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The effect of angiotensin II upon electrogenic ion transport in rat intestinal epithelia

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1 Epithelial sheets from rat jejunum and descending colon have been shown to respond to angiotensin II (AII) when studied under short-circuit conditions and bathed on both sides with Krebs-Henseleit solution.

2 The octapeptide AII elicited increases in short-circuit current (SCC) in preparations of jejunum and decreases in SCC in the descending colon; both responses occurred when the peptide was applied to the basolateral surface, but not when applied to the apical solution.

3 Responses in both tissues were highly specific, being inhibited by a range of AII antagonists with the following order of potency: [Sar^1. Thr^8]-AII > [Sar^1. Leu^8]-AII > [Sar^1. Ile^8]-AII > [Sar^1. Ala^8]-AII > [Des,Asp^1. Ile^8]-AII in rat jejunum. AII responses were not affected by α- or β- adrenoceptor antagonists, atropine or tetrodotoxin.

4 AII responses were totally inhibited by the chloride channel blocker, diphenylamine-2-carboxylate (DPC) while cotransport inhibitors e.g. piretanide and frusemide significantly reduced the size of AII responses in colon and jejunum. These patterns of activity suggest that in the jejunum the responses result from electrogenic chloride secretion. Although AII responses in colon were sensitive to DPC the transporting ions have not yet been identified.

5 Both piroxicam and indomethacin inhibited the increase in SCC elicited by AII in the jejunum, and the reduction in SCC caused by AII in the colon. Taken together these results indicate that eicosanoids are involved in AII responses in both tissues.

6 This is the first study to demonstrate a direct, electrogenic effect for AII on transporting epithelia from the gastrointestinal tract. The responses are most probably initiated by AII interacting with previously identified specific AII receptors within the epithelial membranes.

Introduction

Angiotensin II (AII) has long been known to exhibit a concentration-dependent dual action upon transporting epithelia. In both renal cortex and intestinal epithelia (Munday *et al.*, 1971; Levens *et al.*, 1981) AII, at concentrations lower than 0.1 nM enhances electroneutral Na and Cl reabsorption while at concentrations greater than this, electroneutral Na and Cl secretion occurs. The mechanism by which AII stimulates reabsorption in the intestine has been associated with, but not entirely explained by, the release of noradrenaline from nervous elements present in the lamina propria, which in turn enhances salt and water reabsorption. Whilst significant immunocytochemical evidence is available identifying networks of peptide containing nerve fibres in the mammalian gastrointestinal tract very little is known of AII, although its presence within nerves of the myenteric plexus has been demonstrated (Füxe *et al.*, 1977). In general, however, AII is considered to be a circulating hormone and it is possible this acts directly upon the basolateral surface of intestinal epithelia.

Using an *in vivo* intestinal loop preparation, Levens *et al.* (1979) found that the AII response was inhibited by α- but not β-adrenoceptor antagonists, while a prostaglandin-mediated mechanism was implicated in the secretory responses to higher AII concentrations (Levens *et al.*, 1981). The electroneutral nature of both AII intestinal responses was supported by the lack of any significant changes in potential difference (p.d.) resistance or short-circuit current (SCC) after application of a wide range of AII concentrations to rat jejunum and colon *in vivo* (Bolton *et al.*, 1974; Munday & York, 1976).

The recent identification of high affinity, specific

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[^{125}I]-AII binding sites in intestinal (Cox *et al.*, 1986) and renal cortex epithelia (Cox *et al.*, 1983) and their predominance within basolateral rather than brush-border membranes (Cox, H.M., unpublished; Cox *et al.*, 1984) indicates a direct mechanism of action for AII mediated via receptors within the epithelial cells. The aim of the present study was to determine some of the functional responsibilities of [^{125}I]-AII binding sites present in epithelial cells of rat jejunum and descending colon.

Methods

Short-circuit current recording

Experiments were performed with epithelial preparations obtained from the jejunum and descending colon of male Sprague-Dawley rats, weighing 200–250 g. Preparations of rat jejunum were routinely taken 10–15 cm from the pyloric sphincter while descending colon preparations no further than 2 cm proximal to the terminal Peyer's patch were used. Tissues were freed from as much muscle as possible by dissection and the resulting sheets mounted in Ussing-type chambers with a window area of 0.6 cm^2. Preparations were voltage clamped by a WPI Dual Voltage Clamp and changes in SCC were recorded by standard methods as described previously (Cuthbert & Margolius, 1982).

Normally paired preparations of either jejunum or colon were set up from a single animal. Sheets were bathed on the apical and basolateral surfaces with Krebs-Henseleit solution (15 ml) warmed to 37°C and gassed with 95% O_2 and 5% CO_2. Drugs and peptides could be added separately to the solutions bathing either apical or basolateral surfaces and the mixing time was less than 5 s. The EC_{50} value was calculated as the concentration of AII which elicited a half maximal change in SCC from concentration-response curves constructed over the range, 10^{-9} M–10^{-6} M AII.

