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Induction of bradykinin B_1 receptors in rat colonic epithelium

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1 Des-Arg^9 bradykinin (DAB), a classical B_1-kinin receptor agonist was without effect when applied to the basolateral surface of rat isolated colon epithelium. Three hours after tissues were isolated DAB caused, after a delay of up to 2 min, a maintained increase of short circuit current (SCC).

2 The SCC increase in colonic epithelia, mounted *in vitro* for three hours, caused by DAB was due to electrogenic chloride secretion as the current increase was reversed by frusemide and did not occur in the absence of cystic fibrosis transmembrane conductance regulator (CFTR) chloride channels. The EC_{50} for DAB was approximately 50 nM.

3 An inhibitor of transcription (actinomycin D) and of translation (cycloheximide) prevented the appearance of DAB sensitivity without affecting the responses to another secretagogue (forskolin).

4 The classical B_1-kinin receptor antagonist, Leu^8-des-Arg^9 bradykinin, was shown to be an agonist in rat colon epithelium. Other B_1-kinin receptor antagonists (des-Arg^{10}-Hoe 140 and R-715) inhibited the responses to DAB in 'aged' colonic epithelia, and the inhibition was easily surmounted by increasing the concentration of DAB.

5 Response to DAB did not appear to involve to any significant extent, the formation of prostaglandins, leukotrienes, histamine or nitric oxide. Furthermore, no neuronal involvement was apparent in the stripped colonic preparations. The responses to DAB were not significantly different in epithelia taken from different parts of the distal colon.

6 The differences between the responses of the colonic epithelium to B_1- and B_2-kinin receptor agonists are discussed.

Keywords: B_1-kinin receptors; receptor induction; chloride secretion; colonic epithelium (rat); des-Arg^9 bradykinin; des-Arg^{10} Hoe 140; Leu^8-des-Arg^9 bradykinin

Introduction

A variety of smooth muscle cells respond to B_1-kinin receptor agonists and many different types of cells in culture have been shown to respond by the production of inflammatory mediators, autacoids or increased DNA synthesis (see review by Marceau, 1995). Furthermore there are indications that B_1-receptors are associated with sensory nerves and the mediation of pain (Davis & Perkins, 1994). There are very few studies showing that non-excitable non-contractile tissues are responsive to B_1-agonists. This work arose when it was found that a preparation of mouse colon epithelium from a B_2-kinin receptor knockout mouse, which had been neglected for several hours, responded to a high concentration of bradykinin. Clearly no B_2-kinin receptors were present but the response may have been due to the appearance of B_1-type receptors during the incubation period. A literature survey revealed only two studies of significance. First, Rangachari *et al.* (1993) found the dog colon epithelium responded to the kinin B_1-receptor agonist, des-Arg^9 bradykinin (DAB), but the receptor type was not identified as the classical B_1-antagonists were found to have agonist activity. Secondly, induction of chemical colitis in rats with acetic acid gave epithelia which responded to DAB, but the responses were very small and only twice the response of untreated tissues (Kachur *et al.*, 1986). Specifically in this study we have examined the rat colon epithelium and found that B_1-kinin receptors are induced *in vitro*. In some respects their properties are classical, while in others they are not so.

