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Volume-activated $K^+(Rb^+)$ efflux in lactating rat mammary tissue

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Abstract

The effect of cell swelling, induced by a hyposmotic shock, on $K^+(Rb^+)$ efflux from lactating rat mammary tissue explants has been studied. A hyposmotic challenge increased the fractional release of $K^+(Rb^+)$ from mammary tissue in the absence and presence of the loop-diuretic bumetanide (100 μM). However, the volume-sensitive moiety of $K^+(Rb^+)$ efflux was proportionately larger when bumetanide was present in the incubation medium. On the other hand, a hyposmotic shock appeared to reduce the bumetanide-sensitive component of $K^+(Rb^+)$ efflux. The increase in $K^+(Rb^+)$ efflux, induced by cell swelling, was dependent upon the extent of the hyposmotic challenge. In the presence of bumetanide, substituting Cl^- with NO_3^- reduced the initial increase in volume-sensitive $K^+(Rb^+)$ efflux. However, volume-sensitive $K^+(Rb^+)$ release was prolonged in the presence of NO_3^-. Volume-activated $K^+(Rb^+)$ efflux from rat mammary tissue explants was inhibited by quinine. Cell swelling increased the intracellular concentration of Ca^{2+} in a fashion which depended on the presence of extracellular Ca^{2+}. However, removing extracellular Ca^{2+} did not inhibit volume-activated $K^+(Rb^+)$ efflux from rat mammary tissue explants. The results are consistent with the presence of volume-activated K^+ channels in lactating rat mammary tissue. Volume-activated K^+ efflux may play a central role in mammary cell volume regulation.

Keywords: Mammary; Volume regulation; K^+ efflux

1. Introduction

Cell membranes are very permeable to water which means that cell volume, otherwise termed the cellular hydration state, will be determined by the osmolality of the extracellular fluid and by the cellular content of osmotically active solutes. It is now established that most cell types are able to regulate their volume following swelling induced by anisosmotic media, substrate accumulation and/or changes to the rate of oxidative metabolism (for reviews see [1,2]). Cell swelling activates a variety of membrane transport processes, for example, an increase in the cellular hydration state induces a net efflux of KCl via systems such as (K^+-Cl^-) cotransport and separate K^+ and Cl^- channels [3]. In addition, cell swelling increases the efflux of organic osmolytes such as amino acids [4]. The net efflux of inorganic ions and organic osmolytes allows cells to return towards a normal volume: this process is known as a regulatory volume decrease.

We are interested in the transport mechanisms which the lactating rat mammary gland may use to regulate volume. The mammary gland is essentially a secretory epithelium which transports a large variety of solutes (e.g. lactose, Na^+, K^+, Cl^-) and com-

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pounds (e.g. milk protein and fat) in a vectorial fashion [5]. The uptake of substrates by the mammary epithelium and the process of milk secretion could lead to changes in mammary cell volume. A knowledge of volume-regulatory mechanisms is especially important in the light of the finding that mammary metabolism is affected by perturbations to cell volume. Thus, cell swelling and shrinking respectively increases and decreases mammary protein synthesis [6,7].

We have previously shown that the lactating rat mammary gland expresses a volume-activated transport system which accepts amino acids such as taurine and glycine as substrates [8,9]. This transport system has the characteristics of a channel rather than a carrier and is situated in the blood-facing aspect of the mammary epithelium [10]. The purpose of the present investigation was to identify other pathways in the rat mammary gland which are activated by cell swelling. In particular we have investigated the effect of cell swelling on the efflux of K^+, using $^{86}Rb^+$ as tracer, from rat mammary tissue to test for the presence of volume-sensitive K^+ channels and/or (K^+-Cl^-) cotransport.

2. Materials and methods

2.1. Animals

Lactating Wistar rats, 10–15 days post partum, were used in this study. The animals were suckling between eight and 10 pups. Rats were maintained on a 12 h light:dark cycle and were allowed free access to water and chow.

2.2. Preparation of mammary explants and acini

Mammary tissue explants (each 4–8 mg wet weight) were isolated from the abdominal mammary glands as previously described [11]. Care was taken to remove as much connective tissue as possible. Mammary acini were isolated following collagenase digestion. Briefly, 5 g of mammary tissue was suspended in 10 ml of a buffer containing (mM) 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 10 glucose and 20 Tris–MOPS, pH 7.4, and finely chopped. The tissue was then strained and added to 30 ml of a buffer similar in composition to that just described except that it also contained 1.5 g Ficoll 400, 600 mg bovine serum albumin and 30 mg of collagenase. The tissue was then incubated (at 37°C) for 40 min in a shaking water bath. Following this, the digest was strained and the eluent was centrifuged at $550 \times g$ for approximately 15 s. The pellet was washed ($\times 3$) by centrifugation and resuspension.

