A distinctive electrophysiological signature from the Peyer's patches of rabbit intestine

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1 Rabbit small intestinal segments containing Peyer's patches (PP) were examined in Ussing chambers using short-circuit current (I_{sc}) recording. By comparison with control small intestinal mucosal segments, rabbit PP-containing epithelia exhibited decreased basal I_{sc} , increased transepithelial resistance (TER) and unchanged potential difference (PD).

2 Carbachol caused a decrease in I_{sc} in rabbit PP epithelia. Forskolin, dibutyryl cyclic GMP, histamine and the calcium ionophore, A23187, were without effect. In contrast, control epithelial segments of rabbit intestine responded to carbachol and forskolin with an increased I_{sc} , indicative of electrogenic chloride secretion. The EC_{50} for carbachol was approximately $2\ \mu\text{M}$ in both types of epithelia. Methacholine also caused an outward current in rabbit PP epithelia which had similar properties to that of carbachol. The effect of the cholinomimetics on rabbit PP was basolateral-sided, reversible, and sensitive to low concentrations of the general muscarinic cholinergic blockers, atropine, scopolamine and also to the M_1 cholinergic blocker, pirenzepine.

3 The I_{sc} response to cholinomimetics in rabbit PP was insensitive to bumetanide, amiloride, TEA, barium, acetazolamide, piroxicam and omeprazole, but was attenuated in the presence of ouabain. Using bilaterally-substituted solutions, the carbachol effect on rabbit PP I_{sc} was abolished in chloride/bicarbonate-free, but not in chloride-free solutions, suggestive of stimulation of electrogenic bicarbonate absorption by the agent. Substitution for sodium abolished both the basal current and the I_{sc} response to carbachol. Part of the effect of carbachol on PP I_{sc} appeared to be mediated by submucosal neurones because addition of tetrodotoxin reduced the effect by 60%.

4 As microfold (M) epithelial cells predominate in the PP of the rabbit, the unusual phenotype of cholinomimetic-induced outward current may be used as an electrophysiological marker for these potential sites of oral vaccine delivery, and in particular it may also be of use as a marker for rabbit M cells.

Keywords: Rabbit Peyer's patches; Ussing chamber; carbachol; M cells

Introduction

Peyer's patches (PP) represent concentrations of lymphoid tissue which are distributed throughout mammalian intestine. Functions of these gut-associated lymphoepithelial tissues (GALT) include sampling of luminal nutrients, bacteria and viral particles. The PP are composed of a specialized follicle-associated epithelium (FAE) overlying lymphoid follicular domes (Owen & Jones, 1974). The FAE are sites of intestinal antigen presentation to T lymphocytes which in turn invaginate into the basolateral pocket of the main epithelial cell type of the FAE, i.e. the microfold or M-cell (for review, see Ho *et al.*, 1990).

In marked comparison to the absorptive/secretory columnar epithelial cells of adjoining intestinal tissue, M cells are almost devoid of lysosomes which means that intracellular proteolysis is reduced. The unusual pocket shape of these cells means that peptides only have to travel a short distance from the apical to the basolateral membrane by comparison with adjacent columnar epithelia. Thus intact macromolecules may be delivered across the epithelial barrier. Furthermore, absorbed antigen is also presented to the thoracic duct of the lymphatic system and thus can avoid the first pass effect caused by drainage into the hepatic portal vein (Kreuter, 1991).

PP of the intestine are a potential target site for peptide absorption (Keljo & Hamilton, 1983; Ducroc *et al.*, 1983). Recent research has focused on specific targeting processes for peptides with the PP. Particulate uptake by rabbit PP was demonstrated to be efficient for negatively charged liposomes (Tomizawa *et al.*, 1993), and also for latex microparticles

(Lefevre *et al.*, 1985; Pappo & Ermak, 1989; Jepson *et al.*, 1993), the site appearing to be localized to the M-cell. Indeed there is some evidence that nanoparticles are specifically absorbed by porcine PP using endocytosis (Scherer *et al.*, 1993). While the absorptive capacity of PP may be low even for particles of small size and ideal polymer composition (Ebel, 1990), such a property could be preferable for localized vaccine delivery rather than for delivery of peptides. Recently, M cells were stimulated to secrete monoclonal IgA into the intestine in order to bind a single surface epitope on enteric pathogens and thereby to provide localized immunity (Neutra & Kraehenbuhl, 1993).

