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Changed sensitivity to antigen in a gut epithelium treated with bile salts

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1 Colonic epithelia from guinea-pigs, sensitized by feeding with cow milk, responded to antigen (β-lactoglobulin) challenge when applied to the serosal, but not the mucosal, side of the tissue. The response, under short circuit conditions, was an inwardly directed current due to chloride secretion.

2 Two detergents, deoxycholate and Triton X-100, caused the basal short circuit current to decrease and transepithelial conductance to increase when applied to the mucosal surface.

3 After removing detergents from the bathing solution tissues now responded to antigen challenge from the mucosal side, without impairment of the overall response.

4 There was a correlation between the conductance change induced by detergents and the fraction of the total response which could be elicited form the mucosal side of the tissue.

5 It was concluded that models of local hypersentitivity reactions to ingested foodstuffs require both development of immunological sensitivity plus increased permeability to antigen. The role of bile salts in inducing the latter is discussed.

Introduction

Guinea-pigs given cow milk to drink develop reaginic antibodies to protein components of the milk. Systemic anaphylaxis in response to milk challenge has been demonstrated (Coombs *et al.*, 1978). Cuthbert *et al.* (1983) have described a localized hypersensitivity reaction in colonic epithelia *in vitro* from similarly sensitized animals, in which eicosanoids appear to be mediators of the response. Such epithelia, under voltage clamp conditions, respond to challenge with β-lactoglobulin (βLG) with an inward (that is, mucosal to serosal) short circuit current (SCC), due, largely, to the active secretion of chloride ions. This reaction may be associated with the diarrhoea of food intolerance.

Sensitivity to dietary antigen was apparent *in vitro* only when βLG was added to the solution bathing the serosal side of the colonic preparation, and not when added to the mucosal side. For a model of food intolerance one might expect mucosal side sensitivity to be a feature, and this was considered in a subsequent study (Kessel & Cuthbert, 1984). It was shown that ultraviolet irradiation of the mucosal surface of the tissue made the tissues sensitive to antigen applied from the apical side. It was concluded that lesioning with ultraviolet irradiation conferred apical sensitivity in this model by virtue of increased permeability to antigen.

More appropriate to the situation *in vivo*, we have studied the effects of the bile salt deoxycholate and another detergent (Triton X-100) on colonic resistance and mucosal sensitivity to antigen.

Methods

Female Dunkin-Hartley guinea-pigs were given cow milk to drink instead of water for three weeks after weaning and returned to water drinking for three days before use. Isolated epithelium was dissected from the descending colon and mounted in Ussing-type chambers (window area = 0.6 cm^2) for voltage clamping. Each surface of the tissue was bathed in Krebs-Henseleit solution (20 ml) maintained at 37°C and gassed with 95% O_2; 5% CO_2. SCC was recorded continuously as the current required to clamp the transepithelial potential at zero, using a voltage clamp (WPI DVC-1000). Transepithelial conductance was measured by commanding the membrane voltage to be clamped automatically at +2 mV and calculating conductance from the change in current, by applying the ohmic relationship.

Adjacent segments of colon were randomly designated 'test' and 'control'. Thirty minutes after the start of each experiment detergent was added to the mucosal side bathing solution of the test preparation

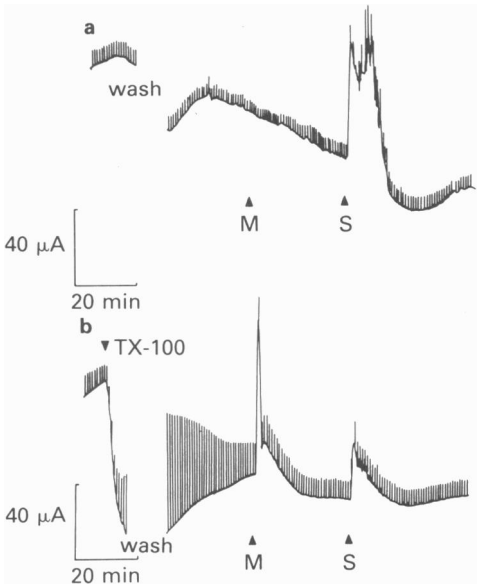

Figure 1 Effects of detergent action on sensitized colonic epithelium and short circuit current (SCC) responses to antigen. SCC recordings from adjacent segments of colon obtained from a cow milk fed guinea-pig are shown. The upward deflections indicate the change in SCC due to altering the membrane clamp voltage by 2 mV, a measure of electrical conductance. Addition of 0.05% Triton X-100 (TX-100) to the mucosal side bathing solution (b) caused SCC to fall and conductance to increase. Following wash-out, both preparations were challenged with β-lactoglobulin (βLG; 1.1×10^{-6} M), first mucosally (M) then serosally (S). In the control preparation (a), mucosal side challenge was without effect. On the detergent treated tissue mucosal side antigen challenge produced a transient inward current. Subsequent serosal side challenge of the treated preparation produce a further small inward current. The sum of the responses to both challenges in the treated preparation were the same as the magnitude of the response to serosal side challenge in the control tissue (see Table 1). The time calibration indicates the position of zero SCC.

for 10 min. Solutions bathing both sides of both test and control tissues were then changed for fresh Krebs-Henseleit solution. Thirty minutes after this solution change each preparation was challenged mucosally with βLG (1.1×10^{-6} M) and again 30 min later on the serosal side.

