ELSEVIER

European Journal of Pharmacology 411 (2001) 255–260

ejp

www.elsevier.nl/locate/ejphar

Direct block of the cystic fibrosis transmembrane conductance regulator Cl^- channel by butyrate and phenylbutyrate

Paul Linsdell *

Department of Physiology and Biophysics, Dalhousie University, Sir Charles Tupper Medical Building, Halifax, Nova Scotia, B3H 4H7, Canada

Received 12 October 2000; received in revised form 29 November 2000; accepted 1 December 2000

Abstract

Chloride permeation through the cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel is inhibited by a broad range of intracellular organic anions. Here it is shown, using patch clamp recording from CFTR-transfected mammalian cell lines, that the fatty acids butyrate and 4-phenylbutyrate cause a voltage-dependent block of CFTR Cl^- currents when applied to the cytoplasmic face of membrane patches, with apparent K_ds (at 0 mV) of 29.6 mM for butyrate and 6.6 mM for 4-phenylbutyrate. At the single channel level, both these fatty acids caused an apparent reduction in CFTR current amplitude, suggesting a kinetically fast blocking mechanism. The concentration-dependence of block suggests that CFTR-mediated Cl^- currents in vivo may be affected by both 4-phenylbutyrate used in the treatment of various diseases, including cystic fibrosis, and by butyrate produced endogenously within the colonic lumen.

Keywords: Cl^- channel; Voltage-dependent block; Fatty acid; CFTR (cystic fibrosis transmembrane conductance regulator); Cystic fibrosis

1. Introduction

Cystic fibrosis is caused by mutations in a single gene, that encoding the cystic fibrosis transmembrane conductance regulator (CFTR). The primary function of CFTR is as a phosphorylation- and nucleotide-dependent Cl^- channel in the apical membrane of a number of different epithelial cells (Sheppard and Welsh, 1999). Over 900 different genetic mutations which cause CFTR channels to be missing or defective have been shown to be associated with cystic fibrosis (see http://www.genet.sickkids.on.ca/cftr/). However, a single allele, ΔF508, accounts for almost 70% of cystic fibrosis chromosomes, with over 90% of cystic fibrosis patients having at least one ΔF508 allele (Kerem et al., 1989; Kopito, 1999). The ΔF508 mutation causes aberrant biosynthetic processing of CFTR in the endoplasmic reticulum, with the result that the CFTR protein fails to reach the plasma membrane (Cheng et al., 1990; Kopito, 1999). ΔF508-CFTR function can be partially restored by culturing cells at reduced temperatures (Denning et al., 1992), by chemical chaperones such as glycerol (Brown et al., 1996; Sato et al., 1996), by disrupting arginine-framed tripeptide motifs which act as signals for endoplasmic reticulum retention (Chang et al., 1999) and by upregulating CFTR expression using sodium butyrate (Cheng et al., 1995) or its orally active analog, sodium 4-phenylbutyrate (Rubenstein et al., 1997). A pilot clinical trial showed that 4-phenylbutyrate altered nasal potential difference in cystic fibrosis patients homozygous for ΔF508-CFTR, in a manner consistent with increased apical membrane CFTR function (Rubenstein and Zeitlin, 1998).

Less is known concerning the effects of butyrate and 4-phenylbutyrate on CFTR function. It has recently been shown that both butyrate and 4-phenylbutyrate increase the expression of wild type CFTR but, paradoxically, reduce Cl^- secretion in renal and airway epithelial cells (Loffing et al., 1999; Moyer et al., 1999). Here it is shown that both butyrate and 4-phenylbutyrate cause a voltage-dependent block of wild type CFTR Cl^- channels when applied to the cytoplasmic face of the membrane. These results suggest that these short chain fatty acids directly inhibit Cl^- permeation through CFTR by blocking the channel pore.

* Tel.: +1-902-494-2265; fax: +1-902-494-1685.
E-mail address: paul.linsdell@dal.ca (P. Linsdell).