Methods

Nearly all experiments were performed with the colonic epithelium of rats (Sprague Dawley) weighing 200–400 g. A few experiments were made with the colonic epithelium of null CF mice (Ratcliff *et al.*, 1993). Animals were killed by exposure to a rising concentration of CO_2, after which the descending colon was rapidly removed into cold Krebs Henseleit Solution (KHS). Pairs of epithelia (usually one but sometimes two pairs) were prepared by dissecting away the muscle layers and some of the sub-mucosa. The tissues were mounted in Ussing chambers with a window area of 20 mm^2 and bathed on both sides with warmed (37°C), circulating KHS (20 ml) and gassed with 95 % O_2/5%CO_2. Short circuit currents (SCC) were recorded by a WPI Dual Voltage Clamp with series resistance compensation. Data were collected continuously by a MacLab and associated computer. The methods have been described in detail elsewhere (Cuthbert *et al.*, 1994). All the experiments were performed in the presence of amiloride, 100 μM, in the apical bathing solution to inhibit electrogenic sodium absorption. The following drugs were obtained from Sigma, amiloride, des-Arg^9-bradykinin (DAB), frusemide, indomethacin, N^G-nitro-L-arginine methyl ester (L-NAME), Leu^8-des-Arg^9-bradykinin (Leu^8-DAB), cycloheximide, actinomycin D and tetrodotoxin (TTX). Hoe 140, which is D-Arg-L-Arg-L-Pro-L-[(4**R**)-4-hydroxyprolyl]-Gly-L-[3-(2-thienylalanyl)]-L-Ser-D-(1, 2, 3, 4-tetraisoquinoline-3′yl-carbonyl)-L-[(3a**S**,7a**S**)-octahydroindol-2-ylcarbonyl[-L-Arg, was a gift from Hoechst AG and des-Arg^9-Hoe 140 was obtained from Bachem Bioscience Inc. R-715 (AcLys[DβNal^7,Ile^8] des-Arg^9-bradykinin), a new B_1-kinin receptor antagonist was a kind gift from Domenico Regoli, and L656,224 (7chloro-4hydroxy 2(4methoxybenzyl)-3methyl-5propylbenzofuran), a potent and specific leukotriene biosynthesis inhibitor was a gift from Merck- Frosst. KHS had the following composition, mM: NaCl 137, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0 and glucose 11.1. This solution had a pH of 7.4 when gassed with 95 % O_2/5% CO_2 at 37°C. Depending on the type of experiment the tests for sig-

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nificance were based upon either paired or non-paired formats. Unless otherwise stated Student's t test was used and $P<0.05$ taken to indicate significance.

Results

Nature of the phenomenon

The phenomenon which forms the basis of this study is illustrated in Figure 1. Shown is a SCC recording from a rat distal colon epithelium exposed on the basolateral side, to DAB, 1 μM, around 15 min after mounting. No effect was seen and the peptide was removed by thorough washing. Three hours later the same epithelium shows, after a delay, an increase in SCC which was maintained until the NaK2Cl cotransport inhibitor, frusemide, was added. The pre-exposure to DAB at time zero was not necessary for the response to be generated at three hours. As the experiment was performed in the presence of amiloride, 100 μM, on the apical side the response cannot be due to electrogenic sodium absorption and inhibition by frusemide indicates electrogenic chloride secretion is responsible for the effect. The effect of frusemide was further explored in a series of experiments (Figure 2). The mean increase in SCC caused by DAB was totally removed by the addition of frusemide. At steady state the SCC after DAB and frusemide was less than the initial basal SCC, but not significantly so (Figure 2).

Does induction require formation of mRNA and protein synthesis?

To examine if the appearance of DAB sensitivity after three hours was due to the synthesis of new receptors, and whether synthesis depended both upon mRNA formation and protein synthesis, inhibitors of transcription and translation were used. It was necessary too to ensure that the effects of inhibitors were selective, rather than having a general depressive effect on responses. Forskolin, an activator of adenylate cyclase, was chosen as an agent which also causes electrogenic chloride secretion in rat colon without preincubation (Cuthbert & Spayne, 1982). The paradigm used for these experiments is illustrated in Figure 3. Forskolin was added at time zero and then washed away. At three hours, DAB, 1 μM, was applied, followed by forskolin without washing. In this way the effects of inhibitors could be differentially assessed on the responses to DAB and forskolin. Paired preparations were used and the responses to forskolin compared in those tissues not exposed to inhibitors with those in their presence. The inhibitors had no acute effect on SCC.

