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RESEARCH PAPER

Sites of action of hydrogen peroxide on ion transport across rat distal colon

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Background and purpose: The aim of this study was the identification of the mechanism of oxidant-induced intestinal secretion.
Experimental approach: The action of H_2O_2 on ion transport across rat distal colon was evaluated in Ussing chambers. Changes in cytosolic Ca^{2+} concentration were measured using fura-2.
Key results: H_2O_2 concentration-dependently induced an increase in short-circuit current (I_{sc}), which was due to a stimulation of Cl^- secretion. The effect of H_2O_2 was dependent on the presence of serosal Ca^{2+}. It was inhibited after emptying of intracellular Ca^{2+} stores by cyclopiazonic acid or blockade of ryanodine receptors by ruthenium red, whereas a blocker of inositol 1,4,5-trisphosphate receptors was less effective. Fura 2-experiments confirmed an increase in the cytosolic Ca^{2+} concentration in the presence of H_2O_2. Measurements of Cl^- currents across the apical membrane at basolaterally depolarized epithelia revealed the activation of a glibenclamide-sensitive, SITS-resistant Cl^- conductance by the oxidant. The activation of this conductance was inhibited after blockade of protein kinases with staurosporine. When the apical membrane was permeabilized with nystatin, two sites of action of H_2O_2 were identified at the basolateral membrane. The oxidant stimulated a basolateral tetrapentylammonium-sensitive K^+ conductance and increased the current generated by the Na^+–K^+ pump. Pretreatment of the tissues with H_2O_2 reduced the action of subsequently administered Ca^{2+}-, cAMP- and cGMP-dependent secretagogues demonstrating a long-term downregulation after the initial secretory response evoked by the oxidant.
Conclusions and implications: H_2O_2 affects colonic anion secretion by action sites at both the apical, as well as the basolateral membrane.
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Keywords: Ca^{2+}; electrolyte transport; oxidants; rat colon

Abbreviations: CFTR, cystic fibrosis transmemembrane regulator; Gt, tissue conductance; I_{sc}, short-circuit current

Introduction

Reactive oxygen metabolites play an important pathophysiological role. For example, during inflammatory bowel diseases, polymorphnuclear leukocytes infiltrate the intestinal mucosa, where they release reactive oxygen metabolites such as superoxide (O_2^-) produced from O_2, for example by nicotinamide adenine dinucleotide phosphate-oxidase, or hydrogen peroxide (H_2O_2), produced from O_2^- by superoxide dismutase (Parkos *et al.*, 1994). The physiological significance of these substances consists in the metal-catalysed conversion into the highly reactive hydroxyl radical, a cytotoxic agent (Pavlick *et al.*, 2002), which is able to damage most biologically important macromolecules such as DNA or proteins (Halliwell and Whiteman, 2004). The consequence of all these reactions is an increased oxidative stress in the gut wall during inflammatory bowel disease (Tüzün *et al.*, 2002). Another situation, where reactive oxygen metabolites are centrally involved, is the ischaemia/reperfusion damage (see Starkov *et al.*, 2004). On the other hand, oxidants might also act as signalling molecules at subtoxic concentrations (Suzuki *et al.*, 1997).

Oxidants such as H_2O_2 induce anion secretion across several intestinal segments, such as rat ileum (Grisham *et al.*, 1990) or rat colon (Tamai *et al.*, 1991). A similar response has been observed at the human colonic tumour cell line, T_{84} (Nguyen and Canada, 1994). In rat colon, the oxidant monochloramine (NH_2Cl), physiologically produced from hypochlorous acid (HOCl) and NH_3, which is present in high concentrations in the lumen of the large intestine due to the bacterial fermentation of proteins (Tamai *et al.*, 1991), induces a Ca^{2+}-dependent Cl^- secretion. The mechanism of action consists in the release of intracellularly stored

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Ca^{2+} by ryanodine and inositol 1,4,5-trisphosphate (IP_3) receptors. The predominant consequence is the activation of basolateral Ca^{2+}-dependent K^+ channels, which increases the driving force for Cl^- exit by apical Cl^- channels (Schultheiss *et al*., 2005a).

Different types of oxidants are known to act by modification of distinct amino-acid residues of proteins: monochloramine is known to oxidize both methionine and cysteine residues, whereas H_2O_2 preferentially oxidizes cysteine residues. These differences in sites of action can lead to profound differences in the effect of both oxidants on voltage-dependent K^+ channels in smooth muscle cells (Prasad and Goyal, 2004). As the mechanism of action of H_2O_2 at rat colonic epithelium is incompletely understood, the aim of this study was to investigate the actions of this oxidant on ion transport across rat colon, the identification of the sites involved at the apical and basolateral membrane and its potential interaction with other secretagogues.

Methods

Solutions

The standard solution for the Ussing chamber experiments was a buffer solution containing the following(mmol L^{-1}): NaCl 107, KCl 4.5, $NaHCO_3$ 25, Na_2HPO_4 1.8, NaH_2PO_4 0.2, $CaCl_2$ 1.25, $MgSO_4$ 1 and glucose 12. The solution was gassed with carbogen (5% CO_2 in 95% O_2, vol/vol); pH was 7.4. For the Na^+-free solution, NaCl was replaced by *N*-methyl-D-glucamine$^+$ chloride. To apply a K^+ gradient in the mucosal to serosal direction, in the mucosal buffer, the KCl concentration was increased to 13.5 mmol L^{-1} while reducing the NaCl concentration to maintain iso-osmolarity. For the depolarization of the basolateral membrane, a 111.5 mmol L^{-1} KCl solution was used, in which NaCl was replaced by equimolar KCl on the serosal side. In the Cl^--free buffers, NaCl and KCl were substituted by Na gluconate and K gluconate (KGluc), respectively. The Ca^{2+} concentration in the Cl^--free buffer was increased to 5.75 mmol L^{-1} to compensate for the Ca^{2+}-buffering properties of gluconate (Kenyon and Gibbons, 1977).

For the experiments carried out with isolated crypts, the following buffers were used. The EDTA solution for the isolation contained the following (mmol L^{-1}): NaCl 107, KCl 4.5, $NaHCO_3$ 25, Na_2HPO_4 1.8, NaH_2PO_4 0.2, glucose 12.2, EDTA 10 and 1 g L^{-1} BSA. It was gassed with carbogen; pH was adjusted by Tris-base (tris(hydroxymethyl)-aminomethane) to 7.4. The isolated crypts were stored in a high potassium Tyrode solution consisting of (mmol L^{-1}) the following: KGluc 100, KCl 30, HEPES 10, NaCl 20, $MgCl_2$ 1, $CaCl_2$ 1.25, glucose 12.2, sodium pyruvate 5 and 1 g L^{-1} BSA; pH was 7.4. For superfusion of the isolated crypts during the fura-2 experiments, the following buffer was used (in mmol L^{-1}): NaCl 140, KCl 5.4, $CaCl_2$ 1.25, $MgSO_4$ 1, HEPES 10 and glucose 12.2; pH was 7.4.