Although the epithelium of PP is structurally continuous with the epithelial cells which regulate absorption and secretion of electrolytes and water, there is little information regarding the ion transport characteristics of these specialised structures. The present study explores features of PP-containing intestinal segments by comparing their ion transport parameters with that of adjacent normal epithelia. Tissues obtained from rabbit were examined by the short-circuit current method in a classic Ussing chamber. An unusual distinctive electrophysiological response to carbachol is described. The response may derive from M cells, the predominant cell type of rabbit PP (Pappo *et al.*, 1988).

Methods

Isolation of PP segments from rabbit small intestine

Male and female New Zealand White rabbits (2–3 kg) were killed by intravenous pentobarbitone sodium injection (150

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mg kg⁻¹). The intestine from duodenum to terminal ileum was removed and placed in oxygenated Krebs-Henseleit solution. Fresh solution was used to irrigate the lumen in order to remove debris. Up to 10 PP were located in the region from jejunum to terminal ileum. The area of the rabbit PP used for studies was approximately 1.2 cm². Upon location of the patch, the intestinal segment was slipped over plastic pipettes where the PP was clearly delineated against the background of a light source. The gut was opened along the line of mesentery attachment in order that the PP remained entire. PP-containing segments and adjacent epithelial (control) segments of epithelia were stripped of associated smooth muscle (Cuthbert & Margolius, 1982).

Short-circuit current recording in Ussing chamber

Rabbit epithelium with or without PP were mounted in Ussing chambers of window areas of 1.13 cm². Measurements of potential difference (PD) (apical side negative), trans-epithelial resistance (TER) and I_{sc} were made as described previously (Cuthbert & Margolius, 1982) using a DVC 1000 Dual Voltage Clamp (World Precision Instruments, U.K.). The voltage was clamped intermittently to 1 mV and the current required was used to calculate TER by applying the Ohmic relationship.

Solutions

The Krebs-Henseleit (KH) solution used to bath the tissues had the following composition (mM): NaCl 117, KCl 4.7, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25, CaCl₂ 2.5 and glucose 11.1. The pH was 7.4 when equilibrated with a mixture of 95% O₂:5% CO₂. Chloride-free KH contained (mM): sodium gluconate 117, potassium gluconate 4.7, and CaSO₄ 2.5, as substitutes for NaCl, KCl and CaCl₂ respectively, and was equilibrated with 95% O₂:5% CO₂ at a pH of 7.4. Chloride/bicarbonate-free KH contained these substitutions and in addition HEPES acid 10, and Tris base 10, in place of NaHCO₃. This solution was bubbled with 95% O₂ which maintained pH at 7.4. Sodium-free KH contained (mM): N-methyl D-glucamine chloride (NMDG) 117, HEPES acid 10, Tris base 10, KCl 4.7, MgCl₂ 1.2, KH₂PO₄ 1.2, CaCl₂ 2.5 and glucose 11.1. The pH was 7.4 when equilibrated with O₂. All solutions were within an osmolarity range of 270–290 mOsmol l⁻¹.

Drugs and chemicals

Forskolin and A23187 were obtained from Calbiochem. Carbachol, methacholine, scopolamine, tetrodotoxin, histamine, dibutyl cyclic GMP, amiloride, bumetanide, pirenzepine, tetraethylammonium (TEA), histamine, ouabain, piroxicam and barium chloride were obtained from the Sigma Chemical Co (UK). Diamox-TM (acetazolamide) was supplied by Lederle Labs (UK). Omeprazole was a gift from Astra-Hassle (Sweden). All other chemicals were reagent grade. Drugs were added in a volume that comprised no more than 1% of the total volume of the Ussing chamber/glass reservoirs.

Statistics

Student's *t* test (paired or unpaired as applicable) was used to test for significance. $P < 0.05$ was considered significant.

