Characterization of L-carnitine transport by rat kidney brush-border-membrane vesicles

Bruno STIEGER, Brigitte O'NEILL and Stephan KRÄHENBÜHL*

Division of Clinical Pharmacology and Toxicology, Department of Internal Medicine, University Hospital, Zürich, Switzerland

In the presence of a 100 mM Na⁺ gradient, transport of L-carnitine into rat renal brush-border-membrane vesicles was linear over 30 s and showed an overshoot at 5 min. The uptake of L-carnitine was clearly less active in the presence of other cations such as Li⁺, K⁺, Cs⁺ or choline. In the presence of a Na⁺ gradient, L-carnitine uptake after 20 s was much higher for chloride as an anion than for SCN[−], NO₃[−], gluconate or SO₄^{2−}. In comparison with conditions with inside positive or no membrane potential, transport was higher in vesicles with an inside

negative membrane potential, suggesting an electrogenic mechanism. The kinetic characterization of the Na⁺-dependent portion of L-carnitine transport revealed two transport systems with K_m values of $17.4 \pm 3.9 \mu\text{M}$ and $15.0 \pm 6.0 \text{ mM}$, respectively. The transport could be inhibited in a concentration-dependent fashion by structural analogues such as butyrobetaine, L-acetyl-carnitine, trimethyl-lysine and D-carnitine, but not by L-arginine or glycinebetaine.

INTRODUCTION

Carnitine (4-*N*-trimethylammonio-3-hydroxybutanoate) is essential for the transport of long-chain fatty acids across the inner mitochondrial membrane [1,2]. Another, well established function of carnitine is the maintenance of the cellular coenzyme-A pool [3,4]. Mediated by carnitine acyltransferases, carnitine can react with acyl-CoAs and form the corresponding acylcarnitines [5]. In this reaction, coenzyme-A is generated and is available for metabolic pathways such as β -oxidation and pyruvate metabolism.

In mammals, the body carnitine stores are maintained by dietary intake and by biosynthesis [2,6]. Carnitine is eliminated as free carnitine or acylcarnitine almost exclusively by the kidneys, where it is filtered and reabsorbed by > 95% in the proximal tubule [7,8]. When carnitine reabsorption is impaired, e.g. in patients with primary carnitine deficiency [9] or patients with Fanconi syndrome [8,10], the plasma and tissue carnitine concentrations decrease and clinical signs of carnitine deficiency can develop, emphasizing the importance of the renal carnitine transport system.

The renal carnitine transport system has been characterized in rat kidney brush-border membrane vesicles [11]. These studies revealed Na⁺-dependent, saturable transport of L-carnitine which could be inhibited by structural analogues such as D-carnitine, butyrobetaine or D,L-acetylcarnitine but not by trimethyl-lysine or choline. The K_m value of this transport system was found to be 55 μM , a value which is close to K_m values of L-carnitine transport in other organs [12]. Interestingly, L-carnitine uptake by kidney brush-border membranes from rats eating a carnitine-supplemented diet was lower than for rats on a regular diet, suggesting that the activity of the renal L-carnitine transport system may be regulated [11].

So far, no L-carnitine transport system has been characterized on the molecular level. Since our group has experience in expression cloning, we expressed rat kidney mRNA in *Xenopus laevis* oocytes and characterized L-carnitine uptake [13]. These

studies revealed Na⁺-dependent transport of L-carnitine in both mRNA-injected and non-injected oocytes, indicating the presence of an endogenous transport system for L-carnitine. Therefore, in order to be able to differentiate between the L-carnitine transport systems in the oocytes and in rat kidney, it seemed necessary to study L-carnitine transport into rat kidney brush-border-membrane vesicles in more detail.

