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The Na/K pump, resting potential and selective permeability in canine Purkinje fibres at physiologic and room temperatures

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Abstract. All mammalian cells maintain a resting potential generated by ions moving down concentration gradients. In excitable cells, the inside potential is negative relative to outside. In order to maintain this electrochemical gradient, the sodium potassium (Na/K) pump actively transports out three sodium ions for every two potassium ions it brings in. This process generates a net outward current and thus hyperpolarizes the resting potential. I employed dihydroouabain (DHO) to inhibit the Na/K pump and thus measure its contribution to the resting potential. It contributed 9.0 mV at 34 °C and 3.8 mV at 25 °C. The P_K/P_{Na} ratios were calculated at both temperatures before and after subtracting the Na/K pump contribution. These ratios also suggested a decreased contribution of the Na/K pump under hypothermia. Taken together, these results suggest that the pump contribution to the resting potential is more significant at physiologic temperatures (34 °C) than at room temperature (25 °C), and that estimates of selective permeability can only be accurately obtained after assessing and eliminating the Na/K pump contribution to the resting potential.
Key words. Na/K pump; resting potential; selective permeability; Purkinje fibres; dihydroouabain.

All cells have a resting potential between the cell interior and the extracellular space. This potential (inside negative) is generated by the electrodiffusion of ions through selectively permeable ion channels. In the Purkinje fibres of the ventricular conducting system this value is usually between about −60 mV to −100 mV depending on the bathing $[K^+]$. The ion gradients responsible for generating the resting potential are sustained by active transport processes like the Na/K pump. This pump maintains both Na^+ and K^+ gradients by extruding three sodium ions and transporting two potassium ions inward in each cycle. Given the net extrusion of charge, a current is generated by the pump which hyperpolarizes the Purkinje cell membrane. The activity of the Na/K pump in Purkinje fibres is confirmed by the phenomenon of overdrive suppression. Rapid activation of Purkinje action potentials loads internal $[Na^+]$, increases Na/K pump activity, hyperpolarizes the cell membrane potential and reduces pacemaker activity[1].

It is the aim of the present study to define the contribution of the Na/K pump to the resting potential of the Purkinje fibre in close to physiologic conditions. The effect of a drop in temperature (which should decrease active transport processes) on this contribution is also assessed. Finally, I determine what effect this pump current has on estimates of the membrane selectivity (P_K/P_{Na} ratio).

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Materials and methods

Adult mongrel dogs were euthanized with pentobarbital (360 mg/ml) and were given doses as per their body weight (1 ml/10 pounds). Free-running Purkinje fibre bundles ranging from 400–800 microns in diameter and 4–5 mm in length were dissected from the heart. Recovery of the fibres was as previously described[2]. Microelectrodes were filled with 3 M KCl and had resistances of 15 MΩ to 30 MΩ. Standard single microelectrode recording with a high impedance amplifier was employed. The experiments were conducted either at 34 °C or at room temperature (25 °C). The solutions I employed contained in mM: KCl 8.0, NaCl 132.0, HEPES 2.5, HEPES, Na 2.5, $MgCl_2$ 1.0, $CaCl_2$ 2.0, dextrose 8.0. The pH was titrated to 7.0.

Dihydroouabain (DHO, 10^{-4} M) was used to totally block the Na/K pump. DHO has been demonstrated to be a specific blocker of the Na/K pump[3], and has a K_d of 3.7 μM in canine Purkinje myocytes[2]. This means that more than 96% of pump current is blocked.

