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Role of the Na^+/K^+-ATPase in regulating the membrane potential in rat peritoneal mast cells

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1 The aim of this study was to investigate the effect of the Na^+/K^+-ATPase on the membrane potential of peritoneal mast cells isolated from male Sprague-Dawley SPF-rats.

2 Experiments were performed at 22–26°C in the tight-seal whole-cell configuration of the patch-clamp technique by use of Sylgard-coated patch pipettes (3–6 MΩ). High-resolution membrane currents were recorded with an EPC-9 patch-clamp amplifier controlled by the 'E9SCREEN' software. In addition, a charting programme on another computer synchronously recorded at low resolution (2 Hz) membrane potential and holding current (low-pass filtered at 500 Hz).

3 Na^+/K^+-ATPase activity was measured as the ouabain-sensitive change in the zero-current potential. The zero-current potential in rat peritoneal mast cells measured 2 min after obtaining whole-cell configuration amounted to 1.7 ± 2.5 mV ($n=21$). Ouabain (5 mM), a Na^+/K^+-ATPase-inhibitor, had only a very minor effect upon the membrane potential under resting conditions ($n=3$).

4 When mast cells were superfused with nominal calcium-free external solution, the cells hyperpolarized (Δ mV: 20.2 ± 3.8 mV ($n=5$)). In addition, when the mast cells were preincubated in nominal calcium-free external solution for 12 ± 1.6 min before whole-cell configuration, the membrane potential amounted to -53.7 ± 9.8 mV ($n=8$). A subsequent superfusion with ouabain (5 mM) depolarized the membrane potential (ouabain-sensitive hyperpolarization (Δ mV): 23.0 ± 8.4 mV ($n=8$)).

5 A high intracellular concentration of Na^+ ($[Na^+]_i$) (26.6 mM) also resulted in hyperpolarization (Δ mV: 20.2 ± 9.1 mV ($n=7$)), but only when ATP was present. A subsequent superfusion with ouabain (5 mM) repolarized these cells to -1.2 ± 14 mV (ouabain-sensitive hyperpolarization (Δ mV): 19.7 ± 7.7 mV ($n=7$)).

6 The size of the $[Na^+]_i$-dependent hyperpolarization was dose-dependent. Low $[Na^+]_i$ (1 mM) had no effect on membrane potential and these cells were unaffected by superfusion with calcium-free external solution.

7 These data thus directly confirm that the stimulant effect of calcium-free external solutions on the ouabain-sensitive changes in the zero-current potential, and hence the Na^+/K^+-ATPase, is mediated through $[Na^+]_i$ and that the activity of the Na^+/K^+-ATPase can have an important influence on the resting membrane potential in rat peritoneal mast cells.

Keywords: Mast cell; Na^+/K^+-ATPase; ouabain; calcium; patch clamp; membrane potential.

Introduction

It was earlier shown that the digitalis glycoside, ouabain, which inhibits the Na^+/K^+-ATPase, increased the stimulation-induced release of histamine from rat peritoneal mast cells in a nominal calcium-free external solution, independently of whether the cells were stimulated immunologically (Frossard *et al*., 1983) or non-immunologically (Amellal *et al*., 1984). These results prompted investigations on the properties of the Na^+/K^+-ATPase in the rat peritoneal mast cell (Bronner *et al*., 1989). They used the potential-sensitive dye *bis*-oxonol and measured the fluorescence intensity as an indicator of the membrane potential. They showed that ouabain increased the fluorescence intensity of *bis*-oxonol only in the absence of extracellular calcium and that the plasma membrane potential was most likely set by the Na^+/K^+-ATPase (Bronner *et al*., 1989). In addition, they showed that the change in the fluorescence of *bis*-oxonol was an ATP-dependent process (Bronner & Landry, 1991).

The Na^+/K^+-ATPase was also investigated by measuring the ouabain-sensitive potassium uptake (with $^{86}Rb^+$ as a K^+-analogue), and these studies demonstrated the presence of a Na^+/K^+-pump in the mast cell (Knudsen & Johansen, 1989a). The pump activity was inhibited by extracellular calcium and by the presence of other divalent cations in the extracellular fluid (Knudsen & Johansen, 1989b; Knudsen *et al*., 1990). Finally, it was shown that the pump had a large reserve capacity (Knudsen & Johansen, 1990). On the basis of these $^{86}Rb^+$-flux studies, it was proposed that the inhibitory effect of divalent cations on the Na^+/K^+-pump occurred through a decreased sodium uptake in the presence of divalent cations followed by a diminished stimulation of the pump by intracellular sodium (Knudsen *et al*., 1990). The ability of the pump to respond to changes in the intracellular concentration of sodium was also suggested to be the reason for the existence of the reserve capacity of the pump (Knudsen & Johansen, 1990).