Tissue preparation and crypt isolation

All animal procedures were approved by the Regierungspräsidium Giessen. Wistar rats of both sexes were used with a weight of 180–240 g. The animals had free access to water and a standard rat diet until the day of the experiment. Animals were killed by a blow on the head followed by exsanguination. The serosa and muscularis propria were stripped away to obtain a mucosa–submucosa preparation of the distal colon. Briefly, the colon was placed on a small plastic rod with a diameter of 5 mm. A circular incision was made near the anal end with a blunt scalpel and the serosa together with the lamina propria were gently removed in a proximal direction.

For the isolation of intact crypts, the mucosa–submucosa was fixed on a plastic holder with tissue adhesive and transferred for about 7 min to the EDTA solution. The mucosa was vibrated once for 30 s to obtain crypts (Schultheiss *et al*., 2002). They were collected in a high-K^+ gluconate Tyrode buffer.

Short-circuit current measurement

The mucosa–submucosa preparation was fixed in a modified Ussing chamber, bathed with a volume of 3.5 ml on each side of the mucosa. The tissue was incubated at 37 °C and short-circuited by a computer-controlled voltage-clamp device (Ingenieur Büro für Mess-und Datentechnik Mussler, Aachen, Germany) with correction for solution resistance. Tissue conductance (Gt) was measured every minute by the voltage deviation induced by a current pulse (±50 μA, duration 200 ms) under open-circuit conditions. Short-circuit current (I_{sc}) was continuously recorded on a chart recorder. I_{sc} is expressed as $\mu Eq\,h^{-1}\cdot cm^{-2}$, that is the flux of a monovalent ion per time and area, with $1\,\mu Eq\,h^{-1}\,cm^{-2} = 26.9\,\mu A\,cm^{-2}$. In the tables, the maximal increase in I_{sc} (ΔI_{sc}) induced by H_2O_2 is calculated as the difference from the baseline just before administration of the oxidant.

For the concentration–response experiments, H_2O_2 was administered in increasing concentrations to an individual tissue. The compartment, where the oxidant had been applied, was exchanged three times with 5 × the chamber volume, before the next concentration of the oxidant was administered.

Measurement of apical and basolateral ion currents

The apical membrane was permeabilized by mucosal administration of nystatin (100 μg ml^{-1}) dissolved in dimethylsulphoxide (final concentration 0.2%, vol/vol). Nystatin was ultrasonicated immediately before use. The I_{sc} response to the ionophore was tested in the presence or absence of a K^+ gradient (13.5 mmol L^{-1} at the mucosal and 4.5 mmol L^{-1} at the serosal side). To depolarize the basolateral membrane, the tissue was exposed to a high K^+ buffer (111.5 mmol L^{-1} KCl) at the serosal side. Under these conditions, the basolateral membrane is electrically eliminated (Fuchs *et al*., 1977; Schultheiss and Diener, 1997) and changes in I_{sc} reflect changes in current across the apical membrane.

Imaging experiments

Relative changes in the intracellular Ca^{2+} concentration were measured using fura-2 (Molecular Probes, Leiden, The

Netherlands), a Ca^{2+}-sensitive fluorescent dye (Grynkiewicz *et al.*, 1985). The crypts were pipetted into the experimental chamber with a volume of about 3 ml. They were attached to the glass bottom of the chamber with the aid of poly-L-lysine (0.1 g L^{-1}; Biochrom KG, Berlin, Germany). The crypts were incubated for 60 min with 2.5 μmol L^{-1} fura-2/acetoxymethyl-ester. Then the dye ester not taken up by the cells was washed away. The preparation was superfused hydrostatically throughout the experiment with 140 mmol L^{-1} NaCl Tyrode. Perfusion rate was about 1 ml min^{-1}.

Changes in the cytoplasmic Ca^{2+} concentration were monitored as changes in the fura-2 ratio (*R*; emission at an excitation wave length of 340 nm divided by the emission at an excitation wave length of 380 nm). Experiments were carried out on an inverted microscope (Olympus IX-50; Olympus, Hamburg, Germany) equipped with an epifluorescence set-up and an image analysis system (Till Photonics, Martinsried, Germany). Several regions of interest, each with the size of about one cell, were placed over an individual crypt. The emission above 420 nm was measured from the regions of interest. Data were sampled at 0.2 Hz. The baseline in the fluorescence ratio of fura-2 was measured for several minutes before drugs were administered.

Statistics

Values are given as means ± s.e. mean. When the means of several groups had to be compared, first ANOVA was performed. If the ANOVA indicated significant differences between the groups investigated, further comparison was carried out by a Student's *t*-test or by the *U*-test. An *F*-test decided which test method was to be used. Both paired and unpaired two-tailed Student's *t*-tests were applied as indicated. $P<0.05$ was considered to be statistically significant.

Drugs

The drug and molecular target nomenclature followed the guide to receptors and channels of the *British Journal of Pharmacology* (Alexander *et al.*, 2007). 2-aminoethoxydiphenylborate (Calbiochem, Bad Soden, Germany), cyclopiazonic acid (Tocris, Bristol, UK), genistein, N–5-isoquinolinesulphonamide (H-89), nystatin and staurosporine were dissolved in dimethylsulphoxide (final maximal concentration 2 mL L^{-1}). Bumetanide, forskolin, glibenclamide (generous gift from Boehringer Mannheim, Mannheim, Germany) and indomethacin were dissolved in ethanol (final maximal concentration 2.5 mL L^{-1}). Scilliroside (generous gift from Sandoz, Basel, Switzerland) was dissolved in methanol (final concentration 2.5 mL L^{-1}). Tetrodotoxin was dissolved in 2×10^{-2} mol L^{-1} citrate buffer. Catalase, 8-4-chlorophenylthio–cGMP, H_2O_2 (30% (wt/vol); Merck, Hohenbrunn, Germany), ruthenium red (Alfa Aesar, Karlsruhe, Germany), 4-acetamido-4′-isothiocyanato-stilbene-2,2′-disulphonic acid sodium salt and tetrapentylammonium chloride were dissolved in aqueous stock solutions. Other drugs were from Sigma, Taufkirchen, Germany, unless stated otherwise.

Results

Effect of H_2O_2 on short-circuit current

Basal I_{sc} across rat distal colon bathed with the standard buffer solution amounted to 0.81 ± 0.28 μEq h^{-1} cm^{-2} at a Gt of 16.4 ± 1.2 mS cm^{-2} ($n=13$). Hydrogen peroxide, when applied at increasing concentrations with an intermediate washing step before the next concentration of the oxidant was administered, induced a concentration-dependent increase in I_{sc} (Figure 1). This increase was more pronounced, when the oxidant was administered at the serosal side of the tissue. A maximal rise in I_{sc} was achieved with a concentration of 5 mmol L^{-1}. In parallel with I_{sc}, there was an increase in Gt by 2.1 ± 0.67 mS cm^{-2} ($P<0.05$, $n=8$) after administration of this concentration to the serosal compartment, and 1.1 ± 1.4 mS cm^{-2} (not significant, $n=5$) after administration to the mucosal compartment. All further Ussing chamber experiments were performed with a concentration of 5 mmol L^{-1} H_2O_2 administered at the serosal side.

