

ing hyperpolarisation produced by such an effect would then act as a stimulus, augmenting  $\text{Cl}^-$  secretion by increasing the driving force for  $\text{Cl}^-$  exit through already active apical  $\text{Cl}^-$  channels. If this  $\text{Cl}^-$  channel is defective in jejunal enterocytes from cystic fibrosis patients then an alternative explanation for the calcium ionophore effect can also be based on activation of  $\text{K}^+$  channels. This activation would in itself be reflected in a transient increase in short circuit current, produced by basolateral  $\text{K}^+$  exit, until a new steady state of intracellular  $\text{K}^+$  is attained. Another possibility is that the hyperpolarisation ensuing from  $\text{K}^+$  channel activation could act to increase  $\text{Na}^+$  absorption by increasing the driving force for an apical electrogenic  $\text{Na}^+$  entry mechanism.

The fact that Necturus small intestine enterocytes possess a channel with comparable properties to those reported to be defective in cystic fibrosis airway epithelium suggests that defective regulation of this channel in mammalian enterocytes could explain the results of Taylor *et al.* This possibility needs to be examined in future work carried out at the molecular level.

F V SEPÚLVEDA, D N SHEPPARD, AND F GIRALDEZ

*AFRC Institute of Animal Physiology and Genetics Research, Babraham, Cambridge CB2 4AT, and Departamento de Bioquímica y Biología Molecular y Fisiología, Facultad de Medicina, 47005- Valladolid, Spain.*

#### References

- 1 Taylor CJ, Baxter PS, Hardcastle J, Hardcastle PT. Failure to induce secretion in jejunal biopsies from children with cystic fibrosis. *Gut* 1988; **29**: 957–62.
- 2 Giraldez F, Sepúlveda FV, Sheppard DN. A chloride conductance activated by adenosine 3'5'-cyclic monophosphate in the apical membrane of Necturus enterocytes. *J Physiol* 1988; **395**: 597–623.
- 3 Giraldez F, Sepúlveda FV, Sheppard DN. An outward rectifying  $\text{Cl}^-$ -selective channel in isolated Necturus enterocytes. *J Physiol* 1988 (In press).
- 4 Welsh MJ, Liedtke CM. Chloride and potassium channels in cystic fibrosis airway epithelia. *Nature* 1986; **322**: 467–70.
- 5 Schoumacher RA, Shoemaker RL, Halm DR, Tallant EA, Wallace RW, Frizzell RA. Phosphorylation fails to activate chloride channels from cystic fibrosis airway cells. *Nature* 1987; **330**: 752–4.
- 6 Li M, McCann JD, Liedtke CM, Nairn AC, Greengard P, Welsh MJ. Cyclic AMP-dependent protein kinase opens chloride channels in normal but not cystic fibrosis airway epithelium. *Nature* 1988; **331**: 358–60.
- 7 Sepúlveda FV, Mason WT. Single channel recordings from basolateral membranes of isolated rabbit enterocytes. *FEBS Lett* 1985; **191**: 87–91.

8 Morris AP, Gallacher DV, Lee JAC. A large conductance, voltage- and calcium-activated  $\text{K}^+$  channel in the basolateral membrane of rat enterocytes. *FEBS Lett* 1986; **206**: 87–92.

9 Sheppard DN, Giraldez F, Sepúlveda FV.  $\text{K}^+$  channels activated by L-alanine transport in isolated Necturus enterocytes. *FEBS Lett* 1988; **234**: 446–8.

#### Reply

SIR,—Sepúlveda *et al* suggest that the ability of the  $\text{Ca}^{2+}$  ionophore A23187 to increase the short circuit current (SCC) across jejunal biopsies from children with cystic fibrosis (CF) may not represent a response of luminal  $\text{Cl}^-$  channels to a rise in intra-cellular  $\text{Ca}^{2+}$ . This is based on patch clamp studies of Necturus enterocytes where luminal  $\text{Cl}^-$  channels fail to respond to changes in cytosolic  $\text{Ca}^{2+}$  concentration.<sup>1</sup> Their view that human  $\text{Cl}^-$  channels behave in the same way is supported by a single patch clamp study of the apical membranes of airway epithelial cells from CF patients where similar  $\text{Ca}^{2+}$ -independent  $\text{Cl}^-$  channels have been demonstrated.<sup>2</sup> Another study however, using the same tissue has identified  $\text{Cl}^-$  channels that can be activated by increased cytosolic  $\text{Ca}^{2+}$  both in the cell attached recording mode (by applying A23187) and in excised cell-free membrane patches.<sup>3</sup> Thus there is as yet no consensus on the properties of the  $\text{Cl}^-$  channels that are activated when secretion is stimulated.

Sepúlveda and colleagues attribute the increase in SCC observed with A23187 in jejunal biopsies to a hyperpolarisation of the basolateral membrane resulting from the activation of  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  channels, as in the airway epithelium patch clamp studies have indicated that these channels operate normally in CF.<sup>2</sup> When the transepithelial SCC of the airway is measured however, no increase is observed in response to secretagogue challenge, nor is a hyperpolarisation of the basolateral membrane detected.<sup>4</sup> In the normal small intestine, secretagogues such as acetylcholine and prostaglandin  $\text{E}_2$  increase basolateral  $\text{K}^+$  conductance,<sup>5</sup> but these agents fail to elicit a SCC response from CF jejunal biopsies.<sup>6</sup> Such observations suggest that  $\text{K}^+$  channel activation alone does not lead to an increase in transepithelial electrical activity and so cannot explain the response of CF jejunal tissue to A23187.