MATERIALS AND METHODS

Materials

Male Sprague–Dawley rats (SUT:SDT) were obtained from the Süddeutsches Tierzuchtinstitut (Tuttlingen, Germany) and were kept under standard conditions. L[³H]-Carnitine was obtained from NEN Du Pont de Nemours (Wilmington, DE, U.S.A.). All other chemicals were of the highest purity available from either Merck (Darmstadt, Germany), Fluka (Buchs, Switzerland) or Sigma (St. Louis, MO, U.S.A.).

Methods

Brush-border-membrane vesicles prepared by an EGTA/magnesium precipitation method as described previously in detail [14] were routinely used for the experiments. The final membrane pellet was resuspended in the transport buffer given in the legends to the Figures or Tables and dispersed using a syringe with a fine needle.

Transport experiments were performed with a rapid filtration technique using cellulose nitrate filters (0.65 μm pore size from Sartorius, Göttingen, Germany) according to a procedure described previously [15].

Protein determinations were performed by the Lowry procedure [16] after precipitation with trichloroacetic acid. The kinetic parameters were fitted using non-linear regression

*To whom correspondence should be addressed.

Figure 1 Time course and Na^+ -dependency of L-carnitine transport

Membrane vesicles were prepared in 400 mM mannitol and 20 mM Hepes adjusted with Tris to pH 7.4. The incubation medium contained (final concentrations) 50 μM $\text{L}^{[3]\text{H}}$ -carnitine (0.2 μCi per assay), 200 mM mannitol, 20 mM Hepes adjusted with Tris to pH 7.4 and 100 mM NaCl (circles, Na^+ -gradient) or 100 mM choline chloride (triangles, choline-gradient). Transport was started by adding 15 μl of membrane suspension to 30 μl of preheated incubation medium (37 $^{\circ}\text{C}$). At the time points indicated, 30 μl of the incubation suspension were withdrawn, diluted with 5 ml of ice-cold stop solution, filtered, the filter rewashed twice with ice-cold stop solution and the radioactivity on the filter was counted.

Table 1 Effect of cations on L-carnitine uptake by rat renal brush-border-membrane vesicles

Membrane vesicles were prepared in 400 mM mannitol and 20 mM Hepes adjusted with Tris to pH 7.4. The incubation medium contained (final concentrations) 50 μM $\text{L}^{[3]\text{H}}$ -carnitine (0.2 μCi per assay), 200 mM mannitol, 20 mM Hepes/Tris, pH 7.4, 100 mM of the cations listed in the Table as chloride. Transport was started by adding 15 μl of membrane suspension to 30 μl of preheated incubation medium (37 $^{\circ}\text{C}$). At the time points given in the Table, 30 μl of the incubation suspension were withdrawn, diluted with 5 ml ice-cold stop solution, filtered, the filter rewashed twice with ice-cold stop solution, and the radioactivity on the filter was counted. Data are presented as mean $\pm$ S.D. Units are pmol/mg of protein.

Cation	L-Carnitine uptake (pmol/mg)			
	10 s	30 s	3 min	120 min
Na^+	24.4 ± 1.3	76.9 ± 1.5	153 ± 7	93.5 ± 4.2
Li^+	15.2 ± 1.1	40.3 ± 1.4	108 ± 2	150 ± 3
K^+	10.4 ± 0.8	29.3 ± 3.9	75.9 ± 3.0	97.2 ± 1.7
Cs^+	18.5 ± 6.2	25.9 ± 1.8	66.6 ± 1.0	91.3 ± 2.0
Choline $^+$	2.3 ± 3.5	5.3 ± 0.3	23.7 ± 2.2	89.3 ± 1.3

(Sigmaplot Windows 1.0, Jandel Scientific, Erkrath, Germany) according to the following equation:

$$v = V_{\max}(1) \times [S]/([S] + K_m(1)) + V_{\max}(2) \times [S]/([S] + K_m(2))$$

where v and V stand for velocity, $[S]$ for L-carnitine concentration and K_m for the Michaelis constant. Unless stated otherwise, all Figures or Tables show representative experiments of at least three individual determinations in triplicate. Data are presented as mean $\pm$ S.D.