The effect of H_2O_2 on I_{sc} (and Gt) was transient; I_{sc} returned spontaneously to baseline values within 15–25 min (Figure 2a). There was no obvious desensitization, when the

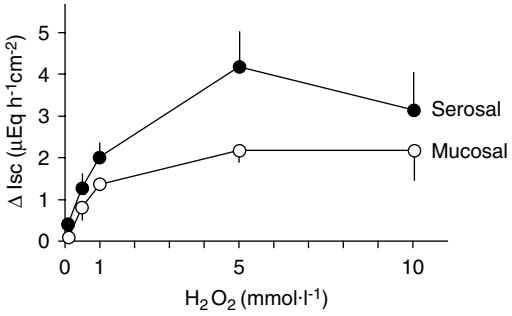

Figure 1 Concentration-dependent increase in I_{sc} across rat distal colon induced by mucosal or serosal administration of hydrogen peroxide (H_2O_2). After each administration, the respective compartment was washed three times with 5 × the chamber volume, before the next concentration of the oxidant was administered. Values are given as difference to the baseline in short-circuit current (I_{sc}) just before administration of H_2O_2 (Δ I_{sc}) and are means ± s.e.m., $n=5$–8.

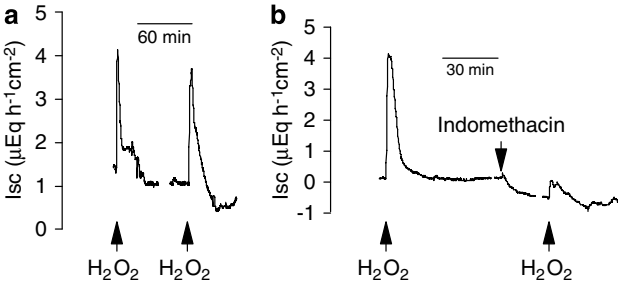

Figure 2 (**a**) Repetitive administration of hydrogen peroxide (H_2O_2) (5 mmol L^{-1} at the serosal side) did not induce a desensitization of the tissue. For mean values, see Table 1. (**b**) In the presence of indomethacin (10^{-6} mol L^{-1} at the mucosal and the serosal side), the response to H_2O_2 (5 mmol L^{-1} at the serosal side) was inhibited; for mean values, see Table 2. Line interruptions are caused by omission of short-circuit current (I_{sc}) artefacts during the washing procedure.

Table 1 Effect of transport inhibitors on H_2O_2-induced I_{sc}

	Δ I_{sc} (μEq h^{-1} cm^{-2})		
Inhibitor	*Response to H_2O_2 alone (control)*	*Response to H_2O_2 in the presence of the inhibitor*	n
None	2.88 ± 0.35^a	2.68 ± 0.30^a	6
DMSO	2.74 ± 0.17^a	2.61 ± 0.23^a	6
Ethanol	2.80 ± 0.31^a	1.96 ± 0.33^a	6
Chloride-free	2.70 ± 0.26^a	1.07 ± 0.12^b	7
Bumetanide	2.63 ± 0.44^a	1.02 ± 0.16^b	6
Glibenclamide	2.74 ± 0.24^a	0.92 ± 0.22^b	6

Abbreviations: H_2O_2, hydrogen peroxide; I_{sc}, short-circuit current.
The effect of H_2O_2 (5 mmol L^{-1} at the serosal side) was first tested under control conditions (left column), and then in the presence of the putative inhibitor (right column). Between the individual applications, the compartments, where drugs had been applied, were washed three times with 5 × the chamber volume, before H_2O_2 was administered again. Inhibitor (or solvent) concentrations were as follows: bumetanide (10^{-4} mol L^{-1} at the serosal side), DMSO (0.3%, vol/vol), ethanol (0.3%, vol/vol), or glibenclamide (5×10^{-4} mol L^{-1} at the mucosal side). For the Cl^--free buffer, Cl^- was replaced by gluconate on both sides of the tissue. Values are given as difference to the baseline in I_{sc} just before administration of H_2O_2 (Δ I_{sc}) and are means ± s.e.m. Different letters (a, b) indicate significant differences ($P<0.05$) between experimental groups, that is statistically homogenous groups were marked with the same letter (ANOVA followed by Tukey's test).

Table 2 Effect of inhibitors of signalling pathways on H_2O_2-induced I_{sc}

	Δ I_{sc} (μEq h^{-1} cm^{-2})		
Inhibitor	*Response to H_2O_2 alone (control)*	*Response to H_2O_2 in the presence of the inhibitor*	n
Catalase	3.33 ± 0.28^a	0.32 ± 0.16^b	9
Tetrodotoxin	3.05 ± 0.30^a	2.26 ± 0.31^a	8
Indomethacin	3.18 ± 0.35^a	1.23 ± 0.22^b	8
Indomethacin + forskolin	3.93 ± 0.38^a	2.91 ± 0.37^a	6
Staurosporine	4.26 ± 0.33^a	2.27 ± 0.20^b	8
H-89	4.26 ± 0.72^a	1.92 ± 0.28^b	8
Genistein	3.88 ± 0.62^a	4.52 ± 0.63^a	8

Abbreviations: H_2O_2, hydrogen peroxide; I_{sc}, short-circuit current.
The effect of H_2O_2 (5 mmol L^{-1} at the serosal side) was first tested under control conditions (left column), and then in the presence of the putative inhibitor (right column). Between the individual administrations, the compartments, where drugs had been applied, were washed three times with 5 × the chamber volume, before H_2O_2 was administered again. Inhibitor concentrations were as follows: catalase (500 U mL^{-1} at the serosal side), genistein (5×10^{-5} at the mucosal and the serosal side), H-89 (2×10^{-5} mol L^{-1} at the mucosal and the serosal side), indomethacin (10^{-6} mol L^{-1} at the mucosal and the serosal side), indomethacin combined with forskolin (5×10^{-7} mol L^{-1} at the mucosal and the serosal side), staurosporine (10^{-6} mol L^{-1} at the serosal side), or tetrodotoxin (10^{-6} mol L^{-1} at the serosal side). Values are given as difference to the baseline in I_{sc} just before administration of H_2O_2 (Δ I_{sc}) and are means ± s.e.m. Different letters (a, b) indicate significant differences ($P<0.05$) between experimental groups, that is statistically homogenous groups were marked with the same letter (ANOVA followed by Tukey's test).

oxidant was administered twice to the same tissue with an intermediate washing step (Figure 2a; Table 1). Consequently, in all subsequent experiments, H_2O_2 was first administered in the absence of any inhibitors to obtain a control response for the individual tissue, followed by an application in the presence of a putative inhibitor.

Ionic nature of the H_2O_2-induced short-circuit current

The increase in I_{sc} induced by H_2O_2 (5 mmol L^{-1} at the serosal side) was reduced by more than 60%, when Cl^- was replaced in the buffer solution by the impermeant anion, gluconate (Table 1). A similar inhibition was achieved in the presence of bumetanide (Table 1), a blocker of the basolateral Na^+–K^+–2Cl^--cotransporter responsible for uptake of Cl^- during Cl^- secretion (Binder and Sandle, 1994), and in the presence of glibenclamide (Table 1), a blocker of the apical cystic fibrosis transmemembrane regulator (CFTR) Cl^- channel, the predominant apical Cl^- channel in the colonic epithelium involved in Cl^- secretion (Greger, 2000). Inhibition was not mimicked by application of the solvents used for solubilization of the inhibitors, that is, dimethylsulphoxide or ethanol (Table 1). Consequently, the current induced by the oxidant mainly represents Cl^- secretion.

