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Involvement of Prostaglandins in Histamine-induced Fluid and Electrolyte Secretion by Rat Colon

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Abstract—Histamine increased the transmural potential difference across rat colon in-vivo and induced a net secretion of fluid. Both effects were inhibited by indomethacin. Histamine increased the potential difference and short-circuit current, and reduced tissue resistance in colonic sheets in-vitro. This response was reduced in the absence of chloride in the bathing medium or in the presence of serosal frusemide, suggesting that histamine stimulated electrogenic chloride secretion by the colon. The rise in short-circuit current induced by histamine was calcium-dependent since it was reduced in the absence of serosal calcium or in the presence of serosal verapamil. Indomethacin, a cyclo-oxygenase inhibitor, and mepacrine, a phospholipase inhibitor, both caused a dose-dependent inhibition of the electrical response of colonic sheets to histamine, without affecting the rise in short-circuit current induced by prostaglandin E_2. The stimulation of chloride secretion induced by histamine in rat colon therefore appears to be mediated by an increased production of prostaglandins.

Electrolyte transport across the colon is known to be influenced by a wide variety of endogenous agents and many of these stimulate the electrogenic secretion of chloride ions into the lumen (Frizzell & Schultz 1979). One substance that is found in significant quantities in the gastrointestinal tract is histamine (Lorenz et al 1973) and this has been shown recently to increase colonic chloride secretion (McCabe & Smith 1984). In other tissues it has been demonstrated that histamine does not act directly, but exerts its effects via the increased production of prostaglandins (Laekeman & Herman 1978; Juan & Sametz 1980), and this also seems to be the case in the small intestine (Hardcastle & Hardcastle 1987). The aim of the present investigation was to assess whether an increased eicosanoid production might mediate the actions of histamine in the colon.

Materials and Methods

Male albino rats, 230–250 g, obtained from the Sheffield Field Laboratories were allowed free access to food (diet 86, Oxoid, London) and water. They were anaesthetized with sodium pentobarbitone (60 mg kg^{-1} i.p.)

Measurement of the transcolonic potential difference in-vivo
In anaesthetized rats, a 2–3 cm segment of proximal colon was isolated by tying it off at the distal end and inserting a cannula at the proximal end. NaCl (154 mM) was added to the loop and the peritoneal cavity, and the potential difference (p.d.) across the wall of the loop was measured using salt bridge electrodes, one in contact with the luminal fluid and the other in contact, via a wick electrode, with the peritoneal fluid. These electrodes were connected via calomel half cells to a differential input electrometer whose output was displayed on a Vitatron chart recorder (MSE Scientific Instruments, 2001 series). A tracheal cannula was inserted and the jugular vein was cannulated for the administration of drugs, each dose of which was washed in with 0·2 mL 154 mM NaCl.

Measurement of colonic fluid transport in-vivo
Fluid movement in-vivo was determined using a modification of the enteropooling assay (Robert et al 1976). The entire colon was washed out with warm 154 mM NaCl and any remaining fluid gently blown out. The distal end of the colon was tied off and approximately 0·5 mL 154 mM NaCl was added to the lumen from a syringe that was weighed before and after the addition. The proximal end of the colon was then tied. After 15 min the colon was removed from the animal and weighed before and after the contents were drained. Fluid transport was taken as the difference between the volume of fluid added to the segment and that recovered from it at the end of the incubation and this was related to the weight of the empty loop. It was expressed as $\mu L\ g^{-1}$ wet weight/15 min. Histamine was administered intraperitoneally at a dose of $1{\cdot}3 \times 10^{-4}$ mol kg^{-1} while control animals received an equivalent volume (2 mL kg^{-1}) of 154 mM NaCl. Indomethacin was administered subcutaneously at a dose of $2{\cdot}6 \times 10^{-5}$ mol kg^{-1} 10 min before the incubation began. Control animals received 1·6 mL kg^{-1} of the vehicle (1 part ethanol:9 parts 0·2% Na_2CO_3). The effect of histamine was calculated by subtracting the mean basal fluid movement from each value obtained in the presence of the amine.