Solutions

Krebs-Henseleit solution was added with the following composition (mM): NaCl 117, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 24.8, KH_2PO_4 1.2 and glucose 11.1. This solution when gassed with 95% O_2:5% CO_2 at 37°C attained a pH of 7.4. All peptides were dissolved in distilled water and solutions were frozen and thawed only once prior to use. Of the drugs and channel blockers used in this study only diphenylamine-2-carboxylate (DPC) was dissolved in 50% dimethylsulphoxide w/v and the pH of the bathing solutions was checked following its addition to both apical and basolateral surfaces.

Results are expressed as means ± s.e.mean. Unpaired Student's *t* tests were used for statistical analysis of data and a *P* value of less than 0.05 was considered statistically significant.

Materials

All AII analogues, amiloride, piroxicam and indomethacin were purchased from Sigma, Poole; piretanide and frusemide from Hoechst U.K. Ltd., Hounslow; acetazolamide from Cyanamid, Gosport and DPC from Lancaster Synthesis Ltd., Morecambe. All other compounds were of analytical grade.

Results

Application of AII in nanomolar concentrations to the fluid bathing the basolateral surface elicited rapid

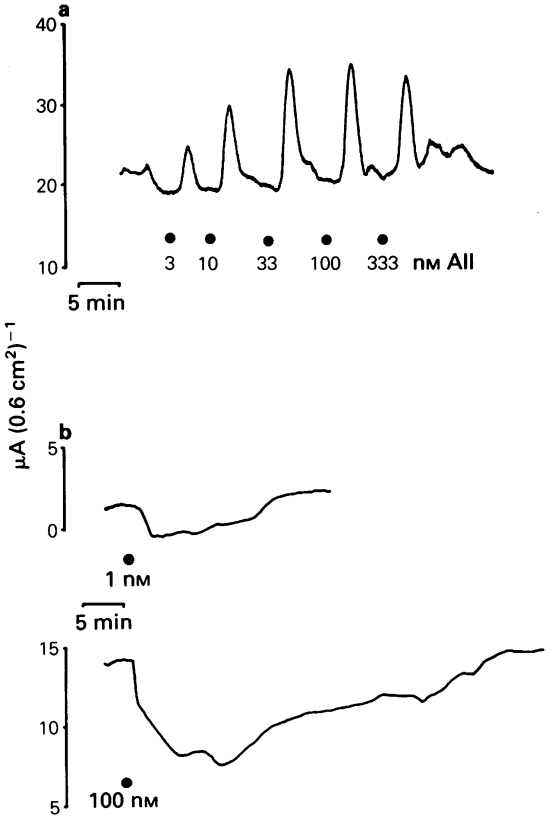

Figure 1 Examples of angiotensin II (AII) responses obtained in (a) rat jejunum and (b) descending colon. Records from 0.6 cm^2 areas of stripped epithelial sheets were obtained after serosal application of AII at the concentrations shown.

changes in SCC whereas additions of the peptide to the mucosal surface were without effect. In preparations of jejunum rapid increases in SCC were obtained and these were transient in nature, the current returning to baseline within 7 min. This behaviour created some problems with respect to the determination of concentration-response relationships. Figure 1 shows a cumulative concentration-response tracing from a single preparation of jejunum while Figure 2 shows the total data from all the preparations used in this study. From these data an EC_{50} value of 10 nM was obtained with a maximal change in SCC of $16.5 \pm 2.0\,\mu A$ $(0.6\,cm^2)^{-1}$. If the peptide was washed away between each addition it took some time for the baseline SCC to become stable again and consequently construction of concentration-response curves took considerably longer. No significant difference was seen between the profiles of curves obtained with or without removal of peptide. An increase in time taken with intermediate washing produced problems of its own as shown in Figure 3. Here the initial concentration-response curve shows a well defined plateau if measured in a cumulative way during the first hour after the tissue had reached basal current. However, subsequent determinations at later times showed enhanced responsiveness and this phenomenon occurred whether or not the tissues were exposed to AII, seemingly only requiring time. Consequently in looking at the effects

