BJP BRITISH JOURNAL OF PHARMACOLOGY

BPS BRITISH PHARMACOLOGICAL SOCIETY

British Journal of Pharmacology (2009), 158, 1313–1321

0007-1188/09
www.brjpharmacol.org

RESEARCH PAPER

Differential activation of the HCO_3^- conductance through the cystic fibrosis transmembrane conductance regulator anion channel by genistein and forskolin in murine duodenum

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Background and purpose: Many cystic fibrosis (CF)-associated mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) anion channels affect CFTR-activated HCO_3^- transport more than Cl^- transport. Targeting the CFTR HCO_3^- conductance, if possible, may therefore be of major therapeutic benefit. In the present study, we examined the effects of genistein and forskolin on duodenal mucosal HCO_3^- and Cl^- secretion.
Experimental approach: Murine duodenal mucosal HCO_3^- and Cl^- secretions were examined *in vitro* in Ussing chambers by the pH stat and short circuit current (I_{sc}) techniques.
Key results: Genistein markedly stimulated duodenal HCO_3^- secretion and I_{sc} in a dose-dependent manner in CFTR wild-type mice, but not in CFTR null mice. $CFTR_{inh}$-172, a highly specific CFTR inhibitor, inhibited genistein-stimulated duodenal HCO_3^- secretion and I_{sc} in wild-type mice. Genistein induced 59% net HCO_3^- increase and 123% net I_{sc} increase over basal value, whereas forskolin, an activator of adenylate cyclase, induced 94% net HCO_3^- increase and 507% net I_{sc} increase, indicating that, compared with forskolin, genistein induced a relatively high HCO_3^-/I_{sc} ratio. Further data showed that CFTR HCO_3^-/Cl^- conductance ratio was 1.05 after genistein stimulation, whereas after forskolin stimulation, the CFTR HCO_3^-/Cl^- conductance ratio was 0.27.
Conclusions and implications: Genistein stimulates duodenal HCO_3^- and Cl^- secretion through CFTR, and has a relatively high selectivity for the CFTR HCO_3^- conductance, compared with forskolin. This may indicate the feasibility of selective targeting of the HCO_3^- conductance of the CFTR channels.
British Journal of Pharmacology (2009) **158,** 1313–1321; doi:10.1111/j.1476-5381.2009.00398.x; published online 25 September 2009

Keywords: CFTR; duodenum; bicarbonate secretion

Abbreviations: CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; I_{sc}, transepithelial short circuit current

Introduction

Cystic fibrosis (CF) is a lethal monogenetic disease characterized by impaired water and ion transport through epithelia (Quinton, 1990). The genetic basis of the disease is a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) (Riordan *et al.*, 1989). CFTR is a phosphorylation-dependent Cl^- channel abundantly expressed in several functionally diverse tissues, such as the pancreas, intestine, kidney, heart, vas deferens, sweat duct and lung (Bradbury, 1999; Sheppard and Welsh, 1999). In addition to its role as a secretory Cl^- and HCO_3^- channel, CFTR also regulates several transport proteins, including the outwardly rectifying chloride channels, epithelial Na^+ channels, K^+ channels, anion exchangers, Na^+-HCO_3^- cotransporters, and aquaporin water channels (Riordan, 2005; Guggino and Stanton, 2006). Thus, CFTR might be central in determining transepithelial salt transport, fluid flow and intracellular ion concentrations.

A consequence of CFTR mutations is defective electrolyte transport, primarily in cells of epithelial origin. Although lung disease is the primary cause of mortality in CF patients, a significant proportion of the morbidity can be directly attributed to gastrointestinal complications. In intestinal

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Received 27 February 2009; revised 3 May 2009; accepted 26 May 2009

epithelial cells, CFTR mediates the regulation of fluid, Cl^- and HCO_3^- transport, and plays an important role in intestinal secretion (Banks and Farthing, 2002). CFTR mutations in intestinal epithelial cells result in decreased fluid secretion, increased mucus viscosity and intestinal obstruction, and intestinal pathophysiology appears to be the hallmark of transgenic CF mouse model (Grubb and Boucher, 1999). As possible therapy for CF, the use of substances that activate CFTR channels has been suggested (Galietta and Moran, 2004), and the Cl^- and HCO_3^- conductances of the CFTR anion channel can be selectively activated (Reddy and Quinton, 2003). In addition, different mutations of CFTR gene have differential effects on Cl^- and HCO_3^- conductance. Some mutations have no effect on Cl^- current but reduce HCO_3^- transport, whereas others markedly reduce Cl^- current but only slightly reduce the ability of CFTR to transport HCO_3^-. The mutations associated with CF with pancreatic insufficiency exhibit severely impaired HCO_3^- transport (Choi *et al.*, 2001). HCO_3^- transport by CFTR-expressing epithelia is critical for normal tissue physiology. Thus, duodenal mucosal HCO_3^- secretion has been accepted as the most important protective factor against damage induced by gastric acid (Flemström and Isenberg, 2001), and impaired HCO_3^- transport can derange pancreatic function (Choi *et al.*, 2001). Targeting the CFTR HCO_3^- conductance, as distinct from its Cl^- conductance, and enhancing HCO_3^- transport by epithelial cells, if possible, may therefore be of considerable therapeutic benefit.

Genistein is a member of the large class of naturally occurring flavonoids, and it activates CFTR channels in the airway, jejunum, colonic and epididymal epithelia (Goddard *et al.*, 2000; Leung and Wong, 2000; Andersson *et al.*, 2003; Baker and Hamilton, 2004). Genistein can also activate the G551D mutant CFTR channel in HeLa cells and in CF patients (Illek *et al.*, 1999), and the ΔF508 CFTR in murine trachea and human airway epithelial cells (Goddard *et al.*, 2000; Andersson *et al.*, 2003). A recent study by Schmidt *et al.* (2008) showed that genistein prolonging treatment to baby hamster kidney cells expressing wild-type CFTR augmented CFTR maturation and increased the localization of CFTR protein to the cell surface. However, the role of genistein in regulating duodenal mucosal HCO_3^- secretion is unclear. In the present study, we examined the effects of genistein on duodenal HCO_3^- and Cl^- secretion in preparations of murine mucosal epithelium *in vitro*.