Patch-clamp measurements of the resting membrane potential of rat peritoneal mast cells were described as early as 1986, where it was shown that rat peritoneal mast cells had neither a defined resting potential nor significant ion conductances in the plasma membrane (Lindau & Fernandez, 1986). It was not possible to record a stable negative resting potential in the whole-cell configuration, and the zero-current potential usually showed slow fluctuations around 0 mV (Lindau & Fernandez, 1986). Due to the lack of significant ion conductances, it was proposed that the zero-current potential was determined by other electrogenic ion translocation systems, such as the Na^+/Ca^{2+}-exchanger or the Na^+/K^+-ATPase. Such ion movements could contribute to inward and outward currents, generating a rather small net current. However, these studies were performed with an internal pipette solution with a low Na^+ concentration (3 mM) and, accordingly, only a rather small current (below the limit of 1 pA) would be expected to be

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mediated by the Na^+/K^+-ATPase (Lindau & Fernandez, 1986).

In the present study, the patch-clamp technique was applied to study more directly the effects of intracellular sodium on the activation of the Na^+/K^+-pump in the rat peritoneal mast cell. Na^+/K^+-ATPase activity was measured as the ouabain-sensitive change in the zero-current potential. The contribution of the Na^+/K^+-pump activity to the membrane potential in these cells was investigated in the presence of different concentrations of intracellular sodium and in the absence or presence of extracellular calcium.

Methods

Isolation of mast cells

Male Sprague-Dawley SPF-rats weighing 150–300 g were used for the experiments. Rats were killed by bleeding from the carotid arteries after asphyxiation in CO_2. Mixed peritoneal cells were collected by injecting a physiological salt solution ('isolation buffer') buffered with 10 mM HEPES (4,(2-hydroxyethyl)-1-piperazine ethane sulphonic acid) through a small incision in the abdominal wall. After gentle massage of the rat abdominal region, the peritoneal cavity was surgically exposed and about 7 to 8 ml of fluid was aspirated. This fluid contained macrophages, neutrophils, erythrocytes and mast cells. After centrifugation at 220 *g* for 10 min, the supernatant was discarded and the mixed cells were resuspended in physiological salt solution buffered with bicarbonate, phosphate and HEPES ('incubation buffer'). Aliquots of about 4 ml were transferred to 6 to 8 coverslips in petri dishes. Cells were incubated at 37°C for at least 60 min before use under constant humidified gas flow (5% CO_2 and 95% O_2).

Patch-clamp experiments

Mixed peritoneal cells on the cover glasses were gently superfused with external physiological solution to remove floating cells and albumin, then quickly transferred to the recording chamber with approximately 150 μl external physiological solution ('experimental buffer'). External solution changes were made by pressure injection from a micropipette (10 to 15 μm in diameter) positioned 10 to 20 μm from the cell. Experiments were performed at room temperature (22 to 26°C) in the tight-seal whole-cell configuration of the patch-clamp technique (Hamill *et al*., 1981), by use of heat-polished Sylgard-coated patch pipettes with resistances of 3 to 6 MΩ. Series resistances were in the range of 5 to 15 MΩ and seal resistances were in the range of 1 to 5 GΩ. High-resolution membrane currents were recorded with an EPC-9 patch-clamp amplifier (HEKA, Lambrecht, Germany) controlled by the 'E9SCREEN' software on an Atari computer (MEGA/STE). High-resolution currents were low-pass filtered at 2.3 kHz and acquired at a sampling rate of 10 kHz, while a charting programme on another computer synchronously recorded at low resolution (2 Hz) parameters such as membrane potential and holding current (low-pass filtered at 500 Hz). Seal, capacitance and other membrane properties were monitored by the response to eight voltage steps of 600 ms duration (covering a range of −80 to +60 mV) applied exactly 60 s after establishment of the whole-cell configuration. Capacitative currents and series resistance were cancelled before each voltage pulse and every second minute during current-clamp experiments by means of the automatic capacitance compensation of the EPC-9. Current-clamp experiments were temporarily interrupted during compensation of capacitative currents and series resistance, because automated compensation does not work in this mode. The reference electrode was an Ag/AgCl pellet connected to the bath solution through a 150 mM NaCl/agar bridge.

All potentials were corrected for the liquid junction potential that develops at the tip of the pipette when it is immersed into the bath solution (Fenwick *et al*., 1982). The liquid junction potential between the normal internal and external solution was about 10 mV, mainly because of the different mobilities of the Cl^- and glutamate anions (Neher, 1992).

For this study, a total of 116 cells was examined. All values are given as means ± s.e.mean (n = number of experiments). These were calculated from values obtained independently, i.e. if $n = 5$, these 5 experiments were performed on 5 mast cells from 5 different rats.

Materials

Bovine serum albumin and heparin were supplied by Sigma Chemical Company (St. Louis, MO, U.S.A.) and glucose by E. Merck (Darmstadt, Germany). Ouabain was dissolved in the experimental buffer (by ultrasound sonification) and was obtained from Mecobenzon, Denmark. All other chemicals were of analytical grade.