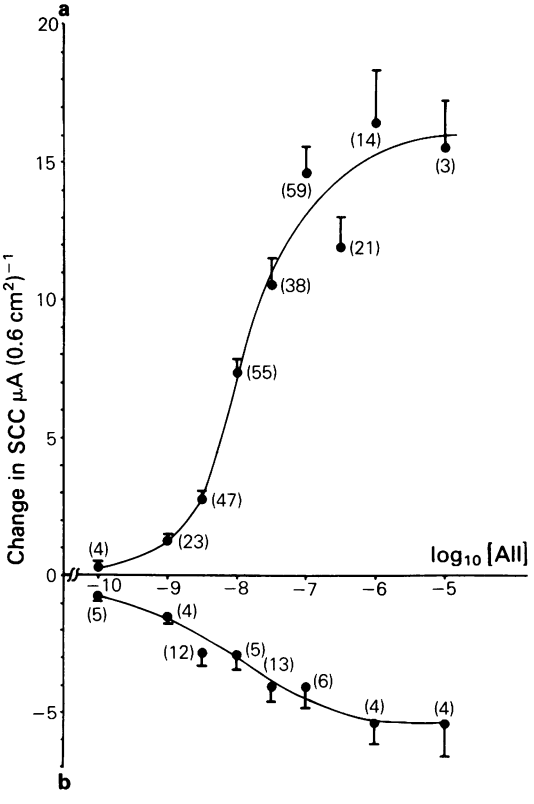

Figure 2 Angiotensin II (AII) concentration-response curves in (a) rat jejunum and (b) descending colon. Responses were recorded from $0.6\,cm^2$ areas of voltage clamped epithelia and pooled from all the preparations used in this study. In jejunum preparations cumulative responses were obtained without removal of peptide by washing, however, in colon preparations where desensitization occurred rapidly, washing was necessary between additions of AII. Values shown are the mean with 1 s.e.mean shown by vertical lines; *n* values are shown in parentheses.

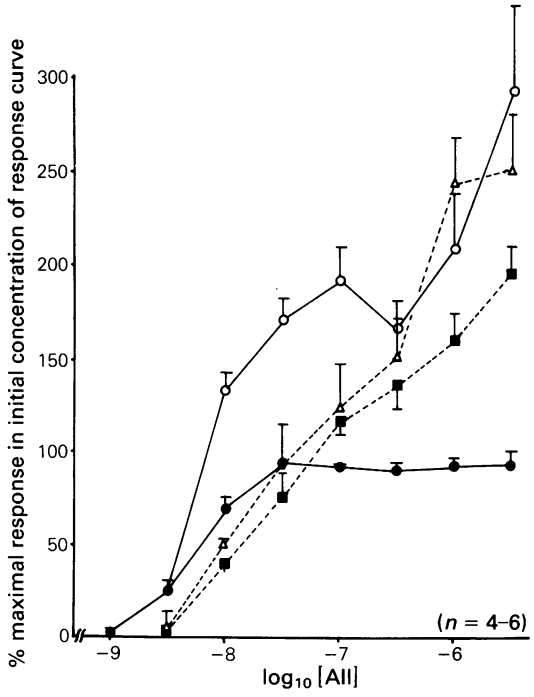

Figure 3 Variation of the angiotensin II (AII) concentration-response curve in rat jejunum with time. An initial cumulative response curve was constructed (●) within an hour of the tissue reaching a steady basal current. The maximal response obtained was designated 100%. Tissues were thoroughly washed with K-H buffer on both sides after completion of each curve. One hour later this procedure was repeated a second time giving increased AII responses (■) at the higher concentrations and a third time (at 3 h) resulting in a further enhancement of the responses (△). These increases in SCC to AII (after 3 h) are compared with responses obtained in tissues that have been washed twice but not exposed to AII (○). Values shown are the means from 4–6 measurements; vertical lines indicate s.e.mean.

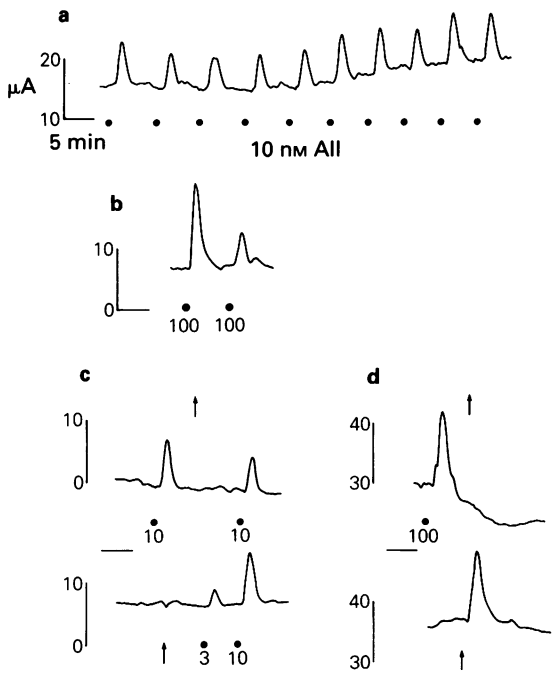

Figure 4 The effect of repeated application of angiotensin II (AII) at concentrations of 10 nM (a) and 100 nM (b) and of transferring bathing fluid containing AII (c and d) to a second untreated preparation of rat jejunum.

The trace shown in (a) was obtained by repeated additions of serosal 10 nM AII every 7 min without removal of peptide by washing. No sign of diminution in the size of the responses were observed. In another preparation of stripped jejunum (b) responses to 100 nM AII were obtained, the second response being only 30% the size of the first. The upper trace of (c) shows the response to 10 nM AII obtained in a preparation of jejunum bathed with 22 ml of K-H buffer each side. Once the response had decayed to the original baseline, 10 ml of bathing fluid was removed (at arrow) from both sides and transferred (at arrow) to a second untreated preparation originally bathed by 12 ml K-H buffer. No response was obtained and to check tissue sensitivity to AII a further 3 and 10 nM AII were applied and the tissue then responded accordingly. A similar protocol was performed in (d) but with 100 nM AII. Here however, the peptide was still present in the transferred bathing fluid and a 10 μA response was obtained in the second untreated tissue. This was approximately the size of response expected of 33 nM AII. Throughout epithelial areas were 0.6 cm^2.

of AII antagonists we used a limited number of AII concentrations and completed experiments in the presence of increasing antagonist concentrations within an hour of reaching a basal current. A second series of AII concentrations was added to one preparation at the end of each experiment, to check for any alteration in tissue responsiveness.