Methods

Animal preparation

All animal care and experimental studies were approved by Committees on Investigations Involving Animals in Zunyi Medical College, China, and Hannover Medical School, Germany. We used CFTR wild-type ($CFTR^{+/+}$) and homozygous ($CFTR^{-/-}$) littermate mice. A murine CF colony, *cftr*^{m1UNC}, was established by mating animals heterozygous for the CFTR gene disruption ($CFTR^{+/-}$; Jackson Laboratories, Bar Harbor, ME, USA). $CFTR^{+/+}$ mice were produced by mating heterozygous ($CFTR^{+/-}$] mice or $CFTR^{-/-}$ mice. Genotyping of CFTR mutant mouse progeny was analysed by PCR. All mice were 6–12 weeks of age. The mice were housed in a standard animal care room with a 12:12 h light–dark cycle, and were allowed free access to food and water. The mice were given electrolyte solution containing polyethylene glycol 4000 (PEG; institutional pharmacy) and fibre-free chow (diet C1013, Altromin, Lage, Germany) to prevent intestinal impaction. Before each experiment, the mice were deprived of food and water for at least 1 h. After brief narcosis with 100% CO_2, the mice were killed by cervical dislocation. The abdomen was opened by midline incision, and the proximal duodenum (a portion stretching approximately from 2 mm distal to the pylorus to the common bile duct ampulla) was removed and immediately placed in ice-cold iso-osmolar mannitol and indomethacin (1 μM) solution (to suppress trauma-induced prostaglandin release). The duodenum was opened along the mesenteric border and stripped of external serosal and muscle layers by sharp dissection in the ice-cold iso-osmolar mannitol and indomethacin solution.

Ussing chamber experiments

For Ussing chamber studies, the mucosal solution contained the following (in mM): Na^+, 140; K^+, 5.4; Ca^{2+}, 1.2; Mg^{2+}, 1.2; Cl^-, 120; gluconate, 25; and mannitol, 10. The serosal solution contained (in mM): Na^+, 140; K^+, 5.4; Ca^{2+}, 1.2; Mg^{2+}, 1.2; Cl^-, 120; HCO_3^-, 25; HPO_4^{2-}, 2.4; $H_2PO_4^-$, 2.4; glucose 10 and indomethacin 0.001. In Cl^--free solution, Cl^- was iso-osmotically replaced by gluconate in both mucosal and serosal solutions. In HCO_3^--free solution, HCO_3^- in serosal solution was iso-osmotically replaced by gluconate. In the Cl^-- and HCO_3^--free solutions, Cl^- and HCO_3^- were iso-osmotically replaced by gluconate. The osmolalities for both solutions were ~300 $mOsm \cdot L^{-1}$.

Ussing chamber experiments were performed as previously described (Tuo and Isenberg, 2003; Tuo *et al.*, 2007). Briefly, the duodenal mucosae were mounted between two chambers with an exposed area of 0.196 cm^2 and placed in a Ussing chamber. Parafilm 'O' ring was used to minimize edge damage to the tissue where it was secured between the chamber halves. The mucosal side was bathed with unbuffered HCO_3^--free modified Ringer's solution (see above) circulated by a gas lift with 100% O_2 to facilitate the measurement of HCO_3^- secretion by pH stat method. The serosal side was bathed with modified buffered Ringer's solution (pH 7.4) containing 25 mM HCO_3^- and gassed with 95% O_2/5% CO_2. In HCO_3^--free serosal solution, both sides were gassed with 100% O_2. Each bath contained 10 mL of the respective solution maintained at 37°C by a heated water jacket. Experiments were performed under continuous short-circuit conditions to maintain the electrical potential difference at zero, except for a brief period (<2 s) at each time point when the open-circuit potential difference was measured. Luminal pH was maintained at 7.40 by the continuous infusion of 0.5 mM HCl under the automatic control of a pH-stat system (PHM290, pH-Stat Controller, Radiometer, Copenhagen, Demmark). The volume of the titrant infused per unit time was used to quantitate HCO_3^- secretion. These measurements were recorded at 5 min intervals. The rate of luminal HCO_3^- secretion is expressed as $\mu mol \cdot cm^{-2} \cdot h^{-1}$. Transepithelial short-circuit current (I_{sc}; reported as $\mu Eq \cdot cm^{-2} \cdot h^{-1}$) was measured via an

automatic voltage clamp (Voltage-Current Clamp, EVC-4000; World Precision Instruments, Sarasota, FL, USA). After a 30 min measurement of basal parameters, the agonist, genistein or control vehicle was added to the serosal side of the tissue in Ussing chambers. Changes in duodenal HCO_3^- secretion and I_{sc} during the 40 min period ensuing after the addition of the agonist were determined. When inhibitor was used, it was added at 30 min before the agonist.

Data analysis

All results are expressed as means ± SEM. ΔHCO_3^- and ΔI_{sc} both refer to stimulated peak responses minus basal levels. Data were analysed by one-way analysis of variance followed by Newman–Keuls's *post hoc* test or, when appropriate, by the two-tailed Student's *t*-tests. $P < 0.05$ was considered statistically significant.

Materials

The reagents, genistein and forskolin, were purchased from Sigma (St Louis, MO, USA). $CFTR_{inh}$-172 was from Calbiochem (San Diego, CA, USA). All other chemicals in solutions were obtained from Sigma and Calbiochem.

Results

Genistein stimulates duodenal mucosal HCO_3^- and Cl^- secretion by activation of CFTR

We first examined the effects of genistein on duodenal mucosal HCO_3^- and Cl^- secretion in CFTR wild-type mice. As shown in Figure 1A and B, the addition of genistein (50 μM) markedly increased duodenal mucosal HCO_3^- secretion and I_{sc} ($P < 0.0001$). The effects of genistein on HCO_3^- secretion and I_{sc} were dose-dependent ($P < 0.0001$) (Figure 2A and B), with the maximal responses to genistein occurring at 50 μM. The findings indicate that genistein stimulates duodenal mucosal HCO_3^- and Cl^- secretion. CFTR is an apical membrane Cl^- channel critical for the regulation of HCO_3^- and Cl^- transport in the duodenal epithelium (Hogan *et al.*, 1997a; Seidler *et al.*, 1997). We tried to determine whether genistein-stimulated duodenal mucosal HCO_3^- and Cl^- secretion is CFTR-dependent or not. The results showed that genistein (50 μM) stimulated HCO_3^- secretion and I_{sc} in $CFTR^{+/+}$ mice, but it failed to stimulate HCO_3^- secretion and I_{sc} in $CFTR^{-/-}$ mice (Figure 3A1 and B1). $CFTR_{inh}$-172 (10 μM), a highly potent and specific CFTR inhibitor (Ma *et al.*, 2002), markedly inhibited genistein-stimulated duodenal mucosal HCO_3^- secretion and I_{sc} in $CFTR^{+/+}$ mice ($P < 0.0001$) (Figure 3A2 and B2). $CFTR_{inh}$-172 reduced the net peak of genistein-stimulated HCO_3^- secretion by 79% and I_{sc} by 84%, respectively ($P < 0.0001$). These results demonstrated that genistein stimulates duodenal mucosal epithelial HCO_3^- and Cl^- secretion through activation of CFTR anion channels.

