purkinje strands in particular, there is substantial literature testifying to the distortions that fluctuations in $[K^+]_o$ confer on time-dependent currents and even the measurement of pump characteristics (Noble & Tsien, 1968; DiFrancesco, 1981*a*, *b*; Attwell, Eisner & Cohen, 1979; Cohen, Falk & Kline, 1982; Eisner & Lederer, 1980; DiFrancesco & Noble, 1985). This is particularly relevant to the dose–response relation for DHO because its binding is markedly K^+-dependent (Daut, 1983; Bachmaier, Ebner & Reitner, 1985).

We have recently reported a technique for the acute dissociation of the canine Purkinje strand into isolated Purkinje myocytes by exposure to enzymes and gentle

trituration (Gintant, Datyner & Cohen, 1985). The isolated myocytes have normal morphology, and their membrane resistance is similar to that of Purkinje strands. Impedance analysis indicates the absence of a physiologically relevant restricted space, as does morphometric analysis (Mathias, Eisenberg, Datyner, Gintant & Cohen, 1985). We therefore chose this preparation to study the properties of the Na^+–K^+ pump in the Purkinje myocyte membrane in the absence of complicating changes in extracellular $[K^+]_o$. Some of these results have been reported in abstract form (Cohen, Datyner, Gintant, Johnson, Mulrine & Pennefather *et al.* 1986; Mulrine, Pennefather, Cohen, Gintant & Datyner, 1986).

METHODS

Preparation of Purkinje strands

Adult mongrel dogs were euthanized with T-61 solution (National Laboratories Corp., Somerville, NJ) and hearts immediately removed and rinsed in cooled, oxygenated (95% O_2–5% CO_2) Tyrode solution containing (mM): NaCl, 140; $NaHCO_3$, 12; NaH_2PO_4, 0·4; $MgCl_2$, 1; dextrose, 9; KCl, 8; $CaCl_2$, 4. Purkinje strands with attached cones of ventricle were excised, placed in a chamber perfused with warmed (35 °C) Tyrode solution and stimulated (1 Hz) until regular contractions were observed. The strands were then dissected free from ventricular muscle, cut into segments, and returned to the superfusion chamber. When vigorous contractions were again observed, the segments were rinsed in a nominally Ca^{2+}-free Tyrode solution and placed in a test-tube for dissociation.

Dissociation procedure

The dissociation procedure employed was identical to that described by Gintant *et al.* (1985). Briefly it consisted of three 20 min 'digests' in which the Purkinje segments were sequentially exposed to decreasing concentrations of collagenase (2·1, 1·8 and 1·6 mg ml^{-1}; Type II, 125–175 u./mg^{-1} activity, Worthington Diagnostics, Freehold, NJ) and increasing concentrations of bovine serum albumin (1·3, 1·5, and 4 mg ml^{-1}; essentially fatty-acid-free, Sigma Chemical Co., St. Louis, MO) added to a digest medium (38 °C) containing (mM): KCl, 140; $NaHCO_3$, 8; NaH_2PO_4, 0·4; $MgCl_2$ 2; dextrose, 10; taurine, 25; β-hydroxybutyric acid, 5; sodium pyruvate, 5; 20–40 μM-Ca^{2+} activity; oxygenated with 95% 0_2–5% CO_2; pH = 7·0. The substitution of KCl for NaCl during the digestion greatly increased the yield of single myocytes with rod-like morphology. During each digestion the segments were 'triturated' by the repeated passage of the solution through the orifice of a pipette. The rate and pattern of agitation was controlled by a Z-80-based microprocessor interfaced to a linear actuator and piston/cylinder (Datyner, Gintant & Cohen, 1985*a*). Following the third digestion, columns of cells and single cells which had separated from the surrounding matrix were 'harvested' and placed on a poly-L-lysine (mol. wt. 55000)-coated glass cover-slip at the bottom of a small-volume (0·5 ml) perfusion chamber heated to 35–37 °C (held within $\pm$1·0 °C in any individual experiment (Datyner, Gintant & Cohen, 1985*b*)). Each harvest typically yielded 25–50 Purkinje myocytes with a rod-like morphology. The chamber was located on the stage of an inverted microscope (Olympus CK series, equipped with Hoffman modulation contrast optics, 200 × magnification).

All reported results were obtained within 8 h following completion of a dissociation procedure. Single cells were usually employed but occasionally two or three cells coupled end-to-end in a linear array were also studied.

Electrophysiological studies

Membrane currents were studied using a one-micro-electrode discontinuous voltage clamp (Axoclamp-2, Axon Instruments, Burlingame, CA; switching frequency 1–6 kHz, leakage current $\leqslant$ 10 pA). We used this technique (rather than a two-micro-electrode voltage clamp or a whole-cell patch-clamp technique) in an attempt to minimize damage to the myocytes and to preserve the intracellular milieu. Bevelled and unbevelled micro-electrodes (filled with 3 M-KCl, d.c. resistances 30–50 MΩ in Tyrode solution) were used for impalements. Current was measured with

a headstage series resistor. Voltage and current traces were recorded on FM tape (Hewlett-Packard, 3964a, 15/32 in/s, 150 Hz band width) for later display and analysis.

The d.c. input resistance of these cells was typically $\geqslant 50$ MΩ. Voltage uniformity determined during voltage-clamp steps in two experiments by the insertion of a second voltage-recording micro-electrode was within the limits of resolution inherent in one-micro-electrode voltage-clamp techniques. Impedance and morphometric studies on these single myocytes have indicated a specific membrane resistance of about 20 kΩ cm^2, similar to that obtained in Purkinje strands by the same techniques (Mathias & Cohen, 1984; Mathias *et al.* 1985). The equivalent circuit obtained from the impedance data suggested a vanishingly small series resistance insufficient to create a physiologically relevant accumulation space, which morphometric analysis confirmed.

The Tyrode solution for the electrophysiological studies of the isolated myocytes was identical with that used for dissection, with a single exception that the concentration of $CaCl_2$ was reduced to 2 mM. Dihydroouabain (DHO; Sigma, U.S.A. or Knoll, W. Germany) was dissolved in Tyrode solution to achieve a final concentration of 10^{-4} M; other concentrations were obtained by serial dilution. Solutions of DHO were stored for less than 3 days and frequently prepared freshly for each experiment.

The total capacitance of the preparation was estimated by measuring the length and radius of the preparation and calculating the surface area as if the myocyte was a right circular cylinder with sealed ends (in cm^2), and then multiplying by 1·54 $\mu F\ cm^{-2}$. The value thus obtained is the average cell capacitance in μF. The factor of 1·54 $\mu F\ cm^{-2}$ was obtained from our impedance studies of the isolated cells (R. Mathias, N. B. Datyner, G. A. Gintant & I. S. Cohen in preparation).

The kinetic studies were performed in a bath of 0·5 ml volume with a flow rate of 1·0–2·0 ml min^{-1}. The exchange properties of the bath were obtained by changing the ionic strength of the bathing solution and observing changes in tip potential of an extracellular micro-electrode. The 20–80% rise time of the solution change was about 20 s.

