Pflügers Arch (1993) 423:406–410

Pflügers Archiv
European Journal of Physiology

Co-expression of an anion conductance pathway with Na^+-glucose cotransport in rat renal brush-border membrane vesicles

C. D. A. Brown, N. King, N. L. Simmons

Department of Physiological Sciences, The Medical School, Framlington Place, The University, Newcastle upon Tyne, NE2 4HH, UK

Received October 30, 1992/Received after revision December 21, 1992/Accepted January 4, 1993

Abstract. Brush-border membrane vesicles were prepared from superficial rat renal cortex by a Mg^{2+}-precipitation technique. The initial (20 s) [^{14}C]glucose uptake rate from solutions containing 100 mmol/l Na (salt) was found to be dependent upon the anion composition of the medium; in comparison to gluconate-containing medium (0.46 ± 0.05 nmol/mg protein), Cl^- accelerated the initial rate to 1.47 ± 0.21 nmol/mg protein ($n = 4$ preparations, ± SEM). This enhancement was reduced by 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB, 0.5 mmol/l), but was unaffected by 4,4′-diisothiocyanatostilbene 2,2′-disulphonate (DIDS, 0.5 mmol/l). When membrane vesicles were pre-equilibrated with 100 mmol/l K (salt) and 100 mmol/l mannitol and glucose uptake was measured from a solution containing 100 mmol/l Na gluconate and 100 mmol/l mannitol in the presence of 80 μmol/l valinomycin (to generate an outward K^+ diffusion electrical p. d.), it was found that intravesicular KCl depressed the initial glucose uptake compared to K gluconate. NPPB (0.5 mmol/l) increased the initial glucose uptake with intravesicular KCl towards values seen in K gluconate vesicles. In conditions where the only driving force for glucose uptake was established by an inward anion gradient ($Na_o = Na_i$) it was found that inward Cl^- gradients could drive uphill glucose transport and that this was sensitive to NPPB (0.5 mmol/l), but insensitive to DIDS. We conclude that a Cl^- conductance co-exists with Na-cotransport in rat renal brush-border membrane vesicles prepared from superficial renal cortex and this may function to regulate the activity of electrogenic transport systems at this membrane.

Key words: Brush border – Membrane vesicle – Cl^- conductance – Cystic fibrosis transmembrane regulator – Na-glucose cotransport

Correspondence to: C. D. A. Brown

Introduction

The existence of a conductance pathway for anions in the proximal tubules is the subject of controversy. A Cl^- conductive pathway sensitive to an imposed K-diffusion potential was identified in brush-border membrane vesicles (BBMVs) isolated from rabbit renal cortex as early as 1981 [29]. The existence of a diphenylamine-carboxylate-inhibitable anion conductance has subsequently been confirmed in rabbit BBMVs using a fluorescent indicator technique [8]. Recently Lipkowitz et al. [18] have shown that parathyroid hormone (PTH) may increase Cl^- permeability in rat renal BBMV. Similarly Suzuki et al. [24] have identified an apical Cl^- channel activated by PTH via the protein kinase A and C pathways in primary cultures of isolated rabbit proximal tubules. The Cl^- channel was outwardly rectifying possessing a conductance of 33 pS at positive and 22.5 pS at negative potentials. It was sensitive to inhibition by the stilbene 4,4′-diisothiocyanatostilbene 2,2′-disulphonate (DIDS, 0.1 mmol/l).

In contrast to these positive data, measurements made with intact preparations argue for a minimal Cl^- conductance pathway, e. g. Cassola et al. [7]. More recently Ishibashi et al. [16] have shown that perfusion of low-Cl^- medium across the luminal surface has a minor effect on intracellular Cl^- activity when compared to basal-lateral perfusion. The effect from the luminal surface was blocked by the stillbene 4-acetamido-4-isothiocyanostilbene 2,2-disulphonic acid (SITS) [16].

Recently renal proximal tubule expression of both P-glycoprotein [multi-drug resistance (MDR)] [12, 13, 27] and the cystic fibrosis transmembrane conductance regulator (CFTR) [10, 11, 20, 26] have been reported. Since both P-glycoprotein [28] and CFTR function as Cl^- channels [1, 30] the debate concerning the expression and functional significance of Cl^- channels in the apical membrane of renal proximal tubule cells has now been reopened.