Differential activation of the duodenal mucosal epithelial CFTR HCO_3^- conductance by genistein and forskolin

Earlier work had demonstrated that forskolin was an CFTR activator in duodenal epithelial cells (Seidler *et al.*, 1997; Tuo *et al.*, 2006). In this study, our results showed that forskolin (10 μM) markedly stimulated duodenal mucosal HCO_3^- secretion and I_{sc} in $CFTR^{+/+}$ mice but failed to induce HCO_3^- secretion and I_{sc} in $CFTR^{-/-}$ mice (Figure 4A1 and B1), and $CFTR_{inh}$-172 (10 μM) markedly inhibited forskolin-stimulated HCO_3^- secretion and I_{sc} in $CFTR^{+/+}$ mice ($P < 0.0001$) (Figure 4A2 and B2), further confirming that forskolin stimulates HCO_3^- and Cl^- secretion in the duodenal mucosal epithelium, through the activation of CFTR channels.

Genistein and forskolin, two very different compounds, both activated CFTR in the duodenal mucosal epithelium. We then analysed these results to look for differences in effects on HCO_3^- and Cl^- conductance through CFTR. As shown in Figure 5A and B, the net maximal increase (maximal response minus basal value) of duodenal HCO_3^- secretion induced by genistein (50 μM) was 0.64 $\mu mol \cdot cm^{-2} \cdot h^{-1}$, which represents a 59% increase over basal value, and the net maximal increase of duodenal I_{sc} was 2.72 $\mu Eq \cdot cm^{-2} \cdot h^{-1}$, which represents a 123% increase over basal value. The ratio of increased net HCO_3^- to net I_{sc} was 0.48. However, the net maximal increase of HCO_3^- secretion induced by forskolin (10 μM) was 1.16 $\mu mol \cdot cm^{-2} \cdot h^{-1}$, which represents a 94% increase over basal value, and the net maximal increase of I_{sc} was

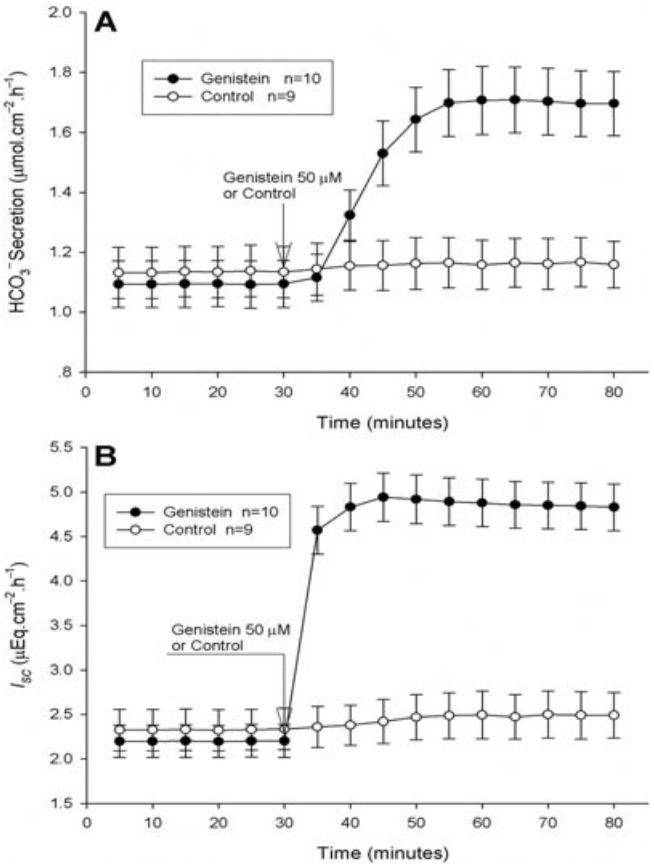

Figure 1 Effects of genistein on duodenal mucosal HCO_3^- secretion (A) and I_{sc} (B) in cystic fibrosis transmembrane conductance regulator wild-type mice. The data represent the time course of changes of HCO_3^- secretion and I_{sc}. Genistein (50 μM) or control vehicle was added at the time indicated by the arrow. Values are expressed as mean ± SEM and n = 9–10 in each series. Genistein markedly stimulated duodenal HCO_3^- secretion and I_{sc} ($P < 0.0001$).