RESULTS

The influence of cations on the transport of L-carnitine into brush-border-membrane vesicles from rat kidney was characterized first. As shown in Figure 1, in the presence of 100 mM

Table 2 Effect of anions on L-carnitine uptake by rat renal brush-border-membrane vesicles

Membrane vesicles were prepared as described in Table 1. Incubation medium contained (final concentrations) 50 μM $\text{L}^{[3]\text{H}}$ -carnitine (0.2 μCi per assay), 200 mM mannitol, 20 mM Hepes/Tris, pH 7.4, and 100 mM of the anions given in the Table as Na^+ - or K^+ -salts. Transport was determined as described in Table 1. Data are presented as mean $\pm$ S.D. Units are pmol/mg of protein.

Anion	Na^+ -salt		K^+ -salt	
	20 s	120 min	20 s	120 min
Cl^-	59.2 ± 6.8	117 ± 20	27.5 ± 1.6	122 ± 12
SCN^-	37.7 ± 4.0	130 ± 17	34.8 ± 4.5	124 ± 10
NO_3^-	36.8 ± 6.9	125 ± 18	36.0 ± 5.3	143 ± 16
Gluconate $^-$	34.6 ± 1.0	105 ± 12	25.3 ± 5.1	87.4 ± 8.8
SO_4^{2-}	29.4 ± 4.0	103 ± 10	26.6 ± 3.8	73.4 ± 8.7

Table 3 Dependency of L-carnitine transport on K^+ /valinomycin-induced diffusion potentials

Membrane vesicles were prepared in 100 mM KCl, 200 mM mannitol and 20 mM Hepes/Tris (pH 7.4) (neutral or inside negative diffusion potential) or 400 mM mannitol and 20 mM Hepes/Tris (pH 7.4) (inside positive). The incubation medium contained (final concentrations) 100 mM KCl, 100 mM NaCl or choline chloride and 20 mM Hepes/Tris (pH 7.4) (neutral), 100 mM KCl, 100 mM NaCl or choline chloride and 20 mM Hepes/Tris (pH 7.4) (inside positive) or 200 mM mannitol, 100 mM NaCl or choline chloride and 20 mM Hepes/Tris (pH 7.4) (inside negative). Incubations contained (final concentration) 50 μM $\text{L}^{[3]\text{H}}$ -carnitine (0.20 μCi). After addition of 15 μl of membrane suspension to 30 μl of preheated incubation medium (37 $^{\circ}\text{C}$), reactions were started by adding 3.5 μg of valinomycin in ethanol (final ethanol concentration 1% v/v). At the time points indicated, 30 μl of the incubation suspension were withdrawn and analysed for transport as described in Table 1. Data are presented as mean $\pm$ S.D. Units are pmol/mg of protein.

Diffusion potential ...	L-Carnitine uptake (pmol/mg of protein)		
	Inside positive	Neutral	Inside negative
Na^+ -gradient			
20 s	30.9 ± 1.9	28.2 ± 2.2	33.6 ± 1.4
60 s	50.4 ± 6.1	49.9 ± 3.9	73.2 ± 5.3
120 min	38.9 ± 2.5	42.2 ± 4.7	44.4 ± 6.6
Choline-gradient			
20 s	5.9 ± 0.5	7.5 ± 0.7	4.4 ± 1.6
60 s	9.2 ± 1.2	10.6 ± 1.5	11.0 ± 2.8
120 min	38.5 ± 2.4	42.6 ± 0.7	41.9 ± 3.5

NaCl, the uptake of L-carnitine into isolated brush-border-membrane vesicles was linear with time for 30 s and showed an overshoot at 5 min, indicating active, Na^+ -dependent uptake. In comparison, in the presence of 100 mM choline, the uptake of L-carnitine was slow and showed no overshoot.