0014-2999/01/$ - see front matter
PII: S0014-2999(00)00928-6

2. Materials and methods

Experiments were performed on baby hamster kidney (BHK) and Chinese hamster ovary (CHO) cell lines stably expressing human CFTR (Hanrahan et al., 1998). BHK cells were used for macroscopic current recordings and CHO cells for single channel recordings (Linsdell and Hanrahan, 1998). Both macroscopic and single channel current recordings were made using the excised, inside–out configuration of the patch clamp technique, as described previously (Linsdell and Hanrahan, 1996a, 1998; Linsdell et al., 1997; Hanrahan et al., 1998). Briefly, channels were activated following patch excision by exposure of the cytoplasmic face of the patch to 30–180 nM protein kinase A catalytic subunit (prepared in the laboratory of Dr. M.P. Walsh, University of Calgary, Alberta, Canada, as described previously; Tabcharani et al., 1991; Hanrahan et al., 1998) plus 1 mM MgATP. Solutions contained (in mM): 150 NaCl, 2 $MgCl_2$, 10 TES, and the pH is adjusted to 7.4 with NaOH. Sodium butyrate and sodium 4-phenylbutyrate were added from freshly prepared stock solutions, made up in this 150 mM NaCl buffer and readjusted to pH 7.4, as required. All chemicals were from Sigma (Oakville, Ontario, Canada) except sodium 4-phenylbutyrate (Aldrich, Milwaukee, WI, USA).

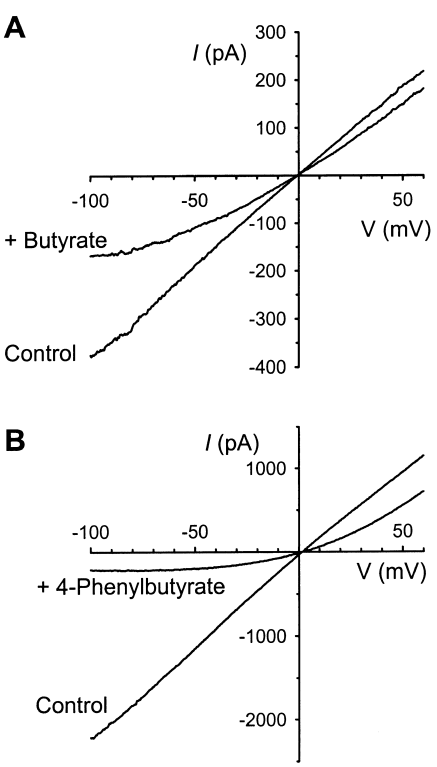

Fig. 1. Block of macroscopic CFTR Cl^- currents by internal butyrate (A) and 4-phenylbutyrate (B). Example CFTR $I–V$ relationships recorded before and immediately following addition of 10 mM butyrate (A) or 10 mM 4-phenylbutyrate (B). Background (leak) currents have been subtracted as described in Section 2.

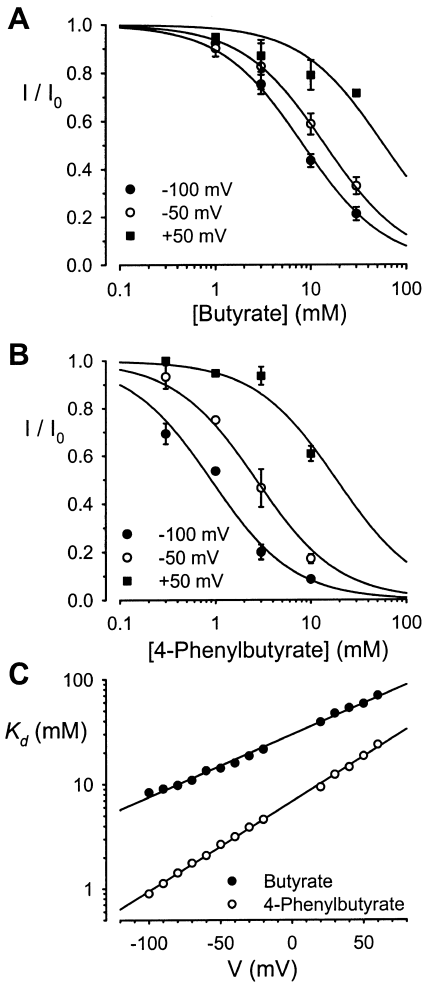

Fig. 2. Concentration- and voltage-dependence of block. (A, B) Mean fraction of control current remaining (I/I_0) following addition of different concentrations of butyrate or 4-phenylbutyrate, at a membrane potential of −100 mV (●), −50 mV (○), or +50 mV (■). The data have been fitted by Eq. (1), as described in the text. (C) Voltage-dependence of K_d, calculated at each voltage from Eq. (1) for both butyrate (●) and 4-phenylbutyrate (○). Mean of data from three to five patches.