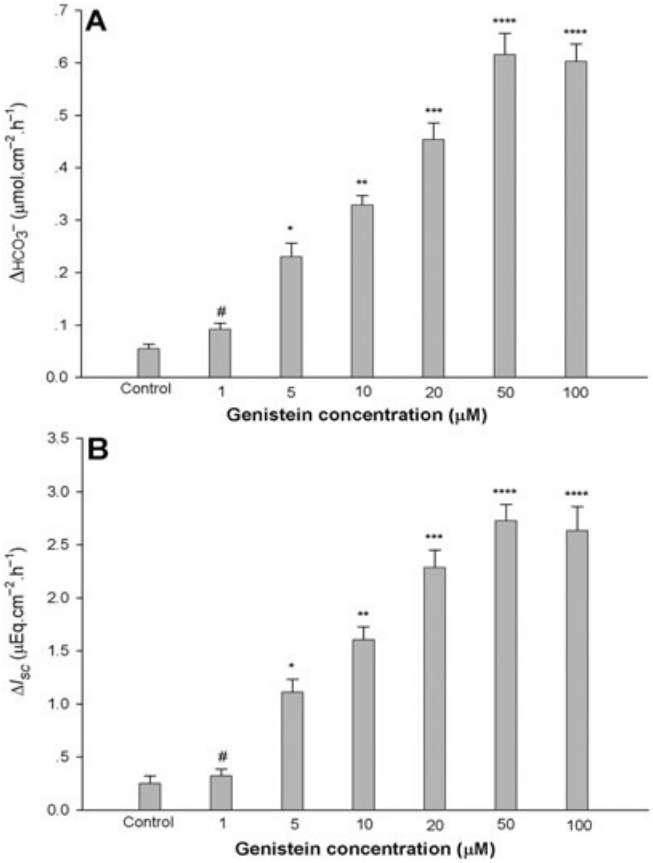

Figure 2 Effects of graded doses of genistein on duodenal mucosal HCO_3^- secretion (A) and I_{sc} (B) in cystic fibrosis transmembrane conductance regulator wild-type mice. Each dose was tested independently in a separate tissue. Results are expressed as mean ± SEM and n = 8–10 in each series. Genistein stimulated duodenal HCO_3^- secretion and I_{sc} in a dose-dependent manner ($P < 0.0001$). The maximal response occurred with genistein 50 μM. $^{\#}P > 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$ (compared with control).

11.19 μEq·cm^{-2}·h^{-1}, which represents a 507% increase over basal value. The ratio of net HCO_3^- to net I_{sc} was 0.19. This analysis indicated that genistein induced a relatively high CFTR HCO_3^-/I_{sc} ratio, compared with forskolin.

We then investigated the effects of omitting Cl^-, HCO_3^- or both Cl^- and HCO_3^- from the bathing solutions on the increased I_{sc} induced by genistein or forskolin. The results showed that in Cl^--free solution, HCO_3^--free solution and both HCO_3^-- and Cl^-- free solutions, the genistein (50 μM)-induced I_{sc} was reduced by 43, 45 and 90%, respectively (Figure 6A), whereas the forskolin (10 μM)-induced I_{sc} was reduced by 70, 19 and 92%, respectively (Figure 6B), further demonstrating that genistein- and forskolin-stimulated I_{sc} mainly result from duodenal epithelial Cl^- and HCO_3^- secretion. In addition, in Cl^--free solution, both genistein (50 μM)- and forskolin (10 μM)-stimulated HCO_3^- secretions were not significantly altered (Figure 7), confirming that genistein and forskolin stimulate HCO_3^- secretion through CFTR channels and not through Cl^-/HCO_3^- exchangers. From these measurements of I_{sc} in Cl^--free solutions and HCO_3^--free solutions, the genistein-stimulated CFTR HCO_3^-/Cl^- conductance ratio in the duodenal mucosal epithelium was 1.05, whereas after forskolin stimulation, the CFTR HCO_3^-/Cl^- conductance ratio was 0.27.

As forskolin (10 μM.) induced a I_{sc} increase more than four-fold greater than that induced by genistein (50 μM) in the experiments above, we then measured CFTR HCO_3^-/Cl^- conductance ratio in the presence of concentrations of genistein or forskolin that induced similar increases in I_{sc}. As shown in Figure 8A, a lower concentration of forskolin (0.5 μM) induced increases of duodenal mucosal epithelial I_{sc}, comparable to those induced by genistein (50 μM). At this concentration of forskolin (0.5 μM), the induced I_{sc} was reduced by 73, 18, and 90% in Cl^--free solution, HCO_3^--free solution, and both HCO_3^-- and Cl^-- free solutions, respectively (Figure 8B). The consequent HCO_3^-/Cl^- conductance ratio was 0.25, and this ratio was similar to that (0.27) obtained with forskolin at the higher concentration (10 μM). Taken together, these results demonstrated that, compared with these effects of forskolin, genistein exerted a relatively selective stimulation of the HCO_3^- conductance, in the duodenal mucosal epithelium.

Discussion and conclusions

In the present study, our results showed that genistein stimulates duodenal mucosal HCO_3^- and Cl^- secretion through the activation of CFTR anion channels. Furthermore, the HCO_3^- and Cl^- conductances of CFTR channels in the duodenal mucosal epithelium are differentially activated by genistein and forskolin. Genistein has a relatively greater effect on the CFTR HCO_3^- conductance, compared with forskolin.

The CFTR is a cAMP-activated epithelial Cl^- channel abundantly expressed in several functionally diverse tissues. Many studies have shown that CFTR functions as both a Cl^- and a HCO_3^- channel (Poulsen *et al.*, 1994; Linsdell *et al.*, 1997; Reddy and Quinton, 2003; Sheheynikov *et al.*, 2004). Most epithelia, including the pancreas, salivary glands, bile duct, intestine, uterus and airway, secrete fluid rich in HCO_3^- (Quinton, 1999). Duodenal mucosal HCO_3^- secretion plays an important role in protecting the duodenal mucosa against damage from gastric acid (Flemström and Isenberg, 2001) and the CFTR channel plays an important role in regulating duodenal mucosal HCO_3^- secretion. A number of neural and humoral factors stimulated duodenal mucosal HCO_3^- secretion through CFTR channels (Hogan *et al.*, 1997a,b; Seidler *et al.*, 1997). Previous studies have shown that genistein stimulated CFTR channel activity in a variety of epithelial and nonepithelial cells as well as in intact tissues that express CFTR (Illek *et al.*, 1999; Goddard *et al.*, 2000; Leung and Wong, 2000; Andersson *et al.*, 2003; Baker and Hamilton, 2004). However, little is known of the effects of genistein on duodenal mucosal HCO_3^- secretion. In this study, we found that genistein dose-dependently stimulated such HCO_3^- secretion in CFTR wild type mice, but not in CFTR knock out mice, and a highly specific CFTR inhibitor, $CFTR_{inh}$-172 inhibited this genistein-stimulated HCO_3^- secretion. The results demonstrated that, in addition to its stimulatory effects on CFTR in tracheal, epididymal and colonic epithelia, genistein also stimulated HCO_3^- secretion through the activation of CFTR anion channels, in the duodenal mucosal epithelium.