Solutions

Various mixtures of standard internal solution (SIS) and high $(Na^+)_i$ (+) internal solution were prepared (see Table 1), in order to obtain a number of different Na^+-concentrations for the dose-response experiments. For the composition of the external solutions see Table 2.

Results

In resting peritoneal mast cells, the membrane potential fluctuated around 0 mV. Two minutes after obtaining whole-cell configuration, the zero-current potential was found to be

Table 1 Composition of internal solutions (in mM)

	Control	*SIS**	*High* $[Na^+]_i(+)$¤	*High* $[Na^+]_i(-)$#	*Low* $[Na^+]_i$
K-glutamate	145	135	134	135	145
NaCl	12.5	2	15	25	–
Na-glutamate	–	–	11	–	–
KCl	–	10	–	–	12.5
$MgCl_2$	1	1.5	–	1	1
HEPES	10	10	10	10	10
Na_2-ATP	0.2	3	–	–	0.2
Mg-ATP	–	–	2	–	–
Na_2-GTP	0.3	0.3	0.3	0.3	0.3
pH (KOH, 25°C)	7.0	7.0	7.02	7.0	7.0
$[Na^+]_i$	13.5	8.6	26.6	25.6	1

SIS* = standard internal solution. (+)¤ = the (+) denotes that this solution contains ATP (2 mM). (−)# = the (−) denotes that this solution is without ATP. Various mixtures of standard internal solution (SIS) and high $(Na^+)_i(+)$ internal solution were prepared, in order to obtain a number of different Na^+-concentrations for the dose-response experiments.

Table 2 Composition of external solutions (in mM)

	Isolation buffer	*Incubation buffer**	*Experimental buffer (control)*	*Calcium-free buffer*
$KH_2PO_4 + Na_2HPO_4$ [Δ]	0.62 + 2.46	0.62 + 2.46	–	–
$NaHCO_3$	–	24	–	–
HEPES	10	10	10	10
NaCl	140	116	140	144
KCl	4	4	2.5	2.5
$MgSO_4$	1.2	1.2	–	–
$MgCl_2$	–	–	5	5
$CaCl_2$	1	1	2	–
Glucose	5.6	5.6	5.6	5.6
BSA	1 mg ml^{-1}	1 mg ml^{-1}	–	–
Heparin	50 μg ml^{-1}	–	–	–
pH (KOH)	7.4 (37°C)	7.4 (37°C)	7.26 (25°C)	7.26 (25°C)

*Equilibrated with 95% O_2 and 5% CO_2. [Δ]pH adjusted to 7.38 (20°C).

1.7 ± 2.5 mV ($n = 21$), and at the end of the experiment (about 10 min), it was -1.3 ± 0.4 mV ($n = 21$). These experiments were performed with control internal and external solutions (Tables 1 and 2). During the current-clamp measurements, some ($n = 11$) mast cells degranulated spontaneously, but the effect on membrane potential was apparently small (Table 3). Spontaneous degranulation occurred generally more frequently during long recordings (> 5 min), and the degranulation was always slow as compared with the degranulation induced by compound 48/80 or GTPγS. The control internal solution for the pipette contained 0.2 mM ATP (Table 1). To ensure that this ATP concentration was sufficient to supply the Na^+/K^+-ATPase with substrate, similar experiments were performed with a pipette solution containing 3 mM ATP (solution SIS, Table 1). These results showed that the membrane potential was unaffected by the higher ATP concentration: the zero-current potential changed only by 0.6 ± 3.1 mV ($n = 15$) during the experimental period lasting 8.3 ± 1.4 min ($n = 15$). Six cells degranulated spontaneously during these experiments.

In another series of experiments, mast cells were superfused by an application pipette that was brought close to the mast cell under investigation. The application pipette contained ouabain (5 mM) dissolved in experimental buffer (control) (Table 2) and positive pressure to the pipette was applied 100 s after whole-cell configuration and lasted for several minutes before the pipette ran empty. Ouabain, a Na^+/K^+-ATPase-inhibitor, only had a very small effect upon the zero-current potential under these experimental conditions (Figure 1). This experiment was repeated 3 times with similar results. No spontaneous degranulation was observed during these experiments.

In another set of experiments, mast cells were superfused with calcium-free external solution (Table 2) in the application pipette. This treatment hyperpolarized the cells from 7.0 ± 4.0 mV before superfusion to -13.2 ± 7.1 mV ($n = 5$). The superfusion lasted approximately 3 to 5 min during which period the zero-current potential was reduced by Δ mV: 20.2 ± 3.8 mV ($n = 5$) (Figure 2a). One of these cells degranulated spontaneously.