In order to examine the Cl^- conductance of BBMVs and to overcome the possible contamination of this ves-

icle population with endosomal Cl^- channels [21] we have chosen to utilise the electrogenicity of the Na^+-glucose cotransproter as an intrinsic intravesicular marker to indirectly report vesicle membrane potential [17, 18]. We have used a standard Mg^{2+}-precipitation technique to prepare highly enriched BBMV in which glucose transport is easily measured using standard techniques [2, 3]. By manipulation of anion composition and the direction of the imposed anion gradients it has proved possible to both demonstrate the presence, and to investigate the nature, of a Cl^- conductance pathway in the apical membrane of the proximal tubule. A preliminary account of the present data has been made [6].

Materials and methods

Isolation of BBMVs. BBMVs were prepared from thin (1−2 mm thick) slices of rat kidney cortex from male Wistar rats (250−300 g body weight) using a two-step Mg^{2+}-precipitation technique [3]. The resultant BBMVs were generally resuspended in (mmol/l): 300 mannitol, 10 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid/tris(hydroxymethyl)aminomethane (HEPES/TRIS) pH 7.4, or with an appropriate medium as detailed in the figure legends. BBMVs isolated using this protocol were enriched approximately 16-fold in alkaline phosphatase and 15-fold in leucine aminopeptidase activity compared with the initial homogenate.

Transport studies. [^{14}C]Glucose uptake was measured at room temperature (20° C) using a rapid filtration technique [2]. Uptake was initiated by the addition of 700 µl uptake buffer, as described in the figure legends, to 120 µl of membrane vesicles. The reaction mix was then taken up into the tip of a 1-ml electronic pipette (Biohit Proline, Helsinki, Finland). At appropriate time points 60-µl aliquots of reaction mix were dispensed directly onto a 0.65-µm pore size nitrocellulose filter under vacuum. Filters were then washed with a total of 3 ml ice-cold stop solution [(mmol/l): 100 K gluconate, 100 mannitol, 10 HEPES/TRIS, 0.2 phloridzin]. The filters were then removed and processed for liquid scintillation counting.

Protein determination. Protein was estimated by the method of Bradford [5] using bovine serum albumin to prepare the standard curve.

Statistics. All data presented are the mean ± SEM from n animals (at least 3) performed in triplicate. Data were analysed for statistical significance using Student's two-tailed t test (for paired or unpaired data, as appropriate to the experiment).

Materials. [^{14}C]D-Glucose was obtained from Amersham International PLC (Amersham, UK). DIDS, valinomycin and other biochemicals were purchased from Sigma (Poole, UK). The Cl^- channel blocker, 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) was a gift from Smith, Kline, Beecham Research (Welwyn Garden City, UK). All other chemicals were obtained from BDH (Poole, UK) or Sigma and were of analytical grade.

Results

Anion dependence of Na^+-glucose cotransport

Figure 1 shows the time course of glucose uptake into rat renal BBMVs under a number of experimental conditions. Glucose uptake is a predominately Na^+-dependent process. This can be seen by comparing the rapid accumulation of glucose into the intravesicular space driven by an inwardly directed 100 mmol/l Na gluconate gradient (1.9- ± 0.3 fold above equilibrium, $n = 4$) to the slow equilibration of glucose into the vesicle in the presence of identical gradients of KCl or K gluconate.

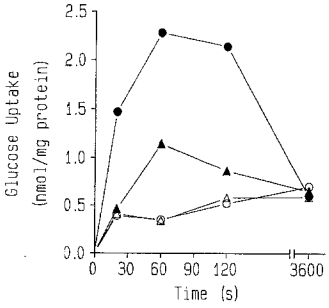

Fig. 1. Time course of glucose uptake in rat brush-border membrane vesicles (BBMV) is dependent upon the anion composition of external meidum. BBMVs were pre-equilibrated with (mmol/l): 300 mannitol, 10 TRIS-HEPES, pH 7.4. Glucose uptake was measured from 0.1 glucose, 100 NaCl (●), Na gluconate (▲), KCl (○) or K gluconate (△) with 100 mannitol, 10 TRIS-Hepes, pH 7.4. Data are the mean ± SEM of four rat preparations where measurements were performed in triplicate