In Figure 4a paired experiments with actinomycin D are illustrated. Actinomycin D, an inhibitor of transcription, significantly reduced the responses to DAB while those to forskolin were unaffected. Similarly, cycloheximide, the protein synthesis inhibitor selectively inhibited the responses to DAB (Figure 4b). It was important to establish in these experiments that pre-exposure to forskolin did not affect the size of the subsequent responses to DAB, three hours later. Pooled responses for DAB at time zero and three hours later, with and without pre-exposure to forskolin, are given in Figure 5; it is evident that forskolin pre-exposure does not affect the response, whereas time makes a striking difference.

It was necessary to establish that the concentration of DAB used throughout these experiments, 1 μM, was a supramaximally effective concentration. Comparisons made at submaximal concentrations may be vulnerable to changes in sensitivity caused, for example, by non-specific effects of inhibitors. The concentration-response relationship to DAB at three hours after mounting is shown in Figure 6, the EC_{50} was around 50 nM. To determine this relationship only 3–4 responses were obtained in cumulative fashion in any one preparation, the last of which was always 1 or 2 μM. Responses were expressed as the percentage of the maximal response, i.e. the response at 1 μM. This procedure was necessary because of the variability of the maximal responses to DAB in preparations from different animals (see also Table 1).

The nature of the receptors responding to DAB

The nature of the receptors involved was investigated with antagonists selective for kinin B_1 and B_2 receptor types. The B_2 antagonist, Hoe 140, had no effect on the response to DAB at 3 h. In 6 experiments with paired preparations the mean response in the presence of Hoe 140, 1 μM, to DAB, 1 μM at 3 h was 92.6 ± 24.9 μA cm^{-2}, whereas in the control tissues the

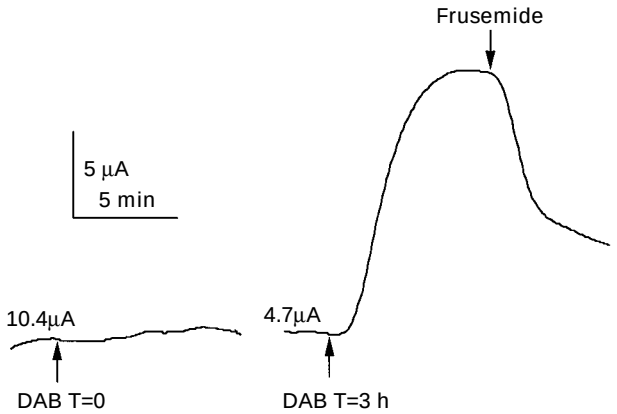

Figure 1 Effect of des-Arg^9 bradykinin (DAB), 1 μM, applied to the basolateral surface of a rat colonic epithelium. Traces show the SCC record measured 15 min after the tissue was mounted (T = 0) and at 3 h (T = 3 hr). Between the two applications the tissue was washed thoroughly. Finally, frusemide, 1 mM, was applied to the basolateral surface of the epithelium. Note the current record is for an area of 20 mm^2, and in later diagrams values are given as μA cm^{-2}. On this and on subsequent traces the figures at the start of the trace indicate the SCC at that time.

