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Efflux of potassium ($^{86}Rb^+$) attenuates the volume-restorative effect of sodium-amino acid cotransport in rat renal inner medullary cells shrunken by exposure to hyperosmotic media

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When the osmolality of the bathing medium was increased from 710 to 2000 mosmol/kg H_2O, cells in incubated slices of rat renal inner medulla lost water and K^+, and the rate of efflux of preloaded $^{86}Rb^+$ (a tracer for K^+) was significantly depressed. Addition of 2-aminoisobutyric acid (AIB, 10 mmol/l) partly restored cell water content but without re-accumulation of K^+; the rate of $^{86}Rb^+$ efflux was greatly increased. The presence of Ba^{2+} (1 mmol/l) or trifluoperazine (50 μmol/l) led to complete recovery of cell volume and K^+ contents, with markedly reduced efflux of $^{86}Rb^+$. Neither additive had any significant effect upon these variables in the absence of AIB or in media of 710 mosmol/kg. Efflux of $^{86}Rb^+$ was pH-sensitive within the physiological range, and was depressed when external AIB was reduced below approx. 5 mmol/l. When external Na^+ was increased from 145 to 500 mmol/l (total osmolality 350 to 2500 mosmol/kg) efflux was retarded only slightly if AIB was present, but markedly if AIB was omitted. Inner medullary cells may contain a class of Ba^{2+}-inhibitable, calmodulin-dependent K^+ conductive pathway which is activated in strongly hyperosmotic media by the operation of an inwardly-directed Na^+-amino acid symport (cf. Law, R.O. (1988) Pflügers Arch. 413, 43–50) and which serves to moderate the volume-restorative effect of this membrane mechanism.

Introduction

Cells in the mammalian renal medulla are confronted with a unique problem in regard to maintenance of volume, since the NaCl and urea concentrations in medullary interstitial fluid, and hence its osmolality, are highly variable depending on the diuretic state of the animal [2–4]. During the transition from normal hydration to antidiuresis, inner medullary cells in the intact animal probably undergo moderate shrinkage (see, for example, Ref. 5); that this is far less than would be predicted on the basis of perfect osmometric behaviour is largely due to the intracellular accumulation of low molecular weight organic solutes [6–8]. It remains unclear to what extent inorganic solutes contribute to medullary cellular osmoprotection in vivo (for discussion see Ref. 7).

Antidiuresis in intact animals (e.g., resulting from dehydration) is often of gradual onset (days), but appropriate experimental manoeuvres can lead to marked increases in inner medullar fluid osmolality and hydropenia within as short a time as 30 min [9,10]. In the acutely oliguric rat this increase is accompanied by significant accumulation of tissue amino nitrogen [10], but other organic solutes, methylamines and polyhydric alcohols, up-regulate too slowly to afford osmoprotection under these conditions [11–13], and the possibility must be considered that, at least in the short-term, entry of extracellular solute (NaCl) contributes to the maintenance of osmotic potential and cell volume. Moreover, cells in incubated slices of rat renal inner medulla display only moderate shrinkage when exposed to media of 2000 mosmol/kg H_2O containing high concentrations of NaCl and urea, which is associated with a large increase in cell Na^+ content and a moderate fall in K^+ content [14]. The extent of cell shrinkage is further reduced if amino acid is present in the incubatory medium, probably through the activa-

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Abbreviations: AIB, 2-aminoisobutyric acid; s.f.d.w., solute-free dry weight; TFP, trifluoperazine dihydrochloride; TMB-8, 3,4,5-trimethoxybenzoic acid-9-(diethylamino)octyl ester; Tris, 2-amino-2-(hydroxymethyl)propane-1,3-diol.

tion of an inwardly-directed Na^+-amino acid symport [1]. This partial recovery of volume is associated with a further increase in cell Na^+ content, but there is no accompanying restoration of cell K^+, and intracellular K^+ concentration decreases. It has been suggested [1] that this failure to re-accumulate osmotically-active K^+ is a mechanism preventing these cells from too rigidly maintaining their volumes under conditions in which, in the intact animal, medullary tissue is required to tolerate marked reduction in overall hydration (see, for example, Ref. 15). In the presence of Ba^{2+} or quinine, there is near-complete restoration of cell volume and K^+ contents, to levels comparable with those observed under mildly hyperosmotic conditions. Failure of cells to re-accumulate K^+ in strongly hyperosmotic media appears to be causally linked to Na^+-amino acid co-transport, since Ba^{2+} is without effect on cell volume or K^+ content if external amino acid is reduced to very low concentrations [1].