The transient SCC response to the antigen was expressed as the total amount of charge transferred, calculated by integration of the area under the SCC response and application of the Faraday relationship. The ratio of the response to mucosal side challenge (M) to the sum of the response to mucosal plus serosal challenge (M+S) may be used as a measure of mucosal side sensitivity (Kessel & Cuthbert, 1984).

Results

A typical experimental trace is shown in Figure 1. Triton X-100 (0.05%) applied in the mucosal side bathing solution caused SCC to fall and transepithelial conductance to increase. After washing out the detergent, SCC remained low while conductance remained elevated. Detergent treated preparations responded to mucosal side antigen challenge while control preparations showed their usual lack of sensitivity. Both types of preparation responded to subsequent addition of βLG to the serosal side bathing solution, the response in the test preparation being relatively small by comparison. Over the duration of the experiment the detergent induced changes in conductance decreased with time.

The results of three series of experiments using Triton X-100 and two concentrations of deoxycholate are given in Table 1. Both agents caused a fall in SCC and an increase in transepithelial conductance, although deoxycholate caused an initial stimulation of SCC. The SCC and conductance changes were statistically significant when compared with control values for the higher concentration of deoxycholate and for the Triton X-100 treated group, and the corresponding M/M+S ratios were also significantly increased, indicating that sensitivity to mucosal challenge was caused by detergent treatment. The lower concentration of deoxycholate did not alter SCC or conductance and was without effect on M/M+S when compared with the control group.

The concentration of βLG used throughout these

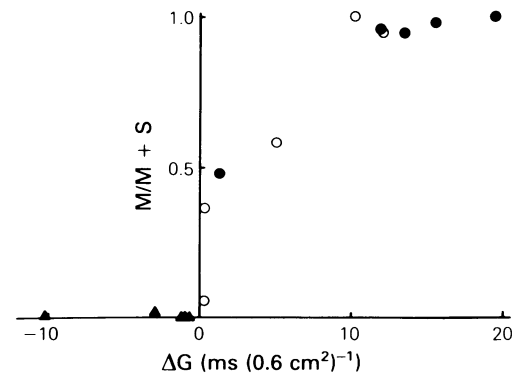

Figure 2 Relationship between detergent induced changes in electrical conductance and mucosal side sensitivity to antigen in guinea-pig colon. Mucosal side sensitivity of colonic epithelium (M/M+S) is shown *versus* the increased conductance (ΔG) due to detergent treatment. The treatments used were 0.05% Triton X-100 (○), 2.4×10^{-4} M deoxycholate (▲) and 1.2×10^{-3} M deoxycholate (●). For non-detergent treated controls, $\Delta G = -3.1 \pm 1$ ms $(0.6\,cm^2)^{-1}$; $M/M+S = 0.03 \pm 0.02$ ($n = 13$). Values are means ± s.e.

Table 1 Effects of Triton X-100 and deoxycholate on sensitized colon and the short circuit current (SCC) responses to β-lactoglobulin (βLG; 1.1×10^{-6} M)

	Initial SCC (μA)	*ΔSCC* (μA)	*Initial G* ($ms(0.6\,cm^2)^{-1}$)	*ΔG* ($ms(0.6\,cm^2)^{-1}$)	*M+S* ($\mu Eq(0.6\,cm^2)^{-1}$)	*M/M+S*
Control ($n = 13$)	55.8 ± 11.5	1.0 ± 4.5	6.4 ± 1.3	−3.1 ± 1.1	0.29 ± 0.05	0.03 ± 0.02
Triton X-100 0.05% ($n = 5$)	49.6 ± 25.2	−41.6 ± 19.7 $P < 0.01$	3.7 ± 0.7	5.5 ± 2.5 $P < 0.01$	0.24 ± 0.07	0.59 ± 0.18 $P < 0.001$
Deoxycholate 2.4×10^{-4} M ($n = 5$)	67.4 ± 8.7	−3.8 ± 5.3	7.5 ± 2.0	−3.5 ± 1.7	0.28 ± 0.06	0.01 ± 0.01
Deoxycholate 1.2×10^{-3} M ($n = 5$)	64.4 ± 8.0	−60.5 ± 9.6 $P < 0.001$	5.7 ± 1.3	12.1 ± 2.9 $P < 0.001$	0.28 ± 0.06	0.86 ± 0.11 $P < 0.001$