Similarly, mast cells were preincubated in a calcium-free external solution for 12 ± 1.6 min ($n = 8$) before whole-cell recording was begun. Under these experimental conditions, the zero-current potential amounted to -53.7 ± 9.8 mV ($n = 8$) two minutes after whole-cell configuration had been obtained. These cells were then superfused with ouabain (5 mM) in an application pipette and this treatment depolarized the cell to -30.7 ± 7.3 mV ($n = 8$). The ouabain-sensitive component of the hyperpolarization (Δ mV) therefore amounted to 23.0 ± 8.4 mV ($n = 8$) (Figure 2b). No spontaneous degranulation was observed during these experiments.

In a further series of experiments, the cytosol of the mast cell was perfused in the whole-cell configuration with a patch pipette filled with an internal solution containing a high concentration of $[Na^+]_i(+)$ (26.6 mM) (Table 1). This treatment hyperpolarized the cell (Δ mV: 20.2 ± 9.1 mV, $n = 7$) (Figure 2c). At this point, an application pipette containing ouabain (5 mM) was used to superfuse the cell and this treatment repolarized the cell back to the initial value. The ouabain-sensitive component of the hyperpolarization therefore amounted to 19.7 ± 7.7 mV ($n = 7$) (Figure 2c). Three of these cells degranulated spontaneously.

These experiments were also performed with no ATP in the patch pipette (high $[Na^+]_i(-)$ (Table 1)). Under these conditions, perfusion of the cytosol with a high concentration of

Table 3 Membrane potential in rat peritoneal mast cells

	Beginning of current clamp	*End of current clamp*	n
Control (all cells)	$+1.7 \pm 2.5$ mV	-1.3 ± 0.4 mV	21
Without degranulation	-3.7 ± 4.2 mV	-2.2 ± 2.0 mV	10
With degranulation	$+6.6 \pm 1.9$ mV	-0.6 ± 0.7 mV	11

Measurements were performed in control internal and external solutions. The cells were not stimulated, and spontaneous degranulation was determined by optical inspection.

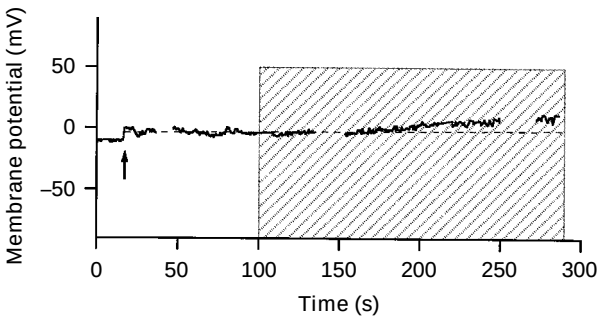

Figure 1 Effect of ouabain on resting membrane potential in rat peritoneal mast cells. This figure shows a single experiment, the experiment was repeated 3 times with similar results. The arrow indicates when the whole cell recording configuration was achieved. The breaks in the recording reflect times during which the current clamp mode was interrupted in order to update cell capacitance (C_{slow}) and series resistance (G_{series}). Pipette resistance was 8 MΩ, seal resistance (whole-cell) was 4.3 GΩ, cell capacitance amounted to 6.2 pF, and the series resistance was 11.7 MΩ. The mast cell was superfused with a solution of ouabain (5 mM) 100 s after whole cell configuration had been obtained. The shaded area denotes the period of time in which the cell was superfused. Pipette solution: solution SIS (Table 1); application pipette solution: ouabain (5 mM); extracellular solution: experimental buffer (Table 2).