This paper describes some features of volume-modulatory loss of K^+ by renal inner medullary cells in vitro, as reflected in rates of efflux of $^{86}Rb^+$ from pre-loaded slices under various incubatory conditions, and correlation of these with steady-state cell volumes and K^+ contents in slices similarly incubated.

The results of a small number of comparable studies have previously been published as abstracts [16,17]. The findings described in the present paper, however, were derived from an entirely separate investigation.

Methods

Slices of inner medulla (thickness approx. 250 μm, weight 3–12 mg) were prepared from the kidneys of normally hydrated adult male Wistar rats, sacrificed by cervical dislocation as previously described [1]. (The term inner medulla is here used to denote that region of the kidney lying between the lower extremity of the inner stripe of the outer (red) medulla and the based of the papillary tip). They were blotted, and weighed to the nearest 50 μg on a torsion balance before being equilibrated for approx. 30 min in a medium of the following composition (mmol/l): Na^+ 203, K^+ 5.9, Ca^{2+} 2.6, Mg^{2+} 1.2, Cl^- 167, HCO_3^- 25, $H_2PO_4^-$ 2.2, SO_4^{2-} 1.2, pyruvate 9.6, fumarate 5.3, glucose 10, urea 266, gassed with 95% O_2/5% CO_2 to pH 7.35 at 37°C. The osmolality of this medium is 710 mosmol/kg H_2O, and approximates to the calculated osmolality of fluids from the inner medullas of normally hydrated rats [18]. Slices were then transferred (except where specified otherwise) for 100 min to media of 2000 mosmol/kg (simulating fluid from inner medullas of antidiuretic rats). This increase in osmolality was achieved by raising concentrations of Na^+ (400 mmol/l), Cl^- (364 mmol/l) and urea (1172 mmol/l).

The following additions and substitutions were made, individually or in combination as specified in the relevant sections of Results:

(i) AIB, 10 mmol/l except where stated otherwise.

(ii) Ba^{2+} (1 mmol/l). In these media HCO_3^-, $H_2PO_4^-$ and SO_4^{2-} were replaced by equimolar NO_3^- and pH adjusted by Tris with 100% O_2 as the gas phase.

(iii) Cd^{2+} (0.1 mmol/l). Anion substitutions and pH adjustments were the same as in the presence of Ba^{2+}.

(iv) TFP (50 μmol/l) (Aldrich Chemical Co.).

(v) TMB-8 (0.5 mmol/l) (Sigma Chemical Co.).

(vi) Verapamil (10 μmol/l) (Sigma Chemical Co.).

(vii) Na^+ and urea were altered so as to give media in the concentration range 350 to 2500 mosmol/kg (Na^+ 145 to 500 mmol/l).

(viii) In some experiments carried out in media of 2000 mosmol/kg, K^+ was increased to 50 mmol/l by equimolar substitution for Na^+.

(ix) Medium pH was altered between 6.85 and 7.85 by addition of Tris, gas phase being 100% O_2.

Experiments were carried out under two general headings. In the first, steady-state cell volumes (water contents) and K^+ contents were determined as previously described in detail [1]. Cell volumes were considered to be the non-sucrose fluid compartment of each slice [14], as determined by the volume of distribution of [^{14}C]sucrose (Amersham International plc) 25 min after addition to media at an activity of approx. 20 kBq/ml. Contents were expressed as μl/mg s.f.d.w. of tissue (volume) and nmol/mg s.f.d.w. (K^+). Where appropriate, K^+ concentrations are given as mmol/l.