Mediation of the H_2O_2 response

The I_{sc} evoked by H_2O_2 (5 mmol L^{-1} at the serosal side) was nearly suppressed in the presence of the enzyme catalase (500 U ml^{-1} at the serosal side; Table 2), which converts H_2O_2 into water and oxygen. Pre-incubation with tetrodotoxin, a neuronal blocker (10^{-6} mol L^{-1} at the serosal side), which inhibits the propagation of action potentials, did not significantly affect the current induced by the oxidant (Table 2), suggesting that submucosal secretomotor neurons were not involved. In contrast, a significant inhibition of the effect of the oxidant was observed in the presence of indomethacin, a cox inhibitor (10^{-6} mol l^{-1} at the mucosal and the serosal side; Figure 2b; Table 2), suggesting that prostaglandins may play a role in the induction of the secretory response. However, when the tissues were pretreated with indomethacin combined with a low concentration of forskolin (5×10^{-7} mol L^{-1} at the mucosal and the serosal side), inhibition by indomethacin was overcome (Table 2), indicating that prostaglandins do not mediate the response to H_2O_2, but that the action of the oxidant depends on the on-going release of prostaglandins within the gut wall, which keep the apical, cAMP-dependent CFTR channel in its open state (Strabel and Diener, 1995).

The CFTR channel, whose involvement in the H_2O_2 response was suggested by the sensitivity to glibenclamide, is predominantly activated by an increase in its phosphorylation state (Greger, 2000). In the human colonic tumour cell line, Caco-2, oxidants have been shown to inhibit a protein phosphatase, the serine/threonine protein phosphatase 2A, which is normally responsible for the dephosphorylation of proteins (Rao and Clayton, 2002). Therefore, we investigated whether inhibition of protein kinases might interfere with the ability of H_2O_2 to induce Cl^- secretion. In the presence of staurosporine (10^{-6} mol L^{-1} at the serosal side), which inhibits a broad range of serine/threonine protein kinases (Tamaoki *et al.*, 1986), the response to H_2O_2 (5 mmol L^{-1} at the serosal side) was significantly reduced (Table 2). The effect of staurosporine was mimicked by H-89 (2×10^{-5} mol L^{-1} at the mucosal and

the serosal side), a specific protein kinase A inhibitor (see for example, Ren *et al*., 2006). In contrast, genistein (5×10^{-5} at the mucosal and the serosal side), a protein tyrosine kinase inhibitor (Akiyama and Ogawara, 1991), was ineffecitve (Table 2). These results would be consistent with the assumption that an inhibition of serine/threonine protein phosphatase activity by H_2O_2, which would only lead to an increase in the phosphorylation status of the CFTR channel when protein kinases are active, that is in the absence of staurosporine, might be involved in the response to the oxidant (see also below, where direct effects of H_2O_2 on apical Cl^- currents were measured in basolaterally depolarized epithelia), although this assumption remains speculative without direct measurement of protein phosphatase activities.

Involvement of Ca^{2+}

In the colonic tumour cell line, T84, the anion secretion evoked by oxidants has been shown to be mediated by intracellular Ca^{2+} (Tamai *et al*., 1992). Therefore, we tested the dependence of the H_2O_2-induced I_{sc} from the presence of extracellular Ca^{2+}. As already reported by Tamai *et al*. (1991), omission of Ca^{2+} from the serosal compartment led to a significant reduction of the H_2O_2 response, by about 55% (Figure 3). Also emptying of intracellular Ca^{2+} stores with cyclopiazonic acid, a blocker of sarcoplasmic-endoplasmic reticulum ATPase (Plenge-Tellechea *et al*., 1997), caused an inhibition of similar amplitude (Figure 3). Release of Ca^{2+} from intracellular stores can be induced by IP_3 as well as by ryanodine receptors (RyRs), which are both expressed in rat colonic epithelium (Siefjediers *et al*., 2007; Prinz and Diener, 2008). In the presence of ruthenium red (5×10^{-4} mol L^{-1} at the mucosal and the serosal side), an inhibitor of RyRs (Xu *et al*., 1999), the effect of H_2O_2 (5 mmol L^{-1} at the serosal side) was inhibited by more than 80%. Also 2-aminoethoxydiphenylborate (10^{-4} mol L^{-1} at the mucosal and the serosal side), an inhibitor of IP_3 receptors (Maruyama *et al*., 1997), reduced the response to the oxidant by 33% (Figure 3), suggesting that both ryanodine and IP_3 receptors are involved.

To investigate the involvement of Ca^{2+} in the H_2O_2 response more directly, isolated colonic crypts were loaded with the Ca^{2+}-sensitive fluorescent dye, fura-2. Hydrogen peroxide (5 mmol L^{-1}) induced a prompt increase in the fura-2 fluorescence ratio ($P<0.05$, $n=218$ cells from $N=10$ crypts), indicating an increase in the cytosolic Ca^{2+} concentration (Figure 4a). In crypts pretreated with ruthenium red (5×10^{-4} mol L^{-1}), the effect of H_2O_2 was reduced by about 70%, if all cells were pooled ($n=273$; Table 3). However, the crypts clearly fell into two categories. In the first category (123 out of 273 cells from $N=6$ crypts), ruthenium red itself evoked already an increase in the fura-2 signal (Figure 4b). In these crypts, the subsequent administration of H_2O_2 induced a prompt decrease in the fura-2 signal (Figure 4b; Table 3). In the second category (150 out of 273 cells from $N=5$ crypts), ruthenium red did not affect the baseline fura-2 signal and did also not affect the response to a subsequent administration of the oxidant (Figure 4c; Table 3).

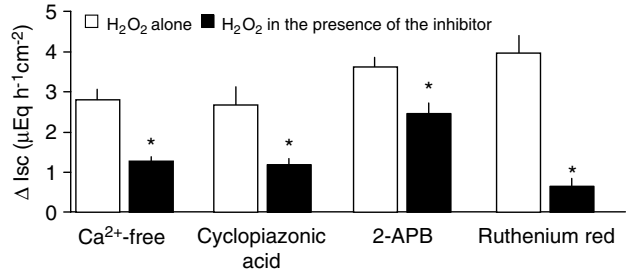

Figure 3 The effect of hydrogen peroxide (H_2O_2) (5 mmol L^{-1} at the serosal side) was first tested under control conditions, and then in the presence of the putative inhibitor. Between the individual administrations, the compartments, where drugs had been applied, were washed three times with 5× the chamber volume, before H_2O_2 was administered again. Inhibitor concentrations were as follows: 2-aminoethoxydiphenylborate (10^{-4} mol L^{-1} at the mucosal and the serosal side), cyclopiazonic acid (10^{-5} mol L^{-1} at the serosal side), ruthenium red (5×10^{-4} mol L^{-1} at the mucosal and the serosal side). For the Ca^{2+}-free buffer, Ca^{2+} was removed from the serosal side. Values are calculated as the difference from the baseline in short-circuit current (I_{sc}) just before administration of H_2O_2 (ΔI_{sc}) and are means ± s.e.m. *$P<0.05$ versus corresponding control response in the absence of the putative inhibitor (ANOVA followed by Tukey's test).