Macroscopic current–voltage ($I–V$) relationships were constructed using depolarizing voltage ramp protocols, with a rate of change of voltage of 100 mV s^{-1} (see Linsdell and Hanrahan, 1996a, 1998). All $I–V$ relationships shown have had the background (leak) current recorded before addition of protein kinase A catalytic subunit subtracted digitally as described previously (Linsdell and Hanrahan, 1996a, 1998). Under these conditions, the leak-subtracted current is carried exclusively by CFTR channels (Linsdell and Hanrahan, 1996a, 1998, 1999; Linsdell et al., 1998, 2000; Linsdell, 2000). Current traces were filtered at 100 Hz (for macroscopic currents, I) or 50 Hz (for single channel currents, i) using an eight-pole Bessel filter, and digitized at 250 Hz. Macroscopic currents were analysed using pCLAMP6 computer software (Axon Instruments, Foster City, CA, USA) while single channel

currents were analysed using custom-written, pCLAMP-compatible DRSCAN software (Hanrahan et al., 1998).

The effects of butyrate and 4-phenylbutyrate on macroscopic CFTR currents were examined by applying these substances to the intracellular solution, once the protein kinase A catalytic subunit-activated current had reached a stable amplitude. A single concentration of butyrate or 4-phenylbutyrate was tested on each patch. The effects of these substances were analysed according to the Woodhull (1973) model of voltage dependent block:

$$I/I_0 = K_d(V)/(K_d(V) + [B]), \quad (1)$$

where I is the amplitude of the current remaining in the presence of blocker B, I_0 is the control, unblocked current amplitude, and $K_d(V)$ is the voltage-dependent dissociation constant at voltage V; the voltage-dependence of which is given by:

$$K_d(V) = K_d(0)\exp(-z'VF/RT), \quad (2)$$

where z' is the apparent valence of the blocking ion, that is, its real valence (assumed to be -1 for both butyrate and 4-phenylbutyrate) multiplied by the fraction of the transmembrane electric field it experiences, and F, R and T have their normal thermodynamic meanings.

Experiments were carried out at room temperature, 20–24°C. Mean values are presented as mean ± S.E.M. For graphical presentation of mean values, error bars represent ± S.E.M., where this is larger than the size of the symbol.

3. Results

The effects of butyrate and 4-phenylbutyrate on macroscopic CFTR Cl^- currents were examined using inside–out membrane patches excised from BHK cells stably expressing CFTR (see Section 2). As shown in Fig. 1, addition of butyrate (10 mM) or 4-phenylbutyrate (10 mM) to the intracellular solution caused a marked reduction in CFTR Cl^- current. In both cases, the block was clearly voltage-dependent, with the reduction in current amplitude being greatest at negative voltages, as would be expected for block of the channel pore within the transmembrane electric field by a negatively charged molecule acting from the inside. Block occurred immediately on application of either butyrate or 4-phenylbutyrate, and in both cases, was readily reversible on washing.

The concentration-dependence of block by both butyrate and 4-phenylbutyrate is shown in Fig. 2A,B. At each concentration of butyrate (A) or 4-phenylbutyrate (B) studied, the mean fraction of control current remaining following the development of block (I/I_0) was calculated from three to five patches at −100, −50 and +50 mV. At each potential, the data have been fitted by Eq. (1) (see Section 2). The fits shown in Fig. 2A suggest that a single butyrate molecule blocks Cl^- permeation with a K_d of 8.3 mM at −100 mV, 14.2 mM at −50 mV, and 58.3 mM at +50 mV, while the fits to Fig. 2B suggest block by a single 4-phenylbutyrate molecule with a K_d of 0.9 mM at −100 mV, 2.7 mM at −50 mV and 18.5 mM at +50 mV. Similar analyses at other potentials allowed estimation of