We have made attempts to understand why the responses to AII are transient in preparations of rat jejunum (Figure 4). Using low concentrations of AII (i.e. 10 nM) repeated responses were obtained with no diminution in the size (Figure 4a). Instead an increase in basal SCC and AII response was achieved after an hour and this continued slowly (data not shown) until the experiment's end (in this case 2 h) by which time the responses were 170% of those obtained originally. In a series of paired preparations (Figure 4c) after the increase in SCC to 10 nM AII had decayed to baseline in one tissue we reapplied that bath fluid to a second untreated tissue but did not record any response. We can, therefore, conclude that at low AII concentrations ($<$ 10 nM) there is no octapeptide left at the time the response has disappeared. However, with higher concentrations of AII ($>$ 100 nM) we were able to detect AII in a second untreated preparation (Figure 4d) after bathing fluid was removed from the first preparation at a time when the SCC there had returned to baseline. The increase in SCC after adding bathing medium to the second preparation was that expected from the concentration-response curve. We conclude that at the lower concentrations of AII the decay of the SCC response represents a loss of peptide from the bathing solution while at higher concentrations there is a desensitization component.

In preparations of rat descending colon, AII caused a long lasting reduction in SCC (Figure 1b), and the concentration-response curve (Figure 2) was made up of a composite of responses from individual tissues. As seen in preparations of jejunum, the EC_{50} value was 10 nM while the maximum reduction in SCC was -5.5 ± 0.8 μA $(0.6\ cm^2)^{-1}$. Colon preparations desensitized rapidly to AII, becoming unresponsive after receiving a single AII addition. It was not possible, therefore, to measure responses cumulatively in the colon and we had to rely on obtaining a few responses in each preparation with extensive washing and re-equilibration between consecutive exposures to AII. For these reasons, detailed studies on the colon were precluded.

In all these studies the height of each response in μA $(0.6\ cm^2)^{-1}$ is taken as the response. When, alternatively, areas under curves (i.e. current × time) were calculated as nEq $(0.6\ cm^2)^{-1}$ very similar profiles were obtained to those shown in Figures 2 and 3; the maxima of the first concentration-response curve (330 nM AII) being 37.0 ± 15.9 nEq $(0.6\ cm^2)^{-1}$ ($n = 4$).

Responses to AII in both the jejunum and colon were inhibited by nanomolar concentrations of specific AII antagonists. With increasing concentrations of [Sar^1. Thr^8]-AII for example (Figure 5) the concentration-response curve to AII was shifted to the right, in a parallel fashion. All the experiments with

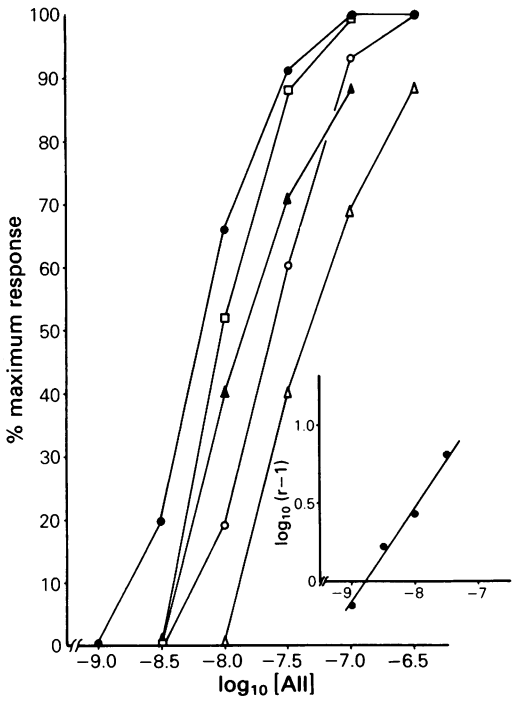

Figure 5 Inhibition of angiotensin II (AII) responses in rat jejunum by increasing concentrations of [Sar¹. Thr⁸]-AII. Results shown are from preparations all from the same animal. Each received 6 additions of AII at increasing concentrations in the absence of antagonist (control curve, ●). Tissues were thoroughly washed and following the addition of either 1 nM (□), 3 nM (▲), 10 nM (○) or 33 nM (△) [Sar¹. Thr⁸]-AII preincubated for 5 min, a further AII concentration-response curve was constructed. Only one concentration of antagonist was tested per preparation. Transformation of data to the Schild Plot (insert) allows the calculation of a pA_2 value (in this case 8.8) from the intercept of a straight line with the x axis.