2.3. Measurement of $K^+(Rb^+)$ efflux from mammary tissue explants

K^+ efflux, using $^{86}Rb^+$ as tracer, was measured according to the method of Shennan [11]. Briefly, mammary tissue explants, prepared as described above, were preloaded with $^{86}Rb^+$ by a 60–90 min incubation at 20°C in a medium containing (mM) 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 10 glucose and 10 Tris–MOPS, pH 7.4 (+7–10 μCi/ml $^{86}Rb^+$). Following the loading incubation, the explants were transferred through a series of tubes containing 2 ml of radioactive-free solutions, maintained at 37°C, at 2 min intervals. The radioactivity remaining in the explants at the end of the wash-out period was determined. The fractional loss of $^{86}Rb^+$ was calculated for each sampling period. This was taken as the ratio of the amount of isotope lost from the tissue per minute to the arithmetic mean radioactive content at the start of each sampling period.

Consistent with previous results [11], we found that $K^+(Rb^+)$ efflux from lactating rat mammary tissue was the sum of at least two components (results not shown). The fast component, which was minimal by 30 min, probably represents isotope originating from the tissue extracellular space whereas the slow component represents membrane-limited transport. $K^+(Rb^+)$ efflux was measured for 40 min before the effect of a hyposmotic shock was examined: this was to minimise the contribution of the extracellular component of $K^+(Rb^+)$ efflux. Previously, it has been shown that $K^+(Rb^+)$ efflux from rat mammary tissue explants occurs via at least two pathways one of which is sensitive to the loop-diuretic furosemide and appears to represent (Na^+-K^+-Cl^-) cotransport [11]. In the present study, bumetanide was chosen in preference to furosemide to characterise the triple cotransporter as the former is a more specific inhibitor [12,13].

2.4. Measurement of taurine efflux from mammary explants

Mammary explants, prepared as described above, were loaded with [^{3}H]taurine by incubating for 60 min at 20°C in a medium containing (mM) 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 10 glucose and 10 Tris–MOPS, pH 7.4 plus 2–3 μCi/ml of [^{3}H]taurine. The efflux of radiolabelled taurine was assayed at 37°C as described for $^{86}Rb^+$ above.

2.5. Measurement of $[Ca^{2+}]_i$

The $[Ca^{2+}]_i$ in mammary acinar cells was measured using the fura-2 dye technique. Acini were loaded with fura-2 by incubating with 5 μM fura-2 acetoxymethyl ester/0.25% pluronic F-127 (w/w) for 45 min at room temperature in the dark. After loading with the dye, acini were washed by centrifugation and resuspension in a buffer containing (mM) 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 10 glucose and 20 Tris–MOPS, pH 7.4. Acini were incubated in a Perkin-Elmer fluorimeter and stirred at 37°C with excitation wavelengths of 340 and 380 nm. Fluorescent light was measured at 510 nm and, following subtraction of the background signal from unloaded acini, the 340/380 ratio was calculated. The intracellular free calcium concentration was calculated using the following equation [14]:

$$[Ca^{2+}]_i = K_d B[(R-R_{min})/(R_{max}-R)] \quad (1)$$

where R is the ratio of light emitted following stimulation at 340/380 nm. The values for R_{min}, R_{max} and B (ratio of the 380 signal in the absence of Ca^{2+} to the 380 signal in the presence of saturating amounts of Ca^{2+}) were obtained by incubating fura-2 free acid with 15 mM EGTA or 10 mM Ca^{2+}. K_d was taken to be 224 nM.

2.6. Materials

$^{86}Rb^+$ (as RbCl) and [^{3}H]taurine were purchased from Amersham International plc, Amersham, Bucks, UK. Collagenase (CLS 3) was purchased from the Worthington Biochemical Corporation. All other chemicals were obtained from Sigma, Poole, UK.

2.7. Statistics

Differences were assessed by Student's paired or unpaired t-test as appropriate and were considered significant when $P < 0.05$.

3. Results

Fig. 1A shows the effect of reducing the osmolality of the incubation medium from 295 to 148 mosmol/kg H_2O on $K^+(Rb^+)$ efflux from rat mammary tissue explants in the absence and presence of bumetanide (100 μM). In the absence of bumetanide a hyposmotic challenge increased the fractional efflux of $K^+(Rb^+)$ (basal-to-peak) from 0.0196 ± 0.0010 min^{-1} to 0.0274 ± 0.0014 ($\pm$S.E.M., $n=4$, $P < 0.05$). The peak response was reached 4 min after exposing the tissue to the hyposmotic buffer. Thereafter, $K^+(Rb^+)$ efflux decreased: the fractional efflux was not significantly different from the pre-stimulated level 20 min after being exposed to the hyposmotic solution. A hyposmotic shock also increased $K^+(Rb^+)$ efflux in the presence of the loop-diuretic bumetanide: the fractional efflux was increased (basal-to-peak) from 0.0110 ± 0.0007 min^{-1} to 0.0250 ± 0.0012 min^{-1} ($\pm$S.E.M., $n=4$, $P < 0.001$). The peak response was reached 4–6 min after the tissue was placed in the hyposmotic medium. The fractional efflux of $K^+(Rb^+)$ rapidly declined after this point but was still significantly above the basal level 20 min after the initial exposure to the hyposmotic conditions. It is evident from Fig. 1A that bumetanide inhibited the fractional loss of $K^+(Rb^+)$ under isosmotic conditions. However, the bumetanide-sensitive component of $K^+(Rb^+)$ efflux was markedly reduced under hyposmotic conditions. All subsequent experiments were performed in the presence of bumetanide.