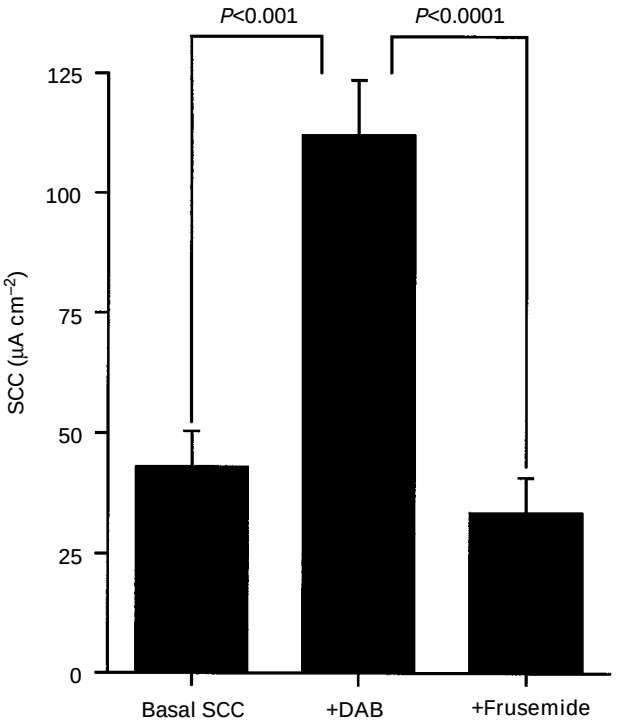

Figure 2 Effects of frusemide on responses to des-Arg^9 bradykinin (DAB). DAB, 1 μM, was applied to epithelia after 3 h incubation, followed by frusemide, 1 mM. The SCC after DAB was compared to the basal SCC and again after frusemide had been added, by use of a two-tailed Student's t test. Data shown are means $\pm$ s.e. mean ($n = 12$).

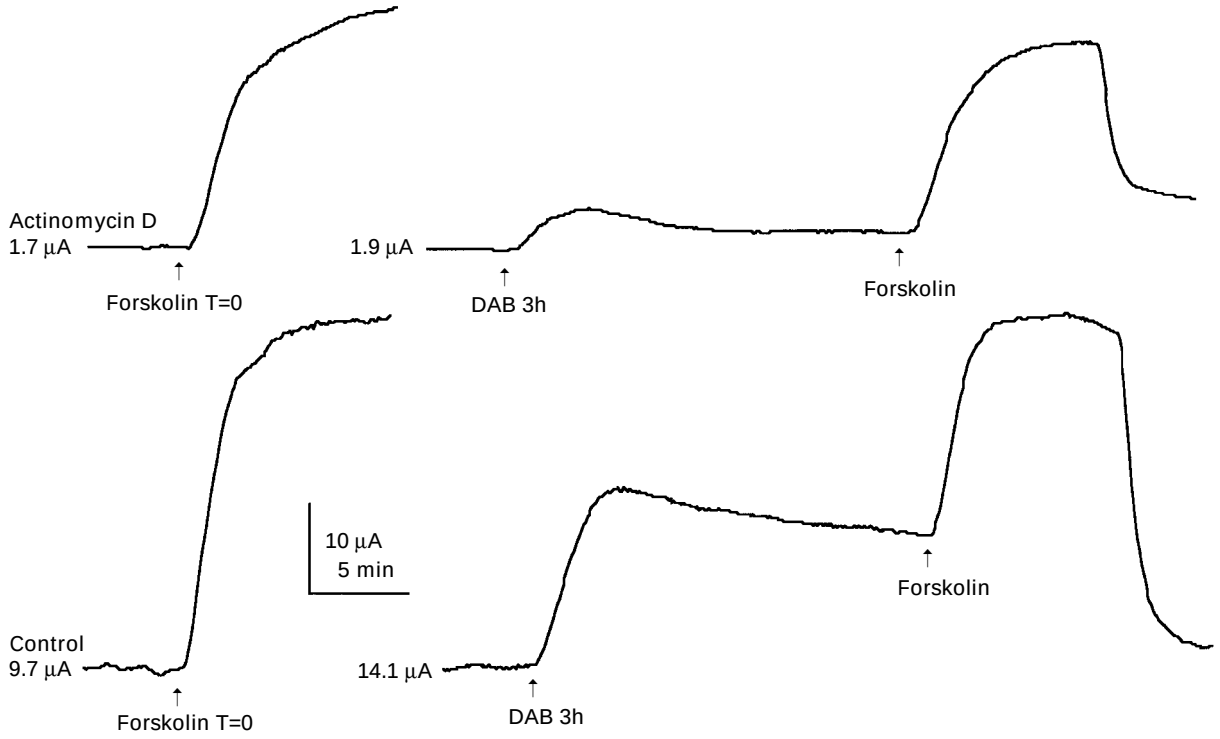

Figure 3 Protocol for examining the effects of inhibitors on the responses to des-Arg^{9}bradykinin (DAB). One of a pair of preparations was treated with actinomycin D, 200 μM, throughout the experiment. Both preparations were exposed simultaneously to forskolin, 10 μM, followed by extensive washing. Three hours later both were treated with DAB, 1 μM, and then forskolin, 10 μM, without washing, and finally frusemide, 1 mM.