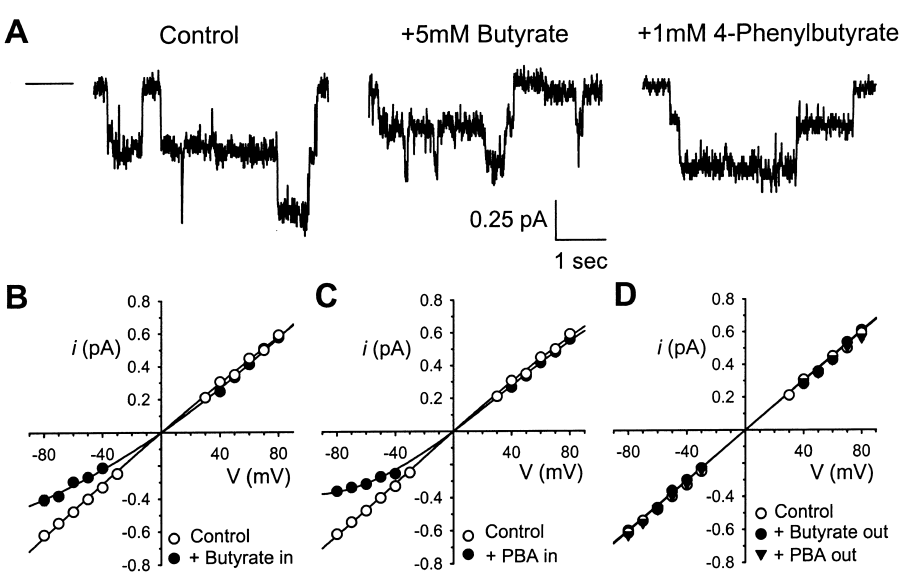

Fig. 3. Block of unitary CFTR Cl^- currents by internal butyrate and 4-phenylbutyrate. (A) Example currents recorded at −50 mV under control conditions, and after addition of 5 mM butyrate or 1 mM 4-phenylbutyrate to the intracellular solution. Both channels closed is indicated by the line to the left in each case. (B, C, D) Mean single channel i–V relationships, under control conditions (○) or in the presence of 5 mM butyrate in the intracellular solution (●, B), 1 mM 4-phenylbutyrate (PBA) in the intracellular solution (●, C), or 5 mM butyrate (■) or 1 mM 4-phenylbutyrate (PBA; ▼) in the extracellular solution (D). Mean of data from three to nine patches in each case.

the voltage-dependence of block by these two fatty acids (Fig. 2C). The fitted straight lines give K_ds at 0 mV of 29.6 mM for butyrate and 6.6 mM for 4-phenylbutyrate, while the slopes of these lines give apparent valencies, z', of −0.36 for butyrate and −0.53 for 4-phenylbutyrate, according to Eq. (2) (see Section 2).

The effects of butyrate and 4-phenylbutyrate on CFTR single channel currents were examined using inside–out membrane patches excised from CHO cells stably expressing CFTR (see Section 2). Addition of 5 mM butyrate or 1 mM 4-phenylbutyrate to the intracellular solution caused a voltage-dependent reduction in single channel current amplitude (Fig. 3A–C), suggesting that both these substances block the channel by a relatively fast mechanism, with individual blocking and unblocking events occurring too rapidly to resolve in the filtered current record at the time resolution used. The reduction in unitary current amplitude was similar to the reduction in macroscopic current amplitude predicted for these concentrations of butyrate and 4-phenylbutyrate (see Fig. 2A,B), suggesting that the fast block observed at the single channel level is sufficient to account for the macroscopic effects of intracellular butyrate and 4-phenylbutyrate. In contrast, inclusion of 5 mM butyrate or 1 mM 4-phenylbutyrate in the pipette (extracellular) solution had no effect on unitary current amplitude (Fig. 3D), suggesting that these substances block the CFTR channel preferentially from its intracellular end.