Fig. 1B shows the effect of a prolonged hyposmotic shock on $K^+(Rb^+)$ efflux in the presence of bumetanide. The fractional release of $K^+(Rb^+)$ was still elevated, compared to that found under isosmotic conditions, 40 min after being exposed to a hyposmotic solution ($P < 0.02$). The fractional efflux as a function of time, following the peak response, could

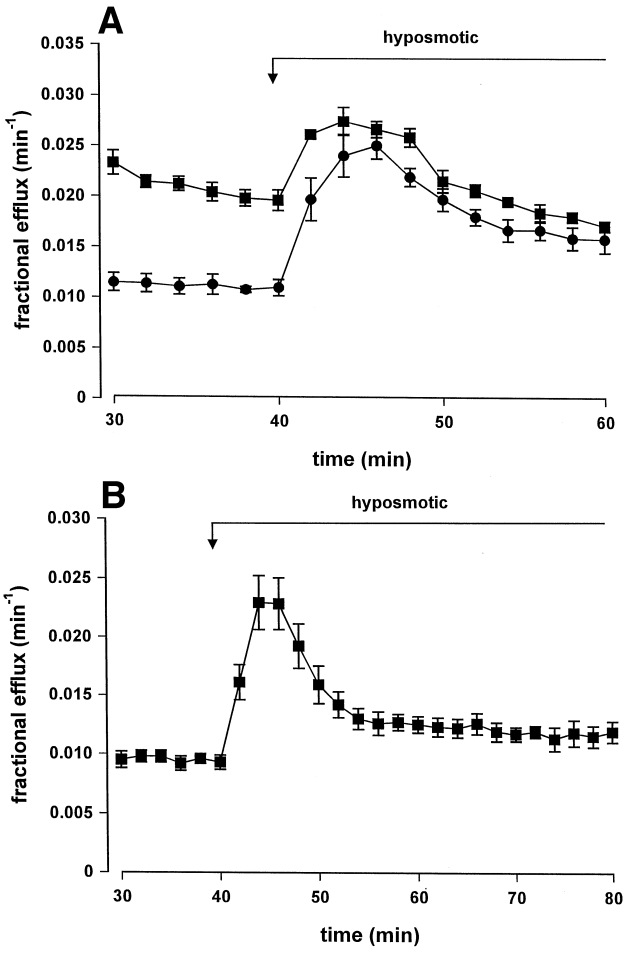

Fig. 1. (A) The effect of a hyposmotic shock on $K^+(Rb^+)$ efflux from rat mammary tissue in the absence (■) and presence (●) of bumetanide. The isosmotic buffer contained (mM) 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 10 glucose and 10 Tris–MOPS, pH, 7.4 (osmolality = 295 ± 5 mosmol/kg H_2O). The hyposmotic buffer was similar in composition except that the NaCl concentration was reduced to 55 mM (osmolality = 148 ± 3 mosmol/kg H_2O). Bumetanide, when required, was used at a concentration of 100 μM. Each point is the mean ± S.E.M. of four experiments using tissue from separate animals. (B) The effect of a prolonged hyposmotic challenge on $K^+(Rb^+)$ efflux from rat mammary tissue in the presence of bumetanide. The composition of the isosmotic (osmolality = 287 ± 2 mosmol/kg H_2O) and hyposmotic (osmolality = 149 ± 3 mosmol/kg H_2O) buffers were the same as that described above. Each point is the mean ± S.E.M. of four separate experiments using tissue from separate animals.

be described by the following exponential association:

$$\text{Fractional efflux } (\text{min}^{-1}) = a(b - e^{-ct}) \quad (2)$$

where a, b and c are constants ($a = -0.011$, $b = -1.067$ and $c = 0.2435$) and t is the time following the peak response.

Fig. 2 shows that volume-activated $K^+(Rb^+)$ efflux was dependent upon the extent of the hyposmotic challenge. Consistent with the results shown in Fig. 1A we found that decreasing the osmolality of the incubation medium by 49% increased $K^+(Rb^+)$ efflux: the fractional efflux was increased (basal-to-peak) from 0.0106 ± 0.0009 min^{-1} to 0.0243 ± 0.0016 min^{-1} (± S.E.M., $n = 5$, $P < 0.001$). Reducing the osmolality of the incubation medium by 31% increased $K^+(Rb^+)$ release from 0.0107 ± 0.0014 to 0.0173 ± 0.0015 (± S.E.M., $n = 5$, $P < 0.01$).