The Na/K pump contribution to the resting potential was defined as $(V_{control} - V_{DHO})$. To get an accurate V_{DHO}, the lowest voltage readings taken every 30 s over a period of 5 min were recorded. The mean of this data was considered V_{DHO}. An experiment was included for further analysis if DHO was applied and washed out with at least 33% recovery towards the original resting potential. All data is presented as mean ± SEM. Comparisons for statistical significance were obtained using Student's *t*-test. Differences were considered to be significant when $p < 0.05$

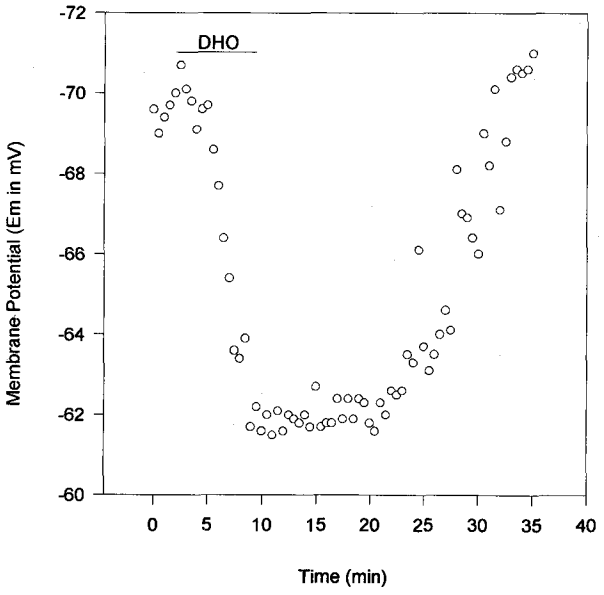

Figure 1. Results of a sample protocol at 34 °C. After obtaining a stable resting potential of −69.0 mV, DHO at 10^{-4} M was added to the bathing Tyrode. The membrane potential depolarized by 7.2 mV to a value of −61.8 mV in DHO containing Tyrode. This fibre showed complete recovery after the DHO was removed.

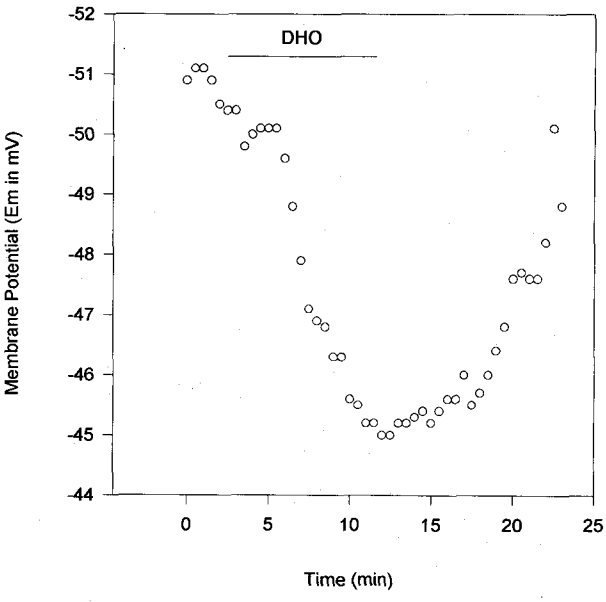

Figure 2. Results of a sample protocol at 25 °C. After obtaining a stable resting potential of −51.0 mV, DHO at 10^{-4} M was added to the bathing Tyrode. The membrane potential depolarized by 6.0 mV to a value of −45.0 mV in DHO containing Tyrode. Partial recovery was obtained when the DHO was removed.

Results

Physiologic temperature. DHO (10^{-4} M) was applied to a Purkinje fibre, and the changes in the resting potential were observed. Results of a sample protocol are illustrated in figure 1, and the data from all experiments are in table 1 (experiments 1 through 6). After obtaining a stable resting potential ($V_{control}$) of −69.0 mV, DHO was added to the bathing Tyrode. The membrane potential depolarized by 7.2 mV (V_p) in the solution containing DHO. The DHO was then washed out, and a complete recovery was obtained.

In six experiments the average value for the voltage change induced by DHO (and thus the pump contribution to the resting potential) was 9.0 ± 1.6 mV (mean ± SEM).