4. Discussion

The CFTR Cl^- channel pore is blocked in a voltage-dependent manner by a broad range of different anionic compounds (Hwang and Sheppard, 1999; Schultz et al., 1999), including sulfonylureas (Sheppard and Robinson, 1997), arylaminobenzoates (McCarty et al., 1993), disulfonic stilbenes (Linsdell and Hanrahan, 1996a), conjugated steroids and bile salts (Linsdell and Hanrahan, 1999) and simple organic anions (Linsdell and Hanrahan, 1996b). All of these pore blockers act preferentially from the intracellular solution, perhaps due to their ability to bind within a large, cytoplasmically accessible vestibule of the channel pore (Linsdell and Hanrahan, 1996a; Hwang and Sheppard, 1999). The present results are consistent with the inhibition of CFTR by the short chain fatty acids butyrate and 4-phenylbutyrate, acting by a similar mechanism. Although the affinity of block is relatively low, it is higher than for other simple organic anions such as gluconate and glutamate (Linsdell and Hanrahan, 1996b; Linsdell et al., 1997) and potentially within a physiologically and therapeutically relevant range (see below). The voltage-dependence of block by both butyrate and 4-phenylbutyrate is similar to that observed for a number of other intracellular anionic blockers that do bind within the pore of CFTR (McCarty et al., 1993; Linsdell and Hanrahan, 1996b, 1999; Linsdell et al., 1997; Sheppard and Robinson, 1997). However, based on the present results, a different mechanism of inhibition by butyrate and 4-phenylbutyrate cannot be ruled out. For example, longer chain fatty acids, including arachidonic acid, have been shown to inhibit CFTR Cl^- currents with a much higher affinity than that estimated for butyrate and 4-phenylbutyrate, but by a mechanism which may involve open channel block and/or alteration of channel gating (Linsdell, 2000).

Sodium 4-phenylbutyrate has been suggested as a treatment in urea cycle enzyme deficiencies (Maestri et al., 1996), sickle cell disease and thalassemia (Collins et al., 1995), cancer (Carducci et al., 1996) and cystic fibrosis (Rubenstein et al., 1997; Rubenstein and Zeitlin, 1998; Andersson and Roomans, 2000). Therapeutic plasma concentrations of up to 2 mM have been suggested (Rubenstein and Zeitlin, 1998). However, it has previously been shown that, although chronic 4-phenylbutyrate treatment upregulates CFTR expression in epithelial cells, it inhibits Cl^- secretion (Loffing et al., 1999). The present results demonstrate that, in addition to these chronic effects on CFTR expression, 4-phenylbutyrate exerts acute effects on channel function by directly inhibiting Cl^- permeation. These acute blocking effects of 4-phenylbutyrate accumulated in the cytoplasm most likely contribute to the paradoxical effects of chronic 4-phenylbutyrate treatment on CFTR-mediated epithelial Cl^- secretion (Loffing et al., 1999). Although Cl^- secretion in Calu-3 airway epithelial cells is inhibited by chronic exposure to high (5 mM) concentrations of 4-phenylbutyrate, it was not affected by lower (therapeutic) concentrations (0.05–2 mM) or by acute exposure (Loffing et al., 1999). Both of these effects are consistent with limited 4-phenylbutyrate accumulation in the cytoplasm of these cells.

Butyrate is present in the colonic lumen in millimolar concentrations (Cummings, 1981; Bugaut, 1987) and is the principal energy source for the colonic epithelium (Roediger, 1980). Butyrate inhibits cAMP-mediated Cl^- secretion in colonic (Dagher et al., 1986) and renal epithelia (Moyer et al., 1999), in spite of its ability to upregulate CFTR expression (Cheng et al., 1995; Moyer et al., 1999). Again, the present results suggest that acute, direct block of CFTR by cytoplasmic butyrate contributes to its chronic effect on Cl^- secretion. It has often been suggested that CFTR currents in vivo may be affected by block by cytoplasmic anions (Tabcharani et al., 1991; Haws et al., 1992; Linsdell and Hanrahan, 1996b, 1999; Linsdell, 2000); butyrate might be one such endogenous CFTR channel blocker, particularly in colonic epithelial cells. Butyrate is known to be readily absorbed by colonic epithelial cells from the colonic lumen (Roediger, 1980).

The identification of short chain fatty acids as blockers of the CFTR Cl^- channel pore increases the range of known modulators of this important channel. Such modulators are of clearly defined experimental and therapeutic importance (Hwang and Sheppard, 1999; Schultz et al.,

1999). The diversity of anions, which have now been shown to inhibit CFTR by a common mechanism, namely by blocking the channel pore from its cytoplasmic end, suggests that entry of large organic anions into the pore from the cytoplasm is a process which shows little specificity.

Acknowledgements

The author is grateful to Dr. John Hanrahan (McGill University, Montréal, Québec, Canada) for providing the CFTR-expressing cell lines used in this study, and to Susan Burbridge for technical assistance. This work was supported by the Canadian Cystic Fibrosis Foundation (CCFF) and the Medical Research Council of Canada. The author is a CCFF scholar.