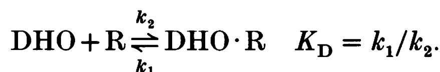
RESULTS

Our first aim was to estimate the steady resting level of current due to the electrogenic transport of Na^+ and K^+. To achieve this aim we needed to block the pump and measure the change in current, I_p. We used DHO to block the pump since experiments on Purkinje fibres (Isenberg & Trautwein, 1974) and ventricular muscle (Daut & Rudel, 1982) suggested it bound to and dissociated rapidly from its receptor (as compared to other cardiotonic steroids). As it was first necessary to determine the concentration of DHO required for total blockade of the Na^+–K^+ pump we investigated the dose–response relation for DHO.

Dose–response relation for DHO

Purkinje myocytes were voltage-clamped near the zero-current potential and concentrations of DHO between 1 and 100 μM were applied. Representative records of three applications of DHO from one experiment are shown in Fig. 1. Increasing the concentration of DHO increased the speed of onset of drug action (simply due to the law of mass action) and also increased the amplitude of the response.

We have analysed our dose–response data in much the same way as Daut & Rudel (1982). If inhibition of the Na^+–K^+ pump results from the reversible association of DHO with a receptor (R) on the pump, then the reaction can be described by the following kinetic scheme:



$$\mathrm{DHO} + \mathrm{R} \underset{k_1}{\overset{k_2}{\rightleftharpoons}} \mathrm{DHO \cdot R} \quad K_D = k_1/k_2.$$

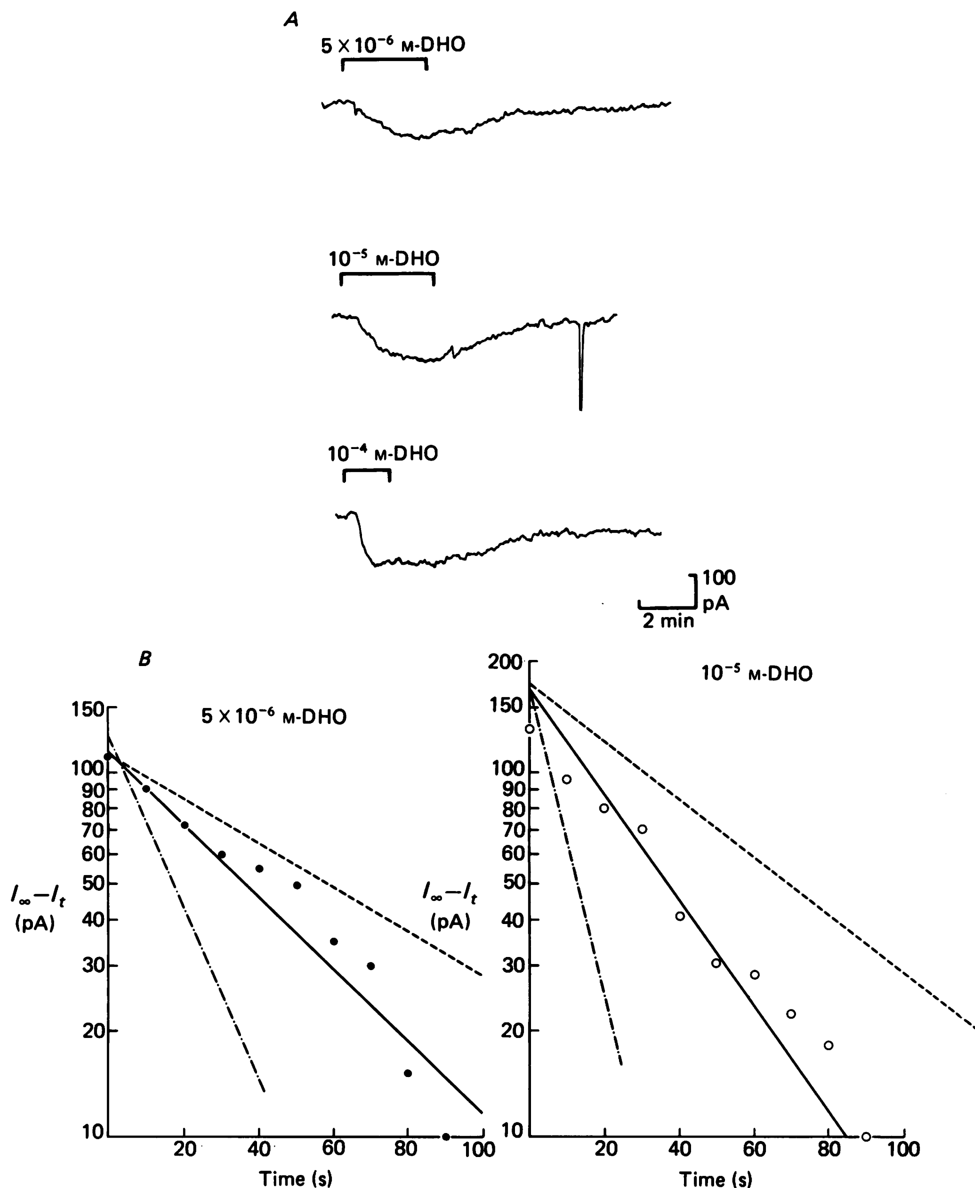


Fig. 1. *A*, representative traces of membrane current responses of a single Purkinje myocyte to three concentrations of dihydroouabain (DHO). Currents filtered at 0·06 Hz; holding potential, −28 mV; holding current, +50 pA before 5×10^{-6} M-DHO, −40 pA before 10^{-5} M-DHO, and −50 pA before 10^{-4} M-DHO. Estimated total capacitance $(C_t) = 3{\cdot}98 \times 10^{-4}$ μF; cell 1, 4/25/85. *B*, semilogarithmic plots of the onset of DHO action for the concentration 5×10^{-6} M (left) and 10^{-5} M (right) illustrated in *A*. The straight lines are drawn according to $k_1 = 0{\cdot}00918$ s^{-1} and $k_2 = 2{\cdot}48 \times 10^3$ M^{-1} s^{-1}, yielding time constants of 46 s (left) and 29 s (right). The expected rates of onset for a K_D of 1 or 10 μM and $k_1 = 0{\cdot}00918$ s^{-1} are indicated by the dot–dashed and dashed lines respectively. These time constants were determined from eqn. (2). For more details see text.

The scheme predicts that at equilibrium, where I_D is the measured change in current produced by DHO and I_p is the maximal change:

$$I_D = I_p \cdot [\mathrm{DHO}]/(K_D + [\mathrm{DHO}]). \tag{1}$$

The scheme also predicts that the system approaches equilibrium with a time constant (τ) such that

$$\tau^{-1} = k_1 + k_2[\mathrm{DHO}]. \tag{2}$$

Eqn. (1) can be linearized (see Scatchard, 1949):

$$I_D/[\mathrm{DHO}] = I_p/K_D - I_D/K_D. \tag{3}$$

Thus a plot of I_D against $I_D/[\mathrm{DHO}]$ gives a slope of $-1/K_D$. Fig. 2 shows the results of this analysis in two different cells. The left-hand example is for the data of Fig. 1 and gives a K_D of 2·4 μM, and the right-hand example is well fitted by a line giving a K_D of 5·8 μM.