The data were further analyzed using the Goldman equation to estimate the P_K/P_{Na} ratio of the fibre with and without the pump contribution. The Goldman equation employed was: $E_m = -61 \log \{(x[K]_i + [Na]_i)/(x[K]_o + [Na]_o)^4$ $(x = P_K/P_{Na})$. If the contribution of the Na/K pump to the resting potential is large, it suggests that estimating the P_K/P_{Na} ratio without removing this contribution can lead to significant errors. It was found that the change in this ratio without the pump was large, decreasing from 33.1 to 13.9 ($p < 0.05$), see table 1. Therefore, the pump contribution does have a significant effect on calculations of selective permeability at physiologic temperature.

Hypothermia. A sample experiment of the same type at room temperature is provided in figure 2. The resting potential is −51.0 mV prior to DHO application and becomes 6.0 mV more positive on application of DHO (10^{-4} M). A partial recovery on washout was obtained. Results of the five hypothermia experiments can be seen in table 1 (hypo-experiments 1 through 5). Under hypothermia, the Na/K pump contributes less to the resting potential than it does at physiologic temperature. This can be seen in table 1.

The mean value the pump contributed (V_p) was 3.8 ± 0.7 mV (mean ± SEM). Again, the data were further analyzed with the Goldman equation to calculate the P_K/P_{Na} ratio. The ratios in these conditions change much less; with the pump, the value is 22.6 ± 4.8 mV (mean ± SEM), and in the absence of the pump contribution, a ratio of 16.6 ± 3.5 mV (mean ± SEM) was calculated. This 1.36-fold difference was not significant. Not surprisingly, the decreased pump contribution to the resting potential results in a much smaller correction when the Na/K pump contribution to the selective permeability ratio is eliminated.

Discussion

All excitable cells contain a resting permeability to Na^+ and K^+. As such, the concentration gradients across the membrane will dissipate if an active transport process does not exist. In Purkinje fibres this active transport generates a current[2]. From their measurements of the current, Cohen et al.[2] expected about a 4.2 mV V_p in isolated canine Purkinje myocytes in 8 mM $[K^+]_o$. My estimate of about 9.0 mV confirms the expectation of a measurable V_p, but exceeds the expected magnitude.

Table 1. This data table summarizes the results from the normothermia and hypothermia experiments. Note the mean pump contribution for normothermia (9.0 mV) as compared to the mean pump contribution for hypothermia (3.8 mV).

Control experiment fibre #	$V_{control}$	V_{DHO}	$V_p = V_{control} - V_{DHO}$	Percent recovery	P_K/P_{NA} ratio (pump present)	P_K/P_{Na} ratio (pump absent)
1: 10/7/93	−52.0 mV	−46.8 mV	5.2 mV	56	12.1	8.8
2: 11/24/93	−62.6 mV	−50.4 mV	12.2 mV	100	28.8	10.9
3: 12/14/93	−60.0 mV	−55.6 mV	4.4 mV	36	22.1	15.6
4: 12/22/93	−67.8 mV	−53.6 mV	14.2 mV	34	53.3	13.5
5: 12/22/93	−56.6 mV	−46.0 mV	10.6 mV	69	16.7	8.4
6: 12/22/93	−69.0 mV	−61.8 mV	7.2 mV	100	65.3	25.9
n = 6	$V_{control} = -61.3$ mV ±2.7	$V_{DHO} = -52.4$ mV ±2.4	$V_p = 9.0$ mV ± 1.6	% recovery = 65.8 ±12.0	x = 33.1 ± 8.8	x = 13.9 ±2.7
Hypo-experiment fibre #						
1: 6/29/94	−54.7 mV	−51.6 mV	3.1 mV	100	16.7	13.3
2: 7/6/94	−51.0 mV	−45.0 mV	6.0 mV	74	12.7	8.7
3: 7/7/94	−54.9 mV	−52.3 mV	2.6 mV	35	17.0	14.0
4: 7/19/94	−63.4 mV	−61.0 mV	2.4 mV	88	39.0	29.5
5: 7/19/94	−60.4 mV	−55.4 mV	5.0 mV	62	27.7	17.6
n = 5	$V_{control} = -56.9$ mV ±2.2	$V_{DHO} = -53.0$ mV ±2.6	$V_p = 3.8$ mV ±0.7	% recovery = 71.8 ±11.2	x = 22.6 ±4.8	x = 16.6 ±3.5