Measurement of the electrical activity of colonic sheets
The potential difference (p.d.), short-circuit (s.c.c.) and resistance (R) were measured in sheets of proximal colon from which the muscle layers had been removed. These were clamped between two Perspex chambers with an exposed tissue area of 1·925 cm^2 and incubated at 37°C in Krebs bicarbonate saline (Krebs & Henseleit 1932), gassed with 95% O_2/5% CO_2. The serosal solution contained 10 mM glucose and the mucosal solution 10 mM mannitol, and each had a volume of 5 mL. The p.d. was measured using salt bridge electrodes connected via calomel half cells to a differential input electrometer. Current was applied across

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the tissue by Ag/AgCl electrodes which made contact with mucosal and serosal solutions via wide-bore salt bridges. When the tissue was short-circuited, a correction was made for the resistance of the medium as described by Field et al (1971). R was calculated from p.d. and s.c.c. measurements using Ohm's Law.

Chloride-free conditions were achieved by replacing all the chloride in the medium with sulphate and maintaining isotonicity with mannitol. The effect of calcium lack was tested by omitting all the calcium from the serosal solution and adding 0·5 mM EGTA to remove interstitial calcium.

The tissues were left to stabilize for 10 min after setting up, by which time the indices of electrical activity had reached steady values. Basal readings were then recorded every min for 5 min, histamine was added and readings taken for a further 10 min. The response to histamine was calculated as the difference between the maximum value obtained in the presence of the amine and the value immediately before its addition. Inhibitors were added to the test sheet as soon as it was set up, while control sheets received an equivalent volume of the appropriate vehicle. The response to 10^{-4}M histamine was unaffected by either dimethylsulphoxide, which was used to dissolve frusemide and verapamil, or the vehicle for indomethacin which was 1 part ethanol:9 parts 0·2% Na_2CO_3 (control response = 58·6 ± 5·5(10) μA cm^{-2}; + dimethylsulphoxide (0·5% v/v) = 66·5 ± 9·6(8) μA cm^{-2}, $P > 0·05$; + indomethacin vehicle (2% v/v) = 59·3 ± 4·7(4) μA cm^{-2}, $P > 0·05$).

Expression of results

Results are expressed as mean values ± 1 s.e. of the mean of the number of observations indicated. Significance was assessed using Student's *t*-test, paired or unpaired as appropriate.

Chemicals

Histamine acid phosphate, glucose and dimethylsulphoxide (DMSO) were obtained from BDH Chemicals Ltd., Poole; mannitol and mepyramine maleate from May & Baker Ltd, Dagenham; EGTA (ethyleneglycol-bis-(β-aminoethylether) *N,N'*-tetraacetic acid), frusemide, indomethacin, mepacrine (quinacrine) and verapamil from the Sigma Chemical Co., St. Louis, MO 63178, USA; prostaglandin E_2 (PGE_2) from the Upjohn Co., Kalamazoo, MI 49001, USA.

Results

Effect of histamine on the transcolonic p.d. in-vivo

The transcolonic p.d. was 5–15 mV, serosa positive, and it was increased transiently following the intravenous administration of histamine (Fig. 1). The magnitude of this effect was dose-dependent, with a sigmoid relationship between the change in p.d. and the logarithm of the histamine dose (Fig. 2a). The response to histamine was inhibited competitively by the H_1 antagonist mepyramine (10^{-6} mol kg^{-1} i.v., Fig. 2a), which increased the Ka value from $1·37 ± 0·33 \times 10^{-6}$(8) to $1·71 ± 0·34 \times 10^{-5}$(8) mol kg^{-1} ($P < 0·01$).

Indomethacin ($4·5 \times 10^{-5}$ mol kg^{-1} s.c.) inhibited the electrical response to histamine (Fig. 2b), reducing the maximum change in p.d. from 4·8 ± 0·6(8) to 2·3 ± 0·7(8) mV ($P < 0·01$).

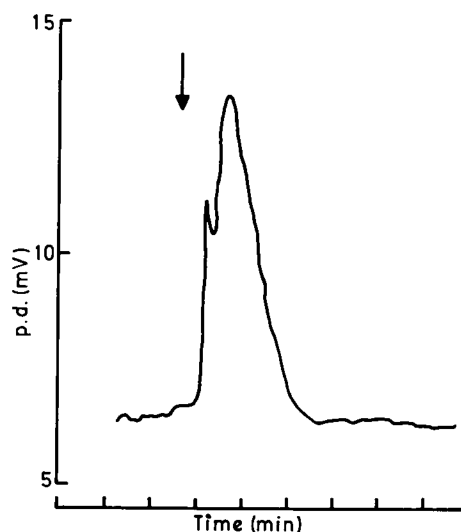

FIG. 1. Typical response of the transcolonic p.d. to the intravenous administration of histamine ($1·3 \times 10^{-5}$ mol kg^{-1}) in-vivo.