Initial SCC was the value immediately before detergent treatment. ΔSCC is the difference between the initial SCC and the value before the first βLG challenge, 30 min after washing out the detergent. Initial conductance (G) and ΔG values were obtained in the same way. M + S is the sum of the responses to both mucosal and serosal side challenge. M/M + S is the ratio of the response to mucosal βLG to the total response. Values given are means ± s.e., n is the number of preparations in each group. The probability values were obtained by use of unpaired Student's t test to compare the control results with each of the treated groups.

experiments was sufficient to elicit the total hypersensitivity reaction when applied to the serosal side, such that no further reaction can be obtained on subsequent challenge (Cuthbert *et al.*, 1983). Consequently the value of M + S should also be equivalent to the total response from the serosal challenge, since in control preparations virtually no reaction occurs with mucosal application. As both detergents lowered the SCC, it was important to establish that the total response to antigen was not changed. Values of M + S for the test and control groups were compared. There were no significant differences, indicating that neither the antigen-antibody reaction nor the secretory potential of the tissues were reduced by detergent action.

The relation between the M/M + S ratio and the change in conductance occurring during the 30 min before the first challenge was investigated in all preparations. Essentially control preparations became more resistive during this period, as did test preparations treated with the lower concentration of deoxycholate. The other two test groups showed both an increase in conductance and in the M/M + S ratio. In a general way, conductance changes were greater in those preparations showing the maximal M/M + S ratio of 1.0 (Figure 2).

Discussion

Electrogenic chloride secretion in response to βLG challenge to the epithelial lining of the colon of cow milk fed guinea-pigs has been demonstrated previously (Cuthbert *et al.*, 1983). Furthermore, this has been proposed to be a model of local anaphylaxis to dietary antigens; chloride secretion being the motive force for the fluid secretion which accompanies these reactions (Baird *et al.*, 1984).

The lack of response in isolated colon to mucosally applied βLG was consistent with the lack of symptoms in the cow milk drinking guinea-pigs. Although mucosal side sensitivity can be conferred by u.v. irradiation (Kessel & Cuthbert, 1984) this procedure has no natural relevance. However, it does suggest that susceptibility of this tissue to mucosal antigen requires the epithelium to be made leaky, which may be part of the gating process which triggers the intestinal symptoms of cow milk intolerance in humans.

Bile salts were used in this study as a more natural method to confer apical sensitivity. Bile salts alter several aspects of colonic function; in addition to increasing mucosal permeability by a detergent-like action (Nell *et al.*, 1975), bile salts may also induce cholerheic enteropathy (Hoffman, 1967). Excess bile salts in the colonic lumen (3 mM deoxycholate in man (Mekhjian *et al.*, 1971)) cause diarrhoea dependent upon active anion secretion mediated by cyclic AMP. Adenylate cyclase activity in rat colon is stimulated by bile salts (Binder *et al.*, 1975) which may explain the transient SCC increase caused by the higher concentration of deoxycholate used in this study. However, Binder *et al.* (1978) have produced evidence dissociating the secretion-promoting activity of bile salts from their action on colonic permeability. Al Dhahir & Zeitlin (1983) have shown that bile salts can activate colonic kallikrein and it is known too that kinins are one of the most potent stimulants of electrogenic

chloride secretion in this tissue (Cuthbert & Margolius, 1982).

In the experiments reported here it is unlikely that any stimulation of adenylate cyclase would persist for 30 min after bile salts were removed. The increased permeability and reduced SCC caused by deoxycholate and by Triton X-100 is likely to have resulted from morphological damage similar to that reported to occur when deoxycholate was applied to the lumen of rat colon (Saunders *et al.*, 1975). It is clear from the results (Figure 2) that only very small changes in conductance are required to raise M/M + S. This is consistent with the report of Kessel & Cuthbert (1984) and may be accounted for by the possibility that mediators released from immunocytes within the lamina propria amplify the response to a very limited amount of antigen.

The antigen induced secretory capacity of the colon was unaffected by these detergents as shown by the lack of effect of such treatment on the total SCC response to βLG i.e. M + S. The detergent induced mucosal side sensitivity to antigen challenge was clearly associated with changes in electrical conductance which probably reflect mucosal leakiness. It is unlikely that, in health, sufficient bile salt is made available within the colon to exert any significant detergent action. Under certain conditions of excess biliary activity or of bile malabsorption as, for example, following ileal resection, the amount of bile entering the colon may increase. This could account not only for the cholerheic diarrhoea induced by the direct secretagogue action of bile acids but may separately increase colonic permeability to allow mucosal antigen to enter the tissue and act upon immunologically sensitized cells, perhaps within the lamina propria. Thus the hypersensitivity reaction may be triggered.

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