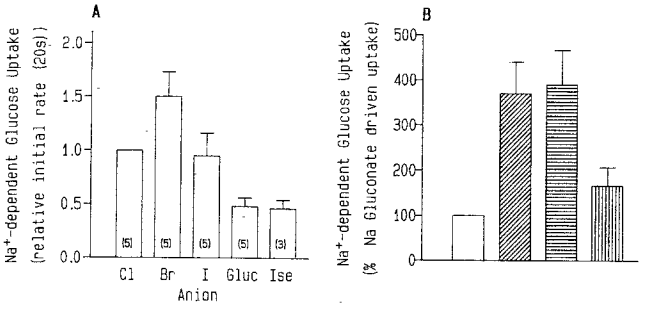

Fig. 2 A. Dependence of the 20-s glucose uptake rate upon anion composition. Details as for Fig. 1. Figures in parentheses are the number of separate preparations. **B** The effect of 0.5 mmol/l DIDS and NPPB in the external medium upon the initial (20 s) glucose uptake. Data are the mean ± SEM of four rat preparations where measurements were performed in triplicate; NaGlu (□), NaCl (▨), NaCl + DIDS (▤), NaCl + NPPB (▥)

Figure 1 also shows that Na^+-dependent glucose uptake exhibits a marked anion dependence; the initial rate of glucose uptake is significantly greater in the presence of an inwardly directed gradient of NaCl than when a Na gluconate gradient is applied (1.47 ± 0.2 vs 0.46 ± 0.05 nmol/mg protein; first 20 s, $P < 0.01$, $n = 4$). In contrast Na^+-independent glucose uptake shows no marked anion dependence.

Characterisation of the anion conductance

In order to characterise the mechanism underlying the anion dependence of Na^+-dependent glucose cotransport in greater detail we constructed an anion selectivity series for the ability of anions to stimulate Na^+-dependent glucose uptake. The results shown in Fig. 2A give an apparent permeability sequence relative to Cl^- of: Br (1.5) > Cl (1) ≃ I (0.95) ≫ gluconate (0.48) ≃ isethion-

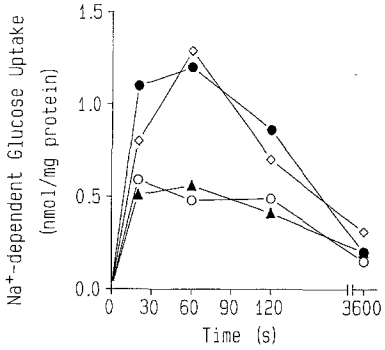

Fig. 3. Time course for the effect of outward Cl gradients ($Cl_i > Cl_o$) upon glucose uptake. BBMVs were pre-equilibrated with (mmol/l): 100 K gluconate, (●) or KCl (○) with 100 mannitol and 10 TRIS-HEPES, pH 7.4; glucose uptake was from 0.1 glucose, 100 Na gluconate, 100 mannitol and 10 TRIS-HEPES, pH 7.4. The effect of external DIDS (▲) and NPPB (△) at 0.5 was tested on vesicles with internal KCl. Data are mean values of three replicates from one representative experiment (of three such experiments)

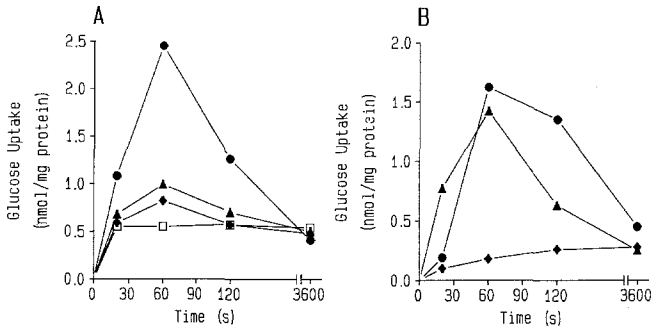

Fig. 4 A, B. Time course of sugar accumulation driven solely by an anion gradient. BBMVs were pre-equilibrated with (mmol/l): 100 K gluconate, 30 Na gluconate, 0.1 glucose and 10 TRIS-HEPES, pH 7.4. **A** Glucose uptake was then measured from an external solution containing: 100 K gluconate (□) (no gradients), 100 KCl (●), 100 KI (◆), 100 KBr (▲), 30 Na gluconate, 0.1 glucose and 10 TRIS-HEPES, pH 7.4. **B** Glucose uptake measured from an external solution as **A** but with 100 KCl (●) plus 0.5 DIDS (▲) or 0.5 NPPB (◆). Data are the mean of three separate rat preparations where measurements were performed in triplicate