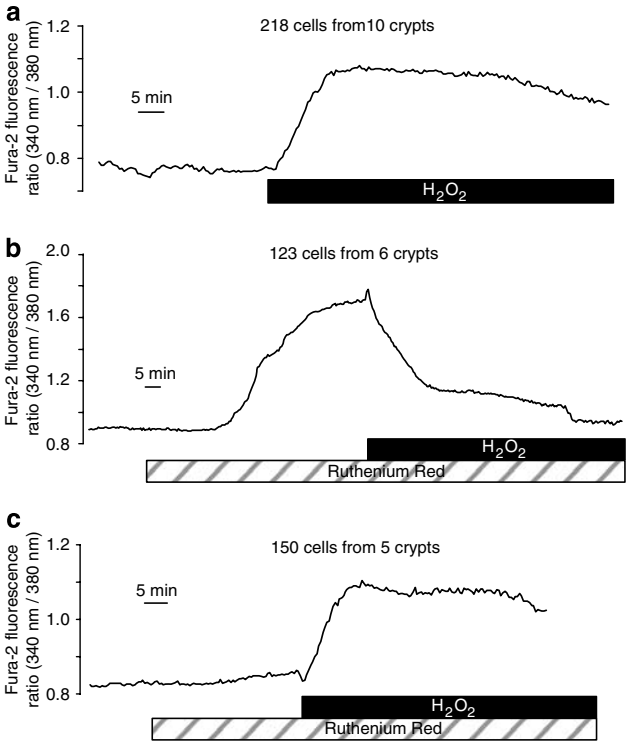

Figure 4 Effect of hydrogen peroxide (H_2O_2) (5 mmol L^{-1}) on the fura-2 ratio in the absence of any inhibitors (**a**), and in the presence of ruthenium red (5×10^{-4} mol L^{-1}; **b** and **c**). In the latter case, the cells were divided in two groups. (**b**) Cells in which ruthenium red itself induced an increase in the fura-2 ratio ($n=123$ cell from 6 crypts), and in which the subsequent administration of H_2O_2 consistently evoked a decrease in the fura-2 ratio. (**c**) Cells in which ruthenium red itself had no effect on the fura-2 ratio ($n=150$ cell from 5 other crypts), and in which the subsequent administration of H_2O_2 consistently evoked an increase in the fura-2 ratio. Typical recordings; for mean values, see Table 3.

Table 3 Effect of H_2O_2 on the fura-2 ratio signal

Inhibitor	*Response to H_2O_2 (Δ fura-2 ratio)*	n
None	0.296 ± 0.0092^a	218
Ruthenium red (all)	0.088 ± 0.048^b	273
Ruthenium red (responder)	-0.351 ± 0.026^c	123
Ruthenium red (non-responder	0.305 ± 0.016^a	150

Abbreviation: H_2O_2, hydrogen peroxide.
Effect of H_2O_2 (5 mmol L^{-1} at the serosal side) on the fura-2 ratio in the absence or presence of ruthenium red (5×10^{-4} mol L^{-1}). The latter group was further divided in two further subgroups: (a) 'responder', in which ruthenium red itself induced an increase in the fura-2 ratio and in which the subsequent administration of H_2O_2 induced a fall in the fura-2 ratio. (b) 'Non-responder', in which ruthenium red did not affect the baseline fura-2 signal and did not inhibit the response to a subsequent administration of H_2O_2. Values are given as difference to the fura-2 ratio just before administration of H_2O_2 and are means ± s.e.m. Different letters (a, b, c) indicate significant differences ($P<0.05$) between experimental groups, that is statistically homogenous groups were marked with the same letter (ANOVA followed by Tukey's test).

Action of H_2O_2 on apical Cl^- currents

To clarify the action of H_2O_2 on colonic ion transport at the apical or the basolateral membrane more directly, tissues were depolarized with a KCl buffer at the basolateral side to bypass the basolateral membrane, which is characterized by a high K^+ conductance (Fuchs *et al*., 1977). The mucosal compartment was filled with a KGluc buffer solution, so that the only ionic gradient at the apical membrane was a serosal-to-mucosal oriented Cl^- concentration gradient. Consequently, all changes in I_{sc} measured under these conditions reflect changes in the apical Cl^- conductance (Schultheiss *et al*., 2005b).

In basolaterally depolarized tissues, H_2O_2 (5 mmol L^{-1} at the serosal side) stimulated a transient Cl^- current across the apical membrane, a response which was followed by a secondary decrease in I_{sc} across the apical membrane (Figure 5a). The current stimulated by H_2O_2 was completely suppressed when the tissues were pretreated with the CFTR blocker, glibenclamide (5×10^{-4} mol L^{-1} at the mucosal side; Figure 5b; Table 4). In contrast to glibenclamide, 4-acetamido-4′-isothiocyanato-stilbene-2,2′-disulphonic acid sodium salt, a blocker of the apical Ca^{2+}-dependent Cl^- channel present in rat colonic epithelium (Schultheiss *et al*., 2005b), was ineffective (Table 4), suggesting a transient activation of a Cl^- channel of the CFTR type by the oxidant. Interestingly, in the presence of the CFTR blocker, glibenclamide, H_2O_2 caused a reduction of I_{sc} across the apical membrane (Figure 5b). As the only current, which should be visible under these conditions (KCl at the serosal side, KGluc at the mucosal side) should be a Cl^- current across apical Cl^- channels, this suggests an inhibition of basal apical Cl^- conductance by the oxidant.

As the predominant mechanism of activation of the CFTR involves phosphorylation (Greger, 2000), the protein kinase inhibitor staurosporine was applied. In the presence of this inhibitor, the activation of apical Cl^- current by H_2O_2 was significantly reduced by about 50% (Table 4). To compare the ability of the oxidant to stimulate CFTR with other secretagogues, forskolin, an activator of the adenylyl cyclase (Seamon and Daly, 1981), was used. The Cl^- current

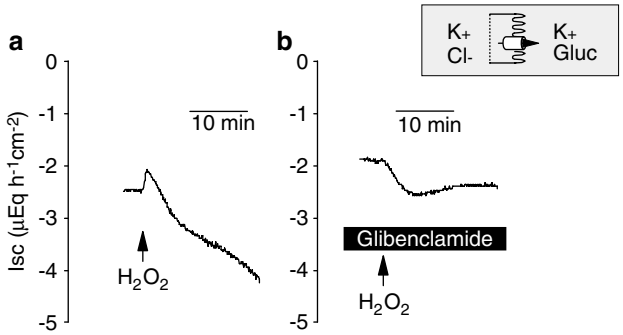

Figure 5 Effect of hydrogen peroxide (H_2O_2) (5 mmol L^{-1} at the serosal side) on Cl^- current across the apical membrane in the absence (**a**) or presence (**b**) of glibenclamide (5×10^{-4} mol L^{-1} at the mucosal side). Tissues were basolaterally depolarized in the presence of a Cl^- gradient (111.5 mmol L^{-1} KCl at the serosal side; 107 mmol L^{-1} KGluc/4.5 mmol L^{-1} KCl at the mucosal side) as indicated by the schematic inset. Typical recordings; for mean values, see Table 4.