The effect of cations on L-carnitine uptake is characterized further in Table 1. In the presence of LiCl, KCl or CsCl, the uptake was between that of NaCl and that of choline and showed no overshoot. The high equilibrium value (120 min) in the presence of LiCl was observed consistently in three experiments with individually prepared brush-border-membrane vesicles and could possibly be explained by increased binding.

Next, a possible influence of anions on the uptake of L-carnitine in the presence of 100 mM Na^+ or K^+ (as a control) was studied. As shown in Table 2, in the presence of Na^+ , the uptake of L-carnitine after 20 s was significantly higher with Cl^- as an anion as compared with SCN^- , NO_3^- , gluconate or SO_4^{2-} . In

Figure 2 Kinetics of Na^+ -dependent L-carnitine transport

Transport of L-carnitine into the membrane vesicles was measured for 20 s in the presence of 100 mM NaCl or choline chloride as described in Figure 1 at the L-[^3H]carnitine concentrations (0.2 μCi per assay) given on the abscissa. The Figure shows a linear plot of the Na^+ -dependent L-carnitine transport rate (transport rate in the presence of NaCl minus transport rate in the presence of choline chloride) and in the insert an Eadie-Hofstee transformation of the transport rates.

contrast, in the presence of K^+ , L-carnitine uptake did not depend on the anion used.

The markedly increased uptake of L-carnitine in the presence of Cl^- as compared with SO_4^{2-} suggested an effect of the membrane potential on the activity of this transport system. Such an influence of the membrane potential on L-carnitine uptake was tested more directly by creating K^+ /valinomycin-induced diffusion potentials (Table 3). In the presence of an inside negative diffusion potential (vesicles preloaded with K^+), Na^+ -dependent L-carnitine uptake was clearly higher as compared with conditions with no potential or with an inside positive diffusion potential. This finding strongly indicates that Na^+ -dependent L-carnitine uptake by rat kidney brush-border-membrane vesicles is potential-dependent.

The kinetic analysis of the Na^+ -dependent L-carnitine transport is shown in Figure 2. In the direct linear and also in the Eadie-Hofstee plots, two K_m values could be distinguished, averaging $17.4 \pm 3.9 \mu\text{M}$ and $15.0 \pm 6.0 \text{ mM}$, respectively. The corresponding V_{max} values were 3.76 ± 0.42 and $231 \pm 54 \text{ pmol/mg of protein per s}$ respectively. Similar results were obtained with brush-border-membrane vesicles isolated by the calcium precipitation method used by Rebouche and Mack [11].

In order to investigate whether both the high- and the low-affinity system represent transport of L-carnitine into the vesicles, L-carnitine uptake was studied in the presence of increasing cellobiose concentrations in the incubation medium with constant mannitol concentrations in the vesicles, thus creating an increasing osmotic gradient. Figure 3 shows a plot of the reciprocal cellobiose concentration against the uptake of L-carnitine. The

Figure 3 Dependency of equilibrium L-carnitine transport on medium osmolarity

Membrane vesicles were prepared in 400 mM mannitol and 20 mM Hepes adjusted with Tris to pH 7.4. The incubation medium contained (final concentrations) 200 mM mannitol, 20 mM Hepes/Tris, pH 7.4, 100 mM NaCl, cellobiose (100, 133, 166, 200, 240, 280, 320, 360 and 400 mM) and 5 mM (■) or 50 μM L-[^3H]carnitine (0.2 μCi per assay) (□). Transport was started by adding 15 μl of membrane vesicles to 30 μl of preheated incubation medium (37 °C). After 60 min of incubation, 30 μl of the incubation suspension were analysed for transport as described in Figure 1.

linear relationships for both 50 μM and 5 mM L-carnitine suggest that there are indeed two kinetically distinct transport systems for L-carnitine present in the vesicle membrane population used for the current studies.