A total of fourteen cells were examined in this aspect of the study. In each case two or more concentrations of DHO were applied and eqn. (3) was used to estimate the K_D. The mean K_D was $3{\cdot}7 \pm 1{\cdot}1$ μM (mean ± S.E. of mean). The data from these fourteen cells is summarized in the dose–response relation of Fig. 3. The smooth curve is drawn for illustrative purposes and is that defined by eqn. (1) when the K_D is set to 3·7 μM (and the highest normalizing concentrations 6×10^{-5} and 10^{-4} M are assumed to lie on the curve). Analysis of the data of Fig. 3 using the Hill equation gave a Hill coefficient of 0·95, and predicts a half-maximal response at 2·8 μM.

Kinetic analysis of the time course of the current change in DHO solution provides a check on the applicability of eqn. (1) to the binding of DHO. We were not always able to resolve kinetic parameters accurately because of transient instabilities of the base-line current. However, in five cells with a quiet base line and a smooth onset and offset of DHO action, the rate of recovery of the pump current from DHO blockade was $9{\cdot}2 \pm 1{\cdot}6 \times 10^{-3}$ s^{-1} (mean ± S.E. of mean). On the assumption that during wash-out, [DHO] quickly falls to zero, eqn. (2) predicts that this rate is equal to k_1. The average association rate constant ($k_2 = k_1/K_D$) was calculated to be $2{\cdot}5 \times 10^3$ M^{-1} s^{-1}. Using eqn. (2) this value can be estimated independently from the rate of development of DHO blockade. The rates of onset of action of the two lowest concentrations (5×10^{-6} and 10^{-5} M) illustrated in Fig. 1*A* predict a value of k_2 of $2{\cdot}5 \times 10^3$ M^{-1} s^{-1} (Fig. 1*B*). The fit of the theory to the data is adequate in this example (compare with the expectations of 1 and 10 μM K_D values respectively and $k_1 = 0{\cdot}0092$), suggesting that the kinetic model is not unreasonable. The more rapid onsets which accompany the application of 10^{-4} M-DHO cannot be resolved from the solution change. Nevertheless, the observation of a much faster onset than recovery with 10^{-4} M-DHO indicates that the drug is approaching its maximum effect at this concentration. However, a more rapid solution change would be required for a complete characterization of DHO binding and unbinding kinetics.

Resting pump current

From the dose–response relation (Fig. 3) we chose 10^{-4} M-DHO as nearly saturating. With a K_D of 3·7 μM, application of 10^{-4} M-DHO should block more than 96 % of the resting pump current.

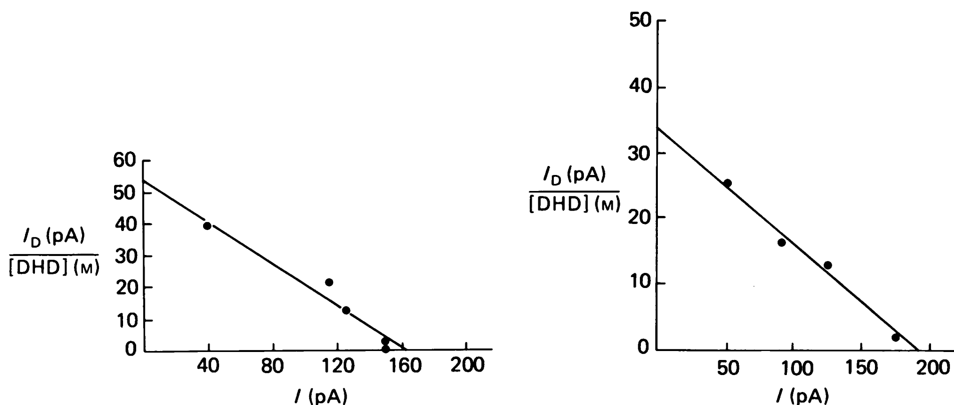


Fig. 2. Two Scatchard plots of dose–response data in isolated myocytes. Left panel same cell as Fig. 1; right panel, holding potential = −35 mV, holding current = +50 pA, $C_t = 4{\cdot}20 \times 10^{-4}$ μF; cell 3, 6/13/85. The straight lines were obtained by linear least-squares regression (see text for details of estimating K_D from these plots).

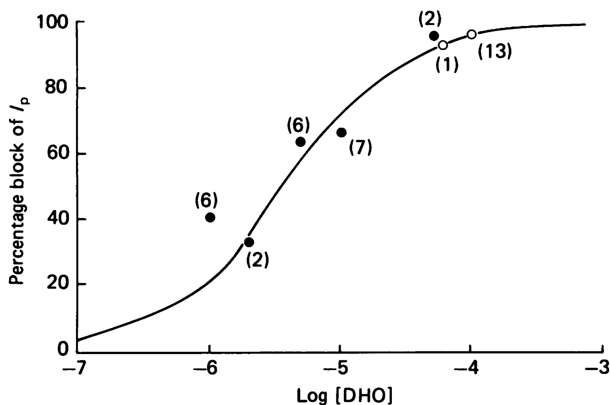


Fig. 3. Dose–response data from a total of fourteen myocytes. In thirteen myocytes 10^{-4} M and in one 6×10^{-5} M was the highest concentration of DHO applied (as indicated by the open circles). The filled circles indicate the average blockage of pump current induced by each of the lower concentrations applied. The numbers in parentheses indicate the number of applications of each concentration (10^{-6}, 2×10^{-6}, 5×10^{-6}, 10^{-5} and 5×10^{-5} M). The average K_D of 3·7 μM was obtained by averaging the K_D values obtained in the fourteen experiments. The graph shows a theoretical dose–response curve with one-to-one binding and a K_D of 3·7 μM. The highest doses (open circles) are assumed to lie on the curve.

We applied 10^{-4} M-DHO to thirty-two preparations in order to estimate the resting pump current. Knowing that the large concentration of cardiotonic steroid would cause an inward shift in holding current, and that the current-passing capabilities of a switch clamp are limited, we usually initiated the experiments with a small net outward holding current. The application of 10^{-4} M-DHO resulted in an increase in inward current which was readily reversible (see Fig. 1). The total amplitude of this current change ranged from 40 to 200 pA depending on the dimensions of the preparation. The mean resting pump current, normalized to capacitance, was $0{\cdot}27 \pm 0{\cdot}02$ μA μF^{-1} (mean ± S.E. of mean, $n = 32$).