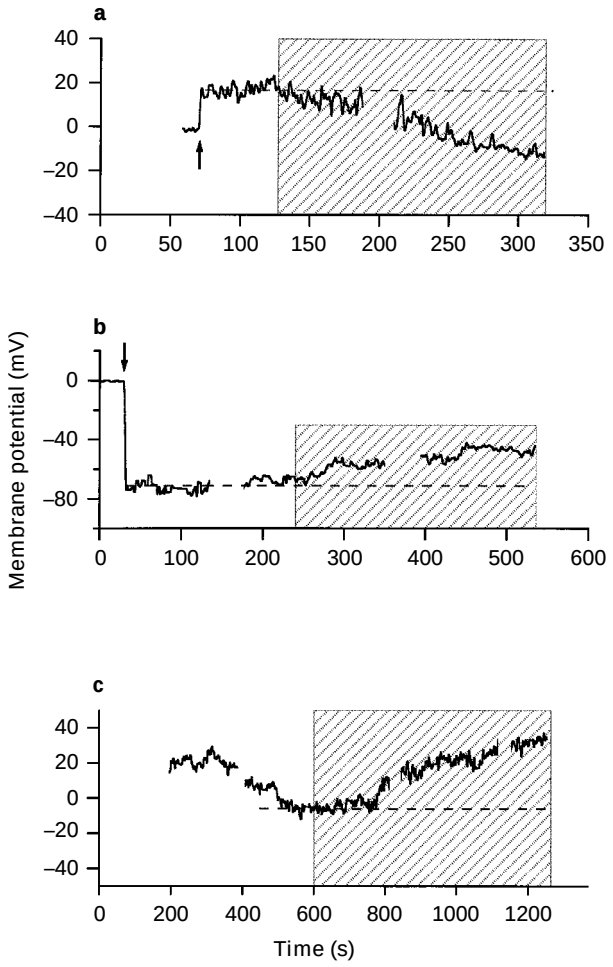

Figure 2 (a) Effect of extracellular calcium on resting membrane potential in rat peritoneal mast cells. This figure shows a single experiment, the experiment was repeated 5 times with similar results. Pipette resistance was 3.2 MΩ, seal resistance (whole-cell) was 3 to 7 GΩ, cell capacitance amounted to 7.1 pF, and the series resistance was 7.1 to 12 MΩ. The mast cell was superfused with a solution of calcium-free buffer (Table 2) 130 s after whole cell configuration had been obtained. Pipette solution: solution SIS (Table 1); application pipette solution: calcium-free buffer (Table 2); extracellular solution: experimental buffer (Table 2). (b) Effect of extracellular calcium on resting membrane potential in rat peritoneal mast cells. This figure shows a single experiment, the experiment was repeated 8 times with similar results. This mast cell was preincubated in a calcium-free buffer (Table 2) for 14 min before whole cell configuration was obtained. Pipette resistance was 4.4 MΩ, seal resistance (whole-cell) was 5 to 10 GΩ, cell capacitance amounted to 5.2 pF, and the series resistance was 7.5 MΩ. The mast cell was superfused with a solution of ouabain 240 s after whole cell configuration had been obtained. Pipette solution: solution SIS (Table 1); application pipette solution: ouabain (5 mM); extracellular solution: calcium-free buffer (Table 2). (c) Effect of high intracellular [Na^+] on resting membrane potential in rat peritoneal mast cells. This figure shows a single experiment; the experiment was repeated 7 times with similar results. This mast cell was perfused in the whole-cell configuration by use of a patch pipette filled with an internal solution containing a high concentration of Na^+. Pipette resistance was 6.0 MΩ, seal resistance (whole-cell) was 1.0 GΩ, cell capacitance amounted to 7.6 pF, and the series resistance was 12.6 MΩ. The mast cell was superfused with a solution of ouabain 600 s after whole cell configuration had been obtained. Pipette solution: ([Na^+](+ATP) (26.6 mM) (Table 1); application pipette solution: ouabain (5 mM); extracellular solution: experimental buffer (Table 2). The arrows indicate when the whole cell recording configuration was achieved. The shaded areas denote the period of time in which the cell was superfused. The breaks in the recording reflect times during which the current clamp mode was interrupted in order to update cell capacitance (C_{slow}) and series resistance (G_{series}).

Na^+ (without ATP) had no effect on the zero-current potential (ΔMP: 1.1 ± 4.6 mV, $n=6$). A subsequent superfusion with ouabain (5 mM) was also without effect (ΔMP: 0.6 ± 0.7 mV, n = 6) (Figure 3). One of these cells degranulated spontaneously.

The dependence of membrane potential on intracellular Na^+ was explored by perfusing the cytosol of a number of mast cells with different concentrations of [Na^+]$_i$ via the patch pipette. Figure 4 shows that when [Na^+]$_i$ was greater than about 12 mM the membrane potential became more negative. At [Na^+]$_i$ of approximately 25 mM, no further increase in hyperpolarization was observed. At this point, the mast cells had hyperpolarized by (Δ mV) 21.5 ± 2.4 mV ($n=8$). Forty seven cells were used to obtain data for this dose-response curve, and 14 of these cells degranulated slowly (especially during experiments in high internal [Na^+]$_i$).

To confirm that the membrane potential was controlled by [Na^+]$_i$ only, and not [Ca^{2+}]$_0$, the cytosol of 8 individual mast cells was perfused with an internal solution containing a low concentration of [Na^+]$_i$ (1 mM). This treatment had no effect on the membrane potential (Δ mV: -2.1 ± 6.9 mV), and when the cells were subsequently superfused with a calcium-free external solution by use of an application pipette, the membrane potential remained largely unchanged (Δ mV: 4.0 ± 5.5 mV).

In order to exclude the possibility that high concentrations of ATP activate ion channels that might contribute to changes in the membrane potential, voltage pulses (current-voltage relationship) were applied to mast cells perfused with either low (0.2 mM) or high (3 mM) concentrations of ATP in the patch pipette. These voltage pulses were applied exactly 60 s after whole cell configuration had been obtained. Figure 5 shows that the effect of ATP on the plasma membrane ion conductance was very small and that the overall conductance in these cells was small. Thus, when the ATP content amounted to 0.2 mM, the slope conductance of the whole cell between -80 and $+60$ mV was 119 ± 21 pS ($n=31$), and similarly, when the ATP content was raised to 3 mM, the slope conductance in the same voltage range was 149 ± 25 pS ($n=8$) (Figure 5).