Results

Basal electrical parameters of rabbit PP epithelia

The basal parameters of PP epithelia compared to control adjacent segments are given in Table 1. There was a statis-

Table 1 Basal electrophysiological parameters from rabbit PP-containing epithelia and intestinal controls

	PP	Control
TER (Ω cm ²)	137.7 $\pm$ 7.9 (37)*	26.9 $\pm$ 3.0 (10)
PD (mV)	-2.0 $\pm$ 0.2 (37)	-2.5 $\pm$ 0.4 (10)
I_{sc} (μ A cm ⁻²)	16.5 $\pm$ 1.5 (37)*	90.2 $\pm$ 8.6 (10)

Numbers in parentheses indicate number of segments examined.

* $P < 0.001$, unpaired *t* test. Values are mean $\pm$ s.e.mean 30 min after mounting in the Ussing chamber. PP = Peyer's patch epithelium. Numbers in parentheses are segments taken from 12 rabbits for PP and 6 rabbits for control intestines.

PD = potential difference; TER = transepithelial resistance.

tically higher TER and statistically lower basal I_{sc} in PP epithelia while the PD was similar to controls.

Pharmacological responses in rabbit PP epithelia

Basolateral additions of carbachol caused a concentration-dependent decrease in I_{sc} in voltage-clamped rabbit PP (Figure 1a). Such an effect may be due to stimulation of outward current (cation secretion and/or anion absorption) or, alternatively, inhibition of inward current (anion secretion and/or cation absorption). The mean net reduction in I_{sc} in response to 10 μ M carbachol was $-5.8 \pm 0.6 \mu$ A cm⁻² ($n = 32$).

In contrast, carbachol stimulated a predominant inward I_{sc} in control rabbit intestine (Figure 1c). The I_{sc} increase in response to 10 μ M carbachol was $34.9 \pm 5.5 \mu$ A cm⁻² ($n = 8$, $P < 0.001$). Responses to carbachol were biphasic, in control preparations; a transient reduction in I_{sc} followed by a sustained net I_{sc} increase (Figure 1c). From the concentration-response curves (Figure 1b,d) the EC₅₀ values were not different (approximately 2 μ M; $n = 4$ in each case).

Responses to carbachol in rabbit PP preparations were basolateral-sided; no effects were observed after apical additions. A second response could be achieved in the same preparation after washing (Figure 2a). Atropine pretreatment (0.1 μ M; added basolaterally) virtually abolished the carbachol effect on I_{sc} . When added at the plateau of the I_{sc} response to carbachol, atropine caused the current to return to basal levels ($n = 6$, see Figure 1a,c). Similar results were obtained with scopolamine ($n = 3$, Figure 2a). Methacholine (MCh; 10 μ M; basolateral) mimicked the response of rabbit PP tissue to carbachol. The reduction in I_{sc} produced by MCh was reversed by pirenzepine, an M₁-selective muscarinic receptor antagonist (Figure 2b).

Histamine (10 μ M) and A23187 (1 μ M), which are shown to elevate intracellular calcium and to increase I_{sc} in intestinal epithelial cells (Wasserman *et al.*, 1988; Wong *et al.*, 1989), were without effect on I_{sc} in rabbit PP epithelia ($n = 3$ for each agent). In the presence of histamine and A23187, responses to carbachol in rabbit PP were not different from untreated controls ($n = 3$, results not shown). Results from these preliminary experiments indicate that elevation of intracellular free calcium does not contribute to cholinomimetic-stimulated outward current in rabbit PP.

Forskolin (10 μ M) had a modest effect on I_{sc} , giving a net mean increase of $1.6 \pm 0.5 \mu$ A cm⁻² in rabbit PP ($n = 6$). By comparison, forskolin stimulated a net I_{sc} increase of $57.0 \pm 12.8 \mu$ A cm⁻² ($n = 6$, $P < 0.001$) in control rabbit intestine (Figure 3). In the presence of forskolin the characteristic carbachol response was maintained in both types of tissue.

Dibutyl cyclic GMP (1 mM) added bilaterally to voltage-clamped rabbit PP was without effect ($n = 3$). Moreover, I_{sc} responses to carbachol were not modified in the presence of

Figure 1 Short-circuit current responses of rabbit PP and intestinal epithelia to carbachol (CCh). In (a) and (c) CCh was added in log concentration steps from 0.01 to 100 μM to a PP and control intestinal epithelium respectively. The horizontal bar indicates zero μA in each case. The plot of percentage CCh-sensitive I_{sc} remaining versus concentration is shown for PP (b) and the plot of percentage maximum CCh-sensitive I_{sc} versus concentration is shown for control epithelia (d). $n = 4$ experiments for each plot. The EC_{50} for CCh was approximately 2 μM in each preparation. Note that the outward current was reversed by atropine (Atr, 0.1 μM) in PP epithelium (a) and that the inward current was reversed by atropine in control rabbit intestine (c).