Table 4 Effect of H_2O_2 on Cl^- currents across the apical membrane

Inhibitor	*Δ I_{sc} (μEq h^{-1} cm^{-2})*		
	Response to H_2O_2 in the presence of the inhibitor	*Response to H_2O_2 in the absence of the inhibitor*	*n*
Glibenclamide	0.02 ± 0.01*	0.29 ± 0.08	6–7
SITS	0.33 ± 0.07	0.30 ± 0.05	8
Staurosporine	0.48 ± 0.07*	0.97 ± 0.19	6–8

Abbreviations: H_2O_2, hydrogen peroxide; I_{sc}, short-circuit current; SITS, 4-acetamido-4′-isothiocyanato-stilbene-2,2′-disulphonic acid sodium salt.
Tissues were basolaterally depolarized in the presence of a Cl^- gradient (111.5 mmol L^{-1} KCl at the serosal side; 107 mmol L^{-1} KGluc/4.5 mmol L^{-1} KCl at the mucosal side). The response to H_2O_2 (5×10^{-3} mol L^{-1} at the serosal side) was tested in concomitantly performed experiments in the absence of inhibitors (right column) or in the presence (left column) of glibenclamide (5×10^{-4} mol L^{-1} at the mucosal side), SITS (10^{-3} mol L^{-1} at the mucosal side), or staurosporine (10^{-6} mol L^{-1} at the serosal side). Values are given as difference to the baseline in I_{sc} just before administration of H_2O_2 (Δ I_{sc}) and are means ± s.e.m. *$P<0.05$ versus response to H_2O_2 in the absence of the respective inhibitor.

stimulated by forskolin in basolaterally depolarized epithelia was more than four times greater than the response evoked by H_2O_2. Under these conditions, forskolin (5×10^{-6} mol L^{-1} at the mucosal and the serosal side) induced an increase in I_{sc} across the apical membrane by 2.57 ± 0.31 μEq h^{-1} cm^{-2} ($n=9$).

Action of H_2O_2 on currents across the basolateral membrane

As the predominant action of an increase in the cytosolic Ca^{2+} concentration in rat colonic epithelium consists in the activation of basolateral Ca^{2+}-dependent K^+ channels (Böhme *et al*., 1991; Bleich *et al*., 1996), which evokes a hyperpolarization of the membrane and thereby increases the driving force for Cl^- efflux across the dominant apical CFTR conductance, the effect of the oxidant on currents across the basolateral membrane was investigated in a further series of experiments. For this purpose, the apical membrane was permeabilized with the ionophore, nystatin

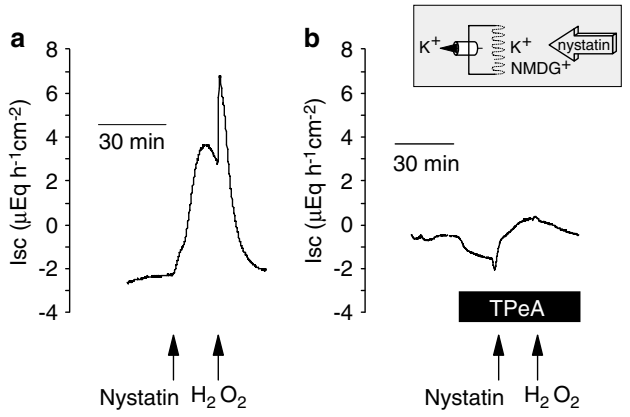

Figure 6 Effect of hydrogen peroxide (H_2O_2) (5 mmol L^{-1} at the serosal side) on K^+ current across the basolateral membrane in the absence (**a**) or presence (**b**) of tetrapentylammonium (10^{-4} mol L^{-1} at the serosal side). The apical membrane was permabilized with nystatin (100 μg mL^{-1} at the mucosal side); a current across basolateral K^+ channels was driven by a mucosal to serosal K^+ concentration gradient in the absence of Na^+ (107 mmol L^{-1} NaCl/4.5 mmol L^{-1} KCl at the serosal side, 98 mmol L^{-1} *N*-methyl-D-glucamine Cl/13.5 mmol L^{-1} KCl at the mucosal side) to avoid a contribution by currents generated by the Na^+–K^+ pump as indicated by the schematic inset. Typical recordings; for mean values, see Table 5.

Table 5 Effect of H_2O_2 on currents across the basolateral membrane

Inhibitor	*Response to H_2O_2 Δ I_{sc} (μEq h^{-1} cm^{-2})*	n
Current across basolateral K^+ channels		
None	6.02 ± 0.63	7
TPeA	1.13 ± 0.53*	6
Pump current		
None	1.93 ± 0.35	5
Scilliroside	0.23 ± 0.06*	6

Abbreviations: H_2O_2, hydrogen peroxide; I_{sc}, short-circuit current; TpeA, tetrapentylammonium chloride.
Effect of H_2O_2 (5 mmol L^{-1} at the serosal side) on currents across the basolateral membrane after permeabilization of the apical membrane with nystatin (100 μg mL^{-1} at the mucosal side) on current across basolateral K^+ channels (measured with 107 mmol L^{-1} NaCl/4.5 mmol L^{-1} KCl at the serosal side, 98 mmol L^{-1} NMDG Cl/13.5 mmol L^{-1} KCl at the mucosal side), or the Na^+–K^+ pump current (measured with 107 mmol L^{-1} NaCl/4.5 mmol L^{-1} KCl at both sides of the tissue). These currents were measured either under control conditions (no inhibitor present), or in the presence of TPeA (10^{-4} mol L^{-1} at the serosal side), or scilliroside (10^{-4} mol L^{-1} at the serosal side), respectively. Values are given as difference to the current extrapolated by linear regression from current data during the last 3 min before administration of the oxidant (Δ I_{sc}) and are means ± s.e.m. *$P<0.05$ versus the response to H_2O_2 in the absence of the inhibitor.

(100 μg mL^{-1} at the mucosal side). Two types of basolateral currents were measured. First, currents across basolateral K^+ channels were measured in the presence of an apical to basolateral oriented K^+ gradient, but in the absence of Na^+ to prevent any contribution of currents generated by the basolateral Na^+–K^+ pump (107 mmol L^{-1} NaCl/4.5 mmol L^{-1} KCl at the serosal side, 98 mmol L^{-1} *N*-methyl-D-glucamine Cl/13.5 mmol L^{-1} KCl at the mucosal side). Under these conditions, H_2O_2 (5 mmol L^{-1} at the serosal side) induced a prompt increase in I_{sc} (Figure 6a; Table 5). This current was strongly reduced after pretreatment of the tissue with tetrapentylammonium (10^{-4} mol L^{-1} at the serosal side; Figure 6b; Table 5). Tetrapentylammonium is a broad K^+ channel blocker with some preferences for Ca^{2+}-dependent K^+ channels (see Lam *et al*., 2004).