The increase in $K^+(Rb^+)$ efflux shown in Figs. 1 and 2 could be due to cell swelling or a reduction in the osmolality of the incubation media per se. To distinguish between these two possibilities we examined the effect of isosmotic swelling on $K^+(Rb^+)$ efflux. Thus, mammary tissue was incubated in an isosmotic buffer containing urea (160 mM). It was predicted that mammary cells would swell on account of the high permeability of urea. For comparison, the effect of a hyposmotic shock on $K^+(Rb^+)$ efflux was examined in parallel experiments. The re-

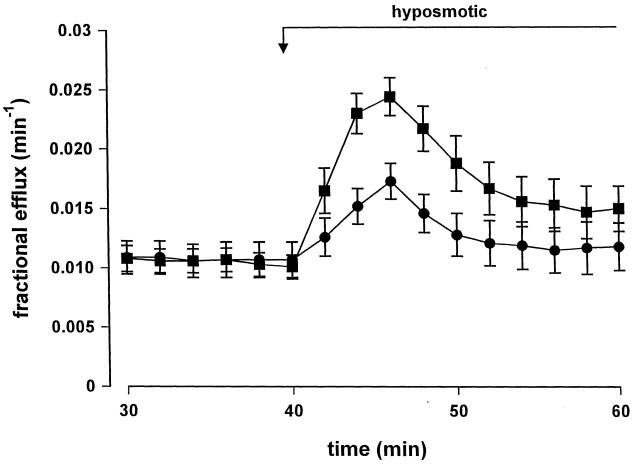

Fig. 2. The effect of cell swelling on $K^+(Rb^+)$ efflux is dependent upon the extent of the osmotic shock. The isosmotic buffer contained (mM) 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 0.1 bumetanide,10 glucose and 10 Tris–MOPS, pH 7.4 (osmolality = 292 ± 3 mosmol/kg H_2O). The hyposmotic buffers were similar in composition except that the NaCl concentration was reduced to 85 mM (●) (osmolality = 149 ± 3 mosmol/kg H_2O) or 55 mM (■) (osmolality = 202 ± 3 mosmol/kg H_2O). Each point is the mean ± S.E.M. of five experiments using tissue from separate animals.

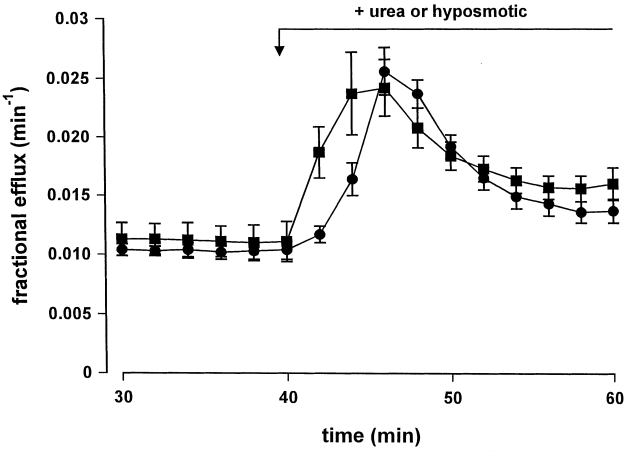

Fig. 3. The effect of isosmotic swelling (●) and a hyposmotic challenge (■) on $K^+(Rb^+)$ efflux from rat mammary tissue explants. The isosmotic buffer contained (mM) 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 0.1 bumetanide, 10 glucose and 10 Tris–MOPS, pH 7.4 (osmolality = 301 ± 4 mosmol/kg H_2O). The hyposmotic buffer was similar in composition except that the NaCl concentration was reduced to 55 mM (osmolality = 147 ± 1 mosmol/kg H_2O). The buffer containing urea was similar in composition to the isosmotic buffer except that the NaCl concentration was 55 mM and the urea concentration was 160 mM (osmolality = 309 ± 4 mosmol/kg H_2O). Each point is the mean ± S.E.M. of three experiments using tissue from separate animals.

sults of these experiments are shown in Fig. 3. It is evident that incubating mammary tissue in an isosmotic buffer which contained urea gives rise to an increase in $K^+(Rb^+)$ efflux: the fractional release was increased (basal-to-peak) from 0.0111 ± 0.0016 min^{-1} to 0.0242 ± 0.0024 (± S.E.M., $n = 3$, $P < 0.01$) Note, however, that the initial increase in $K^+(Rb^+)$ efflux was larger following a hyposmotic shock (Fig. 3).