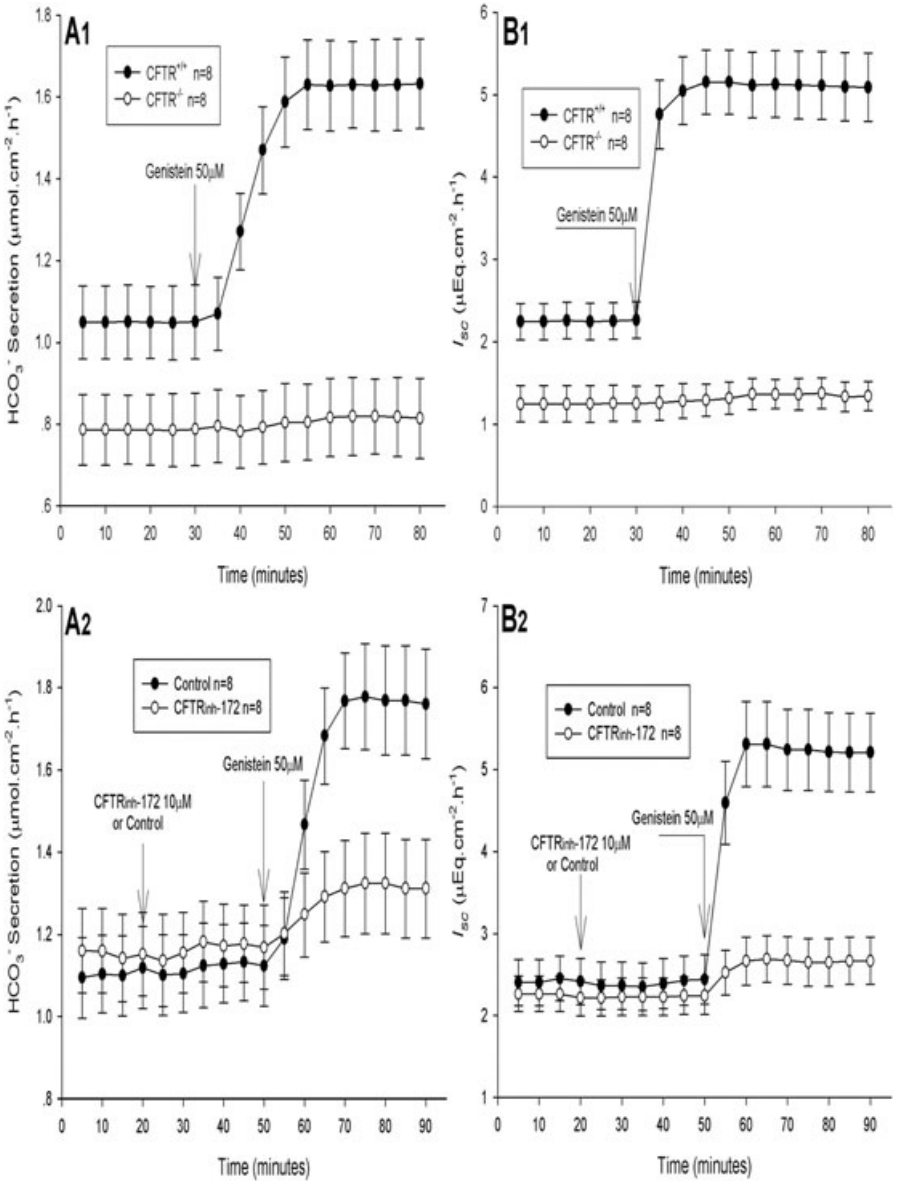

Figure 3 Effects of cystic fibrosis transmembrane conductance regulator (CFTR) gene deficiency and CFTR specific inhibitor, $CFTR_{inh}$-172, on genistein-stimulated duodenal mucosal HCO_3^- secretion (A1, A2) and I_{sc} (B1, B2). The experiments were performed with tissues from $CFTR^{+/+}$ and $CFTR^{-/-}$ mice. When $CFTR_{inh}$-172 was used, $CFTR_{inh}$-172 (10 μM) or control vehicle was added 30 min before genistein (50 μM). Values are expressed as mean ± SEM and $n = 8$ in each series. Genistein stimulated duodenal HCO_3^- secretion and I_{sc} in $CFTR^{+/+}$ mice ($P < 0.0001$), but failed in $CFTR^{-/-}$ mice. $CFTR_{inh}$-172 markedly inhibited genistein-stimulated duodenal HCO_3^- secretion and I_{sc} ($P < 0.0001$).

The CFTR channels conduct both Cl^- and HCO_3^- ions, but the permeability ratios of the CFTR for these anions are different. With the patch-clamp technique, the permeability ratios of HCO_3^-/Cl^- of CFTR were 0.18 in cAMP-stimulated pancreatic duct cells (Gray *et al.*, 1993) and 0.25 in NIH/3T3 fibroblasts expressing recombinant CFTR (Poulsen *et al.*, 1994). With microelectrode methods, HCO_3^- permeability of CFTR channels in the apical membranes of cultured human nasal cells was extremely small (Willumsen and Boucher, 1992). These studies demonstrated that the CFTR channels can exhibit different selectivities for HCO_3^- and Cl^- conductance. Reddy and Quinton (2001; 2003) found that activating CFTR in the apical membranes of the native sweat duct with cAMP and ATP stimulated both HCO_3^- and Cl^- permeability with a HCO_3^-/Cl^- selectivity ratio of 0.2–0.5. However, in the apparent complete absence of cAMP and ATP, cytoplasmic glutamate activates CFTR Cl^- conductance without any HCO_3^- conductance. Glutamate-activated CFTR can be induced to conduct HCO_3^- by the addition of ATP without cAMP. Shcheynikov *et al.* (2004) also found that the HCO_3^-/Cl^- selectivity of CFTR was dynamic and regulated by external Cl^-. These results demonstrated that CFTR can show high selectivity to either HCO_3^- or Cl^- but, more significantly, the HCO_3^-/Cl^- selectivity of CFTR can be altered by changing the stimulation conditions of the CFTR channels.