The second series of experiments followed the efflux of $^{86}Rb^+$ (Amersham International plc), which considered a tracer for K^+ ions, from pre-loaded (100 min) slices into unlabelled but otherwise identically constituted media. The activity of the loading media was approx. 185 kBq/ml. Loading and efflux both took place at 37°C. Serial samples of efflux media were taken at 1, 2, 5, 10, 20, 30, 40, 50 and 60 min intervals. Prior to efflux slices were briefly blotted but not rinsed. $^{86}Rb^+$ remaining within slices at 60 min was leached into 1 ml distilled water overnight at room temperature in order to allow determination of initial total slice $^{86}Rb^+$ content. Efflux media were contained in vigorously agitated polypropylene tubes. Since gassing was found to be impracticable under these conditions, HCO_3^- was replaced, both in loading and efflux media, by equimolar NO_3^-, with pH being adjusted to 7.35 with Tris: media were air-equilibrated. While it was clearly not possible to determine what effect replacement of HCO_3^- by NO_3^- might have had on the rates of $^{86}Rb^+$ efflux, it was found in pilot experiments (not reported here) that this substitution does not significantly affect inner medullary cell volume or K^+ content (in contrast to the significant reduction in cell

volume which does occur when the major anion, Cl^- is replaced by NO_3^- in the presence of 10 mmol/l AIB [1]). Rates of net efflux were expressed as percentage total counts remaining (initially 100%) on a semi-logarithmic basis with respect to time.

Determinations of cell volumes and K^+ contents, and of rate constants for $^{86}Rb^+$ efflux, were made in identical series of media (with the exception of the methods of pH adjustment), except for those designed to examine the effects of AIB and Na^+ concentrations, and of external pH, on the efflux of $^{86}Rb^+$, for which comparable estimations of cell volume and composition were not carried out.

Results are expressed as mean ± S.E. (n = number of individual observations). Statistical comparisons were made on the basis of Student's unpaired t-test, with $P < 0.05$ or better being considered significant.

Results

In Fig. 1 are shown the rates of net efflux of $^{86}Rb^+$ from preloaded slices into media of 710 mosmol/kg, and 2000 mosmol/kg with or without the addition of 10 mmol/l AIB. Standard errors have been omitted because (with a single exception) they would have been obliterated by the symbols. The 2-phase nature of the patterns of efflux was qualitatively identical under all conditions studied. No distinction could be made between the initial rapid phases under differing incubatory conditions, and it is assumed that these represent primarily efflux from the extracellular compartment.

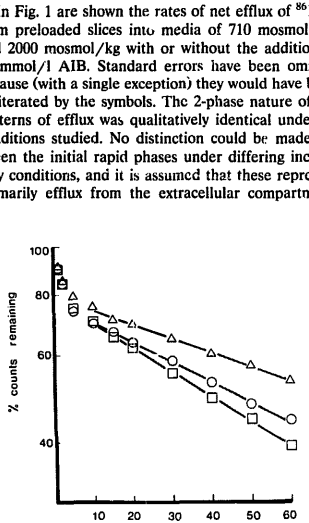

Fig. 1. The efflux of $^{86}Rb^+$ from pre-loaded slices of inner medulla, expressed on a semi-logarithmic basis as percentage counts remaining with respect to time, into media of 710 mosmol/kg (○), 2000 mosmol/kg (△) and 2000 mosmol/kg containing 2-aminoisobutyric acid (10 mmol/l) (□). Each point is the mean of six observations. Rate constants (k) for the second (linear) phases are quantified in Table I.

TABLE I

Rate constants for the 2nd phase of net $^{86}Rb^+$ efflux from pre-loaded rat renal inner medullary slices, and corresponding steady-state cell volumes and K^+ contents

AIB was 10 mmol/l. Values are means ± S.E., n = number of slices studied.

Medium osmolality (mosmol/kg)	k (10^{-4} s^{-1})	Cell volume (μl/mg s.f.d.w.)	Cell K^+ (nmol/mg s.f.d.w.)
710	1.61 ± 0.04 (6)	4.03 ± 0.11 (9)	499 ± 15 (9)
710 + AIB	1.58 ± 0.04 (6)	4.09 ± 0.11 (11)	505 ± 10 (11)
2000	1.20 ± 0.02 (6)	2.85 ± 0.09 (12)	398 ± 11 (12)
2000 + AIB	1.88 ± 0.03 (6)	3.39 ± 0.10 (10)	421 ± 14 (10)

