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# POTASSIUM PERMEABILITY AND VOLUME CONTROL IN ISOLATED RAT HEPATOCYTES

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**(1) The occurrence of volume-regulatory responses in isolated rat hepatocytes was investigated. After hypotonic shock, cells were able to shrink back to their normal volume within 15 min. By contrast, after hypertonic shock cells kept their shrunken volume for at least 15 min. (2) $Na^+$-alanine cotransport increases $K^+$ permeability in rat hepatocytes (Kristensen, L.Ø. (1980) J. Biol. Chem. 255, 5236–5243). It is shown here that this effect is due to cell swelling secondary to alanine accumulation rather than to the $Na^+$-alanine cotransport itself.**

## Introduction

Animal cells react on volume changes with a range of compensatory responses. Notably, cell shrinkage in several cell types activates a bumetanide-sensitive ($Na^+ + K^+ + 2Cl^-$)-cotransport system [1,2] which may function in 'volume-regulatory increase' (VRI) [2,3]. By contrast, cell swelling frequently results in an increase in electrogenic $K^+$ permeability, which may play a role in 'volume-regulatory decrease' (VRD) [3]. In some, but not in all, cell types this increase in $K^+$ permeability is $Ca^{2+}$-dependent (Gardos effect [4]).

The occurrence of volume-dependent permeabilities in isolated rat liver cells has only partly been investigated. Berthon et al. [5] recently reported that a 10% increase in hepatocyte volume produced by hypo-osmotic shock triggered a transient increase in $K^+$ permeability, which was not $Ca^{2+}$-dependent.

The present paper consists of two parts. In the first part I investigate the occurrence of volume-regulatory mechanisms in isolated hepatocytes. The results indicate that volume regulation in these cells was in one direction only, in the sense that cell swelling, but not cell shrinkage elicited a volume response. In the second part I consider an effect recently reported by Kristensen [6]. This author observed that electrogenic $Na^+$-alanine cotransport leads to a sustained increase in $K^+$ permeability in isolated hepatocytes. Since this results in hyperpolarization of the cells, he interpreted this phenomenon as a mechanism for support of the $Na^+$ electrochemical gradient during $Na^+$-coupled amino acid uptake [6]. It occurred to me that the immediate cause of the alanine-induced increase in $K^+$ permeability might be cell swelling secondary to alanine accumulation rather than the $Na^+$-alanine cotransport itself. The results presented here confirm this notion.

## Methods and Materials

Hepatocytes were isolated from male Wistar rats fasted for 20–24 h by collagenase perfusion essentially according to Ref. 7. After isolation the cells were washed, and viability was tested with trypan blue. Cells were stored and incubated in a Krebs-Ringer bicarbonate buffer containing (mM): NaCl, 120; KCl, 4.8; $KH_2PO_4$, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 24. Dialyzed nondefatted bovine serum albumin was added at 2% (w/v).

The pH was kept at 7.4 by equilibration of the cell suspensions with 95% $O_2$/5% $CO_2$. Before the experiments, cells were kept on ice for maximally 3 h.

Cell water was determined on cell suspensions with a cytocrit of approx. 10%. Triplicate 1-ml samples were spun for 1 min in preweighed microfuge tubes. The supernatant was carefully aspirated and the wet weight determined. The samples were dried at 95°C overnight, and weighed again.

For unidirectional $K^+$ efflux measurements, cells at a cytocrit of 5–6% were loaded in Krebs-Ringer buffer for 50 min at 37°C with 0.3 μCi/ml $^{86}Rb^+$ as a tracer for $K^+$. They were subsequently put on ice, spun, and washed once. The pellets were taken up, to a cytocrit of 2%, in precooled media of different composition as indicated. Efflux was started by transferring the cells to a 37°C water bath. At 10, 20, 30 and 40 min after this time point, duplicate 1-ml samples were spun for 20 s in microfuge tubes containing 0.2 ml silicon oil (Wacker Chemie). Supernatants and most of the silicon oil were aspirated. Pellets were lyzed with distilled water, and protein precipitated with trichloroacetic acid (final concentration, 5%). Radioactivity in cell extracts and in identically-treated samples of the supernatants was determined as Cerenkov-radiation in water, in an Isocap or Packard instrument at $^3H$-setting.

For unidirectional $K^+$ influx measurements, cells (at 2% cytocrit) were preincubated for 15–20 min at 37°C. Inhibitors (ouabain, 1 mM; bumetanide, 0.1 mM) were added just before $^{86}Rb^+$ (0.1 μCi/ml at zero time). At 7.5, 15, 22.5 and 30 min, duplicate 1-ml samples were spun down and processed as described above.