In addition, the possible action of the oxidant on the Na^+–K^+ pump was investigated. For this purpose, the apical membrane was permeabilized in the presence of Na^+, but in the absence of a K^+ gradient avoiding currents across basolateral K^+ currents due to the missing driving force (107 mmol L^{-1} NaCl/4.5 mmol L^{-1} KCl at both sides of the tissue). Under these conditions, H_2O_2 (5 mmol L^{-1} at the serosal side) induced an I_{sc} across the basolateral membrane, which was strongly inhibited by prior treatment of the tissue with scilliroside (10^{-4} mol L^{-1} at the serosal side; Table 5), which is a more potent inhibitor of the rat intestinal Na^+–K^+ pump compared to the 'classical' inhibitor, ouabain (Robinson, 1970).

Interaction with other secretagogues

Oxidants are not only known to induce intestinal secretion, but have in addition been reported to exert antisecretory actions under certain conditions (DuVall *et al*., 1998; Ohashi *et al*., 2006). Therefore, the response to secretagogues acting by the Ca^{2+}-, the cAMP- and the cGMP-pathway was tested with and without pretreatment of the tissue with H_2O_2. The cholinergic agonist, carbachol, is known to stimulate a transient Ca^{2+}-dependent Cl^- secretion (Strabel and Diener, 1995). The amplitude of the initial peak in I_{sc} evoked by carbachol was unaffected by prior treatment of the tissue with H_2O_2 (5 mmol L^{-1} at the serosal side; Table 6). However, the transient I_{sc} evoked by the cholinergic agonist showed a faster run-down after prior administration of the oxidant (visible as the current 10 min after administration of carbachol in Table 6).

To stimulate a cAMP-mediated anion secretion, forskolin (5×10^{-6} mol L^{-1} at the mucosal and the serosal side), was used. In the case of forskolin, both the initial peak as well as the long-lasting plateau in I_{sc} (given as I_{sc} 30 min after administration of forskolin in Table 6) were significantly reduced after prior administration of the oxidant (Figure 7; Table 6).

Finally, the ability of H_2O_2 to interfere with the secretion induced by CPT-cGMP, a membrane-permeable derivative of cGMP, was studied. Agonists of the cGMP-pathway induce a secretory response, which only decays very slowly with time (see Nobles *et al*., 1991). However, after pretreatment with H_2O_2 (5 mmol L^{-1} at the serosal side), the I_{sc} induced by CPT-cGMP had declined to values near zero after 30 min in contrast to the stable response in tissues, which had not been pretreated with this oxidant (Table 6).

Discussion

These results demonstrate that the oxidant, H_2O_2, induces a bumetanide- and glibenclamide-sensitive, I_{sc} across rat distal colon, which is mainly due to Cl^- secretion, and thereby confirms the basic observations of Tamai *et al*. (1991),

Table 6 Interaction of H_2O_2 with other secretagogues

Inhibitor	*Δ Isc (μEq h^{-1} cm^{-2})*		
	Response to the secretagogue after pretreatment with H_2O_2	*Response to the secretagogue without H_2O_2pretreatment*	n
Carbachol (initial peak)	11.23 ± 0.91	13.41 ± 1.36	6–8
Carbachol (10 min)	1.02 ± 0.50*	3.43 ± 1.04	
Forskolin (initial peak)	3.79 ± 0.55*	6.79 ± 1.06	6
Forskolin (30 min)	4.74 ± 0.72*	6.28 ± 0.88	
CPT-cGMP (initial peak)	0.42 ± 0.19	1.12 ± 0.34	6–7
CPT-cGMP (30 min)	0.05 ± 0.02*	1.10 ± 0.40	

The response to carbachol (5×10^{-5} mol l^{-1} at the serosal side), CPT-cGMP (10^{-4} mol l^{-1} at the serosal side), or forskolin (5×10^{-6} mol l^{-1} at the mucosal and the serosal side), was tested in the absence of H_2O_2 (right column) or after pretreatment of the tissue with H_2O_2, when the current stimulated by the oxidant had returned to baseline values. H_2O_2 (5×10^{-3} mol l^{-1} at the serosal side) alone induced an increase in Isc by 4.23 ± 0.66 μEq h^{-1} cm^{-2} in the series of experiments with carbachol ($n = 8$), 3.93 ± 0.49 μEq h^{-1} cm^{-2} in the series of experiments with CPT-cGMP ($n = 6$), and 4.91 ± 0.54 μEq h^{-1} cm^{-2} in the series of experiments with forskolin ($n = 6$). Values are given as difference to the baseline in Isc just prior administration of the respective secretagogue (Δ Isc) and are means ± s.e.m. *$P < 0.05$ versus the response to the secretagogue in the absence of the H_2O_2.

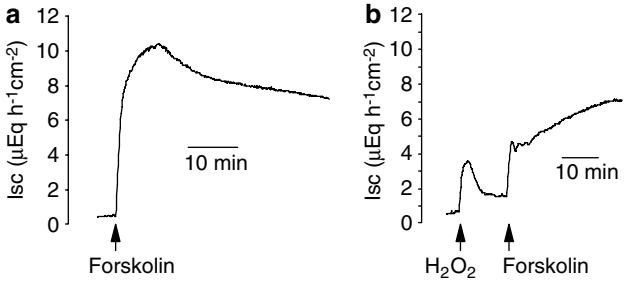

Figure 7 Effect of forskolin (5×10^{-6} mol L^{-1} at the mucosal and the serosal side) alone (**a**) or after pretreatment with hydrogen peroxide (H_2O_2) (5 mmol L^{-1} at the serosal side; (**b**). Typical recordings; for mean values, see Table 6.

although in this earlier study the action of the oxidant proved to be sensitive against the neurotoxin, tetrodotoxin, which was not observed in these experiments (Table 2). The reason for this discrepancy may lie in the fact that Tamai *et al*. (1991) performed their experiments with lower concentrations of the oxidant, where enteric secretomotor neurons may multiply the secretory response to the oxidant. However, these results, also those obtained at isolated crypts (Figure 4; Table 3), that is epithelial cells without an innervation by the enteric nervous system, clearly confirm an epithelial action site of H_2O_2, but do, of course, not exclude a possible additional effect on enteric neurons.

Several mechanisms seem to be involved in oxidant-induced Cl^- secretion. The predominant site of action, by which H_2O_2 induces a strong current response, is the basolateral membrane, where the oxidant stimulates a tetrapentylammonium-sensitive I_{sc} across basolateral K^+ channels (Figure 6; Table 5). Together with the known Ca^{2+}-dependence of the basolateral K^+ conductance (Böhme *et al*., 1991; Bleich *et al*., 1996) and the observed Ca^{2+}-dependence of the H_2O_2-induced I_{sc} (Figure 3), this current presumably represents the activation of Ca^{2+}-dependent K^+ channels, an action that H_2O_2 shares with other Ca^{2+}-dependent secretagogues such as carbachol (Strabel and Diener, 1995). Furthermore, the oxidant also stimulates a scilliroside-sensitive current generated by the Na^+–K^+ pump in this membrane. A similar phenomenon has already been observed for another oxidant, monochloramine, in this tissue (Schultheiss *et al*., 2005a).