These currents seemed to be rather non-specific, as the current-voltage relationship was linear and the reversal potential was close to 0 mV. They were also largely independent of ATP content.

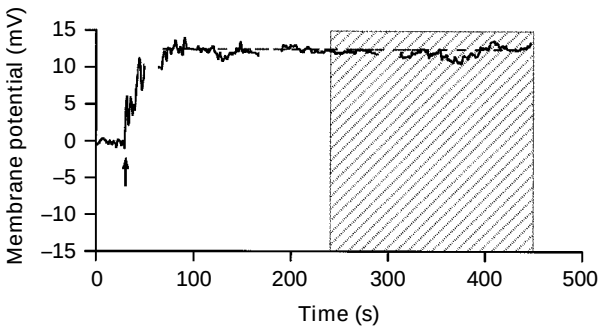

Figure 3 Effect of high intracellular [Na^+] on resting membrane potential in rat peritoneal mast cells in the absence of ATP. This figure shows a single experiment; the experiment was repeated 6 times with similar results. The arrow indicates when the whole cell recording configuration was achieved. The breaks in the recording reflect times during which the current clamp mode was interrupted in order to update cell capacitance (C_{slow}) and series resistance (G_{series}). This mast cell was perfused in the whole-cell configuration with a patch pipette filled with an internal solution containing a high concentration of Na^+ without ATP. Pipette resistance was 3.3 MΩ, seal resistance (whole-cell) was 2.0 GΩ, cell capacitance amounted to 5.4 pF, and the series resistance was 8.4 MΩ. The mast cell was superfused with a solution of ouabain 230 s after whole cell configuration had been obtained. The shaded area denotes the period of time in which the cell was superfused. Pipette solution: ([Na^+] (without ATP) (26.6 mM) (Table 1); application pipette solution: ouabain (5 mM); extracellular solution: experimental buffer (Table 2).

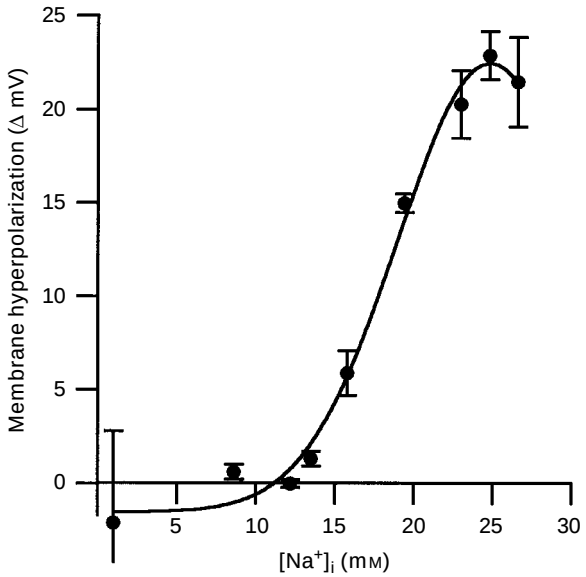

Figure 4 Effect of $[Na^+]_i$ on membrane potential (dose-response curve). The cytosol of a number of mast cells ($n = 47$) were perfused with different concentrations of $[Na^+]_i$ (via the patch pipette) and the change in membrane potential was recorded. Pipette solution: various mixtures of standard internal solution (SIS) and high $[Na^+]_i(+)$ internal solution (Table 1) were prepared, in order to obtain a number of different Na^+ concentrations for the dose-response experiments; external solution: experimental buffer (Table 2).