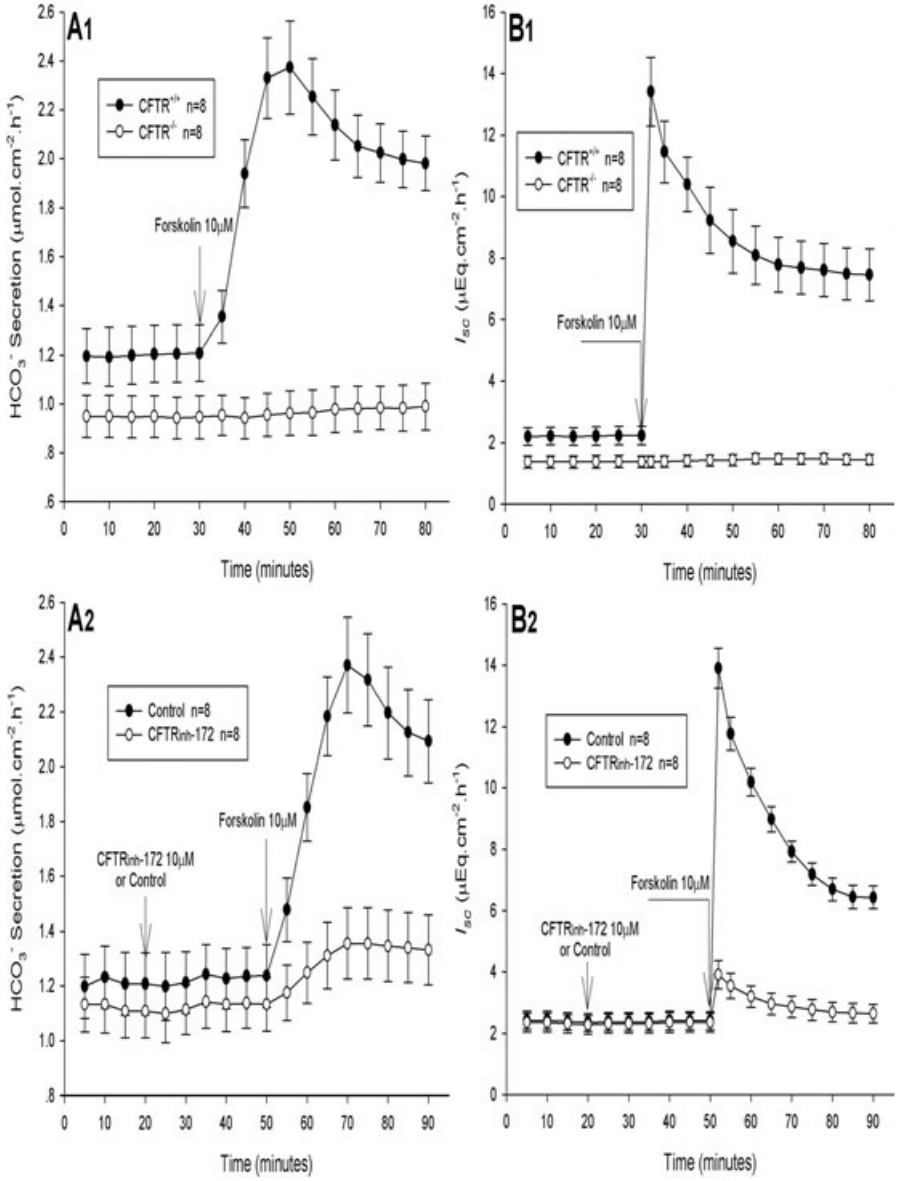

Figure 4 Effects of cystic fibrosis transmembrane conductance regulator (CFTR) gene deficiency and CFTR specific inhibitor, $CFTR_{inh}$-172, on forskolin-stimulated duodenal mucosal HCO_3^- secretion (A1, A2) and I_{sc} (B1, B2). The experiments were performed with tissues from $CFTR^{+/+}$ and $CFTR^{-/-}$ mice. When $CFTR_{inh}$-172 was used, $CFTR_{inh}$-172 (10 μM) or control vehicle was added 30 min before forskolin (10 μM). Values are expressed as mean ± SEM, and $n = 8$ in each series. Forskolin stimulated duodenal HCO_3^- secretion and I_{sc} in $CFTR^{+/+}$ mice ($P < 0.0001$), but failed in $CFTR^{-/-}$ mice. $CFTR_{inh}$-172 markedly inhibited forskolin-stimulated duodenal HCO_3^- secretion and I_{sc} ($P < 0.0001$).

The adenylate cyclase activator, forskolin, is a known CFTR activator and stimulates duodenal mucosal HCO_3^- secretion through CFTR channels (Seidler *et al.*, 1997; Tuo *et al.*, 2006). In this study, our results have shown that genistein also stimulates duodenal mucosal HCO_3^- secretion through CFTR channels. Genistein-induced CFTR activation is known to be cAMP independent, because genistein did not cause a detectable increase in intracellular cAMP levels (Illek *et al.*, 1995; Leung and Wong, 2000). Other studies showed that genistein stimulated anion secretion by direct interaction with CFTR (Weinreich *et al.*, 1997; Leung and Wong, 2000; Al-Nakkash *et al.*, 2001) and modulated cell surface expression of CFTR (Schmidt *et al.*, 2008). Thus, genistein and forskolin are two, mechanistically different, CFTR activators. In the present study, we attempted to compare the HCO_3^-/Cl^- selectivity of CFTR in the duodenal mucosal epithelium, when stimulated by genistein and forskolin. We found that in the duodenal mucosa, genistein stimulated 59% HCO_3^- secretion increase and 123% I_{sc} increase. The ratio of increased HCO_3^- secretion to I_{sc} is 0.48. In contrast, forskolin stimulated 94% HCO_3^- secretion increase and 507% I_{sc} increase. The ratio of increased HCO_3^- secretion to I_{sc} is 0.19. The results indicate that forskolin induced a high I_{sc}, whereas genistein induced a relatively high HCO_3^- secretion ratio. To determine the HCO_3^-/Cl^-