For the determination of alanine accumulation, cells were incubated with [$^{14}C$]alanine (0.1 μCi/ml). Amino-oxyacetate (2.5 mM) was added to inhibit alanine metabolism [6]. Duplicate 1-ml samples were spun and processed as described above. Pellet extracts and supernatants were counted in Packard scintillator 299$^{TM}$, in an Isocap instrument at $^{14}C$-setting.

Collagenase (from *Clostridium histolyticum*) was purchased from Boehringer, radiochemicals from Amersham. Bumetanide was kindly donated by Dr. P.W. Feit, Leo, Ballerup, Denmark. The L-isomer of alanine was used throughout.

*Calculations*

For the calculation of cell water (g/g dry wt.) and alanine accumulation ($[alanine]_i/[alanine]_o$), the extracellular space was taken to be 40% [6]. For the latter parameter, it was furthermore assumed that alanine was excluded from the dry weight compartment.

$^{86}Rb^+$ was taken to be an ideal tracer for $K^+$ in these cells. $^{86}Rb^+$ efflux under all conditions tested followed first-order kinetics, with correlation coefficients for the calculated lines exceeding 0.995.

## Results

*Volume regulation in isolated rat liver cells*

Cells were suspended in either a hypertonic or a hypotonic medium, and cell water determined both immediately after the osmotic shock and after 15 min at 37°C. Table I shows that (i) the cells behaved as ideal osmometers immediately after osmotic shock (see also Ref. 5); (ii) in the hypertonic medium, cells remained shrunken (by contrast, in the hypotonic medium they returned to their original volume within 15 min); (iii) bumetanide (0.1 mM) had no effect on the latter process.

I also checked the effect of bumetanide on unidirectional $K^+$ efflux and ouabain-insensitive

TABLE I

VOLUME-REGULATORY RESPONSES OF ISOLATED HEPATOCYTES AFTER OSMOTIC SHOCK

Cells were spun down at the time points indicated between brackets, zero time being the moment the ice-cold suspensions were transferred to 37°C. Cell water was determined as described in Methods. Hypertonic saline contained, in addition to the standard components, 200 mM sorbitol. Hypotonic saline contained (mM): NaCl, 60; KCl, 3.6; $NaH_2PO_4$, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 24; bovine serum albumin, 2% (w/v).

| Saline | | Cell water (g/g dry wt.) |
|---|---|---|
| Standard (330 mosM) | (15 min) | 1.14 ± 0.02 |
| Hypertonic (530 mosM) | (0 min) | 0.96 ± 0.02 |
| | (15 min) | 0.90 ± 0.03 |
| +0.1 mM bumetanide | (15 min) | 0.93 ± 0.03 |
| Hypotonic (200 mosM) | (0 min) | 2.0 ± 0.1 |
| | (15 min) | 1.21 ± 0.05 |
| +0.1 mM bumetanide | (15 min) | 1.15 ± 0.03 |

TABLE II

ALANINE-INDUCED HEPATOCYTE SWELLING AND ALANINE ACCUMULATION AT NORMAL AND INCREASED OSMOLARITY

Cell water and $[Ala]_i/[Ala]_o$ were determined as described in Methods. The values in brackets give the time points at which the cell suspensions were centrifuged, as in Table I.

| Saline | | Cell water (g/g dry wt.) | | $[Ala]_i/[Ala]_o$ |
|---|---|---|---|---|
| Standard, control | (20 min) | 1.31 ± 0.02 | | – |
| + 10 mM Ala | (20 min) | 1.44 ± 0.01 | (10 min) | 4.8 ± 0.1 |
| | | | (30 min) | 8.0 ± 0.3 |
| Standard + 100 mM NaCl | (20 min) | 0.92 ± 0.02 | | – |
| + 10 mM Ala | (20 min) | 0.99 ± 0.03 | (10 min) | 3.8 ± 0.3 |
| | | | (30 min) | 4.3 ± 0.1 |

$K^+$ influx at normal and elevated osmolarity. At 0.1 mM, bumetanide had no significant inhibitory effect on either of those fluxes (results not shown).

*$Na^+$-alanine cotransport and $K^+$ permeability*

Table II shows that in standard medium rat liver cells increased their volume by approx. 10% upon the addition of 10 mM alanine. The cells accumulated the amino acid by a factor 5–8 under those conditions. In hypertonic medium, cellular volume increased by approx. 8% upon addition of alanine, but was still considerably (30%) below the normal cell volume. The accumulation ratio for alanine was around four under those conditions.