The combined results of the 11 experiments are provided in the table. Fibre # signifies the experiment number. $V_{control}$ is the resting potential for each cell. V_{DHO} is the resting potential after DHO was applied. V_p is the difference between $V_{control}$ and V_{DHO}, therefore giving us the contribution of the Na/K pump. The percent recovery shows how much each cell recovered in the direction of $V_{control}$. All values in the table are given as mean ± SEM. At the far right-hand side of the table, the P_K/P_{Na} ratios can be seen. In the first column (pump present), the values are calculated from measurements prior to pump blockade and assume that the pump contribution to the resting potential is negligible. The second column is calculated from measurements taken after blockade of the Na/K pump by DHO. Note that blocking the pump changes the P_K/P_{Na} ratio from 33.1 to 13.9 at normothermia, but only from 22.6 to 16.6 at hypothermia. In performing these calculations, I used an activity coefficient for K^+ of 0.75, and an activity coefficient for Na^+ of 0.74 for the extracellular solutions. The soldium activities employed in the calculations were $a_iNa = 6.8$ mM and $a_oNa = 100.9$ mM. The potassium activities employed in the calculations were $a_iK = 100.6$ mM and $a_oK = 5.9$ mM[8]. The Goldman equation employed was: $E_m = -61 \log \{(x[K]_i = [Na]_i)/(x[K]_o = Na]_o)\}^4$ $x = (P_K/P_{Na})$. The assumption in employing this equation is that P_{Na} and P_K are the dominant permeabilities at the resting potential.

There are several potential explanations of this somewhat larger observation. First, the Purkinje myocytes may lose some pumping sites in the dissociation procedure due to the collagenase treatment. Second, the Purkinje fibres (unlike the isolated myocytes) contain narrow intercellular spaces between the electrically connected cells. When the Na/K pump is blocked K^+ leaks out of the cells and is not pumped back in. This increased intercellular $[K^+]$ will contribute to the observed depolarization by changing the Nernst equilibrium potential for K^+. This effect is not expected to be too large, since the canine Purkinje fibre contains wide intercellular spaces[5]. Third, at times multiple impalements had to be performed before a stable resting potential was attained. This could have damaged the fibres, causing an increased background Na^+ leak.

The only previous estimate of the pump contribution in canine cardiac Purkinje fibres was obtained by Gadsby and Cranefield in 1979[6] in non-physiologic external solutions (low Cl^- solutions) at more depolarized membrane potentials. Both these differences should have increased membrane resistance and so might be expected to have measured a larger pump contribution, but this experiment also measured 9.0 mV. Thus, taken as a whole, I view the value of 9.0 mV as an upper limit to the pump's contribution to the resting potential. This contribution should increase excitability[7] as well as maintain ion gradients.

At a lower temperature, the resting potential started out at a somewhat more positive value partly because the pump is inhibited under hypothermia. When I blocked the already stunned pump with solutions containing 10^{-4} mM [DHO], the E_m depolarized to a value near the potentials measured with DHO under normal temperatures. This shows that in most cases the resting potential without the Na/K pump is largely unaffected by the change in temperature. Further, the P_K/P_{Na} ratio in the absence of the Na/K pump contri-

bution is nearly identical at the two temperatures ($p > 0.05$). However, if estimates of selective permeability of the Purkinje fibres are made between the two temperatures without first eliminating the Na/K pump contribution, a more noticeable difference is obtained ($p < 0.10$). This calculation demonstrates the inaccuracy of selective permeability measurements when the Na/K pump contribution is not first eliminated.

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