Finally, uptake of L-carnitine by brush-border membrane vesicles was characterized in the presence of various carnitine analogues (Table 4). L-Acetylcarnitine, trimethyl-lysine and butyrobetaine were chosen because both acetylcarnitine and intermediates in carnitine biosynthesis have been shown to compete with Na^+ -dependent L-carnitine transport [11]. L-Arginine, glycinebetaine and choline were chosen to test the possibility that L-carnitine is transported by the respective transport system. L-Carnitine uptake was inhibited in a concentration-dependent fashion in the presence of L-acetylcarnitine, D-carnitine, trimethyl-lysine or butyrobetaine, whereas no significant inhibition was observed in the presence of L-arginine. Interestingly, L-carnitine uptake was stimulated in the presence of 50 or 250 μM glycinebetaine. Choline did not affect Na^+ -dependent L-carnitine uptake up to a concentration of 250 μM . However,

Table 4 Inhibition of L-carnitine uptake into rat kidney brush-border-membrane vesicles by carnitine analogues

Membrane vesicles were prepared as described in Table 1. Incubation medium contained (final concentrations) 50 μM L-[^3H]carnitine (0.2 μCi per assay), 200 mM mannitol, 20 mM Hepes/Tris, pH 7.4, and 100 mM NaCl and the inhibitors at the concentration given in the Table. Transport was determined as described in Table 1. Data are presented as mean $\pm$ S.D. of $n = 3$ independent vesicle preparations. Units are pmol/mg of protein. Values in parentheses represent the inhibition in percent of the control value.

Inhibitor	Incubation time ...	L-Carnitine uptake (pmol/mg of protein)	
		20 s	120 min
No inhibitor (control)		50.0 $\pm$ 3.1	120 $\pm$ 1
L-Acetylcarnitine (50 μM)		37.8 $\pm$ 1.2* (24)	110 $\pm$ 1
L-Acetylcarnitine (250 μM)		28.0 $\pm$ 0.6* (44)	110 $\pm$ 1
D-Carnitine (50 μM)		45.3 $\pm$ 0.9 (9)	107 $\pm$ 1
D-Carnitine (250 μM)		38.2 $\pm$ 1.0* (25)	114 $\pm$ 1
Trimethyl-lysine (50 μM)		48.6 $\pm$ 5.3 (3)	122 $\pm$ 3
Trimethyl-lysine (250 μM)		38.2 $\pm$ 2.0* (25)	119 $\pm$ 1
Butyrobetaine (50 μM)		33.2 $\pm$ 2.3* (34)	112 $\pm$ 2
Butyrobetaine (250 μM)		28.2 $\pm$ 1.2* (45)	107 $\pm$ 6
L-Arginine (50 μM)		48.8 $\pm$ 1.9 (2)	113 $\pm$ 1
L-Arginine (250 μM)		45.0 $\pm$ 2.2 (10)	112 $\pm$ 4
Glycinebetaine (50 μM)		272 $\pm$ 2*	112 $\pm$ 2
Glycinebetaine (250 μM)		315 $\pm$ 19*	113 $\pm$ 1

* $P < 0.05$ versus control incubations by Student's t -test.

in the presence of 100 mM choline, Na^+ -dependent L-carnitine uptake was inhibited by more than 50% (data not shown).

DISCUSSION

In agreement with earlier investigations assessing L-carnitine transport into brush-border-membrane vesicles [11], the current studies revealed saturable, Na^+ -dependent transport of L-carnitine which could be inhibited by structural analogues.

In contrast to an earlier study [11], the kinetic analysis of the L-carnitine uptake clearly revealed two K_m values, suggesting the presence of two Na^+ -dependent transport systems in renal brush-border-membrane vesicles. The high-affinity system has a K_m value in the range of 20 μM which is slightly lower than the value of 55 μM reported earlier [11] but similar to values of L-carnitine transport systems in heart [17,18] or fibroblasts [19,20]. In support of our results, a high- (K_m value 58 μM) and a low-affinity (K_m value 3.7 mM) system for Na^+ -dependent L-carnitine transport have been described in kidney cortex slices from mice [21]. Interestingly, mice having a defective high-affinity carnitine transport system show increased carnitine excretion and systemic carnitine deficiency [21], emphasizing the importance of the renal high-affinity L-carnitine transport system.