ate (0.46). In a second series of experiments we investigated the effect of two Cl^- channel blockers, NPPB and DIDS, upon the Cl^--gradient-driven component of Na^+-dependent glucose uptake. Figure 2B demonstrates that Na^+-dependent glucose uptake was significantly stimulated ($P < 0.01$) by an inwardly directed NaCl gradient compared to a Na gluconate gradient. In addition, Fig. 2B demonstrates that the Cl^--gradient-stimulated glucose uptake was not sensitive to inhibition by DIDS (0.5 mmol/l), but was significantly reduced ($P < 0.02$) by the channel blocker NPPB to a value not significantly different from that measured in the absence of Cl^-. The inhibition by NPPB of glucose uptake (at 30 s) in NaCl medium compared to that level seen in Na gluconate medium is of low affinity; a reduction only being observed at NPPB concentrations greater than 50 μmol/l. At 10 μmol/l NPPB, glucose uptake (in NaCl medium compared to Na gluconate medium) was 114% of control values, at 50 μM NPPB glucose uptake was 77% of control values, at 100 μM, 64% and at 500 μM, 45% for three replicate points in one preparation.

Anion gradient-driven glucose uptake

To test the hypothesis that Cl^- stimulation of Na^+-dependent glucose uptake was a result of the indirect coupling of electrogenic Na^+-glucose cotransport via a specific Cl^- conductance we designed two experimental protocols.

In the first protocol we measured Na^+-dependent glucose uptake into vesicles loaded with either 100 mmol/l KCl or K gluconate in the presence of external valinomycin (Fig. 3). In the presence of the impermeant gluconate anion, glucose accumulation was driven both by an inwardly directed 100 mmol/l Na^+ gradient and by an inside-negative p. d. (generated by a K^+ diffusion potential). In the presence of intravesicular Cl^-, Na^+-dependent glucose accumulation was significantly less as Cl^- efflux through the conductance pathway would reduce the magnitude of the inside-negative p. d. generated by K^+ efflux from the vesicle (Fig. 3). In KCl-loaded vesicles addition of NPPB (0.5 mmol/l), but not DIDS (0.5 mmol/l), blocked Cl^- efflux through the conductance pathway and restored Na^+-dependent glucose accumulation to a level similar to that found with gluconate.

In a second series of experiments we tested the ability of inwardly directed anion gradients to drive glucose accumulation in the absence of transmembrane gradients of either Na^+ or glucose. Under these conditions the anion gradient is the sole driving force for glucose accumulation into the vesicle. Figure 4 A shows that an inwardly directed Cl^- gradient was able to drive a significant accumulation of glucose compared to when no anion gradient was present ($P < 0.02$). Br^- and I^- were also able to drive glucose accumulation, but to a much less extent than Cl^- ($P < 0.03$ and $P < 0.025$ for Br^- and I^- versus Cl^- respectively), suggesting that under these conditions the conductance pathway has a selectivity ratio of $Cl > Br \approx I$. In Fig. 4B we show that, under equilibrium conditions for Na^+ and glucose, Cl^--gradient-driven glucose accumulation was abolished by the addition of NPPB (0.5 mmol/l) to the vesicles ($P < 0.02$) but was unaffected by the addition of DIDS (0.5 mmol/l, $P < 0.8$).