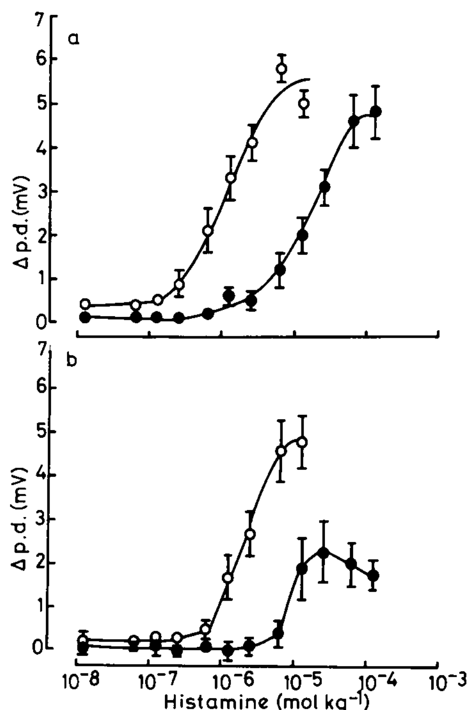

FIG. 2. Dose-response relationship of the rise in transcolonic p.d. (Δp.d.) in-vivo before (○) and after (●) the administration of mepyramine (10^{-6} mol kg^{-1} i.v., Fig. 2a, n = 8) or indomethacin ($4·5 \times 10^{-5}$ mol kg^{-1} s.c., Fig. 2b, n = 8). Each point represents the mean ± 1 s.e. of the mean of the number of observations indicated.

Effect of histamine on colonic fluid transport in-vivo

Histamine ($1·3 \times 10^{-4}$ mol kg^{-1} i.p.) induced a net fluid secretion of 131 ± 40(8) μL g^{-1} wet weight/15 min ($P < 0·05$) and indomethacin ($4·5 \times 10^{-5}$ mol kg^{-1} s.c.) abolished this response (fluid secretion + indomethacin = −3 ± 20(6) μL g^{-1} wet weight/15 min, $P > 0·05$). These two values differed significantly ($P < 0·05$).

Effect of histamine on the electrical activity of colonic sheets

The addition of histamine (10^{-4}M) to the serosal solution increased the p.d. by $4{\cdot}0 \pm 0{\cdot}4(10)$ mV and the s.c.c. by $58{\cdot}6 \pm 5{\cdot}5(10)$ μA cm^{-2}, while there was a small fall in R of $5{\cdot}3 \pm 2{\cdot}9(10)$ ohm cm^2 (Fig. 3). This response was dose-dependent (Fig. 4), with a Ka value of $6{\cdot}5 \times 10^{-5}$M.

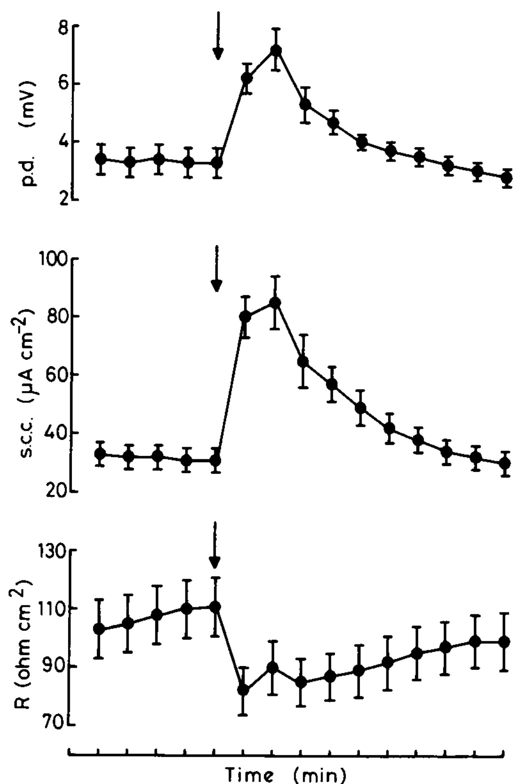

FIG. 3. Effect of histamine on the electrical activity of stripped sheets of rat colon. Histamine was added to the serosal solution to give a concentration of 10^{-4}M at the time indicated by the arrow. Each point represents the mean ± 1 s.e. of the mean of 10 observations.