mean response was 110.1 ± 20.6 μA cm^{-2}, the values not being significantly different. The paired preparation design was also used to investigate the classical B_1 receptor antagonist Leu^8-DAB. In three paired experiments the mean control response to DAB, 1 μM was 97.5 ± 29.7 μA cm^{-2}. Leu^8-DAB had agonist activity at 1 μM when applied to the basolateral face of colonic epithelia, that had been incubated *in vitro* for three hours and caused a SCC increase of 44.1 ± 18.1 μA cm^{-2}. In the presence of Leu^8-DAB a further SCC increase to DAB of 54.8 ± 27.4 μA cm^{-2} was recorded. Thus DAB plus Leu^8-DAB produce the same increase in SCC as DAB alone. To ascertain whether the effects of Leu^8-DAB were exerted on B_2-kinin receptors, a series of non-paired experiments were made in which several drugs were applied sequentially. DAB applied to colonic epithelia after amiloride, Hoe 140 and Leu^8-DAB still caused an increase in SCC of 39.2 ± 5.6 μA cm^{-2}, $n=8$. While this is smaller than the mean DAB response (57.8 ± 5.3 μA cm^{-2}, $n=68$), it was not significantly less (Figure 7). Furthermore, the combined responses to DAB and Leu^8-DAB were equivalent to the mean response to DAB from pooled measurements. Thus it was not possible to conclude that Leu^8-DAB antagonized the effect of DAB. Finally, after all the additions shown in Figure 7, addition of forskolin caused a further increase in SCC of 145.2 ± 20.0 μA cm^{-2}, indicating that the combined effects of DAB with Leu^8-DAB were not limited by the transporting capacity of the tissue. In all experiments the considerable delay between the addition of DAB and the moment when the SCC started to increase was a feature. The set of eight experiments with DAB provided a group in which the delay with DAB and other drugs could be compared. These data are also given in Figure 7. Note that the response time for amiloride was immediate, that to forskolin about 30 s, whereas DAB and Leu^8-DAB took 1–2 min before a response was seen.

Des Arg^{10}-Hoe 140 is known to be a potent B_1-kinin receptor antagonist (Hess *et al*., 1996). At a concentration of 2 μM it caused a significant inhibition of the responses to DAB, 100 nM but the inhibition was readily overcome by increasing the DAB concentration to 10 μM. Des Arg^{10}-Hoe 140 was not without agonist activity, although this was much less than with Leu^8-DAB. The data from these experiments is given in Figure 8.

Finally, a new B_1-kinin receptor antagonist R-715 was investigated, although it has been found not to be as potent in rodent species as in others (Regoli, personal communication). Initially 6 paired experiments were carried out in which both received DAB, 1 μM after three hours incubation, one preparation receiving R-715, 2 μM, 10 min before the agonist was added. R-715 showed no agonist activity whatsoever but caused only modest inhibition, with a mean value of 25%. The pair of preparations showing the greatest inhibition is illustrated in Figure 9a. The traces illustrate other features of note; first, that addition of R-715 after the response to DAB had reached a steady state caused a slow reversal and secondly, that the effects of R-715 did not prevent a swift response to forskolin. In a further 4 paired experiments the DAB concentration was reduced to 100 nM when the responses were only 3.7 ± 2.2 μA cm^{-2}, $n=4$, in the presence of R-715, 2 μM, whereas in controls the SCC increase was 59.3 ± 13.5 μA cm^{-2}, $n=4$, these values being significantly different ($P<0.03$). However, when the DAB concentration was increased to 10 μM in the presence of R-715 responses of 55.3 ± 6.2 μA cm^{-2}, $n=4$, were obtained, not significantly different from the responses to DAB, 100 nM, in the absence of the antagonist, but significantly greater ($P<0.05$) than the response to DAB, 100 nM, in the presence of R-715. An example of one of these paired experiments is given in Figure 9b.