antagonist were completed within the period during which the maximal response obtainable was unchanged. An order of potency: [Sar¹. Thr⁸]-AII > [Sar¹. Leu⁸]-AII > [Sar¹. Ile⁸]-AII > [Sar¹. Ala⁸]-AII > [Des,Asp¹. Ile⁸]-AII was obtained for inhibition of AII induced increases in SCC. This was not significantly different from the order of potency obtained from displacement data from both specific [^{125}I]-AII binding (Cox *et al.*, 1986) and [^{125}I]-[Sar¹. Thr⁸]-AII binding (Cox & Munday, unpublished data) in membranes from rat jejunum epithelia (Table 1). All the antagonists quoted above inhibited AII-induced decreases in SCC in rat colon (data not shown) but because of tissue desensitization, estimations of pA_2 values were not obtained. Preparations of colon that had been voltage clamped for longer than 3 h showed a reversal in response to AII that was only partially blocked by AII antagonists. Whilst an explanation for this phenomenon is not currently available it may be linked in some way with the time-dependent changes AII responses in the jejunum and referred to earlier.

The decapeptide AII precursor, AI and the heptapeptide AIII were similarly effective but less potent than AII in the jejunum and colon. Both peptides produced rapid, transient increases in SCC in jejunum while prolonged decreases in SCC were recorded in colon. The threshold concentrations in the jejunum were 3 nM for both AI and AIII with maximal responses of $10.2 \pm 1.8\,\mu A\ (0.6\,cm^2)^{-1}$ ($n = 7$) and $13.0 \pm 1.7\,\mu A\ (0.6\,cm^2)^{-1}$ ($n = 11$) to 1 μM AI and 1 μM AIII respectively. EC_{50} values of 20 nM for AI and 100 nM for AIII were obtained in preparations of jejunum. In the colon both agonist peptides were less potent with threshold concentrations of 10 nM and maximal decreases in SCC of $-2.2 \pm 0.2\,\mu A\ (0.6\,cm^2)^{-1}$ for 1 μM AI and $-1.7 \pm 0.4\,\mu A\ (0.6\,cm^2)^{-1}$ for 1 μM AIII.

The electrogenic responses to AII in both tissues were insensitive to atropine (10 μM) and to α- and β-adrenoceptor antagonists. Phentolamine (35 μM), prazosin (50 μM), yohimbine (50 μM) and propranolol (50 μM) did not alter the jejunal increase in SCC to AII. However, the reduction in SCC elicited by NA (1 μM) in this preparation was blocked by phentolamine and yohimbine but not by prazosin at the concentrations given above. In a corresponding fashion, the AII antagonist [Sar¹. Thr⁸]-AII did not alter NA responses. Additions of tetrodotoxin (10 nM and 100 nM) to the basolateral surface 10 min before AII application did not affect either electrogenic responses (data not shown).

Identification of the ionic species responsible for AII-induced changes in SCC was performed with the use of various ion channel and cotransport blockers. DPC a mucosal chloride channel blocker significantly reduced AII responses in both jejunum and colon (Figure 6). In the jejunum, 30 μM DPC added to both apical and basolateral compartments completely blocked the AII response ($P < 0.0005$, Student's unpaired t test) and after washing the response was recovered. The reductions in SCC to AII in the colon were also significantly reduced from control responses $(-5.10 \pm 0.94 \pm \mu A\ (0.6\,cm^2)^{-1}$ to $-0.60 \pm 0.12\,\mu A\ (0.6\,cm^2)^{-1}$, $P < 0.0025$). Piretanide (200 μM) and frusemide (100 μM) added basolaterally reduced AII responses by 56.0% and 62.6% respectively in the jejunum and by 56.1% and 75.0% in the colon. These reductions were significant, $P < 0.0025$ and $P < 0.01$ in the jejunum and $P < 0.01$ and $P < 0.005$ in the colon. Addition of piretanide or frusemide to the apical surface had no significant effect on either AII responses.

Table 1 A comparison of angiotensin II (AII) antagonist pA_2 values with K_i values obtained from displacement of specific [^{125}I]-AII and [^{125}I]-[Sar^1. Thr^8]-AII binding in rat jejunum

AII analogue	*pA_2*	*Schild slope*	*$-\log_{10} K_i$ [^{125}I]-AII binding*	*$-\log_{10} K_i$ [^{125}I]-[Sar^1. Thr^8] -AII binding*	Hill coeff. *(n)*
[Sar^1. Leu^8]-AII	9.35 ± 0.24	0.89 ± 0.05	9.03 ± 0.10	8.37	0.88
[Sar^1. Thr^8]-AII	9.18 ± 0.14	0.88 ± 0.07	8.85 ± 0.16	8.48 ± 0.07	0.86 ± 0.05
[Sar^1. Ile^8]-AII	9.03 ± 0.30	0.98 ± 0.11	8.19 ± 0.03	8.20 ± 0.06	1.02 ± 0.06
[Sar^1. Ala^8]-AII	8.41 ± 0.21	0.93 ± 0.03	ND	7.96	0.84
[Des, Asp^1. Ile^8]-AII	7.71 ± 0.32 ($n = 4$)	1.04 ± 0.09	7.93 ($n = 2/3$)	7.39 ± 0.19 ($n = 2/3$)	0.84 ± 0.08

pA_2 values were obtained using at least three concentrations of antagonist that caused a right shift of the AII concentration-response curve. These experiments were repeated four times with preparations of jejunum obtained from different animals. K_i values were calculated from IC_{50} data using the Cheng-Prussof equation:

$$K_i = IC_{50} / \frac{1 + [\text{ligand}]}{K_D}$$

Specific [^{125}I]-AII binding data in rat jejunum membranes has been published previously and includes Hill coefficients for the analogues listed (Cox *et al.*, 1986) whereas the characteristics of [^{125}I]-[Sar^1. Thr^8]-AII binding data in the same membrane preparations (Manning *et al.*, 1982) is currently in preparation. The numbers in parentheses denote *n* values and ND means not determined.