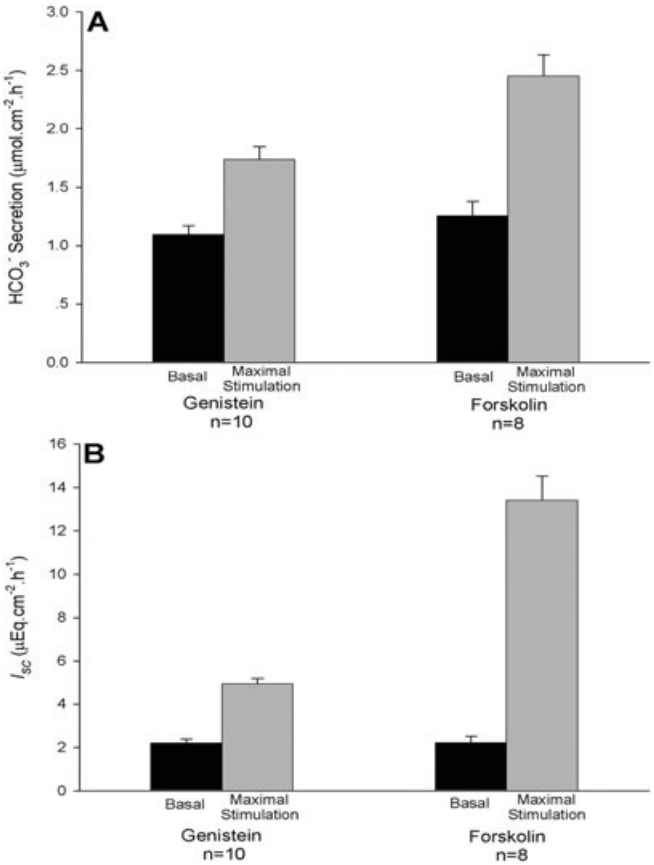

Figure 5 The comparisons of effects of genistein (50 μM) and forskolin (10 μM) on duodenal mucosal HCO_3^- secretion (A) and I_{sc} (B) in cystic fibrosis transmembrane conductance regulator wild-type mice. Values are expressed as mean ± SEM, and n = 9–10 in each series. Genistein induced a 59% increase of HCO_3^- secretion and 123% increase of I_{sc} over basal values, and the derived ratio of increased HCO_3^-/I_{sc} was 0.48, whereas forskolin induced a 94% increase of HCO_3^- secretion and 507% increase of I_{sc} over basal values, and the ratio of increased HCO_3^-/I_{sc} was 0.19.

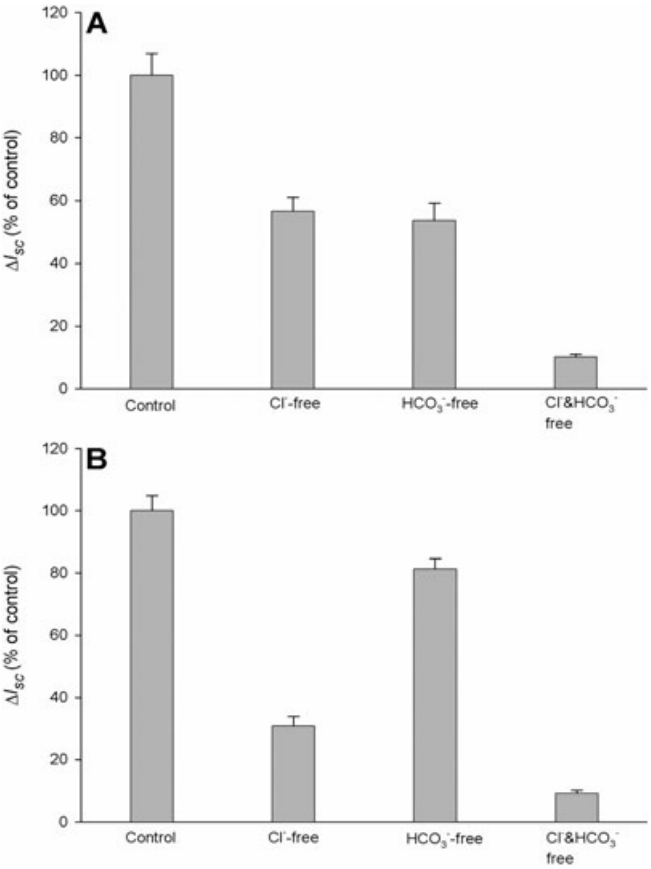

Figure 6 The HCO_3^-/Cl^- selectivity of genistein (50 μM)- (A) and forskolin (10 μM)- (B) stimulated cystic fibrosis transmembrane conductance regulator (CFTR) channels in the duodenal mucosal epithelium. The experiments were performed with tissues from $CFTR^{+/+}$ mice. Values are expressed as mean ± SEM, and n = 8–9 in each series. HCO_3^- and Cl^- currents were estimated by the removal of HCO_3^- or Cl^-. Compared with control (normal solution with HCO_3^- and Cl^-), in Cl^--free solution, HCO_3^--free solution and both HCO_3^-- and Cl^-- free solutions, genistein (50 μM)-induced I_{sc} were reduced by 43, 45 and 90%, respectively, whereas forskolin (10 μM)-induced I_{sc} were reduced by 70, 19 and 92%, respectively. From the changes of I_{sc} in Cl^--free solution and HCO_3^--free solution, the genistein-stimulated CFTR HCO_3^-/Cl^- conductance ratio was 1.05, whereas after forskolin stimulation, the CFTR HCO_3^-/Cl^- conductance ratio was 0.27.

selectivity of stimulated CFTR, we further measured HCO_3^- and Cl^- currents by removal of HCO_3^- or Cl^- in the solution. The results showed that genistein-stimulated CFTR HCO_3^-/Cl^- conductance ratio was 1.05, whereas the forskolin-stimulated CFTR HCO_3^-/Cl^- conductance ratio was 0.27. These results demonstrated that genistein- and forskolin-stimulated CFTR channels had different HCO_3^- and Cl^- conductance, and genistein had a relatively greater effect on the HCO_3^- conductance, further demonstrating that the HCO_3^-/Cl^- selectivity of CFTR can be altered by changing the stimuli activating the CFTR.

The differential activation of CFTR HCO_3^- conductance by genistein and forskolin may be of some interest for selective targeting of the CFTR by drugs. Genistein is an isoflavonoid that is classed as a plant oestrogen and is found in some food plants and, in particular, in soya beans (Barnes and Petersen, 1995). Previous studies have shown that genistein activated not only wild-type CFTR, but also G551D-CFTR and ΔF508 CFTR (Goddard *et al.*, 2000; Andersson *et al.*, 2003). HCO_3^- is an ion with particular physiological importance, as it acts as a biological buffer to control pH and it affects the solubility of macromolecules and ions in biological fluids. Secretion of HCO_3^- is a crucial function of the stomach, pancreas and small and large intestines (Quinton, 1999; 2008), and aberrant HCO_3^- secretion has long been associated with CF. Furthermore, many CF-associated mutations in CFTR affect CFTR-activated HCO_3^- transport more than Cl^- transport, and the severity of the pathogenesis in CF is closely related to the phenotypic ability of a mutant CFTR to express a HCO_3^- conductance (Choi *et al.*, 2001). These results demonstrate that targeting the CFTR HCO_3^- conductance and enhancing HCO_3^- transport in affected tissues may be effective therapy for CF with impaired HCO_3^- conductance. Therefore, our finding of high HCO_3^- conductance in CFTR, induced by genistein suggests that this compound is an interesting lead in the development of the pharmacological treatment of CF with impaired HCO_3^- secretion.