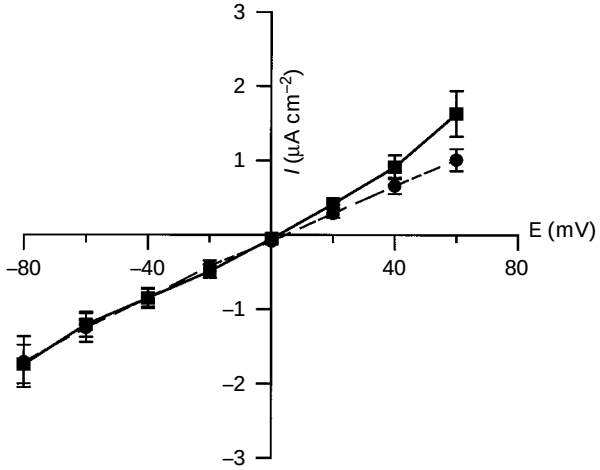

Figure 5 Effect of intracellular ATP on mast cell membrane conductance. The current-voltage relationship was measured by applying eight voltage pulses of 600 ms duration (covering a range of -80 to $+60$ mV) exactly 60 s after establishment of the whole-cell configuration. Current is expressed in terms of $\mu A\ cm^{-2}$, since cell membranes are parallel-plate capacitors with specific capacitances near 1 μFcm^{-2}. Squares: the patch pipette contained 3 mM ATP (=SIS-solution (Table 1)) ($n=8$). Cell size: 6.2 ± 0.4 pF ($n=8$). Whole cell conductance: $(3.37\ \mu A\ cm^{-2} \times 6.2 \times 10^{-6}\ cm^2)/140\ mV = 149$ pS. Circles: the patch pipette contained 0.2 mM ATP (=control-solution (Table 1)) ($n=31$). Cell size: 6.1 ± 0.2 pF ($n=31$). Whole cell conductance: $(2.72\ \mu A cm^{-2} \times 6.1 \times 10^{-6}\ cm^2)/140\ mV = 119$ pS.

Discussion

In a microelectrode study of rat peritoneal mast cells, resting potentials between -6 and -24 mV, with an average of -14.3 mV, have been demonstrated (Tasaka *et al*., 1970). In patch-clamp measurements of the resting membrane potential of rat peritoneal mast cells, it was shown that the mast cells had neither a defined resting potential nor significant ion conductances in the plasma membrane (Lindau & Fernandez, 1986). Similar results were obtained by Matthews *et al*. (1989a), who showed that the zero-current potential of an unstimulated mast cell was near 0 mV. In this study, we measured resting membrane (zero-current) potential in rat peritoneal mast cells by means of an internal pipette solution containing 8.6 mM Na^+, and we recorded zero-current potentials in the same range, i.e. around 0 mV. Similarly, we were unable to demonstrate significant ion conductances in the plasma membrane under resting conditions: the slope conductance of the whole cell was only 120–150 pS and seemed to be rather non-specific.

When these mast cells were exposed to a calcium-free extracellular solution, the zero-current potential decreased, i.e. the membranes hyperpolarized. Such hyperpolarization can only be the result of either anion influx or cation efflux. Chloride influx via chloride channels is not very likely under resting conditions, since only two chloride channels have been described in these cells: (1) a large chloride channel with a slope conductance of around 350 pS (activated by Ca^{2+}) (Lindau & Fernandez, 1986), and (2) a small chloride channel with a single channel conductance of around 1 pS (activated by cyclic AMP, GTPγS, or externally applied secretagogues). Activation of this channel resulted in a large, but slowly developing membrane current (Matthews *et al*., 1989b).

All zero-current potentials in this study were recorded under resting conditions, and mast cell membrane hyperpolarization could be induced by either high $[Na^+]_i$ or low $[Ca^{2+}]_0$ (about 10–20 μM). However, these hyperpolarizations were reversible with ouabain, thus indicating that the Na^+/K^+-ATPase must have been activated under the experimental conditions used. It is therefore very unlikely that the membrane hyperpolarizations were due to activation of chloride channels.

The Na^+/K^+-ATPase can only be activated by increases in $[Na^+]_i$ or $[K^+]_0$. Since $[K^+]_0$ was held constant in this study, the activation of the Na^+/K^+-ATPase must be due to an increase in $[Na^+]_i$. The only (non-calcium) cation channel described in rat peritoneal mast cells is the 30–50 pS non-specific cation channel. It is activated by receptor stimulation probably via an increase in $[Ca^{2+}]_i$, has a reversal potential around 0 mV and is independent of membrane potential (Lindau & Fernandez, 1986; Penner *et al*., 1988; Matthews *et al*., 1989a; Kuno *et al*., 1989; Kuno & Kimura, 1992; Fasolato *et al*., 1993). In the unstimulated cell, the addition of EGTA slightly increased the conductance of this channel and, furthermore, in the absence of extracellular calcium, the unitary amplitude of the cation-selective channel was unaffected, but the opening of the channels was enhanced (Kuno *et al*., 1989). Finally, it was shown that the amount of current flowing through the 30–50 pS channel declined with increasing external calcium-concentrations (Fasolato *et al*., 1993). It is therefore likely that in the absence of extracellular calcium, Na^+ enters the mast cell cytosol via the 30–50 pS non-specific cation channel and the resulting increase in $[Na^+]_i$ subsequently activates the Na^+/K^+-ATPase leading to the ouabain-sensitive membrane hyperpolarization.

The possibility that the electrogenic Na^+/Ca^{2+} exchanger contributes to the setting of the membrane potential, under certain conditions favoured by the ion gradients for Na^+ and Ca^{2+}, cannot be resolved by the results described in this paper, but this issue is addressed specifically in another study.