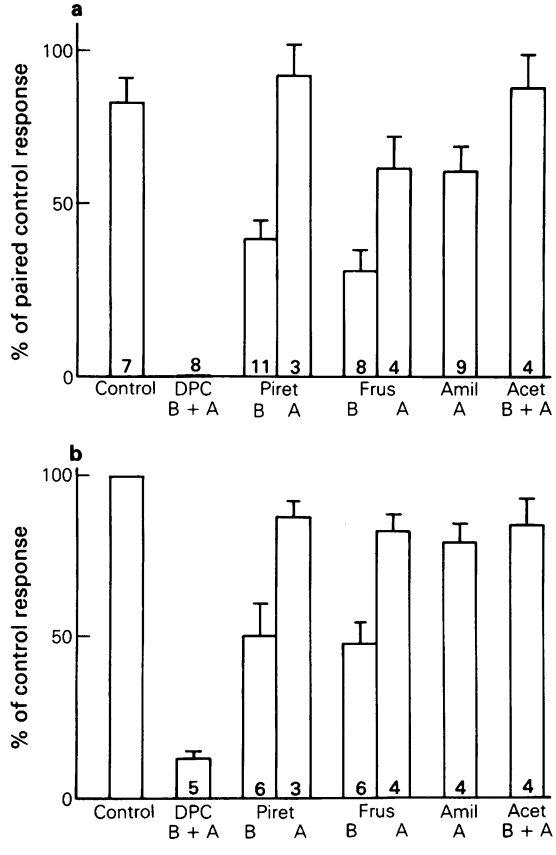

Figure 6 The effect of a range of transport inhibitors on angiotensin II (AII) responses in (a) rat jejunum and (b) descending colon. In preparations of jejunum control responses to 33 nM AII were initially obtained; the antagonist was then added to the apical (A) or basolateral surface (B) as shown and without washing another 33 nM AII added 15 min later once a steady current had been reached. The second response was calculated as a percentage of the first. In control experiments where two AII additions were made 15 min apart, the second response (15.3 ± 3.9 μA 0.6 cm^{-2}) was not significantly different from the first (18.8 ± 4.8 μA 0.6 cm^{-2}); $P < 0.3$, unpaired Student's *t* test). Although values are plotted as percentages, statistical significance was calculated with values of μA 0.6 cm^{-2} only.

In preparations of descending colon the protocol was different due to rapid tissue desensitization to AII. Here control responses were first obtained to 33 nM AII (100%, −4.98 ± 0.58 μA 0.6 cm^{-2}, $n = 32$). Tissues were then washed and once a steady current had been achieved the antagonist was added and a further addition of 33 nM AII was made. The second AII responses were calculated as percentages of the initial control response.

DPC = diphenylamine-2-carboxylate; Piret = piretanide; Frus = frusemide; Amil = amiloride; Acet = acetazolamide.

The apical sodium channel blocker, amiloride (20 μM) when added apically did slightly reduce AII responses in the jejunum ($10.6 \pm 1.4\,\mu A\ (0.6\,cm^2)^{-1}$ to $7.3 \pm 1.6\,\mu A\ (0.6\,cm^2)^{-1}$, $P < 0.05$) but not in the colon ($-2.85 \pm 1.1\,\mu A\ (0.6\,cm^2)^{-1}$ to $-1.90 \pm 0.42\,\mu A\ (0.6\,cm^2)^{-1}$, $P < 0.4$). The carbonic anhydrase inhibitor, acetazolamide (450 μM to both sides) had no significant effect on either tissue ($P < 0.3$ in jejunum and $P < 0.25$ in the colon).