Other influences on the responses to DAB

A number of agents which may have affected the responses to DAB were investigated. None of these produced significant changes in the responses to DAB (Table 1). The measurements were carried out in paired preparations and in a given set of experiments the animals all came from the same

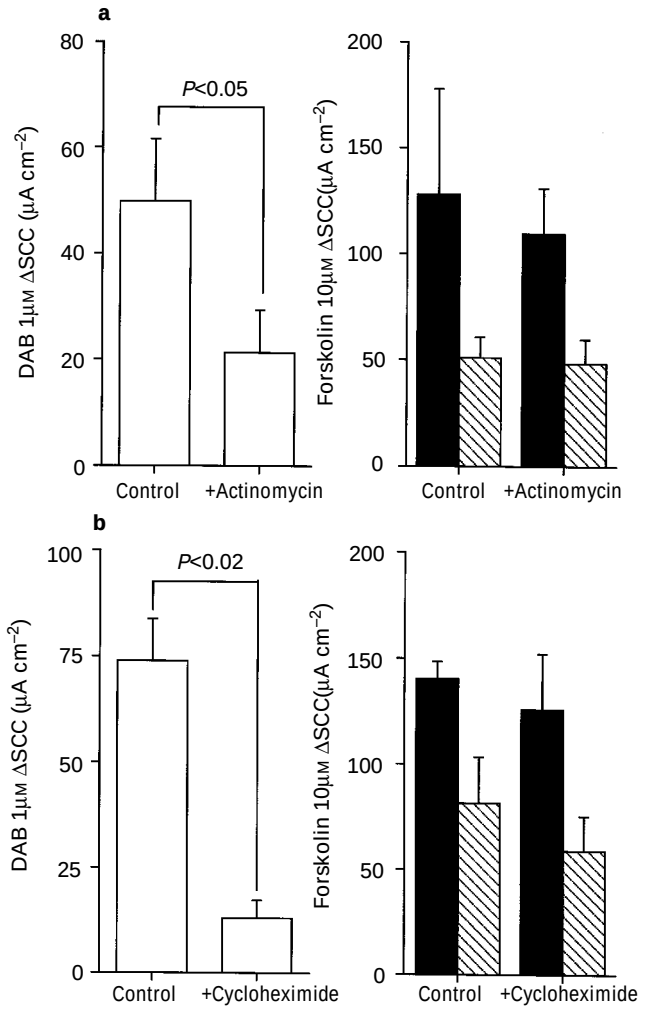

Figure 4 Effects of actinomycin D and cycloheximide on responses to des-Arg^{9}bradykinin (DAB). One of each pair of tissues was exposed to actinomycin D, 200 μM (a) or cycloheximide, 5 μM (b). Both agents caused a significant reduction in the responses to DAB when tested by a two-tailed Student's t test. The responses to forskolin at T = 0 (solid columns) and at 3 h (hatched columns) were not different in test and control tissue. n values were 14 in (a) and 4 in (b).