Discussion

In this study we have used the electrogenic Na^+-glucose cotransporter (SGLT1) [9, 19] as an intrinsic sensor of membrane potential to demonstrate the presence of a specific anion conductance pathway in rat renal superficial cortical BBMVs. The approach followed is indirect and consists of manipulating membrane potential with anion gradients to see if this alters electrogenic Na^+-coupled glucose transport. Only if the anion conductance pathway and the electrogenic glucose transport mechanism are present in the same membrane vesicle

would glucose uptake be affected. The major advantage of this approach is that it pinpoints the location of the conductance pathway unequivocally to the apical membrane by excluding basolateral and endosomal Cl^- conductance pathways [17, 18]. Though endosomal membranes may be induced to fuse together under special conditions [21], BBMVs could not be induced to fuse with endosomes under identical conditions [21]. Furthermore there is no evidence that the majority vesicle population prepared under standard conditions may be contaminated by fusion with other endomembranes present as a minor contaminant [3]. It should be noted that the superficial cortex will contain the Na^+-glucose cotransporter (SGLT1) expressed in the renal proximal tubule (S1 segment) [9, 19]. This carrier has an apparent stoichiometry of 1 Na:1 glucose and is associated with the generation of transmembrane current in voltage-clamped oocytes expressing SGLT1 [4]. Furthermore the sugar-dependent current mediated by the cotransporter is voltage sensitive [4]. Though the nature of these effects is complex and precludes a simple standardisation of glucose transfer and vesicle membrane potential [4], the gross dependence of Na^+-glucose cotransport on membrane potential does allow us to make qualitative conclusions concerning major effects observed with the anion gradients used in the present study.

The effect of anions in the modulation of the initial rate and extent of overshoot of glucose transport in BBMVs was noted in an initial description of the method [15]. This result has been described in renal BBMVs where more permeant anions stimulate the glucose overshoot [3]. Such results have been usually interpreted in terms of an anion permeability rather than an anion conductance pathway per se. That a Cl^- gradient can enhance or reduce Na^+-dependent glucose transport dependent upon the direction of the imposed anion gradients (using relatively impermeant anions such as gluconate and isethionate) is good evidence that there is a Cl^- conductance pathway per se. In addition we have devised conditions where, in Na^+-equilibrated conditions, the only driving force for glucose transport is the anion gradient. With a Cl^- gradient a substantial initial acceleration of glucose uptake and overshoot is observed. In these conditions the only plausible explanation is the presence of an anion conductance which, together with the imposed gradient, may generate an intravesicular negative membrane potential.

Using RNA gel-blot hybridization with a cDNA probe, the tissue-specific expression of the cystic fibrosis conductance regulator (CFTR) has been shown in the kidney [20]. Cloning of the mouse homologue of the human CTFR gene has involved cDNA isolated from total mouse kidney RNA [26], suggesting that renal expression of CFTR in mammals is common. Crawford et al. [10] have used antibodies raised against peptides comprising amino acids 724–746 and 415–427 from the region of the R domain and preceding the first nucleotide binding domain respectively, to localise CFTR expression by immunohistochemistry in human tissues. They found that in addition to expression in those epithelial cells lining sweat ducts, pancreatic ducts and intestinal crypts there was abundant expression in kidney tubules. Within the renal cortex there was CFTR expression in both proximal and distal tubules; the staining was restricted to the apical poles of the epithelial cells facing the lumen, suggesting a proportion is present within the apical membrane [10]. In rabbit BBMVs it has been reported that CFTR levels are enriched compared to homogenates [11].

Immunocytochemical evidence suggests that P-glycoprotein is expressed in proximal tubules [27]. Analysis of mRNA suggests that renal medullary tissue possess P-glycoprotein mRNA levels in excess of renal cortex [13]. Recently it has been suggested that P-glycoprotein may function as a volume-activated Cl^- channel [28].

There is now overwhelming evidence that CFTR is a small-conductance Cl^- channel [1, 30]. The channel is sensitive to inhibition by external NPPB with low affinity but is insensitive to DIDS [1, 23, 30]. The anion selectivity sequence is $Br > Cl > I$ [30], but this may depend upon the species [14] and the exact experimental conditions [25]. The channel is regulated mainly via the actions of protein-kinase-A-dependent phosphorylation [1, 30]. In contrast to CFTR, the whole-cell currents associated with P-glycoprotein are outwardly rectifying [28]; in addition these whole-cell currents show sensitivity to external DIDS reminiscent of the properties of the ORDIC Cl^- channel [22]. The Cl^- channel activity associated with P-glycoprotein is stimulated in whole cells by hypotonic cell swelling [28].