To investigate the contribution made by prostaglandins to both AII responses, the fatty acid cyclooxygenase inhibitors, piroxicam and indomethacin were used. Addition of 5 μM indomethacin to both apical and basolateral surfaces of jejunum significantly ($P < 0.0025$) reduced responses to 100 nM AII by 65.5% from $15.9 \pm 1.9\,\mu A\ (0.6\,cm^2)^{-1}$ to $5.5 \pm 1.6\,\mu A\ (0.6\,cm^2)^{-1}$ ($n = 6$, Figure 7). This inhibition was reversible in 3 of the 6 preparations tested. Piroxicam (5 μM) completely and irreversibly inhibited AII responses (control; $18.5 \pm 3.4\,\mu A\ (0.6\,cm^2)^{-1}$ and with piroxicam; $0.0 \pm 0.0\,\mu A\ (0.6\,cm^2)^{-1}$ ($n = 4$)); small increases in SCC being obtained to AII only after 3 or more washes with Krebs-Henseleit buffer. The AII induced decreases in SCC in the colon were also sensitive to both indomethacin and piroxicam. Addition of 5 μM indomethacin apically and basolaterally significantly reduced the control responses to 33 nM AII from $-3.53 \pm 0.48\,\mu A\ (0.6\,cm^2)^{-1}$ to $-0.93 \pm 0.20\,\mu A\ (0.6\,cm^2)^{-1}$ ($n = 6$, $P < 0.0005$). Piroxicam was similarly effective decreasing AII-induced reductions in SCC from $-3.95 \pm 0.54\,\mu A\ (0.6\,cm^2)^{-1}$ to $-1.51 \pm 0.44\,\mu A\ (0.6\,cm^2)^{-1}$ ($n = 8$, $P < 0.0025$).

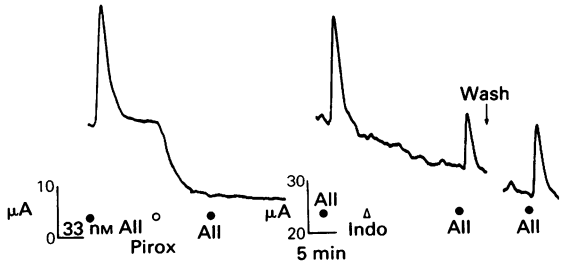

Figure 7 The effect of 5 μM piroxicam (Pirox) and 5 μM indomethacin (Indo) added apically and basolaterally on angiotensin II (AII) responses in rat jejunum. Control responses were first obtained to 33 nM basolateral AII. Either piroxicam or indomethacin were added to apical (A) and basolateral (B) surfaces and once the current was steady a further addition of AII made. The traces shown are typical examples of those seen with these prostaglandin synthesis inhibitors. The arrow indicates the removal of indomethacin by washing.

Discussion

These results identify, for the first time, a direct electrogenic action for AII in rat intestinal epithelia. A concentration-dependent increase in SCC was observed upon basolateral application of AII to sheets of jejunum while reductions in SCC were recorded from preparations of descending colon. These responses are most probably elicited by interaction of the peptide with specific AII receptors located within the basolateral surface of intestinal epithelia. However, it has recently been shown that prostaglandin-mediated kinin responses in intestinal epithelia may involve a non-epithelial site of action (Hoult & Phillips, 1986; Warhurst *et al.*, 1986). While the action of AII upon subepithelial cells in the lamina propria cannot be overlooked, the evidence presented in this study and previous receptor binding studies strongly suggests an epithelial action for AII. In addition in recent experiments (MacVinish, Cox & Cuthbert, unpublished), we have identified a rapid increase in SCC to both serosal and apical AII in confluent monolayers of a human colonic epithelial cell line, HCA-7 (Kirkland, 1985). The presence of AII responses in a cell line devoid of neuronal, lymphatic or cardiovascular elements provides good evidence for a direct epithelial action. The absence of an effect on AII responses by TTX in either the jejunum or colon also reinforces the thesis for a direct, postjunctional effect of the peptide. High affinity binding sites for [^{125}I]-AII have previously been identified in epithelial membranes (Cox *et al.*, 1986) exhibiting affinities (K_D) of 0.64 ± 0.16 nM in preparations from the jejunum and 0.48 ± 0.06 nM in descending colon. More recent data obtained with the radiolabelled antagonist, [^{125}I]-Sar^1. Thr^8-AII provide further evidence that AII receptors are indeed preferentially located on the basolateral surface of epithelia (Cox & Munday, unpublished) from gastrointestinal areas where an AII physiological response has been characterized. The order of affinity of a range of AII antagonists as blockers of the AII jejunal response was very similar to the order obtained from K_i values for displacement of specific [^{125}I]-AII and [^{125}I]-Sar^1. Thr^8-AII binding (Table 1). The pA_2 values compared well with the negative $\log_{10} K_i$ values indicating that the AII receptors identified by physiological and biochemical techniques are the same population of receptor proteins. It should be noted that none of the AII analogues tested exhibited any agonist activity at all. This contrasts with reports that the analogue, [Sar^1. Leu^8]-AII possesses full agonist activity in rat jejunum (Levens *et al.*, 1981). Only AI and AIII were similar in action to AII, but less potent. Both peptides increased SCC in the jejunum and reduced it in colon preparations.

The electrogenic responses characterized above were achieved in tissues prepared from rats that had

neither undergone adrenalectomy nor nephrectomy. Such surgical removal of the kidneys and adrenals has, in the past, been necessary to increase tissue sensitivity to AII (Crocker & Munday, 1970; Davies *et al.*, 1970) and the evidence presented in these earlier studies indicated that AII stimulated electroneutral NaCl cotransport. Inconsistencies in the effects of AII reported by different groups have been further complicated by the use of different epithelial preparations and the widely varying hormone concentrations used. Nevertheless in 1973, Hornych *et al.* identified a direct action for AII in everted colon sacs from normal rats. Net fluxes of sodium and water were stimulated maximally by 0.01 nM AII in ascending colon preparations and inhibited in descending colon segments. After adrenalectomy and nephrectomy, tissue preparations exhibited a biphasic effect in response to AII. We too concluded from our own findings, that the area of intestine taken for experimentation was critical, particularly in the colon. If segments more than 4 cm proximal to the Peyer's patch of descending colon preparations were used, AII was found to stimulate SCC.