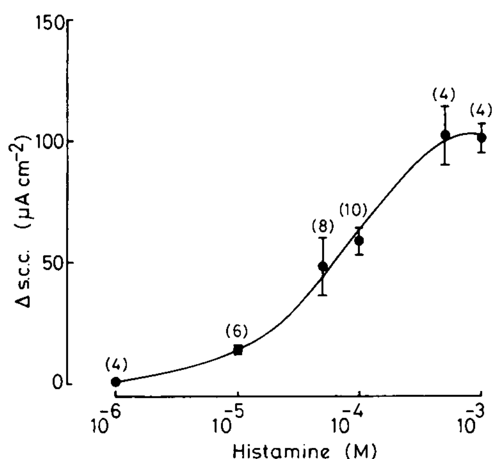

FIG. 4. Relationship between the concentration of histamine in the serosal solution and the increase in short-circuit current (Δs.c.c.) in colonic sheets. Each point represents the mean ± 1 s.e. of the mean of the number of observations indicated.

The histamine-induced rise in s.c.c. was reduced in the absence of chloride in the bathing medium or in the presence of serosal frusemide (Fig. 5), suggesting that a stimulation of chloride secretion was responsible for the changes observed. The omission of calcium from the serosal solution or the addition of serosal verapamil reduced the response to histamine (Fig. 5), indicating that a calcium-dependent process, requiring an extracellular source of calcium, was involved.

Indomethacin caused a dose-dependent reduction in the magnitude of the electrical response to histamine (Fig. 6),

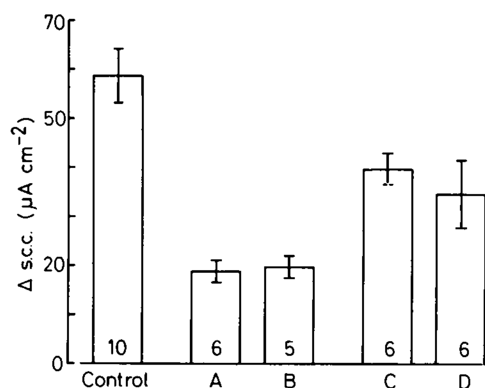

FIG. 5. Increase in short-circuit current (Δs.c.c.) generated by colonic sheets in response to 10^{-4}M histamine in the serosal solution. Chloride-free conditions were achieved by replacing all the chloride in both mucosal and serosal solutions with sulphate and maintaining isotonicity with mannitol. Calcium-free conditions were achieved by omitting calcium from the serosal solution and adding 0·5 mM EGTA. Frusemide was added to the serosal compartment to give a concentration of 10^{-3}M while verapamil was added serosally to give a concentration of 10^{-4}M. Both drugs were dissolved in DMSO which, at the concentration present (0·5% v/v), had no effect on colonic electrical activity. Each value represents the mean ± 1 s.e. of the mean of the number of observations indicated and significance was assessed using an unpaired *t*-test. Key: A, Cl-free ($P < 0{\cdot}001$); B, frusemide ($P < 0{\cdot}001$); C, Ca-free ($P < 0{\cdot}05$); D, verapamil ($P < 0{\cdot}05$).

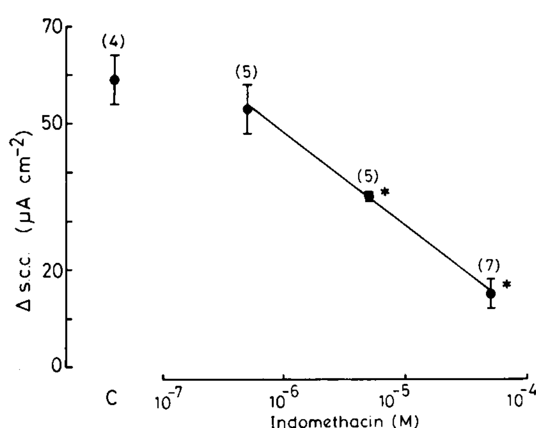

FIG. 6. Effect of indomethacin on the rise in short-circuit current (Δs.c.c.) induced by serosal histamine (10^{-4}M) in colonic sheets. Indomethacin was added to the serosal solution to give the concentrations indicated, while the control sheets received an equivalent volume (20 μL mL^{-1}) of the vehicle (1:9 ethanol:0·2% Na_2CO_3). Each point represents the mean ± 1 s.e. of the mean of the number of observations in parentheses and the significance of indomethacin action was assessed using an unpaired *t*-test (*$P < 0{\cdot}001$).

with a K_i value of 9.5×10^{-6}M. The highest concentration used (5×10^{-5}M) also reduced the basal s.c.c. from 30·6±4·2(4) to 20·9±2·1(7) μA cm^{-2} ($P<0.05$). However, even at this concentration (5×10^{-5}M), the rise in s.c.c. induced by PGE_2 (2.8×10^{-6}M) was not impaired by indomethacin (control = 53·7±6·8(6) μA cm^{-2}, +indomethacin = 61·1±2·8(6) μA cm^{-2}, $P>0.05$).