Since some of our resting potentials were low (between −20 and −30 mV), we considered it possible that the micro-electrodes could have damaged the membrane on penetration, inducing an additional Na^+-leak component. This addition to the inward background current would add to the normally present background Na^+ current, and increase the pump current necessary to maintain a steady state. To test this hypothesis we divided our preparations into four groups based on holding potential. The results of this analysis are shown in Table 1. The highest resting pump current is observed in the group with holding potentials between −30 and −39 mV. The value obtained for these nine cells of 0·34 $\mu A\ \mu F^{-1}$ was 26 % above the mean value for all cells. Similarly, the lowest value was obtained at the most negative potentials, −50 to −61 mV. This value of 0·21 $\mu A\ \mu F^{-1}$ was 22 % lower than the mean value for all cells. We compared each group of pump currents in Table 1 with those obtained at the lowest holding potentials between −20 and −30 mV by *t* tests. No significant difference ($P > 0.05$) was obtained in all cases.

TABLE 1. Resting pump current as a function of voltage-clamp holding potential

Holding potential	$I_p \pm$ S.E. ($\mu A\ \mu F^{-1}$)	*n*
−20 to −29 mV	0·28 ± 0·03	11
−30 to −39 mV	0·34 ± 0·06	9
−40 to −49 mV	0·25 ± 0·05	4
−50 to −61 mV	0·21 ± 0·03	8

I_p is the resting pump current, S.E. is the standard error of the mean, *n* is the number of myocytes studied in the stated range of holding potentials. I_p can be converted to $\mu A\ cm^{-2}$ by multiplying by 1·54. See Methods for details.

Kinetic properties of the Na^+ pump and a second estimate of resting pump current

Previous investigators (Gadsby, 1980; Eisner & Lederer, 1980) measured the kinetic properties of the Na^+–K^+ pump in Purkinje strands following brief exposure to K^+-free Tyrode solution. We performed similar experiments using isolated myocytes with the added aim of obtaining a second measure of the resting pump current.

Fig. 4*A* illustrates how resting pump current can be estimated from the pump current transient in K^+-containing solution. During the exposure to K^+-free solution, Na^+ enters the cell, but is not extruded by the Na^+–K^+ exchange pump. On re-exposure to 8 mM-K^+, there is an outward current transient that decays to base line, reflecting an increase in the pumping rate resulting from the previous Na^+ load. If we assume that the amount of Na^+ that enters does not change with $[K^+]_o$ (see next section), that the Na^+ that enters does not significantly alter the Na^+ equilibrium potential and that the increase in outward current is solely due to pump current, the resting pump current normalized to myocyte capacitance, is

$$I_{p,K\text{-free}} = Q/TC, \quad (4)$$

where Q is the integral of the pump current transient, T is the time period of exposure to 0 mM-K^+, and C is the membrane capacity of the preparation. In four myocytes we compared this new measure of resting pump current with the measure obtained with high concentrations of DHO (two with 10^{-4} M-DHO, and two with

6×10^{-5} M-DHO). The average ratio of these two measures of resting pump current was $I_{p,DHO}/I_{p,K\text{-free}} = 1{\cdot}76 \pm 0{\cdot}15$ (mean ± S.E. of mean, $n = 4$).

In order to assess the kinetic response of the Na^+–K^+ pump in the myocytes as compared to that in Purkinje strands, we also measured the time constant of decay in eight preparations that were not previously exposed to DHO (see Fig. 4*B*). The time constant of this pump current transient decay was 87 ± 8 s (mean ± S.E. of mean, $n = 8$).

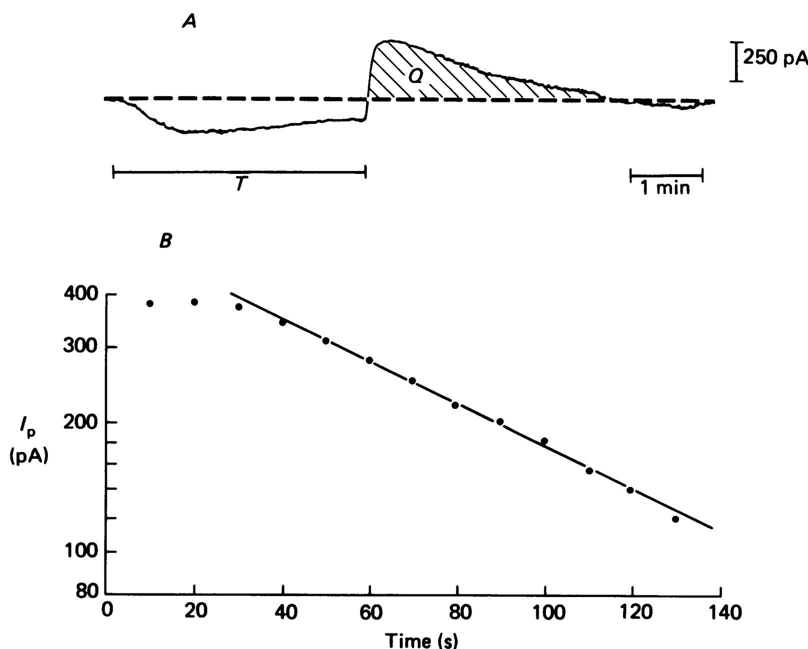


Fig. 4. An illustration of pump current estimation (I_p) using exposure in K^+-free solutions. *A*, for a period of 200 s (T), the myocyte was exposed to K^+-free Tyrode solution, resulting in an increased net inward holding current. Upon return to K^+-containing Tyrode solution net outward current appears, representing additional pump current. Outward current slowly declines towards base-line level. *Q* represents the total charge measured (hatched area). *B*, a semilogarithmic plot of the decaying pump current yields a time constant of 89 s. Holding potential, −24 mV; holding current, = 0 pA; $C_t = 3{\cdot}1 \times 10^{-4}$ μF; $Q = 3{\cdot}73 \times 10^{-8}$ C; cell 2, 4/4/85.

Resting pump current in reduced $[K^+]_o$ *and an estimate of the* K_m *for external* K^+

We examined the changes in resting pump current that occur when $[K^+]_o$ is reduced from 8 to 1 mM. According to models of Na^+–K^+ pump activity and the inward background current, upon reducing $[K^+]_o$ the pump current should decline initially (due to the reduced K^+ activation) and then slowly return to its original level (due to increased Na^+–K^+ pump activity, caused by an increase in $[Na^+]_i$). Results from one such experiment are shown in Fig. 5. The resting pump current in 8 mM-K^+ was 90 pA. Upon reduction of $[K^+]_o$ to 1 mM there was an inward shift in current which resulted in part from the reduced conductance to K^+ at lower $[K^+]_o$. This more than compensated for the increased driving force on K^+ at potentials in the plateau range.