Although accurate calculations cannot be made on the basis of so few experimental points, rate constants during the initial 5 min can be shown to lie within the range $(1.3–1.5) \cdot 10^{-3}$ s^{-1}. They thus exceed by approximately one order of magnitude the rate constants for the 2nd phase (10–60 min) effluxes reported in Tables I–IV, and it is reasonable to infer that they do not significantly influence the latter, which were in all instances rectilinear and are assumed to represent loss of cellular $^{86}Rb^+$. It can be seen that whereas an increase in incubatory osmolality from 710 to 2000 mosmol/kg led to a marked slowing of net 2nd phase $^{86}Rb^+$ efflux, k decreasing from $(1.61 \pm 0.04) \cdot 10^{-4}$ s^{-1} (6) to $(1.20 \pm 0.02) \cdot 10^{-4}$ s^{-1} (6) ($P < 0.001$), the rate of efflux was significantly accelerated ($(1.88 \pm 0.03) \cdot 10^{-4}$ s^{-1} (6); $P < 0.001$) when AIB was added to the medium, i.e., under conditions when the Na^+-amino acid symport is presumed to be activated. These data are included in Table I, in which they are correlated with cell volumes and K^+ contents under identical incubatory conditions. It can be seen that the presence of AIB was without effect on $^{86}Rb^+$ efflux, cell volume or cell K^+ contents in media of 710 mosmol/kg. Conversely, while imposition of several hyperosmotic conditions predictably caused marked losses or both cell volume and K^+ contents, the presence of AIB led to highly significant partial restoration of cell volume ($P < 0.001$). K^+, however, failed to reaccumulate significantly, and this was associated with the increased rate of $^{86}Rb^+$ efflux. The accompanying fall in mean intracellular K^+ concentrations from 138 to 122 mmol/l may be ascribed to dilution of the residual cellular K^+ by the osmotically obligated water entering the cells secondarily to activation of the Na^+-amino acid symport.

Table II shows that Ba^{2+} ions, which block certain classes of K^+ channels (for review, see Ref. 19), did not affect any of the three variables studied in media of 710 mosmol/kg or of 2000 mosmol/kg in the absence of AIB. When AIB was added to the latter media, $^{86}Rb^+$ efflux was markedly decreased ($P <$

TABLE II

Rate constants for the 2nd phase of net $^{86}Rb^+$ efflux from pre-loaded rat renal inner medullary slices, and corresponding steady-state cell volumes and K^+ contents in the presence of Ba^{2+} (1 mmol/l)

AIB was 10 mmol/l. Values are means ± S.E., n = number of slices studied.

Medium osmolality (mosmol/kg)	k (10^{-4} s^{-1})	Cell volume (μl/mg s.f.d.w.)	Cell K^+ (nmol/mg s.f.d.w.)
710 + Ba^{2+}	1.55 ± 0.02 (6)	3.92 ± 0.13 (11)	489 ± 12 (11)
2000 + Ba^{2+}	1.27 ± 0.02 (6)	2.92 ± 0.07 (13)	402 ± 10 (13)
2000 + Ba^{2+} + AIB	1.06 ± 0.03 (6)	3.95 ± 0.12 (12)	488 ± 9 (12)

0.001) and cell volumes and K^+ contents increased to levels comparable with those observed in media of 710 mosmol/kg.

The effects of TFP, an inhibitor of calmodulin activation [20], were qualitatively comparable to those of Ba^{2+}, and are shown in Table III. Like Ba^{2+}, TFP was without significant effect on $^{86}Rb^+$ efflux or cell composition in media of 710 or 2000 mosmol/kg in the absence of AIB, but when AIB was present caused marked enhancement of cell volume and K^+ contents, while significantly depressing $^{86}Rb^+$ efflux.

Table IV summarizes the effects of agents known to block the entry of Ca^{2+} into certain cell types, viz. Cd^{2+} ions and verapamil (for references, see Refs. 21 and 22) and of TMB-8, which is reported to inhibit the release of Ca^{2+} from internal stores [23]. At the concentrations used in this investigation, no single agent had any effect in isolation, but when all three were present there were highly significant decreases in the rate of efflux of $^{86}Rb^+$ as well as increases in cell volumes and K^+ contents.