Mepacrine inhibited the increase in s.c.c. induced by histamine in a dose-dependent manner (Fig. 7), with a K_i value of 3.0×10^{-5}M. It also reduced the basal s.c.c. from 35·9±6·0(10) μA cm^{-2} to 14·1±2·7(8) μA cm^{-2} at 10^{-5}M

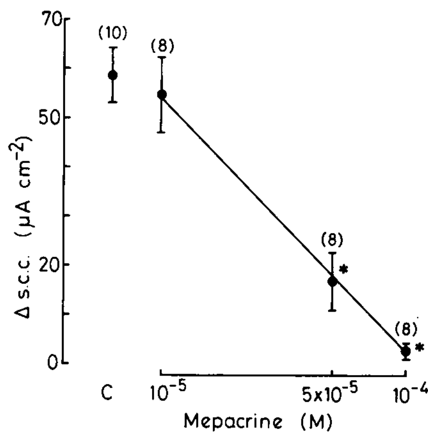

FIG. 7. Effect of mepacrine on the rise in short-circuit current (Δs.c.c.) induced by serosal histamine (10^{-4}M) in colonic sheets. Mepacrine was added to the serosal fluid to give the concentrations indicated and each point represents the mean ± 1 s.e. of the mean of the number of observations in parentheses. The significance of mepacrine action was assessed using an unpaired *t*-test (*$P<0.001$).

($P<0.01$), to 15·3±2·3(8) μA cm^{-2} at 5×10^{-5}M ($P<0.01$) and to 13·4±1·5(14) μA cm^{-2} at 10^{-4}M ($P<0.001$). However, even the highest concentration used (10^{-4}M) did not affect the response to 2.8×10^{-6}M PGE_2 (control = 56·9±5·5(4) μA cm^{-2}, +mepacrine = 56·5±5·2(6) μA cm^{-2}, $P>0.05$).

Discussion

The ability of histamine to increase colonic electrical activity was observed both in-vivo (Figs 1, 2) and in-vitro (Figs 3, 4), suggesting that the amine was having a direct action on the colon. This effect was mediated by H_1 receptors as it was inhibited competitively by the H_1 antagonist, mepyramine. This confirms data obtained in the rabbit colon where histamine also increases the s.c.c. by an interaction with H_1 receptors (McCabe & Smith 1984). The fact that histamine induces a net secretion of fluid by the colon suggests that the electrical response results from a stimulation of anion secretion. The ion involved appears to be chloride since the histamine-induced rise in s.c.c. was reduced by the removal of chloride or by the presence in the serosal fluid of frusemide (Fig. 5), an agent that inhibits active chloride secretion by preventing the coupled uptake of sodium and chloride at the basolateral membrane (Heintze et al 1983). Direct flux determinations were not undertaken due to the transient nature of histamine action, which would render steady-state conditions impossible to achieve during the period of the response. It appears that histamine can induce a net secretory state in the colon, an effect that is similar to its actions in the small intestine (Lee & Silverberg 1976; Linaker et al 1981; Cooke et al 1984; Hardcastle & Hardcastle 1987).

The activation of secretion by histamine involves a calcium-dependent mechanism since the rise in s.c.c. was reduced by the absence of serosal calcium or by the presence of the calcium channel blocker verapamil (Fig. 5). In this respect it resembles the behaviour of other secretagogues both in the small intestine (Hardcastle et al 1984) and the colon (Zimmerman et al 1983). Calcium-dependent regulation of cell function is currently thought to involve a change in the metabolism of inositol lipids located at the inner leaflet of the plasma membrane (Nishizuka 1984; Majerus et al 1985). One of the products of this system is diacylglycerol and this promotes the release of arachidonic acid, so allowing an increase in eicosanoid production. It has been suggested that the stimulation of intestinal secretion may be mediated by such an increase in phosphoinositide turnover (Chang et al 1985) and that an increase in prostaglandin levels could contribute to the secretory response (Martens et al 1985), since the prostanoids are potent stimulants of intestinal secretion (Matuchansky & Coutrot 1978). It has already been established that bradykinin-induced intestinal secretion is mediated by prostaglandins (Hardcastle et al 1978; Musch et al 1983) and more recently it has been reported that the intestinal secretion induced by 5-HT also involves the prostaglandins (Beubler et al 1986).