Additional evidence for our suggestion that the mechanism for raising intracellular  $\text{Ca}^{2+}$  might be impaired in CF comes from experiments with  $\text{BaCl}_2$ . This agent, in spite of acting as a  $\text{K}^+$  channel blocker, elicits a net fluid secretion and an associated rise in SCC in the normal intestine,<sup>7,8</sup> an effect that can be attributed to its ability to release  $\text{Ca}^{2+}$  from intracellular stores.  $\text{BaCl}_2$  also increases the SCC genera-

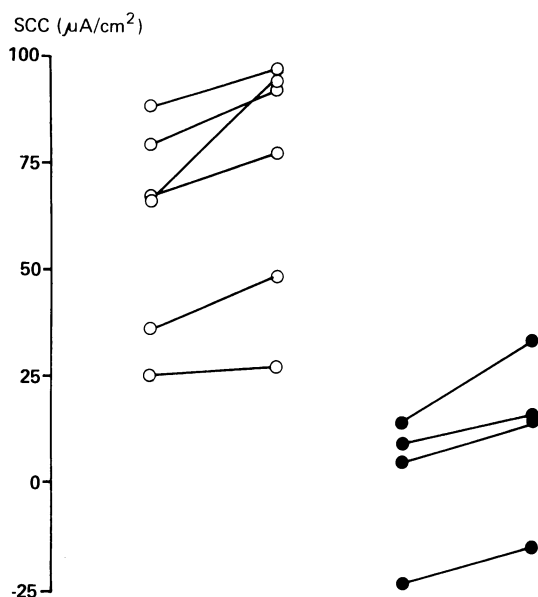


Figure Responses of jejunal biopsies from control (○) and CF (●) children to 5 mM BaCl<sub>2</sub>. The SCC for each tissue is shown before the addition of BaCl<sub>2</sub> and at the peak of the response. The mean (1 SE of the mean) was 12.5 (3.6) (6) μA/cm<sup>2</sup> for control biopsies and 10.5 (3.1) (4) μA/cm<sup>2</sup> for CF biopsies ( $p > 0.05$ ).

ted by jejunal biopsies from CF children (Figure), an effect that was not significantly different from the response obtained in biopsies from a control group.

It therefore appears that the increased SCC across CF jejunum induced by A23187 is unlikely to result from the opening of basolateral K<sup>+</sup> channels alone, but could represent a Cl<sup>-</sup> secretory response brought about by the elevation of intracellular Ca<sup>2+</sup>. Patch clamp analysis of the luminal membranes of enterocytes affected by CF would provide a more definitive answer to this problem.

J HARDCASTLE, P T HARDCASTLE, J GOLDHILL,  
C J TAYLOR, AND P S BAXTER

Department of Biomedical Science,  
The University,  
Sheffield S10 2TN, and  
Department of Paediatrics,  
Children's Hospital,  
Sheffield S10 2TH.

#### References

- Giraldez F, Sepúlveda FV, Sheppard DN. An outward rectifying Cl<sup>-</sup>-selective channel in isolated Necturus enterocytes. *J Physiol* 1988 (In press).

- Welsh MJ, Liedtke CM. Chloride and potassium channels in cystic fibrosis airway epithelia. *Nature* 1986; **322**: 467–70.
- Frizzell RA, Rechkemmer G, Shoemaker RL. Altered regulation of airway epithelial cell chloride channels in cystic fibrosis. *Science* 1986; **233**: 558–60.
- Boucher RC, Cotton CU, Gatzky JT, Knowles MR, Yankaskas JR. Evidence for reduced Cl<sup>-</sup> and increased Na<sup>+</sup> permeability in cystic fibrosis human primary cell cultures. *J Physiol* 1988; **405**: 77–103.
- Hardcastle J, Hardcastle PT. The involvement of basolateral potassium channels in the intestinal response to secretagogues in the rat. *J Physiol* 1986; **379**: 331–45.
- Taylor CJ, Baxter PS, Hardcastle J, Hardcastle PT. Failure to induce secretion in jejunal biopsies from children with cystic fibrosis. *Gut* 1988; **29**: 957–62.
- Hardcastle J, Hardcastle PT, Noble JM. The effect of barium chloride on intestinal secretion in the rat. *J Physiol* 1983; **344**: 69–80.
- Hardcastle J, Hardcastle PT, Noble JM. The secretory action of barium chloride in rat colon. *J Physiol* 1985; **361**: 19–33.

## News

### International Bilirubin Workshop

This workshop will be held in Trieste, Italy, 6–8 April, 1989. For inquiries and registration forms contact either of the Directors: Claudio Tiribelli, MD, Istituto Patologia Medica, University of Trieste, 34100 Trieste, Italy. Tel: (40)-776-4525, Fax: (40)-910-690; or J Donald Ostrow, MD, V.A. Lakeside Medical Center, 333 East Huron Street, Chicago, IL 60611, USA. Tel: (312)-943-6600, Ext 358, Fax: (312)-908-0365.

### 31st International Congress of Physiological Sciences

Will be held in Helsinki, Finland, on 7–14 July, 1989. Information from The Congress Secretariat, PO Box 722, SF-00101 Helsinki, Finland. (Telefax: 358–0–611 188).

### Correction

**24 h Intra-gastric acidity and plasma gastrin concentration . . .** Lanzon-Miller *et al.* October 1988 issue pp 1364–9. The fourth column in Figures 3 and 4 should be correctly labelled ranitidine 300 mg.