3.1. The effect of Cl^- replacement on volume-sensitive $K^+(Rb^+)$ efflux

An increase in $K^+(Rb^+)$ efflux from mammary tissue following swelling could be due to an increase in (K^+-Cl^-) cotransport or the activation of a $K^+(Rb^+)$ conductance pathway. In an attempt to establish if either of these two pathways is activated by cell swelling we tested the effect of replacing Cl^- with NO_3^- on volume-activated $K^+(Rb^+)$ efflux. Fig. 4 shows that replacing Cl^- with NO_3^- did not affect the magnitude of the peak response of $K^+(Rb^+)$ release to a hyposmotic shock. In Cl^--rich media the fractional efflux was increased (basal-to-peak) from 0.0105 ± 0.0011 min^{-1} to 0.0263 ± 0.0036 min^{-1} (± S.E.M., $n = 5$, $P < 0.002$). In the presence of NO_3^-, the fractional release of $K^+(Rb^+)$ was increased (basal-to-peak) from 0.0103 ± 0.0005 min^{-1} to 0.0266 ± 0.0016 min^{-1} (± S.E.M., $n = 5$, $P < 0.001$). However, it is apparent that the peak response took longer to reach when the tissue was suspended in a NO_3^- buffer (10 min vs. 4 min). Moreover, it is evident that the fractional efflux of $K^+(Rb^+)$ remained elevated in a NO_3^- buffer compared to that found in a Cl^- medium. Replacing Cl^- with NO_3^- did not inhibit the bumetanide-insensitive efflux of $K^+(Rb^+)$ efflux under isosmotic conditions (Fig. 4).

3.2. Inhibition of volume-sensitive $K^+(Rb^+)$ efflux by quinine

The effect of quinine on $K^+(Rb^+)$ efflux was examined (Fig. 5). In this set of experiments quinine, when

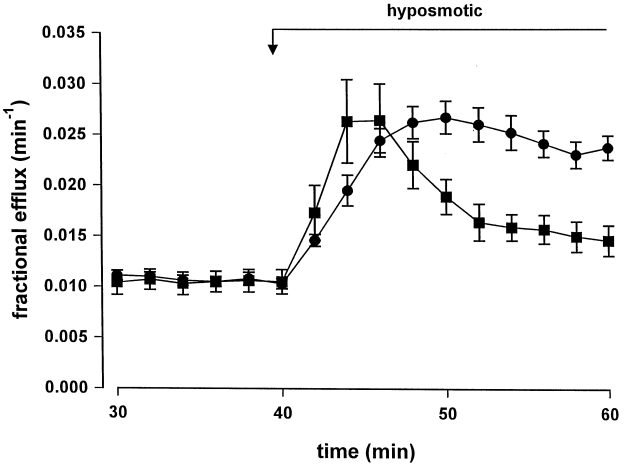

Fig. 4. The effect of a hyposmotic challenge on $K^+(Rb^+)$ efflux from lactating rat mammary tissue explants suspended in a Cl^--rich (■) or NO_3^--rich (●) buffer. The isosmotic buffers contained (mM) 135 NaX, 5 KX, 2 CaX_2 (where X = Cl^- or NO_3^-), 1 $MgSO_4$, 10 glucose and 10 Tris–MOPS, pH 7.4 plus 100μM bumetanide (osmolality = 288 ± 8 and 278 ± 5 mosmol/kg H_2O for Cl^- and NO_3^- buffers respectively). The hyposmotic buffers were similar in composition except that the NaCl or $NaNO_3$ concentrations were reduced to 55 mM (osmolality = 149 ± 2 and 144 ± 1 mosmol/kg H_2O for Cl^- and NO_3^- buffers respectively). Each point is the mean ± S.E.M. of five experiments using tissue from separate animals.

required, was present throughout the entire time course at a concentration of 1 mM. A hyposmotic challenge increased the fractional release of $K^+(Rb^+)$ (basal-to-peak) from 0.0114 ± 0.0001 min^{-1} to 0.0247 ± 0.0012 min^{-1} ($\pm$S.E.M., $n=3$, $P<0.01$) in the absence of quinine. When the drug was present, a hyposmotic shock increased the fractional efflux from 0.0082 ± 0.0001 min^{-1} to 0.0153 ± 0.0006 min^{-1} ($\pm$S.E.M., $n=3$, $P<0.01$). Thus, quinine inhibited (basal-to-peak) the volume-sensitive moiety of $K^+(Rb^+)$ efflux by 47% ($P<0.02$). In addition, quinine blocked $K^+(Rb^+)$ efflux under isosmotic conditions by 27% ($P<0.01$).

3.3. The effect of Ca^{2+} on volume-activated $K^+(Rb^+)$ release

The effect of removing extracellular Ca^{2+} (+EGTA) on $K^+(Rb^+)$ efflux was investigated (Fig. 6). Reducing the osmolality of the incubation buffer by 48% in the presence of extracellular Ca^{2+} increased the fractional release of $K^+(Rb^+)$ (basal-to-peak) from 0.0105 ± 0.0009 min^{-1} to 0.0246 ± 0.0020 min^{-1} ($\pm$S.E.M., $n=5$, $P<0.001$). A similar osmotic shock increased the fractional loss of $K^+(Rb^+)$ from 0.0146 ± 0.0015 min^{-1} to 0.0262 ± 0.0029 min^{-1} ($\pm$S.E.M., $n=5$, $P<0.01$) in the absence of extracellular Ca^{2+}. Thus, removing extracellular Ca^{2+} (+EGTA) had no significant effect on the volume-activated moiety of $K^+(Rb^+)$ efflux. However, the fractional efflux of $K^+(Rb^+)$ from rat mammary explants was increased in the absence of extracellular Ca^{2+} (+EGTA) under isosmotic conditions ($P<0.02$). We found that an isosmotic Ca^{2+}-free (+EGTA) buffer increased the fractional release of radiolabelled taurine from rat mammary explants. Thus, in the presence and absence of Ca^{2+} (+EGTA) the fractional release of taurine (at $t=40$ min) was respectively 0.0013 ± 0.0003 min^{-1} and 0.0050 ± 0.0001 min^{-1} ($\pm$S.E.M., $n=3$, $P<0.01$).