The presence of more than one transport system for a substrate in the kidney is not unique for L-carnitine. Heterogeneity of tubular transport processes has been demonstrated for different substrates in perfused tubules as well as in isolated vesicles [15,22–25]. These findings have now been extended to the molecular level, since two types of renal Na^+ -dependent transport systems for inorganic phosphate have been identified [26].

The reasons for the divergence of the carnitine transport kinetics between the current and earlier studies [11] remain unclear. Differences in the preparation of the membrane vesicles can be excluded since we obtained evidence for a high- and a low-affinity transport system also with the calcium precipitation method used by Rebouche and Mack [11]. A major difference between the current and earlier studies lies in the uptake values

used for the kinetic analysis [11]. While we used values obtained from the difference between uptake in the presence of sodium and choline, Rebouche and Mack used values corrected by subtraction of uptake in the presence of high concentrations of unlabelled substrate [11,27].

In addition to its dependence on sodium, the uptake of L-carnitine was stimulated by an inside negative K^+ diffusion potential, suggesting that the membrane potential represents an additional driving force and that L-carnitine is transported as a cationic complex. The higher uptake after 20 s in the presence of chloride as compared with other anions is not easily explained. While a chloride conductance has been demonstrated in renal membrane vesicles [28,29], uptake of D-glucose [14] and of L-lysine [15] have been shown to be higher in the presence of SCN^- or NO_3^- than in the presence of chloride in the same vesicle preparation. These latter findings suggest that chloride has a specific effect on L-carnitine uptake into renal brush-border membrane vesicles.

In agreement with an earlier study [11], L-acetylcarnitine, intermediates in carnitine biosynthesis and D-carnitine inhibited L-carnitine transport in a concentration-dependent manner. In contrast, no significant inhibition was observed by L-arginine, arguing strongly against the possibility that L-carnitine is transported by a transport system for cationic amino acids. Interestingly, a stimulation of L-carnitine transport was observed in the presence of glycinebetaine. Since a transport system for glycinebetaine in rat kidney has recently been characterized and cloned [30,31], the lack of an inhibition of L-carnitine transport suggests the existence of different transport systems for these structurally related substances. In agreement with earlier studies [11], choline did not affect Na^+ -dependent L-carnitine transport up to a concentration of 250 μM but showed a significant inhibition at a concentration of 100 mM. While these findings argue against the possibility that L-carnitine is transported by the high-affinity choline transport system, it cannot be excluded that L-carnitine is transported by the low-affinity transport system for choline [24].

In conclusion, the characterization of L-carnitine transport into rat renal brush-border-membrane vesicles revealed low- and high-affinity transport systems and identified a Na^+ -gradient and the membrane potential as major driving forces.

We thank Professor P. J. Meier and Dr. B. Hagenbuch for fruitful discussions. The work was supported by grant 32-37280.93 from the Swiss National Science Foundation to S.K.