Figure 2 Carbachol I_{sc} responses in rabbit PP epithelia are sided and reversible. In (a) carbachol was added first to the apical (Ap) side without result and then to the basolateral side (Bl) where a decrease in I_{sc} was noted. Upon washing (W), the response to basolateral additions of carbachol was re-established and was inhibited by scopolamine (Scop, 0.1 μM). Example is typical of 4 experiments. In (b) a response is shown to basolateral addition of methacholine (MCh, 10 μM), followed by a reversing of the response with pirenzepine (Pzp, 0.1 μM), also added to the basolateral side. Example is typical of 4 experiments. Horizontal lines indicate zero μA .

dibutyryl cyclic GMP, suggesting that cyclic GMP plays little part in ion transport responses to cholinergic agonists (Figure 4). In contrast, in the rabbit small intestine cyclic GMP is well-known as the powerful intracellular mediator of the electrogenic chloride secretory action stimulated by heat stable enterotoxin (Field *et al.*, 1978).

Ionic basis of the basal I_{sc}

The following inhibitors were without effect on basal I_{sc} in rabbit PP tissue: bumetanide (200 μM), amiloride (0.1 mM), barium (3 mM), tetraethyl ammonium chloride (3 mM), piroxicam (10 μM), ouabain (1 μM) and omeprazole (0.1 mM). At least 4 separate experiments were carried out using bilateral additions of each agent. The only manoeuvre tested which abolished or reduced the basal I_{sc} was replacement of KH with sodium-free solution. In 6 paired experiments sodium-free solution abolished basal I_{sc} , reduced PD and increased TER with respect to controls (Table 2).

Ionic basis of the carbachol-stimulated I_{sc} in rabbit PP

The carbachol-evoked outward current was not altered by pretreatment with bumetanide (200 μM ; serosal) or amiloride (0.1 mM; mucosal) which inhibit the Na/K/2Cl cotransporter and the Na/H exchanger respectively. Bilateral addition of the potassium channel blockers, barium (3 mM) or tetraethyl ammonium chloride (3 mM), was without effect on carbachol responses in rabbit PP. The results with these inhibitors are summarized in Table 3.

In 6 paired preparations from 6 different rabbits, serosal incubation with 1 μM ouabain for 30 min inhibited by 60% the outward current stimulated by carbachol in rabbit PP. The mean control value for the CCh-stimulated I_{sc} was $-3.6 \pm 0.7 \mu\text{A cm}^{-2}$ while the mean value for the ouabain-

Figure 3 I_{sc} responses of rabbit PP and control intestinal epithelia to forskolin. In (a) and (b) forskolin (F, 10 μM) was added to the basolateral side of PP and control epithelia respectively. The responses for forskolin are pooled in (c), where $P < 0.002$ ($n = 6$). In each case carbachol (CCh, 10 μM) was added as a second agent in order to demonstrate pharmacological responsiveness and to define the tissue segment. The horizontal line indicates zero μA for recording (a).

Figure 4 Absence of dibutyryl-cyclic GMP I_{sc} response in rabbit PP epithelium. 1 mM dibutyryl-cyclic GMP (cGMP) was added bilaterally followed by basolateral addition of carbachol (CCh, 10 μM) and atropine (Atr, 0.1 μM). The presence of cyclic GMP had no effect on the subsequent I_{sc} responses to carbachol.