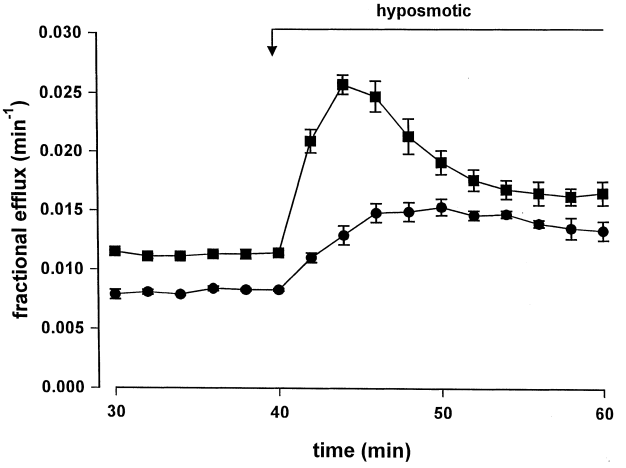

Fig. 5. The effect of a hyposmotic challenge on $K^+(Rb^+)$ efflux from lactating rat mammary tissue in the absence (■) and presence (●) of quinine. The isosmotic buffer contained (mM) 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 0.1 bumetanide, 10 glucose and 10 Tris–MOPS, pH 7.4. (osmolality = 305 ± 3 mosmol/kg H_2O). The hyposmotic buffer was similar in composition except that the NaCl concentration was reduced to 55 mM (osmolality = 151 ± 1 mosmol/kg H_2O). Quinine, when required, was used at a concentration of 1 mM. Each point is the mean ± S.E.M. of three experiments using tissue from separate animals.

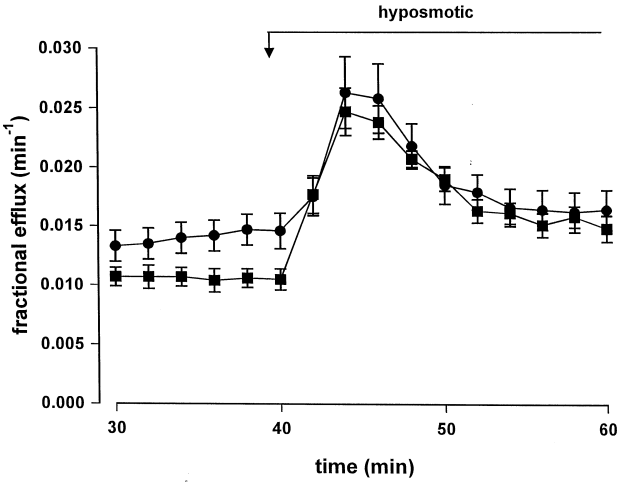

Fig. 6. The effect of a hyposmotic shock on $K^+(Rb^+)$ efflux from rat mammary tissue in the presence (■) and absence (●) of extracellular Ca^{2+}. The isosmotic buffers contained (mM) 135 NaCl, 5 KCl, 1 $MgSO_4$, 10 glucose, 0.1 bumetanide and 10 Tris–MOPS, pH 7.4 ± 2 $CaCl_2$ (osmolality = 292 ± 4 mosmol/kg H_2O). The hyposmotic buffers were similar in composition except that the NaCl concentration was reduced to 55 mM (osmolality = 153 ± 3 mosmol/kg H_2O). The Ca^{2+}-free buffers also contained 1 mM Tris–EGTA. Each point is the mean ± S.E.M. of five experiments using tissue from separate animals.

3.4. The effect of a hyposmotic shock on the $[Ca^{2+}]_i$ in mammary acinar cells

Fig. 7 illustrates the effect of a hyposmotic shock, in the presence and absence of extracellular Ca^{2+}, on $[Ca^{2+}]_i$ in rat mammary acinar cells. Shown for comparison is the $[Ca^{2+}]_i$ under isosmotic conditions