It has previously been shown that the Na^+/K^+-ATPase is inactive under resting conditions and is activated in the absence of extracellular calcium (Bronner *et al*., 1989; Knudsen & Johansen, 1989a,b; Knudsen *et al*., 1990). In those studies different methods were used ($^{86}Rb^+$-flux studies or *bis*-oxonol-fluorescence studies), nevertheless, the electrophysiological measurements of the Na^+/K^+-ATPase activity described in this study confirmed the results in those studies. In addition, our study indirectly conveys information about the physiological intracellular concentration of Na^+. The Na^+/K^+-

ATPase is inactive under resting conditions (Knudsen & Johansen, 1989a,b; Knudsen *et al*., 1990), and the present study has shown that the pump is activated by an intracellular $[Na^+]$ larger than 12 mM. This means that the $[Na^+]_i$ in resting rat peritoneal mast cells must be below 12 mM.

Since all changes in the zero-current potential were caused by changes in the activity of the Na^+/K^+-ATPase, it seems reasonable to conclude that the activity of the Na^+/K^+-ATPase can have an important effect on the membrane potential of rat peritoneal mast cells.

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References

AMELLAL, M., BINCK, M., FROSSARD, N., ILIEN, B. & LANDRY, Y. (1984). Sodium-potassium ATPase inhibition potentiates compound 48/80-induced histamine secretion from mast cells. *Br. J. Pharmacol*., **82,** 423–430.

BRONNER, C. & LANDRY, Y. (1991). The use of the potential-sensitive fluorescent probe bisoxonol in mast cells. *Biochim. Biophys. Acta*, **1070,** 321–331.

BRONNER, C., MOUSLI, M., ELENO, N. & LANDRY, Y. (1989). Resting plasma membrane potential of rat peritoneal mast cells is set predominantly by the sodium pump. *FEBS Lett*., **255,** 401–404.

FASOLATO, C., HOTH, M., MATTHEWS, G. & PENNER, R. (1993). Ca^{2+} and Mn^{2+} influx through receptor-mediated activation of nonspecific cation channels in mast cells. *Proc. Natl. Acad. Sci. U.S.A*., **90,** 3068–3072.

FENWICK, E.M., MARTY, A. & NEHER, E. (1982). A patch-clamp study of bovine chromaffin cells and of their sensitivity to acetylcholine. *J. Physiol*., **331,** 577–597.

FROSSARD, N., AMELLAL, M. & LANDRY, Y. (1983). Sodium-potassium ATPase, calcium and immunological histamine release. *Biochem. Pharmacol*., **32,** 3259–3262.

HAMILL, O.P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F.J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch*., **391,** 85–100.

KNUDSEN, T., BERTELSEN, H.C. & JOHANSEN, T. (1990). Inhibition of Na^+/K^+-pump activity by divalent cations in intact peritoneal mast cells of the rat. *Br. J. Pharmacol*., **100,** 453–456.

KNUDSEN, T. & JOHANSEN, T. (1989a). Na^+/K^+-pump activity in rat peritoneal mast cells: inhibition by extracellular calcium. *Br. J. Pharmacol*., **96,** 773–778.

KNUDSEN, T. & JOHANSEN, T. (1989b). The mode of inhibition of the Na^+/K^+-pump in mast cells by calcium. *Br. J. Pharmacol*., **98,** 1119–1126.

KNUDSEN, T. & JOHANSEN, T. (1990). Regulation of the Na^+/K^+-pump activity and estimation of the reserve capacity in intact rat peritoneal mast cells. *FEBS Lett*., **269,** 7–10.

KUNO, M. & KIMURA, M. (1992). Noise of secretagogue-induced inward currents dependent on extracellular calcium in rat mast cells. *J. Membr. Biol*., **128,** 53–61.

KUNO, M., OKADA, T. & SHIBATA, T. (1989). A patch-clamp study: secretagogue-induced currents in rat peritoneal mast cells. *Am. J. Physiol*., **256,** (*Cell Physiol*., **25,**) C560–C568.

LINDAU, M. & FERNANDEZ, J.M. (1986). A patch-clamp study of histamine-secreting cells. *J. Gen. Physiol*., **88,** 349–368.

MATTHEWS, G., NEHER, E. & PENNER, R. (1989a). Second messenger-activated calcium influx in rat peritoneal mast cells. *J. Physiol*., **418,** 105–130.

MATTHEWS, G., NEHER, E. & PENNER, R. (1989b). Chloride conductance activated by external agonists and internal messengers in rat peritoneal mast cells. *J. Physiol*., **418,** 131–144.

NEHER, E. (1992). Correction for liquid junction potentials in patch clamp experiments. *Methods Enzymol*., **207,** 123–131.

PENNER, R., MATTHEWS, G. & NEHER, E. (1988). Regulation of calcium influx by second messengers in rat mast cells. *Nature*, **334,** 499–504.

TASAKA, K., SUGIYAMA, K., KOMOTO, S. & YAMASAKI, H. (1970). Dissociation of degranulation and depolarization of the rat mesentery mast cell exposed to compound 48/80 and ATP. *Proc. Jpn. Acad*., **46,** 826–830.

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