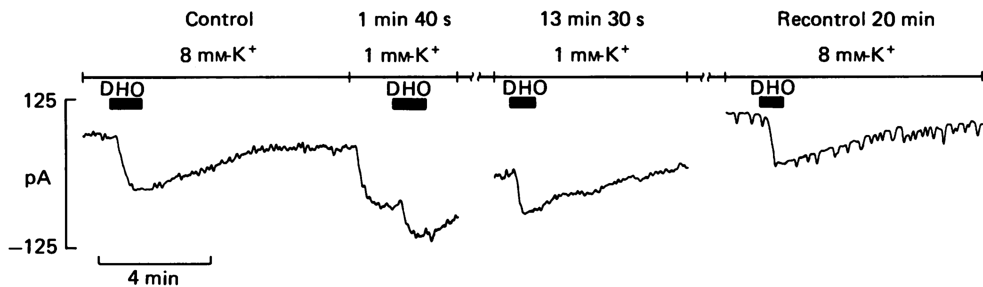


Fig. 5. The effects of reducing $[K^+]_o$ on I_p. The control I_p was 90 pA. On exposure to lowered $[K^+]$ (1 mM) there is an inward shift in current. After 1 min 40 s, 100 μM-DHO was applied. I_p was 50 pA. This concentration should be saturating at the reduced $[K^+]$ since it was saturating at the higher $[K^+]$ (Daut & Rudel, 1982). During prolonged exposure to 1 mM-K^+ the holding current moves more outward. This is at least in part due to an increase in $[Na^+]_i$ and a rise in I_p since at 13·5 min in 1 mM-K^+ I_p has increased to 75 pA. The right-hand panel illustrates a recontrol I_p of 85 pA after 20 min in 8 mM-K^+. The holding current is 30 pA more outward than control. The holding potential is −30 mV and $C_t = 3{\cdot}3 \times 10^{-4}$ μF; cell 4, 11/20/85.

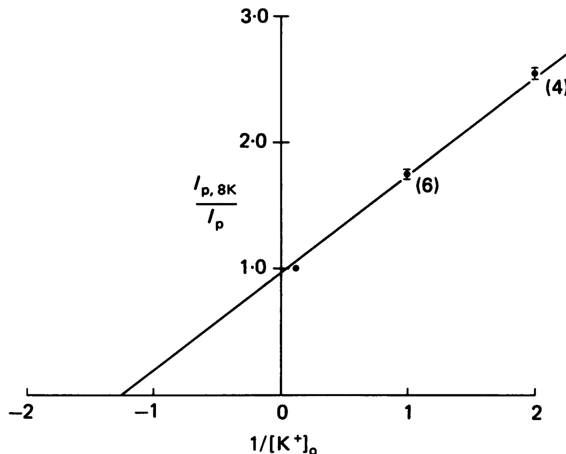


Fig. 6. A Lineweaver–Burk plot is used to estimate K_m for K^+. The inverse of $[K^+]_o$ is plotted against the inverse of the pump current normalized to the value in 8 mM-K^+ ($I_{p,8K}/I_p$). The bar indicates the standard error for the number of experiments indicated in parentheses.

Immediately following reduction to 1 mM-K^+ the resting pump current was 50 pA. During continued exposure to 1 mM-K^+ for 13·5 min the holding current became more outward (presumably due, at least in part, to Na^+ loading and the resulting pump activation), and the resting pump current increased to 75 pA. A recontrol in 8 mM-K^+ Tyrode solution yielded an I_p of 85 pA. At least partial recovery of I_p was obtained in all six preparations studied. In some myocytes full recovery occurred but took up to 30 min.

In three preparations partial recovery of I_p in 1 mM-K^+ Tyrode solution was followed (at $t > 20$ min) by a reduction in both the rate of onset and recovery of the response to DHO application. Upon return to 8 mM-K^+ Tyrode solution, these rates returned to their initial values.

Since the increase in I_p upon reduction to 1 mM-K^+ takes more than 13 min to approach steady state, it is likely that the I_p obtained immediately after changing to 1 mM-K^+ Tyrode solution ($t < 1{\cdot}75$ min) largely represents the effects of reducing $[K^+]_o$ on the external K^+ site of the Na^+–K^+ pump. On the basis of this assumption the K_m for K^+ can be obtained by comparing I_p in low (0·5 or 1 mM) to high (8 mM) K^+ Tyrode solution. A Lineweaver–Burk plot of the results is shown in Fig. 6. The value obtained for K_m for K^+ is 0·8 mM

DISCUSSION

We initiated these experiments to examine the properties of the Na^+–K^+ pump in a preparation where accumulation or depletion of extracellular K^+ was largely eliminated. Our experiments examined some of the steady-state and kinetic properties of the pump as well as the effects of cardiotonic steroid application.

The dose–response relation for DHO

We constructed the dose–response relationship for DHO on the basis of a simple drug–receptor interaction, one drug molecule per receptor, with a saturable pool of receptors. The results were consistent with a K_D of 3·7 μM. There was, however, a potential source of artifact in the experiments, the increase in $[Na^+]_i$ due to partial blockade of the Na^+–K^+ pump. In the absence of drug the inward flux of Na^+ through the inward background current is exactly balanced by its extrusion through the Na^+–K^+ pump. When a fraction of the pump sites are blocked Na^+ efflux is reduced and a net influx of Na^+ results. This net influx increases $[Na^+]_i$, reactivating the Na^+–K^+ pump until the efflux of Na^+ again equals the influx. The time course of this increase in $[Na^+]_i$ is determined by the pump time constant (Falk & Cohen, 1984). Thus, superimposed on the pump inhibition, is a reactivation of the pump initiated by the rise in $[Na^+]_i$. The more rapid the onset of drug action the more minimal is the contamination. For example, on application of 10^{-4} M-DHO, peak current changes occur within 20 s. However, as long as 100 s may be required for the peak change in current following application of 10^{-6} M-DHO. In this latter case appreciable increases in $[Na^+]_i$ could occur before the peak drug-induced current change, reducing the apparent effect of the cardiotonic steroid.

A number of heart preparations exhibit apparent negative co-operativity for the binding of cardiotonic steroids (see Noel & Godfraind, 1984; Lazdunski, Kazazoglou, Renaud & Rossi, 1984; Erdmann, Werdon & Brown, 1984). The origin and significance of the shallow binding curves remains controversial. Despite the possibility that the action of low concentrations of DHO might be underestimated, the action of 10^{-6} M-DHO was somewhat greater than expected. For the moment, however, there is no other evidence to support apparent negative co-operativity in our system. The linear Scatchard plots shown in Fig. 2 and the Hill coefficient of near unity argue against this alternative.

The K_D of 3·7 μM confirms the difference in sensitivity to DHO between Purkinje strands of several species (Ellis, 1977; Lee, Kang, Sokol & Lee, 1980; Falk & Cohen, 1984) and guinea-pig ventricular muscle (Daut & Rudel, 1982). This confirmation is

important because in using the present procedure we have removed both extracellular ion fluctuations in the Purkinje strand and differences in protocol as possible artifactual sources of this difference in sensitivity.