Figs. 2, 3 and 4 illustrate certain characteristics of net 2nd phase $^{86}Rb^+$ efflux. A relationship between efflux and cell water and K^+ contents having been established in Tables I–IV, the correlation between these variables was not studied under the conditions whose effects on $^{86}Rb^+$ efflux are reported below.

TABLE III

Rate constants for the 2nd phase of net $^{86}Rb^+$ efflux from pre-loaded rat renal inner medullary slices, and corresponding steady-state cell volumes and K^+ contents in the presence of TFP (50 μmol/l)

AIB was 10 mmol/l. Values are means ± S.E., n = number of slices studied.

Medium osmolality (mosmol/kg)	k (10^{-4} s^{-1})	Cell volume (μl/mg s.f.d.w.)	Cell K^+ (nmol/mg s.f.d.w.)
710 + TFP	1.60 ± 0.03 (6)	4.09 ± 0.14 (9)	521 ± 16 (9)
2000 + TFP	1.25 ± 0.03 (6)	2.88 ± 0.08 (13)	383 ± 10 (13)
2000 + TFP + AIB	0.90 ± 0.03 (6)	4.21 ± 0.13 (12)	516 ± 14 (12)

TABLE IV

Rate constants of the 2nd phase of net $^{86}Rb^+$ efflux from pre-loaded rat renal inner medullary slices and corresponding steady-state cell volumes and K^+ contents in media of 2000 mosmol/kg containing additives potentially affecting the availability of intracellular free Ca^{2+} (viz. Cd^{2+} (0.1 mmol/l), verapamil (10 μmol/l), TMB-8 (0.5 mmol/l) and a combination of all three (at the same concentrations))

All media contained AIB (10 mmol/l). Values are means ± S.E., n = number of slices studied.

	k (10^{-4} s^{-1})	Cell volume (μl/mg s.f.d.w.)	Cell K^+ (nmol/mg s.f.d.w.)
Cd^{2+} ions	1.79 ± 0.04 (6)	3.49 ± 0.12 (12)	429 ± 13 (12)
Verapamil	1.84 ± 0.04 (6)	3.48 ± 0.14 (9)	418 ± 16 (9)
TMB-8	1.83 ± 0.03 (6)	3.36 ± 0.09 (11)	420 ± 13 (11)
Cd^{2+} + verapamil + TMB	1.13 ± 0.03 (6)	4.63 ± 0.08 (15)	511 ± 12 (15)

Fig. 2 shows the relationship between $^{86}Rb^+$ efflux and ambient pH in media of 2000 mosmol/kg containing 10 mmol/l AIB. Efflux displays a marked dependence on pH which is most pronounced in the range from approximately 7.25 to 7.4, i.e., close to normal physiological levels. Fig. 3 relates efflux to AIB concentration in media of 2000 mosmol/kg. Raising the concentration markedly stimulated efflux: half-maximum stimulation occurred at about 2 mmol/l. Increasing the concentration above 5 mmol/l produced no further significant effect.

In Fig. 4 are shown the relationships between $^{86}Rb^+$ efflux and medium Na^+ concentration with and without the addition of 10 mmol/l AIB. The Na^+ concentration ranged from 145 to 500 mmol/l, and, as ex-

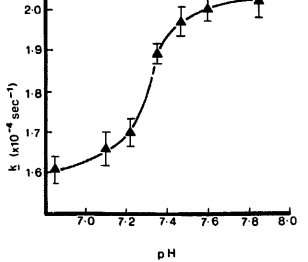

Fig. 2. The relationship between extracellular pH and the rate constant of 2nd phase of efflux of $^{86}Rb^+$ from pre-loaded slices of inner medulla into medium of 2000 mosmol/kg containing 2-aminoisobutyric acid (10 mmol/l). Points are mean ± S.E. (n = 6).