Table III shows the rate constant, $k$, for unidirectional $K^+$ efflux under a comparable set of conditions (The alanine concentration was increased from 10 to 20 mM in this experiment, but this is not expected to make any significant difference; compare Ref. 6, Fig. 9.) In standard medium, alanine increased $k$ by a factor of approximately 2; also passive $K^+$ influx (measured in the presence of 1 mM ouabain) was enhanced (not shown). This confirms the observation by Kristensen [6]. (The latter paper reports higher factors, of 3–4; this is possibly due to the fact that the rats used for those experiments had been fasted for 48 h rather than for 20–24 h, as here.) Strikingly, the effect of alanine on $k$ was suppressed, or even reversed, upon hypertonic treatment of the cells (Table III; in another experiment, $k$ in the hypertonic medium was $3.95 \cdot 10^{-4}$ $s^{-1}$ in the absence-, and $3.55 \cdot 10^{-4}$ $s^{-1}$ in the presence of alanine). It is possible to estimate the relative $K^+$ permeabilities under the different conditions. It appeared that only in standard saline with 20 mM alanine the $K^+$ permeability was significantly higher than the control value (Table III).

TABLE III

EFFECT OF ALANINE ON THE RATE CONSTANT FOR UNIDIRECTIONAL $K^+$ EFFLUX, $k$, AND THE RELATIVE $K^+$ PERMEABILITY, $P_K/P_K$ (CONTROL), AT NORMAL AND ELEVATED OSMOLARITY

$k$ was determined as described in Methods. Relative $K^+$ permeabilities were calculated [6] assuming the values for cell water given in Table II, and membrane potentials of ([a]) −30 mV and ([b]) −45 mV, respectively [6].

| Saline | $10^4 \times k$ ($s^{-1}$) | $P_K/P_K$ (control) |
|---|---|---|
| Standard, control | 1.45 | 1.0 [a] |
| +20 mM Ala | 3.03 | 2.6 [b] |
| Standard + 100 mM NaCl | 3.08 | 1.4 [a] |
| +20 mM Ala | 1.83 | 0.9 [a] |

## Discussion

*Volume regulation in isolated rat liver cells*

The data of Table I indicate that isolated rat hepatocytes did not have a mechanism to swell back after hyperosmotic shrinkage. In other cells, this type of volume control is frequently based on the action of a bumetanide-sensitive ($K^+$ +

$Na^+ + 2Cl^-$)-cotransport system [2,3]. Consistent with this notion, I did not find evidence of bumetanide-sensitive $K^+$ transport activity in these cells. It can of course not be excluded that a bumetanide-sensitive system may be operative in intact liver, but is largely inactivated upon collagenase digestion. Such inactivation has been observed [5] for at least one other transport system, a $Na^+$-$Cl^-$ cotransport system involving vesicle-membrane fusion [8].

By contrast, the isolated hepatocytes did show a compensatory response after a hypo-osmotic shock. The fact that the cells shrunk back to their original volume within 15 min is consistent with the observation [5] that the increase in $K^+$ permeability induced by hypotonic swelling was transient within the same time course. Conversely, the increase in $K^+$ permeability probably plays a quantitatively important role in the shrinkage process. This permeability apparently is neither $Ca^{2+}$-dependent [5] nor is it sensitive towards bumetanide (Table I). A volume-dependent $K^+$ permeability with similar negative attributes has been reported for human lymphocytes [9].

*$Na^+$-alanine cotransport and $K^+$ permeability*

The conclusion that $Na^+$-alanine cotransport increases $K^+$ permeability through its effect on cell volume is based om two observations: (i) cells increased their volume under conditions where they accumulated the amino acid (Table II). Alanine accumulation in its turn is indicative of $Na^+$-alanine cotransport through the A system [10]. The increase in cell volume can be quantitatively accounted for by the amino acid accumulation. E.g., at 10 mM alanine, an accumulation factor of 5 results in a difference in amino acid concentration across the plasma membrane of 40 mM. In the absence of compensatory shifts in the concentrations of other cellular solutes, the cells at normal osmolarity would indeed have to swell by some 14% to reach a steady state in which to accomodate this concentration difference. Conversely, the data in Ref. 5 indicate that a volume increase of 10% may easily enhance the $K^+$ permeability by a factor 5 or more. (ii) Under conditions where the cells did accumulate alanine, but the cell volume was kept under its normal value by a raise in medium osmolarity, the $K^+$ permeability was not enhanced (Table III).

These two observations clearly indicate that cell swelling above the normal cell volume is required for the effect of $Na^+$-alanine cotransport on $K^+$ permeability to become apparent. The problem of how $Na^+$-amino acid cotransport may affect $K^+$ permeability is hereby reduced to the more general problem of how cellular volume affects $K^+$ permeability. Of course, this does not exclude the possibility [6] that under certain conditions volume-dependent changes in $K^+$ permeability, in addition to or even rather than serving as a volume-controlling device, may have an important function in hyperpolarizing the cells.

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