Under Na^+ gradient conditions, and an imposed inwardly directed anion gradient, the conductance exhibited a selectivity sequence for anions of $Br > Cl \simeq I \gg$ -gluconate $\simeq$ isethionate. In contrast, imposition of an inwardly directed anion gradient with Na^+ equilibrated across the vesicle membrane gave an anion selectivity sequence of $Cl > Br \simeq I$. The reasons for this apparent difference in anion selectivity are not yet clear but may relate to the anion composition of both intravesicular and extravesicular compartments. It has been demonstrated that cytosolic I may affect the properties of the CFTR conductance [25] and a similar effect of other intravesicular ions cannot be excluded. The effects of NPPB and the stilbene DIDS were tested on the Cl^- conductance in rat BBMVs; for each experimental paradigm, the effect of the anion gradient was blocked by extravesicular NPPB but was insensitive to extravesicular DIDS (both at 0.5 mmol/l). The blocking actin of NPPB was of low affinity. This is identical to pharmacological blockade of CFTR expressed in oocytes [23]. The properties of the Cl^- conductance pathway associated with rat renal superficial BBMVs are thus more reminiscent of CFTR rather than P-glycoprotein.

How can the present observation of a Cl^- conductance pathway in rat renal BBMVs be reconciled with the inability of most workers to demonstrate a Cl^- conductance pathway in the apical membrane of the intact proximal tubule? It is important to note that the Cl^- conductance pathway of renal BBMVs can be regulated. Inclusion of appropriate physiological regulators, e. g. PTH and angiotensin, in the initial homogenate has been reported to increase the apparent Cl^- permeability of

BBMVs subsequently prepared [18]. It is also pertinent to note that measurements of intracellular p. d. and Cl^- activity in proximal tubule cells have been selected according to rigorous and predetermined values of membrane potential [7]. If a substantial regulated Cl^- conductance pathway were evident, cell p. d. is likely to be held at more depolarised values towards E_{Cl}.

In conclusion we have provided new evidence arguing for the existence of a Cl^- conductance pathway in rat BBMV. It is possible that the operation of such a conductance pathway could serve to regulate electrogenic transport at the apical membrane of the proximal tubule and to regulate fluid absorption. We hope that our findings, combined with other recent work in renal BBMVs, will provide a sufficient stimulus for reopening the question of the physiological significance of such a Cl^- conductance pathway in the proximal tubule.

Acknowledgement. N. King was supported by Lucock studentship of the Faculty of Medicine, University of Newcastle upon Tyne.