Kinetics of DHO action

Our estimate of parameters describing the kinetics of DHO interaction with the Na^+–K^+ pump are rather rudimentary and subject to a number of confounding variables. For example, enhancement of Na^+–K^+ pump activity resulting from a rise of $[Na]_i$ will tend to distort both the 'on' and 'off' rate constants. The time taken for [DHO] to reach a steady level in the bath will also complicate our estimates of kinetic parameters. Despite such distortions, the agreement between steady-state and kinetic estimates of the K_D implies that the calculated rate constants were not unreasonable. The values of k_1 and k_2 were found to be $9{\cdot}2 \times 10^{-3}$ s^{-1} and $2{\cdot}5 \times 10^3$ M^{-1} s^{-1} respectively. The values of k_1 and k_2 calculated by Daut & Rudel (1982) for blockade of the pump current in the guinea-pig were respectively $3{\cdot}4 \times 10^{-2}$ s^{-1} and $7{\cdot}4 \times 10^2$ M^{-1} s^{-1} for DHO and 6×10^{-3} s^{-1} and 4×10^3 M^{-1} s^{-1} for ouabain obtained in 3 mM-K^+. Thus, the interaction between DHO and the Na^+–K^+ pump was characterized by a faster association rate and a slower dissociation rate in Purkinje myocytes as compared to ventricular muscle. It is likely that these differences reflect differences in the complimentarity between ligand and receptor in the two preparations. Indeed the change in the complimentarity occurring when a different ligand (e.g. ouabain) interacts with the receptor is associated with a comparable change in the binding rate constants.

The pump current measured here is thought to reflect the activity of a well-characterized enzyme, the Na^+- and K^+-dependent ATPase. A number of investigators have determined kinetic constants for the interaction of cardiac glycosides with the Na^+–K^+-ATPase using binding assays involving [^{3}H]ouabain. Typically the reported rates of association and dissociation are even slower than those observed with electrophysiological assays such as the one used in the present study. However, it has become clear that the binding rates of cardiac glycosides are influenced by a variety of factors including the levels of Na^+, K^+, Mg^{2+}, ATP and Ca^{2+} (Schwartz, Lindenmayer & Allen, 1975). Repke, Hermann & Portius (1984) have presented evidence that these effectors control the equilibrium between two conformations of the Na^+–K^+-ATPase. Cardiac glycosides dissociate much faster from one conformer as compared to the other. Differences observed between DHO affinity in the preparation used here, and that used by Daut & Rudel (1982), could presumably reflect differences in local factors that can modify the cardiac glycoside binding site. Alternatively, the primary structure of the ATPase found in the two preparations may be different. Different tissues have been found to contain varying levels of at least two isosymes of the Na^+–K^+-ATPase that differ in their affinity for cardiac glycosides (see Sweadner, 1985).

The resting pump current

Our results demonstrate an appreciable difference in the resting pump current in canine Purkinje myocytes ($0{\cdot}27$ μA μF^{-1}) (this report) and guinea-pig ventricular muscle ($0{\cdot}8$ μA μF^{-1}) (Daut & Rudel, 1982). This difference is well correlated with the appreciable difference in resting membrane resistance (5–7 kΩ cm^2 for ventricle

(Daut, 1982; Sakmann & Trube, 1984) *vs.* 21 kΩ μF for these myocytes (Mathias *et al.* 1985). It is possible that there is an appreciable voltage dependence to the inward background current and our value of I_p in Purkinje myocytes is lower because our resting potentials were lower (−20 to −60 mV *vs.* −90 mV found by Daut & Rudel, 1982), reducing the driving force for the inward background current (Eisner, Lederer & Vaughan-Jones, 1981*a*). Certainly in sheep Purkinje strands $[Na^+]_i$ is higher at more negative potentials (Eisner *et al.* 1981*a*; January & Fozzard, 1984). Evidence against this interpretation is the finding of increased pump current in the steady state in canine and rabbit Purkinje fibres at depolarized potentials, presumably due to tetrodotoxin-sensitive and D-600-sensitive 'window' currents (Cohen *et al.* 1982; Verdonck, 1982; Gadsby, 1983; Verdonck & Bosteels, 1985). Our results indicate a small but non-significant decrease in the resting pump current recorded at the most negative potentials (see Table 1). Any damage induced by the microelectrode would tend to increase the Na^+ influx and the measured I_p. Thus 0·27 $\mu A\ \mu F^{-1}$ can be taken as an upper estimate of I_p. For these reasons it seems most reasonable to assume that the differences in resting pump current in canine Purkinje myocytes and guinea-pig ventricular trabeculae represent real differences in membrane properties between the two preparations.

We compared resting pump current measured in DHO with those estimated by integrating the pump current transient following exposure in K^+-free solution. We chose to compare only those cells where exposures to DHO and to K^+-free solutions were employed because some cells provided very small pump current transients which could not be integrated meaningfully. The ratio, $I_{p,\,DHO}/I_{p,\,K\text{-free}}$ equals 1·76. There are several reasons why $I_{p,\,K\text{-free}}$ could be an underestimate. We know that our solution change is not instantaneous, taking up to 20 s to be nearly complete. This suggests that the time period T of exposure in K^+-free solution could be over-estimated, and also the integral of pump current might be underestimated (since integration is initiated only when the outward current exceeds the original base line). The assumption that no Na^+ is extruded during the period of exposure in K^+-free solution may not be strictly true. K^+ leaking through background channels could activate the Na^+ pump and be responsible for the slow increase in outward current often seen during prolonged exposure to K^+-free solution as internal Na^+ increases (see Fig. 4*A*). Certainly in sheep Purkinje strands it is thought that K^+-free Tyrode solution does not entirely inhibit the pump. However, in this case, cleft K^+ accumulation was the assumed source of residual activation (Ellis, 1977; Eisner *et al.* 1981*b*; DiFrancesco & Noble, 1985). It is therefore interesting that our estimate of resting pump current (0·15 $\mu A\ \mu F^{-1}$; i.e. 0·27/1·76) from exposure in K^+-free solution is similar to that obtained by Eisner *et al.* (1981*a*) employing the sheep Purkinje strand using a different procedure.

The assumption that the outward current transient is pump current was confirmed in a single experiment by applying the entire K^+-free procedure in the presence of 10^{-4} M-DHO. The transient response in K^+-containing Tyrode solution following exposure to K^+-free Tyrode solution was eliminated.

Changes in $[K^+]_o$ and I_p

We tested the assumption that the inward background current is independent of $[K^+]_o$ by varying $[K^+]_o$ from 8 to 1 mM. Our results do suggest (as in guinea-pig

ventricle (Daut & Rudel (1982)) that during long exposures to low K^+ concentrations the $[Na^+]$ rises, causing I_p to return toward initial levels. This compensation was slow, taking more than 15 min for I_p to reach values near the original level. This gradual rise allowed us to estimate the K_m for K^+ from I_p measured at early times. Our value of 0·8 mM is in good agreement with previous kinetic estimates in canine Purkinje strands of 0·9 and 1·2 mM (Gadsby, 1980; Falk & Cohen, 1984).

The pump time constant following exposure to K^+-free solution

The time constant of decay of pump current in 8 mM-K^+ Tyrode solution following a 200 s exposure to K^+-free solution was 87 s. Results from intact canine Purkinje strands are about 75 s in 8 mM-K^+ (Gadsby, 1980; Falk & Cohen, 1984). We cannot rule out changes in V_{max} (due to the effects of the dissociation). However, the similarity in values of time constants provides evidence for the physiological state of the Na^+–K^+ pump in these isolated Purkinje myocytes.