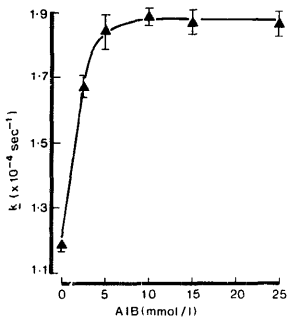

Fig. 3. The relationship between extracellular concentration of 2-aminoisobutyric acid and the rate constant of the 2nd phase of efflux of $^{86}Rb^+$ from pre-loaded inner medullary slices into media of 2000 mosmol/kg. Points are mean ± S.E. ($n = 6$).

plained in Methods, accompanying urea concentrations were adjusted to vary the osmolality from 350 to 2500 mosmol/kg. Thus, in terms of Na^+ and urea concentrations, and total osmolality, the media used in this series of experiments simulated those in the medullary tissues of rats under conditions ranging form diuresis to severe antidiuresis. It can be seen that the decrease

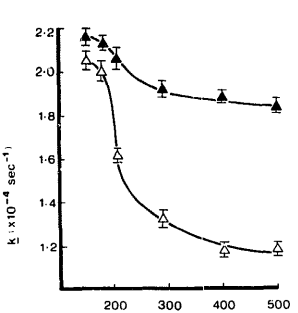

Fig. 4. The relationship between extracellular Na^+ concentration and the rate constant of the 2nd phase of $^{86}Rb^+$ efflux from pre-loaded inner medullary slices into media with (solid triangles) or without (open triangles) the addition of 2-aminoisobutyric acid (10 mmol/l). The calculated osmolalities of the incubation media ranged between 350 and 2500 mosmol/kg (see text). Points are mean ± S.E. ($n = 6$).

in the rate of $^{86}Rb^+$ efflux with increasing Na^+ concentration is far less steep in the presence of AIB than when AIB was omitted.

In confirmation of a previous report [16], raising external K^+ to 50 mmol/l in media of 2000 mosmol/kg, with or without addition of AIB, significantly increased steady-state cell volumes (with AIB 4.04 ± 0.14(8) μl/mg s.f.d.w.; AIB-free, 3.35 ± 0.11(9) μl/mg s.f.d.w., $P < 0.005$). Cell K^+ contents increased (with AIB, 457 ± 10(8) nmol/mg s.f.d.w.; AIB-free, 433 ± 9(9) nmol/mg s.f.d.w.) but remained not significantly different. The rate constants for net efflux of $^{86}Rb^+$ remained far higher in the presence of AIB than in AIB-free media ((1.95 ± 0.04) · 10^{-3} s^{-1} (6) and (1.26 ± 0.03) · 10^{-4} s^{-1} (6), respectively, $P < 0.001$) and did not differ significantly from those in media containing 5.9 mmol/l K^+.

Discussion

The present findings are consistent with the suggestion that rat renal inner medullary cells contain a population of K^+ (Rb^+) channels which influence cell volume (water content) and are activated by the operation of inwardly-directed Na^+-amino acid cotransport under strongly hyperosmotic conditions. The latter requirement is inferred from the finding that Rb^+ efflux, cell volume and K^+ contents are unaffected by the presence of AIB in relatively dilute medium (710 mosmol/kg) (Table I). Although the behaviour of medullary cells are described in this paper is consistent with the presence of such channels, two cautionary points must be made. Firstly, there is no certainty that in this tissue the cellular fluxes of K^+ are necessarily identical with those for tracer Rb^+. Secondly, it is conceivable that some of the experimental manoeuvres under taken could have influenced the electrical driving force for net cellular K^+ extrusion. No relevant data is presently available which could enable this possibility to be pursued further. K^+ efflux is blocked by Ba^{2+} ions (Table II), requires the activation of calmodulin (Table III), and the availability of Ca^{2+} ions. (Since no attempt has here been made to characterize cellular Ca^{2+} entry pathways, inferences regarding the source of these ions must clearly be made with caution; but the data summarized in Table IV suggest that they may be derived from the external medium or from intracellular stores). Enhanced medullary cell K^+ efflux appears to be unique in that it is activated under conditions in which cells have undergone a reduction in their volume (by comparison with volumes in media of lower osmolality (Table I)), in contrast to the well-documented K^+ channels in other cell types which are responsive to swelling or stretching of the plasma membranes (e.g., Refs. 24–27). Medullary cells probably also contain K^+ of the latter type (cf. the data in