References

Andersson, C., Roomans, G.M., 2000. Activation of ΔF508 CFTR in a cystic fibrosis respiratory epithelial cell line by 4-phenylbutyrate, genistein and CPX. Eur. Respir. J. 15, 937–941.

Brown, C.R., Hong-Brown, L.Q., Biwersi, J., Verkman, A.S., Welch, W.J., 1996. Chemical chaperones correct the mutant phenotype of the ΔF508 cystic fibrosis transmembrane conductance regulator protein. Cell Stress Chaperones 1, 117–125.

Bugaut, M., 1987. Occurrence, absorption and metabolism of short chain fatty acids in the digestive tract of mammals. Comp. Biochem. Physiol. B. 86B, 439–472.

Carducci, M.A., Nelson, J.B., Chan-Tack, K.M., Ayyagari, S.R., Sweatt, W.S., Campbell, P.A., Nelson, W.G., Simons, J.W., 1996. Phenylbutyrate induces apoptosis in human prostate cancer and is more potent than phenylacetate. Clin. Cancer Res. 2, 379–387.

Chang, X.-B., Cui, L., Hou, Y.-X., Jensen, T.J., Aleksandrov, A.A., Mengos, A., Riordan, J.R., 1999. Removal of multiple arginine-framed trafficking signals overcomes misprocessing of delta F508 CFTR present in most patients with cystic fibrosis. Mol. Cell 4, 137–142.

Cheng, S.H., Gregory, R.J., Marshall, J., Paul, S., Souza, D.W., White, G.A., O'Riordan, C.R., Smith, A.E., 1990. Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. Cell 63, 827–834.

Cheng, S.H., Fang, S.L., Zabner, J., Marshall, J., Piraino, S., Schiavi, S.C., Jefferson, D.M., Welsh, M.J., Smith, A.E., 1995. Functional activation of the cystic fibrosis trafficking mutant ΔF508-CFTR by overexpression. Am. J. Physiol. 268, L615–L624.

Collins, A.F., Pearson, H.A., Giardina, P., McDonagh, K.T., Brusilow, S.W., Dover, G.J., 1995. Oral sodium phenylbutyrate therapy in homozygous beta thalassemia: a clinical trial. Blood 85, 43–49.

Cummings, J.H., 1981. Short chain fatty acids in the human colon. Gut 22, 763–779.

Dagher, P.C., Egnor, R.W., Taglietta-Kohlbrecher, A., Charney, A.N., 1986. Short-chain fatty acids inhibit cAMP-mediated chloride secretion in the rat colon. Am. J. Physiol. 271, C1853–C1860.

Denning, G.M., Anderson, M.P., Amara, J.F., Marshall, J., Smith, A.E., Welsh, M.J., 1992. Processing of mutant cystic fibrosis transmembrane conductance regulator is temperature-sensitive. Nature 358, 761–764.

Hanrahan, J.W., Kone, Z., Mathews, C.J., Luo, J., Jia, Y., Linsdell, P., 1998. Patch-clamp studies of cystic fibrosis transmembrane conductance regulator chloride channel. Methods Enzymol. 293, 169–194.

Haws, C., Krouse, M.E., Xia, Y., Gruenert, D.C., Wine, J.J., 1992. CFTR channels in immortalized human airway cells. Am. J. Physiol. 263, L692–L707.

Hwang, T.-C., Sheppard, D.N., 1999. Molecular pharmacology of the CFTR Cl^- channel. Trends Pharmacol. Sci. 20, 448–453.

Kerem, B., Rommens, J.M., Buchanan, J.A., Markiewicz, D., Cox, T.K., Chakravarti, A., Buchwald, M., Tsui, L.-C., 1989. Identification of the cystic fibrosis gene: genetic analysis. Science 245, 1073–1080.

Kopito, R.R., 1999. Biosynthesis and degradation of CFTR. Physiol. Rev. 79, S167–S173.

Linsdell, P., 2000. Inhibition of cystic fibrosis transmembrane conductance regulator chloride channel currents by arachidonic acid. Can. J. Physiol. Pharmacol. 78, 490–499.

Linsdell, P., Hanrahan, J.W., 1996a. Disulphonic stilbene block of cystic fibrosis transmembrane conductance regulator Cl^- channels expressed in a mammalian cell line and its regulation by a critical pore residue. J. Physiol. 496, 687–693.