pretreated tissues was $-1.1 \pm 0.77 \mu\text{A cm}^{-2}$ ($P < 0.05$). Ion substitution experiments were performed in order to help identify the charge-carrying ion(s) responsible for the carbachol-stimulated I_{sc} . Replacement of chloride in the bath solutions had no effect on carbachol responses (Table 4) confirming the result with bumetanide. In contrast, responses were inhibited by 50% in chloride/bicarbonate-free KH ($P < 0.001$). TER values were maintained throughout chloride/bicarbonate substitution experiments, indicating that the integrity of the tissue was not compromised. Likewise, there were no statistical differences in basal electrical parameters in these substituted solutions. The large effect of chloride/bicarbonate-free KH on the carbachol response implied that bicarbonate absorption may contribute to the outward I_{sc} . Incubation with acetazolamide (0.45 mM) in either KH or in chloride-free KH was without effect on responses to subsequent challenge with carbachol ($n = 4$, paired in each case). In 6 paired preparations from 6 rabbits, sodium-free solution caused abolition of subsequent responses to carbachol (Table 2). The increased TER values obtained in sodium-free solution indicated that the tissues were still viable.

Incubation with the inhibitor of Na/H ATPase, omeprazole (0.1 mM), was without effect on subsequent I_{sc} responses to carbachol in rabbit PP. In 4 paired preparations the mean responses were $-4.2 \pm 0.9 \mu\text{A cm}^{-2}$ (Control) and $-3.6 \pm 1.0 \mu\text{A cm}^{-2}$ (Test). These values were not statistically different. The indirect evidence thus indicates that bicarbonate absorption stimulated by carbachol does not appear to be linked to proton secretion.

The possibility that prostaglandins might be involved in

Table 2 Effects of sodium-free solution on electrophysiological parameters from rabbit PP-containing epithelia and intestinal controls

	KH	Na-free solution
TER ($\Omega \text{ cm}^2$)	168.0 ± 7.8 (6)	265.2 ± 31.3 (6)*
PD (mV)	-1.1 ± 0.2 (6)	0.0 ± 0.1 (6)**
I_{sc} ($\mu\text{A cm}^{-2}$)	11.3 ± 3.3 (6)	0.6 ± 0.8 (6)*
ΔI_{sc} after CCh	-3.6 ± 0.7 (6)	0.0 ± 0.0 (6)**

Numbers in parentheses indicate number of segments examined.

For comparison each PP came from a different rabbit and was paired. * $P < 0.05$, ** $P < 0.005$, paired t test. Basal values are mean $\pm$ s.e.mean 30 min after mounting in the chamber. TER = transepithelial resistance; PD = potential difference.

the mediation of the CCh-stimulated I_{sc} was investigated by preincubation of 6 paired PP tissues from 6 rabbits with piroxicam (10 μM) for 30 min. The mean control value for the CCh-stimulated I_{sc} was $-3.6 \pm 0.7 \mu\text{A cm}^{-2}$ while the mean value for the piroxicam-pretreated tissues was $-4.0 \pm 1.2 \mu\text{A cm}^{-2}$ (NS).

Neuronal involvement in the carbachol response in rabbit PP

Acetylcholine stimulates chloride secretion in small intestinal epithelium of rabbit both by a direct effect on intestinal epithelial tissues and also by an indirect effect mediated by cholinergic neurones in the lamina propria (Diener *et al.*, 1988). In rabbit PP, tetrodotoxin, an inhibitor of neuronal sodium conductance, reduced the carbachol stimulated outward current on average by 60.1% in 5 paired experiments on adjacent patches from the same animal for each experiment (Figure 5a–c). Thus, the cholinergic response can be estimated as 39.9% due to a direct effect on the patch-containing epithelium. These results are comparable with those of Diener *et al.* (1988), in which tetrodotoxin also substantially reduced the I_{sc} response in rabbit intestine to a number of secretagogues. The effects of tetrodotoxin were not reversible. However, unchanged TER measurements indicated that attenuation of the I_{sc} response to carbachol was not due to toxicity.

Table 3 The effects of ion transport inhibitors on the I_{sc} response to carbachol in rabbit PP epithelia

	ΔI_{sc} in response to CCh
Control	$-5.78 \pm 0.56 \mu A cm^{-2}$ (32)
Bumetanide	$-7.05 \pm 0.78 \mu A cm^{-2}$ (4)
Amiloride	$-6.46 \pm 0.77 \mu A cm^{-2}$ (3)
Barium	$-5.90 \pm 0.65 \mu A cm^{-2}$ (3)
TEA	$-5.56 \pm 0.40 \mu A cm^{-2}$ (3)
Ouabain	$-1.11 \pm 0.77 \mu A cm^{-2}$ (6)*

Numbers in parentheses indicate number of segments examined. Values are mean $\pm$ s.e. mean for the peak reduction in I_{sc} 2 min after addition of $10 \mu M$ carbachol. The concentrations and side of addition of the inhibitors are given in the text. Inhibitors were present for at least 15 min before addition of carbachol. * $P < 0.05$, unpaired.