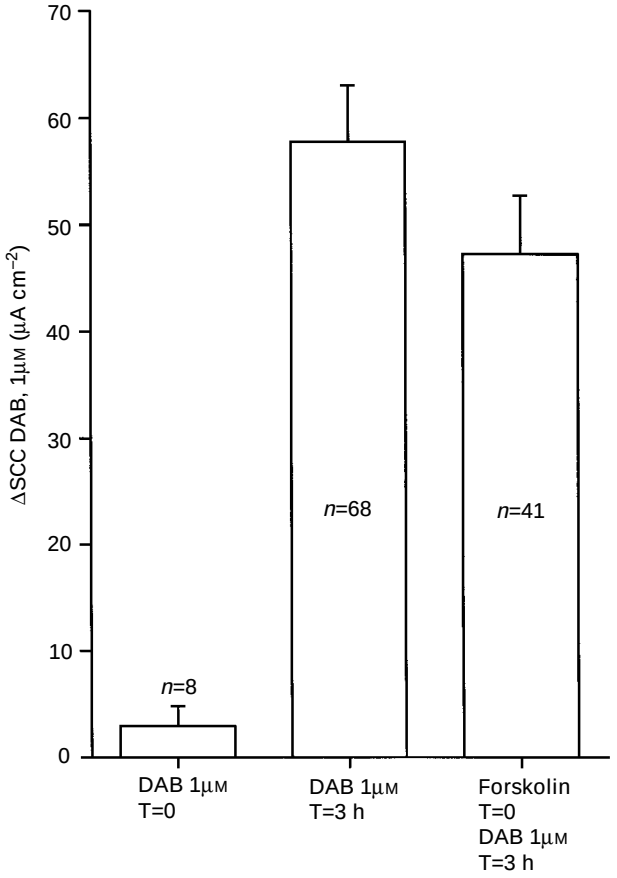

Figure 5 Effects of time on responses to des-Arg^9 bradykinin (DAB). DAB, 1 μM, was applied at T = 0 or 3 h, the latter with or without pre-exposure to forskolin, 10 μM, at T = 0–30 min followed by washing. The values at 3 h were significantly greater than at T = 0 ($P < 0.001$, two-tailed Student's t test), but were not significantly different from each other.

batch. The responses to DAB were quite variable from batch to batch, but we were unable to tie the variation to either the age or weight of the animals. The results with indomethacin, L-NAME and mepyramine were used to investigate the possible involvement of prostaglandins, nitric oxide or histamine in the genesis of the responses. After indomethacin responses to DAB were larger, but not significantly so. If arachidonic acid metabolites were involved in the responses to DAB this substrate may be diverted to the leukotriene pathway in the presence of indomethacin. However, the specific leukotriene synthesis inhibitor, L-656,224 had no significant effect on the responses to DAB. We cannot conclude that any of autacoids for which inhibitors have been used do not have a minor role in the responses generated by DAB. The variability of the responses to DAB precludes detecting anything but major involvement of mediators. Similarly, TTX was used to investigate whether the B_1-receptors were located on nerve terminals left within the submucosa. Finally, it was shown that there was little variation in response between the distal and more proximal parts of the distal colon. The effects of Hoe 140 were given earlier and are added again in the table to indicate the extent of the variation of the control responses between different groups of animals.

The absolute requirement for CFTR chloride channels

In cystic fibrosis (CF) animals chloride secretion to the standard secretagogues does not occur because of the absence of CFTR (cystic fibrosis transmembrane conductance regulator) chloride channels in the apical membrane. However, as most secretagogues also stimulate potassium secretion in addition to chloride the former is revealed in CF tissues (Cuthbert *et al.*, 1994). An unequivocal way of demonstrating that DAB stimulates chloride secretion in the colon is to show this does not occur if CFTR is absent. It was only possible to do this with the mouse colon and Figure 10 gives one of four similar examples of the result. DAB caused a reduction in SCC which was reversed by frusemide, the typical fingerprint of a mammalian CF colon. Note the size of response was relatively small compared to the chloride secretory responses to DAB. In normal mouse colon the chloride secretory response to forskolin is around 150 μA cm^{-2}, as it is for the rat (see Figure 4). Note too that the response to DAB was delayed after addition, as with the response in normal tissues. No responses to DAB were obtained at zero time in CF colons (data not shown).