Fig. 4, in which efflux progressively increases as tonic stress on cells is reduced by decreasing the external Na^+ concentration). But from inspection of Tables I–IV it is clear that K^+ efflux is not simply dependent upon cell volume per se. Thus while an increase in external osmolality from 710 to 2000 mosmol/kg, in the absence of amino acid, leads to parallel decreases in cell volume and K^+ contents and slowing of K^+ efflux (Table I and Fig. 4) which are unaffected by Ba^{2+} ions (Table II) or inhibition of calmodulin activation by TFP (Table III), addition of amino acid to media of 2000 mosmol/kg reveals a strong negative correlation K^+ efflux and cell K^+ and water contents, both under control conditions and in the presence of Ba^{2+} ions, TFP, and agents potentially influencing the availability of Ca^{2+} ions (Table IV). Inner medullary tissue comprises a heterogeneous population of cell types, and although they are faced with common problems of volume regulation, there is no certainty that they necessarily utilize identical strategies in surmounting them. However, as has been stressed previously in a similar experimental context [1], if the responses described in this study are lacking in part or parts of the cell population, it is reasonable to infer that they are present in a more pronounced form in other cells.

The problem arises of placing the present observations into a realistic physiological context. As suggested in the Introduction, efflux of K^+ under strongly hyperosmotic conditions – i.e., the shedding of solute and hence osmotically obligated water – may represent cells' contribution to the generalized reduction in tissue hydration which occurs during severe antidiuresis. If the loss of K^+ is blocked by Ba^{2+}, TFP or restricted availability of Ca^{2+}, cells exposed to media of 2000 mosmol/kg retain volumes and K^+ contents comparable with those observed in media of 710 mosmol/kg. Enhanced K^+ efflux in hyperosmotic media appears to be significantly dependent upon the presence of external amino acid (Fig. 4). Amino acid-dependent K^+ efflux has been observed in a variety of epithelial cells (e.g., refs. 28–31) and maybe interpreted as a regulatory volume decrease in response to transient cell swelling caused by entry of excess osmotically active solute. While cells incubated in media of 2000 mosmol/kg clearly cannot be regarded as 'swollen' in the normal sense of the word, those exposed to amino acid nevertheless display relative augmentation of cell water content, by comparison with cells in amino acid-free media (Table I), which is associated with increased cellular entry of Na^+ (and secondarily Cl^-) [1]. Low concentrations of AIB (< 5 mmol/l) have less stimulatory effect on inward Na^+ transport [1] and associated K^+ efflux (Fig. 3). Significant concentrations of amino acids are believed to be present in the intact medullary interstitium [32,33], and although when the concentration of Na^+ is increased from 200 to 400 mmol/l incubatory media (simulating conditions of severe antidiuresis in vivo) there are large increases in medullary cell Na^+ concentration and contents [14], amino acid cotransport may represent one means whereby these cells can *further* augment their Na^+ content (hence osmotic potential).

Incubations in media containing 50 mmol/l K^+ were performed in view of reports that medullary interstitial K^+ concentrations increase markedly in antidiuresis (e.g., Refs. 5 and 34). Although high concentrations of external K^+ led to increases in cell volumes and K^+ contents, the same triad of relationships persisted as in low-K^+ media, viz. the presence of AIB (i) significantly increased cell volumes, (ii) failed to elevate cell K^+ contents, and (iii) greatly accelerated K^+ efflux.

The question now remains as to how hyperosmotic, amino acid-dependent cellular entry of Na^+ is causally linked to the loss of cell K^+ which effectively attenuates the volume-restorative effect of such entry. One possibility, currently under investigation in this laboratory, is that medullary cells possess a class of K^+ conductive pathway activated by high internal Na^+ concentration, of the type which has been identified in certain excitable cells (for review see Ref. 35). This idea is attractive, but unlikely to provide a complete answer. Na^+-activated K^+ channels appear to be dependent upon an *increase* in intracellular Na^+ concentration. Medullary cells incubated in high-Na^+ media have high internal concentrations regardless of whether amino acid (which is necessary for activation of enhanced K^+ efflux, Fig. 1 and Table I) is present or not [14]. Cotransport activity increases cell Na^+ *content* but not *concentration*, since additional Na^+ entry is accompanied by parallel entry of osmotically-obligated water [1].

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