REFERENCES

- Fritz, I. B. (1955) *Acta Physiol. Scand.* **34**, 367–385
- Bremer, J. (1983) *Physiol. Rev.* **63**, 1420–1480
- Brass, E. P. and Hoppel, C. L. (1980) *Biochem. J.* **190**, 495–504
- Bieber, L. L., Emaus, R., Valkner, K. and Farrell, S. (1982) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41**, 2858–2862
- Ramsay, R. R. and Arduini, A. (1993) *Arch. Biochem. Biophys.* **302**, 307–314
- Hoppel, C. L. and Davis, A. T. (1983) *Biochem. Soc. Trans.* **14**, 673–674
- Brass, E. P. and Hoppel, C. L. (1978) *J. Biol. Chem.* **253**, 2688–2693
- Steinmann, B., Bachmann, C., Colombo, J. P. and Gitzelmann, R. (1987) *Pediatr. Res.* **21**, 201–204
- Treem, W. R., Stanley, C. A., Finegold, D. N., Hale, D. E. and Coates, P. M. (1988) *N. Engl. J. Med.* **319**, 1331–1336
- Gahl, W. A., Bernardini, I., Dalakas, M., Rizzo, W. B., Harper, G. S., Hoeg, J. M., Hurko, O. and Bernat, J. (1988) *J. Clin. Invest.* **81**, 549–560
- Rebouche, C. J. and Mack, D. L. (1984) *Arch. Biochem. Biophys.* **235**, 393–402
- Brass, E. P. (1991) in *L-Carnitine and its Role in Medicine: From Function to Therapy* (Ferrari, R., DiMauro, S. and Sherwood, G., eds.), pp. 21–36, Academic Press, San Diego, London
- Berardi, S., Hagenbuch, B., Carafoli, E. and Krähenbühl, S. (1995) *Biochem. J.* **309**, 389–393

- 14 Biber, J., Stieger, B., Haase, W. and Murer, H. (1981) *Biochim. Biophys. Acta* **647**, 169–176
- 15 Stieger, B., Stange, G., Biber, J. and Murer, H. (1983) *J. Membr. Biol.* **73**, 25–37
- 16 Bensadoun, A. and Weinstein, D. (1976) *Anal. Biochem.* **70**, 241–250
- 17 Böhmer, T., Eiklid, K. and Jonsen, J. (1977) *Biochim. Biophys. Acta* **465**, 627–633
- 18 Vary, T. and Neely, J. R. (1982) *Am. J. Physiol.* **242**, H585–H595
- 19 Carnicero, H. H., England, S. and Seifter, S. (1982) *Arch. Biochem. Biophys.* **215**, 78–88
- 20 Stanley, C. A., Treem, W. R., Hale, D. E. and Coates, P. M. (1990) *Prog. Clin. Biol. Res.* **321**, 457–464
- 21 Horiuchi, M., Kobayashi, K., Yamaguchi, S., Shimizu, N., Koizumi, T., Nikaido, H., Hayakawa, J., Kuwajima, M. and Saheki, T. (1994) *Biochim. Biophys. Acta* **1226**, 25–30
- 22 Turner, R. J. and Moran, A. (1982) *Am. J. Physiol.* **242**, F406–F414
- 23 McKinney, T. D. (1982) *Am. J. Physiol.* **243**, F404–F407
- 24 Wright, S. H., Wunz, T. M. and Wunz, T. P. (1992) *J. Membr. Biol.* **126**, 51–65
- 25 Berry, C. A. (1982) *Annu. Rev. Physiol.* **44**, 181–201
- 26 Murer, H. and Biber, J. (1994) *Curr. Opin. Nephrol. Hypertens.* **3**, 504–510
- 27 Diamond, I. and Kennedy, E. P. (1969) *J. Biol. Chem.* **244**, 3258–3263
- 28 Burnham, C., Munzesheimer, C., Rabon, E. and Sachs, G. (1982) *Biochim. Biophys. Acta* **685**, 260–272
- 29 Sabolic, I. and Burckhardt, G. (1983) *Biochim. Biophys. Acta* **734**, 210–220
- 30 Robey, R. B., Kwon, H. M., Handler, J. S., Garcia-Perez, A. and Burg, M. B. (1991) *J. Biol. Chem.* **266**, 10400–10405
- 31 Yamauchi, A., Uchida, S., Kwon, H. M., Preston, A. S., Robey, R. B., Garcia-Perez, A., Burg, M. B. and Handler, J. S. (1992) *J. Biol. Chem.* **267**, 649–652