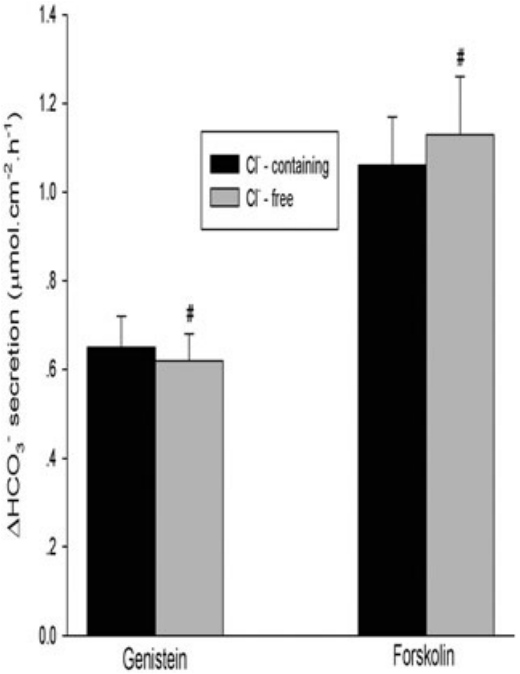

Figure 7 Effects of Cl^- free solutions on genistein (50 μM)- and forskolin (10 μM)-stimulated HCO_3^- secretion in duodenal mucosa. The experiments were performed with tissues from cystic fibrosis transmembrane conductance regulator $(CFTR)^{+/+}$ mice. Values are expressed as mean ± SEM, and n = 8–9 in each series. Compared with Cl^-- containing solutions, genistein- and forskolin-stimulated HCO_3^- secretion was not significantly altered in Cl^-- free solution ($^{\#}P > 0.05$).

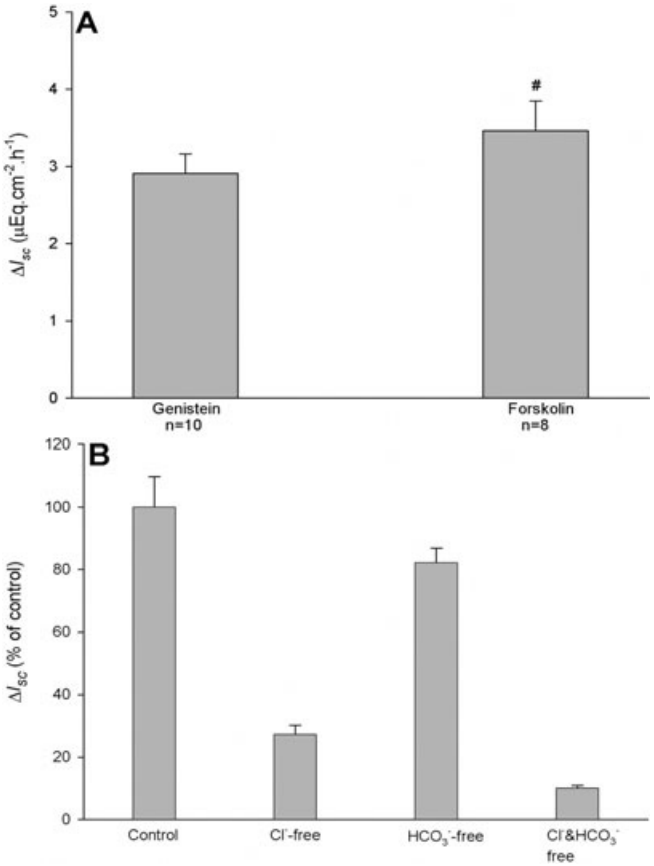

Figure 8 (A) Comparison of effects of genistein (50 μM) and forskolin (0.5 μM) on I_{sc} in duodenal mucosa. The experiments were performed with tissues from cystic fibrosis transmembrane conductance regulator $(CFTR)^{+/+}$ mice. Values are expressed as mean ± SEM, and n = 8–10 in each series. Forskolin (0.5 μM) induced a I_{sc} response similar to that induced by genistein (50 μM) ($^{\#}P > 0.05$). (B) The HCO_3^-/Cl^-selectivity of forskolin (0.5 μM)-stimulated CFTR channels. The experiments were performed with tissues from $CFTR^{+/+}$ mice, as in Figure 6. Values are expressed as mean ± SEM, and n = 8–9 in each series. Compared with control (normal solution with HCO_3^- and Cl^-), in Cl^--free solution, HCO_3^--free solution and both HCO_3^-- and Cl^--free solutions, forskolin (0.5 μM)-induced I_{sc} were reduced by 73, 18 and 92%, respectively. From the changes of I_{sc} in Cl^--free solution and HCO_3^--free solution, the CFTR HCO_3^-/Cl^- conductance ratio was 0.25, following stimulation by forskolin (0.5 μM).

In conclusion, in this study, we have demonstrated that genistein stimulated duodenal mucosal HCO_3^- and Cl^- secretion through CFTR anion channels. Genistein- and forskolin-stimulated CFTR channels have different HCO_3^-/Cl^- selectivity, and genistein induces a relatively high CFTR HCO_3^- conductance, further demonstrating that HCO_3^-/Cl^- selectivity of CFTR can be altered by changing the conditions of stimulating CFTR. This suggests a therapeutic potential for selective targeting of the HCO_3^- conductance of the CFTR.

Acknowledgements

This work was supported by the International Scientific and Technological Cooperation Program of China (2008DFA32260), Scientific Research Fund of Affiliated Hospital of Zunyi Medical College (G-046) and Doctor Fund of Zunyi Medical College, China (F-023) to B. Tuo, and the Deutsche Forschungsgemeinschaft (Se 460/13-1/2 and Se 460/13-6) to U. Seidler.

Conflicts of interest

None.

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