The data obtained with indomethacin and mepacrine in the present study suggest that prostaglandins contribute to the colonic secretion induced by histamine. The fact that PGE_2, like histamine, increases the s.c.c. in colonic sheets is consistent with this view. Indomethacin inhibits the enzyme cyclo-oxygenase which is responsible for the first stage in the conversion of arachidonic acid to prostanoids (Vane 1971). This agent reduced the rise in p.d. (Fig. 2b) and the fluid secretion caused by histamine in-vivo. This effect appeared to originate at the level of the colon since indomethacin also inhibited the histamine-induced increase in s.c.c. in isolated colonic sheets (Fig. 6). The ability of indomethacin to inhibit the colonic response to histamine has been described previously but the effect was interpreted as demonstrating the calcium-dependence of histamine action (McCabe & Smith 1984). Eicosanoid production is normally limited by the availability of the precursor, arachidonic acid. On stimulation, phospholipase A_2 is activated, causing the release of arachidonic acid for subsequent eicosanoid production (Flower & Blackwell 1976). Mepacrine inhibits this enzyme and hence reduces eicosanoid levels (Lapetina et al 1981). The fact that this agent also reduced the response to histamine in colonic sheets (Fig. 7) is further evidence for the involvment of prostaglandins in this response. Both indomethacin and mepacrine were applied at concentrations known to cause a marked inhibition of intestinal prostaglandin production (Hojvat et al 1983), yet neither of these agents affected the rise in s.c.c. induced by PGE_2 and hence their actions on the histamine response could not be attributed to a non-specific inhibition of the secretory process.

In addition to their effects on histamine-induced colonic secretion, mepacrine and the highest concentration of indo-

methacin reduced the basal s.c.c. in colonic sheets, suggesting that endogenous prostaglandin production may contribute to basal transport activity in this tissue. This effect of indomethacin was not observed at the dose used in-vivo.

It has been suggested that secretagogues that increase cyclic(c)AMP production in the colon may act independently of external calcium (Frizzell 1977). Since prostaglandins stimulate cyclic nucleotide levels in the colon (Simon & Kather 1978), the calcium-dependence of histamine action (Fig. 5) could argue against the involvement of prostanoids in the histamine response. It is now considered, however, that at physiological doses prostaglandins activate intestinal secretion by a calcium-dependent mechanism rather than by a rise in cAMP levels (Beubler et al 1986) and moreover, it has been shown in rat colon that cAMP-mediated secretion is reduced by a lack of external calcium (Zimmerman et al 1983). Thus the calcium-dependence of histamine action does not rule out its mediation by prostaglandins. As phospholipase A_2 is a calcium-dependent enzyme, the decreased response to histamine when calcium availability is limited (Fig. 5) could result from a reduced formation of prostaglandins in addition to a lack of calcium for the secretory process.

The amount of histamine normally present in rat colon has been estimated to be in the order of 2×10^{-5} mol kg^{-1} (Lorenz et al 1973), which exceeds the dose of histamine required to produce a half maximal rise in the transcolonic p.d. in-vivo (Fig. 2) by a factor of 10. This quantity of histamine would give a concentration of approximately 3×10^{-5}M in the tissue water (assumed to be 80% wet weight), sufficient to give a significant response in colonic sheets (Fig. 4). This raises the possibility that histamine may play a physiological role in the regulation of colonic secretion, although it should be remembered that the histamine may not be uniformly distributed throughout the tissue. The ability of histamine to induce a net secretion by the colon could be of relevance in certain disease states which are characterized by an increase in the level of this amine in the intestinal tract, e.g. intestinal allergies, systemic mastocytosis (Linaker et al 1981) and it is possible that histamine could contribute to the observed alterations in colonic transport function. A recent report (Russell 1986) has demonstrated that histamine is involved in the ion transport changes that result from the antigenic challenge of guinea-pig jejunum, suggesting that histamine may play a role in the response of the intestine to immunological stimulation.

The colonic response to histamine involves a stimulation of fluid secretion that appears to result from an increased electrogenic movement of chloride into the lumen. This change in the transport activity of the tissue is mediated, at least in part, by an increase in prostanoid production by the colon.

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