Estimating the rate of increase of a^i_{Na} during pump blockade

Our resting pump current of 0·27 $\mu A\ \mu F^{-1}$ suggests an inward background current of 0·8 $\mu A\ \mu F^{-1}$ (on the assumption of a 3:2 pump coupling ratio). A typical cell of dimensions $180 \times 30\ \mu m$ has a total capacitance of $2{\cdot}83 \times 10^{-4}\ \mu F$ and an inward background current of $2{\cdot}29 \times 10^{-10}$ A. This yields a Na^+ entry of $1{\cdot}4 \times 10^{-13}$ mol min^{-1}. The volume of such a cell is $1{\cdot}27 \times 10^{-10}$ l, yielding an estimated rate of rise of $[Na^+]_i$ of 1·1 mM min^{-1} or of a^i_{Na} (assuming an activity coefficient of 0·75) of $\sim$0·8 mM min^{-1}. This assumes dilution of the Na^+ gained by the cell in the entire cell volume. A more reasonable assumption might be to dilute the Na^+ in the osmotically active volume fraction of 0·61 (Hauser & Freeman, 1980, for canine Purkinje strands). The rate of increase of a^i_{Na} in the osmotically active fraction is 1·3 mM min^{-1}. Considering the slightly higher surface to volume ratio of the canine as compared to the sheep Purkinje myocyte (Callewaert, Carmeliet & Vereecke, 1984) this value compares favourably with values reported by Ellis (1977) (0·5 mM) and Eisner *et al.* (1981 *b*) (1·1 mM) for sheep Purkinje fibres obtained by direct measurement with Na^+ electrodes.

Physiological significance of the results

The physiological significance of the increased sensitivity to cardiotonic steroids and lower resting pump current in Purkinje myocytes as compared to guinea-pig ventricle should be discussed. There has long been discussion about why the Purkinje system becomes digitalis-toxic before working ventricular muscle (Vassalle, Karis & Hoffman, 1962; Somberg, Barry & Smith, 1981). This differential sensitivity could have two origins: (1) similar affinities for cardiotonic steroids in the two preparations, but the same pump blockade causes a more severe alteration of electrical activity in Purkinje fibres or (2) the K_D for DHO could be lower in Purkinje fibres. Our results provide some support for the second of the two alternatives. The possibility that our dissociation procedure altered the K_D is strongly argued against by previous results in intact Purkinje strands (Ellis, 1977; Lee *et al.* 1980; Falk & Cohen, 1984).

The remaining alternative that DHO affinity in guinea-pig and canine ventricular muscle differ cannot be ruled out. In fact, studies involving the binding of [^{3}H]ouabain

argue for this interpretation. In sheep heart no difference was observed between the affinity of [^{3}H]ouabain for sites in ventricle or Purkinje strands (Brown, Hug, Wagner & Erdmann, 1985). The same group has found that [^{3}H]ouabain affinity for guinea-pig heart is much lower than for sheep or dog ventricle (Erdmann, 1981). Further experiments involving pump current measurements should resolve this question.

For the most fully polarized cells (holding potential −50 to −61 mV, Table 1) a pump current magnitude of 0·21 μA μF^{-1} should hyperpolarize the cell membrane by about 4·2 mV if the membrane resistance is about 20 kΩ μF. The membrane should sit 8 mV positive to the K^+ equilibrium potential in steady state under these conditions.

We would like to thank Stephen Johnson for help with the dissociation and Judy Samarel for micro-electrodes and manuscript preparation. This work was supported by HL20558 and PPG HL28958 to I.S.C., and an NRSA and an AHA grant to G.A.G. P.P. is a career scientist of the Ontario Ministry of Health.

REFERENCES

ATTWELL, D., EISNER, D. A. & COHEN, I. (1979). Voltage clamp and tracer flux data: effects of a restricted extracellular space. *Quarterly Review of Biophysics* **12**, 213–261.

BACHMAIER, A., EBNER, F. & REITER, M. (1985). Potassium changes the relationship between receptor occupancy and the inotropic effect of cardiac glycosides in guinea-pig myocardium. *British Journal of Pharmacology* **85**, 755–765.

BROWN, L., HUG, E., WAGNER, G. & ERDMANN, E. (1985). Comparison of ouabain receptors in sheep myocardium and Purkinje fibres. *Biochemical Pharmacology* **34**, 3701–3710.

CALLEWAERT, G., CARMELIET, E. & VEREECKE, J. (1984). Single cardiac Purkine cells: general electrophysiology and voltage clamp analysis of the pace-maker current. *Journal of Physiology* **349**, 643–662.

COHEN, I. S., DATYNER, N. B., GINTANT, G. A., JOHNSON, S. J., MULRINE, N. K. & PENNEFATHER, P. (1986). Na/K (electrogenic) pump current in isolated canine cardiac Purkinje myocytes. *Journal of Physiology* **371**, 190*P*.

COHEN, I., FALK, R. & KLINE, R. P. (1982). Voltage clamp studies on the canine Purkinje strand. *Proceedings of the Royal Society* B **217**, 215–236.

DATYNER, N., GINTANT, G. & COHEN, I. (1985*a*). Microprocessor controlled trituration device for the dissociation of cardiac and other tissues. *Pflügers Archiv* **403**, 105–108.

DATYNER, N., GINTANT, G. & COHEN, I. (1985*b*). Versatile temperature controlled tissue bath for studies of isolated cells using an inverted microscope. *Pflügers Archiv* **403**, 318–323.

DAUT, J. (1982). The passive electrical properties of guinea-pig ventricular muscle as examined with a voltage-clamp technique. *Journal of Physiology* **330**, 221–242.

DAUT, J. (1983). Inhibition of the sodium pump in guinea-pig ventricular muscle by dihydro-ouabain: effects of external potassium and sodium. *Journal of Physiology* **339**, 643–662.

DAUT, J. & RUDEL, R. (1982). The electrogenic sodium pump in guinea-pig ventricular muscle: inhibition of the pump current by cardiac glycosides. *Journal of Physiology* **330**, 243–264.

DIFRANCESCO, D. (1981*a*). A new interpretation of the pace-maker current in calf Purkinje fibres. *Journal of Physiology* **314**, 359–376.

DIFRANCESCO, D. (1981*b*). A study of the ionic nature of the pace-maker current in calf Purkinje fibres. *Journal of Physiology* **314**, 377–393.

DIFRANCESCO, D. & NOBLE, D. (1985). A model of cardiac electrical activity incorporating ionic pumps and concentration changes. *Philosophical Transactions of the Royal Society B***307**, 353–398.

EISNER, D. A. & LEDERER, J. (1980). Characterization of the electrogenic sodium pump in cardiac Purkinje fibres. *Journal of Physiology* **303**, 441–474.