Linsdell, P., Hanrahan, J.W., 1996b. Flickery block of single CFTR chloride channels by intracellular anions and osmolytes. Am. J. Physiol. 271, C628–C634.

Linsdell, P., Hanrahan, J.W., 1998. Adenosine triphosphate-dependent asymmetry of anion permeation in the cystic fibrosis transmembrane conductance regulator chloride channel. J. Gen. Physiol. 111, 601–614.

Linsdell, P., Hanrahan, J.W., 1999. Substrates of multidrug resistance-associated proteins block the cystic fibrosis transmembrane conductance regulator chloride channel. Br. J. Pharmacol. 126, 1471–1477.

Linsdell, P., Tabcharani, J.A., Hanrahan, J.W., 1997. Multi-ion mechanism for ion permeation and block in the cystic fibrosis transmembrane conductance regulator chloride channel. J. Gen. Physiol. 110, 365–377.

Linsdell, P., Zheng, S.-X., Hanrahan, J.W., 1998. Non-pore lining amino acid side chains influence anion selectivity of the human CFTR Cl^- channel expressed in mammalian cell lines. J. Physiol. 512, 1–16.

Linsdell, P., Evagelidis, A., Hanrahan, J.W., 2000. Molecular determinants of anion selectivity in the cystic fibrosis transmembrane conductance regulator chloride channel pore. Biophys. J. 78, 2973–2982.

Loffing, J., Moyer, B.D., Reynolds, D., Stanton, B.A., 1999. PBA increases CFTR expression but at high doses inhibits Cl^- secretion in Calu-3 airway epithelial cells. Am. J. Physiol. 277, L700–L708.

Maestri, N.E., Brusilow, S.W., Clissold, D.B., Bassett, S.S., 1996. Long-term treatment of girls with ornithine transcarbamylase deficiency. N. Engl. J. Med. 335, 855–859.

McCarty, N.A., McDonough, S., Cohen, B.N., Riordan, J.R., Davidson, N., Lester, H.A., 1993. Voltage-dependent block of the cystic fibrosis transmembrane conductance regulator Cl^- channel by two closely related arylaminobenzoates. J. Gen. Physiol. 102, 1–23.

Moyer, B.D., Loffing-Cueni, D., Loffing, J., Reynolds, D., Stanton, B.A., 1999. Butyrate increases apical membrane CFTR but reduces chloride secretion in MDCK cells. Am. J. Physiol. 277, F271–F276.

Roediger, W.E.W., 1980. Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. Gut 21, 793–798.

Rubenstein, R.C., Zeitlin, P.L., 1998. A pilot clinical trial of oral sodium 4-phenylbutyrate (Buphenyl) in ΔF508-homozygous cystic fibrosis patients: partial restoration of nasal epithelial CFTR function. Am. J. Respir. Crit. Care Med. 157, 484–490.

Rubenstein, R.C., Egan, M.E., Zeitlin, P.L., 1997. In vitro pharmacologic restoration of CFTR-mediated chloride transport with sodium 4-phenylbutyrate in cystic fibrosis epithelial cells containing ΔF508-CFTR. J. Clin. Invest. 100, 2457–2465.

Sato, S., Ward, C.L., Krouse, M.E., Wine, J.J., Kopito, R.R., 1996. Glycerol reverses the misfolding phenotype of the most common cystic fibrosis mutation. J. Biol. Chem. 271, 635–638.

Schultz, B.D., Singh, A.K., Devor, D.C., Bridges, R.J., 1999. Pharmacology of CFTR chloride channel activity. Physiol. Rev. 79, S107–S144.

Sheppard, D.N., Robinson, K.A., 1997. Mechanism of glibenclamide inhibition of cystic fibrosis transmembrane conductance regulator Cl^- channels expressed in a murine cell line. J. Physiol. 503, 333–346.

Sheppard, D.N., Welsh, M.J., 1999. Structure and function of the CFTR chloride channel. Physiol. Rev. 79, S23–S45.

Tabcharani, J.A., Chang, X.-B., Riordan, J.R., Hanrahan, J.W., 1991. Phosphorylation-regulated Cl^- channel in CHO cells stably expressing the cystic fibrosis gene. Nature 352, 628–631.

Woodhull, A.M., 1973. Ionic blockage of sodium channels in nerve. J. Gen. Physiol. 61, 687–708.