Diez de los Rios *et al.* (1980) also identified AII responses in preparations of rat proximal colon from adrenalectomized and nephrectomized animals. In contrast with earlier studies 1 pM AII stimulated fluid and sodium transport associated with a decrease in potential difference and SCC while preparations from normal rats were insensitive to AII. No significant changes in p.d. or SCC could be measured in response to a wide range of AII concentrations in preparations of normal rat jejunum or distal colon *in vivo* (Bolton *et al.*, 1974; 1975; Munday & York, 1976). However, upon closer examination of the values quoted by Munday & York (1976) comparing control SCC and p.d. with electrical measurements during infusions of 7.0 ng kg^{-1} min^{-1} AII, a definite decrease in both parameters was observed in the colon while increases were obtained in the jejunum. These changes, although not significant, do compare favourably with the data presented above.

While substantial evidence is available indicating noradrenergic mediation of AII-induced ion transport *in vivo* (Levens *et al.*, 1979; 1981) none of the α- or β-adrenoceptor antagonists tested in the present study affected AII responses in either the jejunum or colon. Yohimbine and phentolamine did, however, block NA-induced reductions in SCC in preparations of jejunum in agreement with an α_2-adrenoceptor mechanism of action described by Chang *et al.* (1982).

The enhanced sodium and water secretion seen in jejunal sacs *in vivo* after application of higher doses of AII has been inhibited by pretreating animals with either meclofenamate or indomethacin (Levens *et al.*, 1981). In the present study, addition of either 5 μM piroxicam or indomethacin to both apical and basolateral surfaces of intestine significantly reduced AII responses. Thus the electrogenic responses identified in the jejunum are mediated by prostaglandins and may in fact correspond to the secretory responses previously described for AII which, until now, have been thought to be nonelectrogenic (Bolton *et al.*, 1974; Munday & York, 1976; Levens *et al.*, 1981). The AII-induced reduction in SCC in the colon was also inhibited by prevention of eicosanoid formation. However, we do not know which eicosanoids are formed when AII acts on the colon. In some species even PGE_2 can inhibit SCC when added in low concentrations (Baird *et al.*, 1984). Nevertheless the consistency of the effects of piroxicam and indomethacin argue for a prostaglandin involvement in AII responses in the colon. Indeed it remains obscure why AII should cause a fall in SCC, especially as other agents which stimulate eicosanoid formation, such as kinins, actually increase SCC in this tissue (Cuthbert & Margolius, 1982). Until the mediators are identified, nothing further about the mechanism of AII action in the colon can be added other than to remark that the reduction in SCC could be due to inhibition of basal Cl secretion, increase in cation secretion or stimulation of anion absorption. It is possible that a combination of more than one of these could be responsible.

Rather than perform ion replacement studies, omitting either sodium or chloride or bicarbonate from the K-H buffer, specific epithelial transport inhibitors were used to identify the ionic species responsible for the altered SCC. The most effective inhibitor of AII responses was the apical chloride channel blocker, DPC (Distefano *et al.*, 1985). Jejunal responses to AII were completely blocked by addition of 5 μM DPC to both surfaces. We can rule out the probability that AII stimulated glucose-dependent cation absorption which was blocked by DPC, as responses to AII were not in any way attenuated by removing glucose from the bathing K-H solution. Additionally the evidence obtained with two loop diuretics, frusemide and piretanide, which are known to block the Na, K, 2Cl triporter in the basolateral surface also inhibited the effects of AII. Overall the pharmacological evidence that AII responses in the jejunum are due to electrogenic Cl secretion is strong. The marginal action of amiloride and the lack of effect of acetazolamide on AII responses argues that the peptide causes neither the stimulation of electrogenic Na absorption nor of HCO_3 secretion.

However, the problem remains that a similar profile for the inhibitors was obtained in the colon where AII causes a reduction in SCC. Here, when DPC or the loop diuretics were added to the colon there was often a reduction in SCC (see Cuthbert & Margolius, 1982, Figure 4) indicating a basal level of Cl secretion which could not then be further reduced by AII. In this way the attenuation of inhibitory responses to AII may be

explained. It should also be emphasized that the responses in the colon (shown as percentages in Figure 6) are indeed only one third the size of AII responses seen in the jejunum (see Figure 1).

In summary we have identified rapid electrogenic responses to nanomolar concentrations of AII in preparations of rat jejunum and descending colon under SCC conditions. The selective inhibition of these responses in the jejunum by AII antagonists, cyclo-oxygenase inhibitors and DPC strongly indicate a direct epithelial action for the peptide which subsequent to receptor activation stimulates prostaglandin biosynthesis leading to chloride secretion.

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