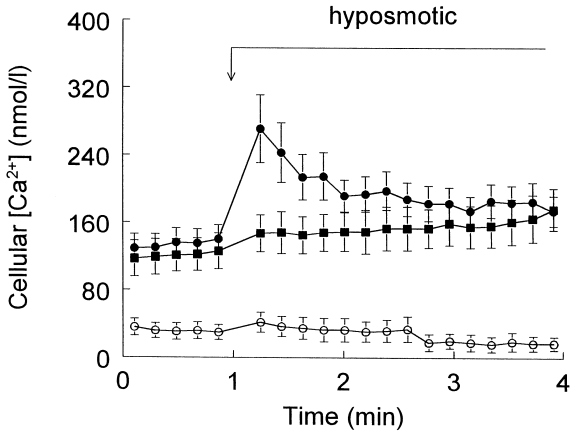

Fig. 7. The effect of a hyposmotic shock in the presence (●) and absence (○) of extracellular Ca^{2+} on the intracellular Ca^{2+} concentration in mammary acinar cells. The points denoted (■) represent the $[Ca^{2+}]_i$ measured under isosmotic conditions (plus extracellular calcium) throughout. The isosmotic buffer (osmolality 327 mosmol/kg H_2O) contained (mM) 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgSO_4$, 1 KH_2PO_4, 10 glucose and 20 Tris–MOPS, pH 7.4. The buffer was made hyposmotic by adding an equal volume of distilled H_2O containing 2 mM $CaCl_2$. The experimental conditions were similar when the effect of a hyposmotic challenge was examined in the absence of Ca^{2+} except that the isosmotic buffer contained Tris–EGTA (15 mM) and the hyposmotic buffer buffer contained Tris–EGTA (8.5 mM) and no $CaCl_2$. Each point is the mean ± S.E.M. of 6–10 experiments using acini prepared from separate animals.

throughout. Under isosmotic conditions, cytosolic $[Ca^{2+}]$ was 140 ± 17 nM ($n = 10$) in the presence of extracellular Ca^{2+}. Reducing the external osmolality, whilst maintaining the extracellular Ca^{2+} concentration constant, increased the $[Ca^{2+}]_i$ to a value of 271 ± 40 nM ($n = 10$) within 23 s. Thereafter, $[Ca^{2+}]_i$ declined with time and was not significantly different from the value measured under isosmotic conditions by the end of the time course. In contrast, a similar osmotic perturbation failed to increase $[Ca^{2+}]_i$ in the absence of extracellular Ca^{2+} (+EGTA). Note, however, that $[Ca^{2+}]_i$ was markedly reduced in isosmotic and hyposmotic conditions when the buffers contained EGTA.

4. Discussion

Cell swelling, induced by a hyposmotic shock, increased the fractional efflux of $K^+(Rb^+)$ from lactating rat mammary tissue explants. Although mammary explants prepared from lactating rats are comprised of more than one cell type it must be borne in mind that the vast majority of cells are alveolar secretory cells. Previous work from this laboratory has shown that a significant portion of the unidirectional $K^+(Rb^+)$ efflux from rat mammary tissue is via (Na^+-K^+-Cl^-) cotransport [11]. The finding that bumetanide inhibited $K^+(Rb^+)$ efflux under isosmotic conditions is in accordance with the earlier findings. However, it is apparent that volume-activated $K^+(Rb^+)$ efflux persisted in the presence of bumetanide suggesting that the increase in transport cannot be attributed to the triple cotransporter. It is notable that the increase in $K^+(Rb^+)$ efflux in response to cell swelling was proportionately larger in the presence of bumetanide. At present, we have no clear explanation for this although it is interesting to note that bumetanide has been found to potentiate cell swelling, induced by a hyposmotic challenge, in rat cardiomyocytes [15]. The bumetanide-sensitive component of $K^+(Rb^+)$ efflux from rat mammary tissue explants was reduced under hyposmotic conditions. In this connection, bumetanide-sensitive K^+ efflux from vascular endothelial cells is attenuated following cell swelling [16]. In addition, bumetanide-sensitive K^+ uptake in trabecular meshwork cells and *Xenopus* oocytes is markedly reduced by a hyposmotic challenge [17,18].

$K^+(Rb^+)$ efflux was increased by isosmotic swelling (using urea) suggesting that the increase induced by a hyposmotic shock is not a consequence of reducing the external osmolality per se but reflects a decrease in the internal osmotic pressure. It is apparent, however, that the initial increase in $K^+(Rb^+)$ efflux was higher following hyposmotic swelling than isosmotic swelling: this probably reflects that mammary cell membranes are more permeable to water than they are to urea.

Volume-activated $K^+(Rb^+)$ efflux in other cell types has been been shown to be mediated by (K^+-Cl^-) cotransport and/or $K^+(Rb^+)$ conductance pathways [3]. Replacing Cl^- with NO_3^- did not inhibit the peak response of $K^+(Rb^+)$ efflux from mammary tissue to a hyposmotic shock. Furthermore, replacing Cl^- did not inhibit the bumetanide-insensitive $K^+(Rb^+)$ efflux under isosmotic conditions. This suggests that (K^+-Cl^-) symport does not make a significant contribution to $K^+(Rb^+)$ efflux from lactating

rat mammary tissue explants under isosmotic and hyposmotic conditions because it is established that NO_3^- cannot support K^+ transport via (K^+-Cl^-) co-transport. The finding that NO_3^- did not diminish volume-activated $K^+(Rb^+)$ efflux is consistent with the notion that $K^+(Rb^+)$ transport in mammary tissue may be mediated via a conductance pathway(s). However, replacing Cl^- with NO_3^- did decrease the initial rise in $K^+(Rb^+)$ efflux in response to a hyposmotic shock and prolonged volume-activated $K^+(Rb^+)$ release. Both of these events may reflect that mammary cell membranes have different permeabilities to Cl^- and NO_3^-.