Table 4 The effects of substituted Krebs-Henseleit solutions on the I_{sc} response to carbachol in rabbit PP epithelia

	ΔI_{sc} after CCh
Normal KH	$-6.20 \pm 0.90 \mu A cm^{-2}$ (16)
Cl-free KH	$-6.94 \pm 0.82 \mu A cm^{-2}$ (10)
Cl/HCO ₃ -free KH	$-1.01 \pm 0.56 \mu A cm^{-2}$ (10)*
Na-free KH	$0.00 \pm 0.00 \mu A cm^{-2}$ (6)*

Numbers in parentheses indicate number of segments examined. ** $P < 0.005$, unpaired t test. Values are mean $\pm$ s.e. mean for the peak reduction in I_{sc} 2 min after addition of $10 \mu M$ carbachol.

Discussion

The objective of this investigation was to compare basal and stimulated electrophysiological parameters of PP-derived intestinal epithelial tissues from the rabbit with adjacent, histologically normal intestinal controls. To our knowledge this is the first study describing transepithelial ion transport processes in PP-containing epithelium of the intestine. The basal electrical characteristics of control rabbit small intestine compare favourably with those obtained in previous studies (Smith & Field, 1980), respectively. Measurements made with PP tissues indicated that the basal I_{sc} was lower, and the TER was higher than the corresponding controls. Even with the higher TER, the PP regions should be classified as leaky epithelia (Frizzell & Schultz, 1972). The lower basal I_{sc} in the PP may be a consequence of activity of a smaller number of columnar epithelial cells which are identical to those in adjacent (control) tissue or, alternatively, of distinct populations of cells which contribute to basal I_{sc} in PP. Of the range of inhibitors used, amiloride, bumetanide, barium, TEA, piroxicam or ouabain all failed to inhibit the basal I_{sc} . Replacement of sodium abolished basal I_{sc} and increased TER, indicating that maintenance of the current is sodium-dependent and not due to voltage offset.

In response to established secretagogues, control ileal tissues behaved in a predictable manner with inward current responses to carbachol, A23187 and histamine, which were accounted for by electrogenic chloride secretion as previously reported (Bolton & Field, 1977; Frizzell, 1977). During treatment of PP epithelia with cholinomimetics we discovered a characteristic outward I_{sc} in response to carbachol and methacholine. No inward current was detected. These cholinomimetics caused a sided, reversible, receptor-dependent decrease in I_{sc} , which may or may not be identical to the cholinergic-induced transient outward I_{sc} reported in mouse colonic studies (Sagmanligil & Levin, 1993). Of importance is that carbachol caused a predominantly inward I_{sc} in control intestine, as reported here and in the latter study.

Figure 5 Tetrodotoxin inhibited the carbachol-stimulated outward current in rabbit PP epithelium. In (a) a typical PP response to carbachol (CCh, $10 \mu M$) is shown. In (b) a PP-containing epithelium from the same segment of the same rabbit was pretreated with tetrodotoxin (TTX, $1 \mu M$ basolateral) followed by CCh. In (c) the pooled responses are shown from 5 paired preparations using the same protocol. $P < 0.02$, paired.

The carbachol response in rabbit PP did not appear to be mediated by calcium because the I_{sc} was unresponsive to A23187 and to histamine, agents known to increase intracellular Ca^{2+} in intestinal epithelia (Wong *et al.*, 1989; Wasserman *et al.*, 1988). Another likely candidate as mediator of the electrogenic process in response to muscarinic activation is cyclic GMP. Unlike carbachol, the dibutyl ester of cyclic GMP had no effect on I_{sc} and, moreover, did not prevent subsequent responses to carbachol. Previous studies on T84 cells showed that occlusivity in the I_{sc} response demonstrates the presence of a common intracellular pathway, while synergy indicates separate intracellular pools (Cartwright *et al.*, 1985; Brayden *et al.*, 1993). According to this model, the lack of occlusivity between cyclic GMP and the carbachol response (and the lack of effect of cyclic GMP on I_{sc} *per se*) indicates that cyclic GMP is not involved in the action of carbachol in rabbit PP epithelia.