EISNER, D. A., LEDERER, J. & VAUGHAN-JONES, R. (1981*a*). The effects of rubidium ions and membrane potential on the intracellular sodium activity of sheep Purkinje fibres. *Journal of Physiology* **317**, 188–205.

EISNER, D. A., LEDERER, J. & VAUGHAN-JONES, R. (1981*b*). The dependence of sodium pumping

and tension on intracellular sodium activity of voltage-clamped sheep Purkinje fibres. *Journal of Physiology* **317**, 163–187.

ELLIS, D. (1977). The effects of external cations and ouabain on the intracellular sodium activity of sheep heart Purkinje fibres. *Journal of Physiology* **273**, 211–240.

ERDMANN, E. (1981). Influence of cardiac glycosides on the receptor. In *Handbook of Experimental Pharmacology*, vol. 56, sect. I, ed. GREEF, K., p. 337. Berlin: Springer Verlag.

ERDMANN, E., WERDAN, K. & BROWN, L. (1984). Evidence for two kinetically and functionally different types of cardiac glycoside receptors in the heart. *European Heart Journal* **5**, suppl. F, 297–302.

FALK, R. & COHEN, I. (1984). Membrane current following activity in canine cardiac Purkinje fibers. *Journal of General Physiology* **83**, 771–799.

FORBUSH, B. (1983). Cardiotonic steroid binding to Na, K-ATPase. *Current Topics in Membrane and Transport* **19**, 169–201.

GADSBY, D. (1980). Activation of electrogenic Na^+/K^+ exchange by extracellular K^+ in canine cardiac Purkinje fibres. *Proceedings of the National Academy of Sciences of the U.S.A.* **77**, 4035–4039.

GADSBY, D. (1983). Adrenoceptor agonists increase membrane K^+ conductance in cardiac Purkinje fibres. *Nature* **306**, 691–693.

GADSBY, D. C. (1984). The Na/K pump of cardiac cells. *Annual Review of Biophysics and Bioengineering* **13**, 373–398.

GADSBY, D. & CRANEFIELD, P. (1979). Direct measurement of sodium pump current in canine Purkinje fibers. *Proceedings of the National Academy of Sciences of the U.S.A.* **76**, 1783–1787.

GINTANT, G. A., DATYNER, N. & COHEN, I. (1985). Gating of delayed rectification in acutely isolated canine cardiac Purkinje myocytes: evidence for a single voltage-gated conductance. *Biophysical Journal* **48**, 1059–1064.

GLITSCH, H. & PUSCH, H. (1980). Correlation between changes in membrane potential and intracellular sodium activity during K activated response in sheep Purkinje fibres. *Pflügers Archiv* **384**, 189–191.

HAUSER, S. & FREEMAN, A. (1980). Volumetric properties of intracellular compartments in canine cardiac Purkinje cells. *American Journal of Physiology* **238**, H561–568.

ISENBERG, G. & TRAUTWEIN, W. (1974). The effect of dihydroouabain and lithium ions on the outward current in cardiac Purkinje fibres: evidence for electrogenicity of active transport. *Pflügers Archiv* **350**, 41–54.

JANUARY, C. T. & FOZZARD, H. A. (1984). The effects of membrane potential, extracellular potassium and tetrodotoxin on the intracellular sodium activity of sheep cardiac muscle. *Circulation Research* **54**, 652–665.

LAZDUNSKI, M., KAZAZOGLOU, T., RENAUD, J. F. & ROSSI, B. (1984). Digitalis receptors in cardiac cells and their relation with positive inotropic and cardiotoxic effects. *European Heart Journal* **5**, suppl. F, 281–290.

LEE, C. O., KANG, D. H., SOKOL, J. H. & LEE, K. S. (1980). Relation between intracellular Na ion activity and tension of sheep cardiac Purkinje fibres exposed to dihydro-ouabain. *Biophysical Journal* **29**, 315–330.

MATHIAS, R. & COHEN, I. (1984). Linear impedance of Purkinje strands. *Biophysical Journal* **45**, 278a.

MATHIAS, R. T., EISENBERG, B., DATYNER, N. B., GINTANT, G. A. & COHEN, I. S. (1985). Impedance and morphology of isolated canine cardiac Purkinje myocytes: comparison with intact strand preparations. *Biophysical Journal* **47**, 499a.

MULRINE, N. K., PENNEFATHER, P., COHEN, I., GINTANT, G. & DATYNER, N. (1986). Resting pump current and the dose–response relationship for dihydro-ouabain in isolated canine cardiac Purkinje myocytes. *Biophysical Journal* **49**, 402a.

NOEL, F. & GODFRAIND, T. (1984). Heterogeneity of ouabain specific binding sites and (Na^+ + K^+)-ATPase inhibition in microsomes from rat heart. *Biochemical Pharmacology* **33**, 47–53.

NOBLE, D. & TSIEN, R. W. (1968). The kinetics and rectifier properties of the slow potassium current in cardiac Purkinje fibres. *Journal of Physiology* **195**, 185–214.

REPKE, K. R. H., HERMANN, I. & PORTIUS, H. J. (1984). Interaction of cardiac glycosides and Na, K-ATPase is regulated by effector-controlled equilibrium between two limit enzyme conformers. *Biochemical Pharmacology* **33**, 2089–2099.

SAKMANN, B. & TRUBE, G. (1984). Conductance properties of single inwardly rectifying potassium channels in ventricular cells from guinea-pig heart. *Journal of Physiology* **347**, 641–657.

SCATCHARD, G. (1949). The attractions of proteins for small molecules and ions. *Annals of the New York Academy of Sciences* **51**, 660–672.

SCHWARTZ, A., LINDENMAYER, G. & ALLEN, J. (1975). The sodium–potassium adenosinetriphosphatase: pharmacological, physiological and biochemical aspects. *Pharmacological Reviews* **27**, 3–134.

SOMBERG, J., BARRY, H. & SMITH, T. W. (1981). Differing sensitivities of Purkinje fibers and myocardium to inhibition of monovalent cation transport by digitalis. *Journal of Clinical Investigation* **67**, 116–123.

SWEADNER, K. J. (1985). Enzymatic properties of separated isozymes of the Na,K-ATPase: subtrate affinities, kinetic cooperativity and ion transport stoichiometry. *Journal of Biological Chemistry* **260**, 11508–11513.

THOMAS, R. C. (1972). Electrogenic sodium pump in nerve and muscle cells. *Physiological Reviews* **52**, 563–594.

VASSALLE, M., KARIS, J. & HOFFMAN, B. F. (1962). Toxic effects of ouabain on Purkinje fibers and ventricular muscle fibers. *American Journal of Physiology* **203**, 433–439.

VERDONCK, F. (1982). Activation of sodium pump current in rabbit Purkinje fibers. *Journal of General Physiology* **80**, 24a.

VERDONCK, F. & BOSTEELS, S. (1985). The effects of membrane potential and extracellular K^+ on Na^+ pump activity and intracellular Na^+ activity in rabbit cardiac Purkinje fibers. *Journal of General Physiology* **86**, 30a.