Previous work from this laboratory has failed to detect volume-activated $Cl^-(I^-)$ efflux from rat mammary tissue [8,9,19]. Furthermore, we have failed to find evidence for volume-sensitive glutamate, aspartate, sulphate and formate transport [8,10,19,20]. This appears to be at odds with the idea that volume-activated $K^+(Rb^+)$ efflux is via a conductance pathway as the efflux of an anion will be required during a regulatory volume decrease. There are at least two possibilities which could account for the apparent lack of volume-sensitive $Cl^-(I^-)$ efflux. First, it could be that our method for measuring ion transport in rat mammary explants is not sensitive enough to detect volume-activated $Cl^-(I^-)$ transport. Second, volume-activated anion transport may occur at the apical aspect of the mammary gland. In our opinion, mammary explants, on account of their cellular architecture, allow transport to be measured across the blood-facing aspect of mammary cells. The apical membranes, on the other hand, are not readily accessible, therefore, transport events occurring at the apical pole of the mammary epithelium would not be easily detected.

The suggestion that volume-sensitive $K^+(Rb^+)$ efflux is mediated via a conductance pathway is strengthened by the finding that swelling-induced $K^+(Rb^+)$ efflux is inhibited by the K^+ channel blocker quinine. $K^+(Rb^+)$ efflux from mammary tissue under isosmotic conditions was also inhibited by quinine suggesting that the pathway responsible for volume-activated $K^+(Rb^+)$ efflux is operating under isosmotic conditions. However, it must be borne in mind that quinine and related analogues are relatively non-specific (e.g. see [21–23]) so there is the possibility that quinine is inhibiting more than one K^+ transport system in rat mammary tissue.

Reducing the external osmolality increased the intracellular Ca^{2+} concentration in a fashion which was consistent with increased uptake from the medium. This is consistent with the results of Sudlow and Burgoyne [24] who found that cell swelling, induced by a hyposmotic shock, increased intracellular Ca^{2+} in mouse mammary acinar cells. In spite of the presence of volume-sensitive Ca^{2+} uptake we found that volume-activated $K^+(Rb^+)$ efflux from rat mammary tissue explants was not dependent upon external Ca^{2+} suggesting that Ca^{2+}-activated K^+ channels are not involved. Similarly, Morales-Mulia et al. [25] found that volume-activated $K^+(Rb^+)$ efflux from cerebellar granule neurones is not dependent upon Ca^{2+} even though cell swelling increases cytosolic Ca^{2+} as a consequence of increasing Ca^{2+} uptake from the medium and Ca^{2+} release from internal stores. There is the possibility that the volume-sensitive increase in intracellular Ca^{2+} in mammary cells is not large enough to activate Ca^{2+}-sensitive K^+ channels. However, ruling out a role for Ca^{2+}-dependent, volume-activated K^+ channels in mammary cells may be premature because cell swelling may increase the affinity of such channels to intracellular Ca^{2+}. On the other hand, volume-activated $K^+(Rb^+)$ efflux from rat mammary tissue may be via by voltage-sensitive or stretch-activated K^+ channels rather than Ca^{2+}-activated channels. Confirmation of this awaits a detailed electrophysiological study of rat mammary tissue. Removing Ca^{2+} (+EGTA) increased $K^+(Rb^+)$ and taurine efflux from mammary tissue under isosmotic conditions suggesting that Ca^{2+}-free (+EGTA) buffers may induce a general increase in mammary cell membrane permeability. It should be noted that under these conditions intracellular Ca^{2+} would have been markedly reduced (see Fig. 7).

4.1. *Physiological significance of volume-sensitive K^+ efflux*

In this study we have shown that a hyposmotic shock markedly increases $K^+(Rb^+)$ efflux from lactating rat mammary tissue explants. It must be stressed that under normal physiological conditions mammary cells will never be exposed to such hypos-

motic challenges. However, it can be predicted that mammary cells will swell on account of substrate uptake particularly via Na^+-dependent transport systems. In addition, a change to the rate of oxidative metabolism within mammary cells will affect the cellular hydration state. Furthermore, the presence of high concentrations of impermeable solutes in milk (e.g. lactose, citrate and phosphate) will pose a challenge to mammary cell volume. We predict that mammary tissue could utilise volume-activated K^+ transport to volume regulate following swelling and in turn could regulate mammary metabolism [6,7]. The finding that volume-sensitive $K^+(Rb^+)$ transport could be detected in mammary explants suggests that this mechanism is situated in the blood-facing aspect of the mammary epithelium. Volume-activated K^+ efflux could act in parallel with other volume-sensitive pathways, such as the volume-activated amino acid transport mechanism [8,9,19], to regulate mammary cell volume following swelling.

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