Ouabain inhibition of the outward I_{sc} response in PP implicated participation of the Na/K ATPase on the basolateral membrane. Electrogenic chloride transport processes did not contribute to the I_{sc} response to carbachol in

PP as shown by the failure of serosal bumetanide and of chloride-free solution to alter the response. Sodium transport did not contribute to the outward I_{sc} , as shown by the lack of effect of mucosal amiloride in concentrations high enough to inhibit Na/H exchange. The inhibition of the outward I_{sc} by sodium-free solutions is difficult to interpret since the Na/K ATPase may also be blocked under such conditions. Electrogenic potassium absorption did not make a major contribution as seen by the lack of effect of mM concentrations of serosal barium and TEA.

When chloride/bicarbonate-free solution was substituted for KH, the carbachol-induced I_{sc} was indeed reduced. As the basal electrical parameters were similar in the two solutions, it seems that the reduced effect of carbachol in the substituted solution was specifically related to the absence of bicarbonate and not to loss of cellular integrity. Bicarbonate substitution alone was not investigated since this leaves the possibility of the stressed tissue reversibly switching to chloride transport, as has been shown already for rabbit colon in the case of adenosine (Grasl & Turnheim, 1984). However, as acetazolamide was without effect on the outward current, our working hypothesis is that carbachol stimulates bicarbonate absorption which is carbonic anhydrase-independent. It is known that carbonic anhydrase inhibitors do not alter transport of bicarbonate derived from metabolism (Hopfer & Liedtke, 1987). Similarly, the lack of effect of omeprazole suggests the bicarbonate absorption stimulated by carbachol in PP appears to be proton-independent. The assumption was made that these inhibitors can reach their enzyme binding sites in this system.

Tetrodotoxin, which blocks neuronal sodium channels but does not alter electrogenic chloride secretion in intestinal epithelia (Hubel, 1978), attenuated the outward current response to carbachol in PP. Thus, carbachol may have distinct, additive indirect actions on mucosal nerves and direct effects on epithelial cells (Diener *et al.*, 1988). TTX-sensitive carbachol-induced outward I_{sc} has been reported in colon

from rat (Nzegwu & Levin, 1990) and mouse (Sagmanligil & Levin, 1993).

In this study, the neuronal component may mask direct epithelial effects of carbachol. In the presence of tetrodotoxin the residual response to carbachol was only $2-3 \mu A cm^{-2}$, not enough upon which to carry out quantitative research. Thus, we cannot discern what degree of muscarinic receptor involvement in the carbachol response is divided between the PP epithelial cells and the pre- and postsynaptic myenteric neurones.

The majority of cells in PP of the rabbit are of the M cell type (Pappo *et al.*, 1988). Features include truncated microvilli on the apical membrane, low levels of intracellular lysosomes (Owen, 1977) and high levels of vimentin (Jepson *et al.*, 1992). M cells are ideal candidates for oral peptide targeting because they appear to have bioadhesive properties for peptide-encasing polymers, namely latex microspheres (Lefèvre *et al.*, 1985; Pappo & Ermak, 1989), and cyanoacrylate nanoparticles (Scherer *et al.*, 1993). More importantly, the basolateral side of the M cells opens to the lymphatic system rather than to the hepatic portal vein. As PP make up as much as 1% of the total absorptive area of the intestine of the rabbit, it would be useful to specifically target the M cells using markers such as negative staining for alkaline phosphatase (Owen & Bhalla, 1983), mannan coated microspheres (Tomizawa *et al.*, 1993) or with monoclonal antibodies to apical membrane surface antigens (Pappo *et al.*, 1991).

Many of the characteristics of rabbit PP appear to have common features with those of human PP (Cornes, 1965). It is not unlikely that the carbachol response located in rabbit PP in this study may be used as new electrophysiological marker for the M cell. The carbachol-stimulated outward I_{sc} may be of use as a marker for characterization of primary cultures of subtypes of cells which make up the PP epithelium.

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