References

1. Bear CE, Li C, Kartner N, Bridges RJ, Jensen TJ, Ramjeesingh M, Riordan JR (1992) Purification and functional reconstitution of the cystic fibrosis transmembrane regulator (CFTR). Cell 68:809–818
2. Berner W, Kinne R, Murer H (1976) Phosphate transport into brush-border membrane vesicles isolated from the rate small intestine. Biochem J 160:467–474
3. Biber J, Steiger B, Haase W, Murer H (1981) A high yield preparation for rat kidney brush-border membranes: Different behaviour of lysosomal markers. Biochim Biophys Acta 647:169–176
4. Birnir B, Loo DDF, Wright EM (1991) Voltage-clamp studies of the Na/glucose cotransporter cloned from rabbit small intestine. Pflügers Arch 418:79–85
5. Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein using the principle of dye binding. Anal Biochem 72:248–254
6. Brown CDA, King N, Simmons NL (1992) Coexistence of a Cl^- conductance and Na-glucose cotransport in rat renal brush-border membrane vesicles in vitro. Proceedings of the Physiological Society, J. Physiol (Lond) (in the press)
7. Cassola AC, Mollenhauer M, Fromter E (1983) The intracellular chloride activity of rat proximal tubular brush-border membrane vesicles. Pflügers Arch 400:309–317
8. Chen PY, Illsely NP, Verkman AS (1988) Renal brush-border chloride transport mechanisms characterised using a fluorescent indicator. J Physiol 254:F114–F120
9. Coady MJ, Pajor AM, Wright EM (1990) Sequence homologies among intestinal and renal Na-glucose cotransporters. Am J Physiol 259:C605–C610
10. Crawford I, Maloney PC, Zeitlin PL, Guggino WB, Hyde SC, Turley H, Gatter KC, Harris A, Higgins CF (1991) Immunocytochemical localisation of the cystic fibrosis gene product CFTR. Proc Natl Acad Sci USA 88:9262–9266
11. Crawford I, Beardmore C, Beck JC, Guggino WG, Maloney PC (1992) Identification of CFTR-immunoreactive material in renal endosomes. FASEB J 6:A1192, 1476
12. Croop JM, Raymond M, Haber D, Devault A, Arceci RJ, Gros P, Housman DE (1989) The three mouse multi-drug resistance (mdr) genes are expressed in a tissue specific manner in normal mouse tissues. Mol Cell Biol 9:1346–1350
13. Gottesman MM, Wllingham MC, Thiebaut F, Pastan I (1991) In: Roninson IB (ed) Molecular and cellular biology of multidrug resistance in tumor cells. Plenum Press, New York, pp 279–288
14. Gray MA, Greenwell JR, Argent BE (1988) Secretin-regulated chloride channel on the apical membrane of pancreatic duct cells. J Membr Biol 105:131–142
15. Hopfer U, Nelson K, Perotto J, Isselbacher KJ (1972) Glucose transport in isolated brush-border membrane from rat small intestine. J Biol Chem 248:25–32
16. Ishibashi K, Sasaki S, Yoshiyama N (1988) Intracellular chloride activity of rabbit straight tubule perfused in vitro. Am J Physiol 255:F49–F56
17. Lipkowitz MS, Abramson RG (1989) Modulation of the ionic permeability of renal cortical brush-border membranes by cAMP. Am J Physiol 257:F769–F776
18. Lipkowitz MS, London RD, Beck JC, Abramson RG (1992) Hormonal regulation of rat renal proximal tubule brush-border membrane ionic permeability. Am J Physiol 263:F144–F151
19. Morrison AM, Panayotova-Heiermann M, Feigl G, Schoelermann B, Kinne RH (1991) Sequence comparison of the sodium-D-glucose cotransport system in rabbit renal and intestinal epithelia. Biochim Biophys Acta 1089:121–123
20. Riordan JR, Rommens JM, Kerem B-S, Alon N, Rozmahel R, Gbzelczak Z, Zielenski J, Lok S, Plavsic N, Choul JL, Drumm M, Lannuzzi MC, Collins FS, Tsiu LC (1989) Identification of the cystic fibrosis gene: Cloning and characterisation of complementary DNA. Science 245:1066–1073
21. Schmid A, Burckhardt G, Gogelein H (1989) Single chloride channels in endosomal vesicle preparations from rat kidney cortex. J Membr Biol 111:265–275
22. Solc CK, Wine JJ (1991) Swelling-induced and depolarization-induced Cl^- channels in normal and cystic fibrosis epithelial cells. Am J Physiol 261:C658–C674
23. Sullivan S, Schick R, Gregory R, Paul S, Agellon L, Field M (1992) Pharmacology of CFTR channels expressed in oocytes. Pediatr Pulmonol [Suppl] 8:127P
24. Suzuki M, Morita T, Hanaoka K, Kawaguchi Y, Sakai O (1991) A Cl^- channel activated by parathyroid hormone in rabbit renal proximal tubule cells. J Clin Invest 88:735–742
25. Tabcharani JA, Chang X-B, Riordan JR, Hanrahan JW (1992) The cystic fibrosis transmembrane conductance regulator chloride channel: iodide block and permeation. Biophys J 62:1–4
26. Tata F, Stanier P, Wicking C, Halford S, Kruyer H, Lench NJ, Scambler PJ, Hansen C, Braman JC, Williamson R, Wainwright BJ (1991) Cloning of the mouse homolog of the human cystic fibrosis transmembrane conductance regulator gene. Genomics 10:301–307
27. Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC (1987) Cellular localisation of the multi-drug resistance gene product P-glycoprotein in normal human tissues. Proc Natl Acad Sci USA 84:7735–7738
28. Valverde MA, Diaz M, Sepulveda FV, Gill DR, Hyde SC, Higgins CF (1992) Volume-regulated chloride channels associated with human multidrug resistance P-glycoprotein. Nature 355:830–833
29. Warnock DG, Lee VJ (1981) Chloride uptake by brush-border membrane vesicles isolated from rabbit renal cortex. Coupling to proton gradients and K diffusion potentials. J Clin Invest 67:103–115
30. Widdicombe JH, Wine JJ (1991) The basic defect in cystic fibrosis. Trends Biochem Sci 16:474–477