Surprisingly, the oxidant did not stimulate a Ca^{2+}-dependent Cl^- current across the apical membrane, which can transiently be observed after administration of carbachol (Schultheiss *et al*., 2005b; Hennig *et al*., 2008). This is probably caused by the (unexpected) activation of the apical, glibenclamide-sensitive CFTR Cl^- channel by H_2O_2 demonstrated in basolaterally depolarized epithelia (Figure 5; Table 4). This response is hard to explain by the increase in the cytosolic Ca^{2+} concentration, as the predominant mode of activation of CFTR consists of a cAMP-dependent phosphorylation (Greger, 2000). However, oxidants, which are believed to play also a physiological role for the regulation of many cellular processes (Suzuki *et al*., 1997), are known to inhibit protein phosphatases (Rao and Clayton, 2002). Thus, a shift in the equilibrium between phosphorylation and dephosphorylation of the CFTR might be responsible for the observed action of H_2O_2. In contrast, in another epithelium, that is human nasal epithelial cells, the activation of Ca^{2+}-dependent Cl^- channels by oxidants has been clearly demonstrated (Jeulin *et al*., 2005).

Intracellular Ca^{2+} plays a prominent role in the anion secretion evoked by H_2O_2. This is demonstrated by the increase in the cytoplasmic Ca^{2+} concentration observed in the fura-2 experiments (Figure 4a) and by the inhibition of the H_2O_2-induced I_{sc} by manoeuvres assumed to interfere with intracellular Ca^{2+} signalling (Figure 3). For example, the sarcoplasmic-endoplasmic reticulum ATPase blocker, cyclopiazonic acid, strongly reduced the I_{sc} induced by the oxidant (Figure 3). The concomitantly observed inhibition of the H_2O_2 response in the absence of serosal Ca^{2+} does not contradict the assumption that the dominant mechanism of action of the oxidant consists of the release of Ca^{2+} from intracellular stores, as manoeuvres leading to store depletion are known to initiate a so-called capacitative Ca^{2+} inflow from the extracellular space (Parekh and Penner, 1997). The colonic epithelium possesses two main types of intracellular Ca^{2+} release channels. Independently, from their differentiation state, all enterocytes express nuclear IP_3 receptors of the subtype IP_3R2, supported by apically localized cytoplasmic IP_3R3 in the mature surface epithelial cells

(Siefjediers *et al*., 2007). All over the crypt axis, RyR of the subtype RyR1 are found, which do not obviously colocalize with the IP_3 receptors (Prinz and Diener, 2008). The strong inhibitory action of ruthenium red (and the poor effectiveness of the IP_3 receptor blocker, 2-aminoethoxydiphenylborate; Figure 3), suggests a predominant involvement of RyRs in the mediation of the H_2O_2 response. This distinguishes the effect of H_2O_2 from that of another oxidant, monochloramine, which causes a release of Ca^{2+} to which RyRs and IP_3 receptors contribute equally (Schultheiss *et al*., 2005a), a difference that might be explained by a difference in the amino-acid residues oxidized by these two types of oxidants (Prasad and Goyal, 2004).

Fura-2 experiments designed to directly investigate the presumed interaction of H_2O_2 with RyRs were not completely conclusive. In about half of the crypts tested, ruthenium red induced an increase in the fluorescence ratio, which is probably explained by the light absorbing properties of this dye (Schultheiss *et al*., 2005a). Under these conditions, the stimulatory effect of H_2O_2 on the fura-2 ratio signal, that is on the cytosolic Ca^{2+} concentration, was completely suppressed and even reversed into a paradoxical fall of the fura-2 signal (Figure 4b). A similar paradoxical fall in the fura-2 ratio signal after prevention of Ca^{2+} store release has already been observed with an other oxidant, monochloramine (Schultheiss *et al*., 2005a), which might suggest that oxidants may in addition stimulate a Ca^{2+} efflux pathway for Ca^{2+} extrusion out of the cell.

In contrast, in about the other half of the crypts tested, ruthenium red was ineffective (Figure 4c). As in these cells the dye, ruthenium red, did not affect the basal fura-2 signal and due to the striking observation that all cells in a crypt either responded to ruthenium red or not, the most plausible explanation for this variability might be that a diffusion barrier, for example a mucus layer, might have hindered the blocker to reach its site of action, that is the intracellular Ca^{2+} release channels. Another reason might be the known cell heterogeneity within the crypt, which contains enterocytes, goblet cells and enterochromaffin cells, and even within the enterocytes, there are pronounced differences within their state of differentiation, depending on the localization along the longitudinal axis of the crypt. However, if cell type heterogeneity would be the reason for the inconsistent response to ruthenium red, we should have observed divergent responses within one individual crypt, which was not the case. Nevertheless, from many cell types, redox regulation of RyRs by sulphydryl groups is well known. The result of the oxidation of these thiol groups is an activation of the receptor, a reduction leads to an inhibition of this Ca^{2+} channel (Zima *et al*., 2004). The redox state of these sulphydryl groups can alter the channel conductance as well as the open probability of the RyRs (Oba *et al*., 2002).

Beside the activation of ion transporters involved in transepithelial secretion, oxidants have been shown to exert further actions on intestinal epithelia. For example, in Caco-2 cells, oxidative stress has been shown to increase the tyrosine phosphorylation of occludin, zonula occludens-1 (ZO-1) or other components of the tight junctional apparatus and thereby to increase the paracellular permeability (Rao *et al*., 2002). The enzyme PI 3-kinase has been proposed to be involved in this process too (Sheth *et al*., 2003). Only a small increase in Gt was observed in this study, in rat colon, which was completely reversible upon wash-out of the oxidant, which makes a prominent alteration of paracellular permeability unlikely.

Reactive oxygen metabolites play a central role for leukocytes, as they use cytotoxic oxygen radicals as defence mechanisms against bacteria (Pavlick *et al*., 2002). The infiltration of the mucosa with leukocytes during inflammatory bowel diseases causes an enhanced oxidative stress in the gut wall (Tüzün *et al*., 2002). Consequently, the increase in the cytoplasmic Ca^{2+} concentration and the activation of ion transporters involved in anion secretion might contribute to the induction of a secretory diarrhoea. On the other hand, the downregulation of intestinal secretion by H_2O_2 (Figure 7; Table 6), which has already been observed at colonic tumour cells (DuVall *et al*., 1998), might limit the secretory response. One target involved in this downregulation seems to be the apical Cl^- conductance, as H_2O_2 induces a fall in I_{sc} across the apical membrane in basolaterally depolarized epithelia after the transient activation of the apical Cl^- current (Figure 5a). Hydrogen peroxide shares this property with other agonists of the Ca^{2+} signalling pathway, such as carbachol. In T_{84} cells, the mechanisms of action of this downregulation has been shown to involve the stimulation of extracellular signal-regulated kinase isoforms of mitogen-activated protein kinase (Chow *et al*., 2000; Keely and Barrett, 2003), although it is not clear, whether this model can be transferred completely to rat colonic mucosa (Schultheiss and Diener, 2005).

Taken together, these results demonstrate that the oxidant, H_2O_2, evokes intestinal anion secretion by several mechanisms of action: in the basolateral membrane, a K^+ conductance and the Na^+–K^+ pump are stimulated, whereas in the apical membrane a Cl^- conductance of the CFTR type is activated. This stimulation of anion secretion is followed by a downregulation, which may explain the observation that, in chronic colitis, responses to secretagogues are in general impaired (Kachur *et al*., 1995).

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Conflict of interest

The authors state no conflict of interest.

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