Discussion

There can be little doubt that the SCC responses to DAB in colonic epithelia mounted *in vitro* for 3 h are due to electrogenic chloride secretion; they occurred in the presence of amiloride, were reversed by frusemide and were not present in tissues lacking CFTR chloride channels. All parts of the distal

colon were shown to be capable of responding equally well to DAB, whereas amiloride sensitivity is confined to the most distal parts (Will *et al.*, 1980). Responses to DAB were not increased by pre-exposure to forskolin, as is found in the mouse colon (Cuthbert, unpublished).

The responses to DAB are classical in that induction is prevented by inhibitors of transcription and translation, as shown for various smooth muscle preparations (Regoli *et al.*, 1978). Few B_1-kinin receptors are present in fresh rat colon tissues, whereas in the dog colon B_1 receptors appear to be constitutive (Rangachari *et al.*, 1993), although it should be remembered that no definitive evidence was obtained to indicate the receptors were B_1. Quite wide variations in the responses to DAB were obtained despite all preparations being treated in an equivalent manner. This suggests that some unknown inducing factors, such as bacterial products, peptidases etc. may be present to different extents in different batches of animals. For this reason we have used paired tissues for most experiments, as closely adjacent tissue pieces generally respond in an equivalent manner. Nevertheless, the pD_2 for DAB was 2×10^7 M^{-1}, comparable to the values given by smooth muscle

Table 1 Agents having no effect on the responses to des-Arg^9 bradykinin

Control	*Test*	*No of pairs*	P	*Treatment*
24.7±4.5	45.5±12.5	8	NS	Indomethacin, 50 μM
68.8±20.3	51.5±27.6	4	NS	L-656,224, 1 μM
110±20.6	92.6±24.9	6	NS	Hoe 140, 1 μM
30.3±13.4	54.1±14.9	6	NS	L-NAME, 200 μM
24.2±7.3	20.2±7.5	6	NS	TTX, 100 nM
55.1±22.9	81.3±22.7	4	NS	Mepyramine, 0.1 μM
62.7±12.2	51.9±14.2	5	NS	Most distal versus 5 cm more proximal

Data show responses to DAB, 1 μM, in μA cm^{-2}; means ±s.e.mean.

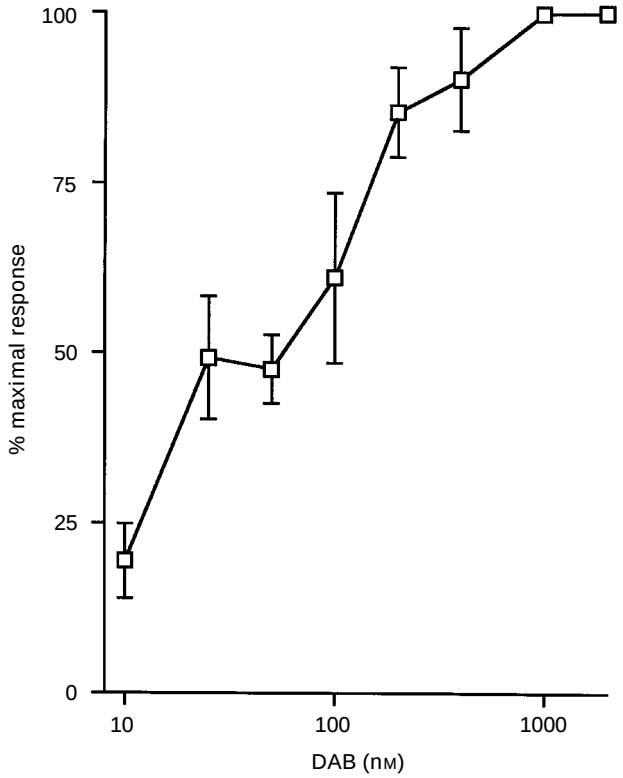

Figure 6 Concentration-response curve for des-Arg^9 bradykinin (DAB). Curve was constructed from 14 preparations, each was exposed to 3–4 concentrations of DAB, applied cumulatively at 3 h after mounting. The response to 1(or 2) μM DAB, applied last, was taken as the maximal effect and smaller responses expressed as a percentage of this response. Vertical lines show s.e.mean.

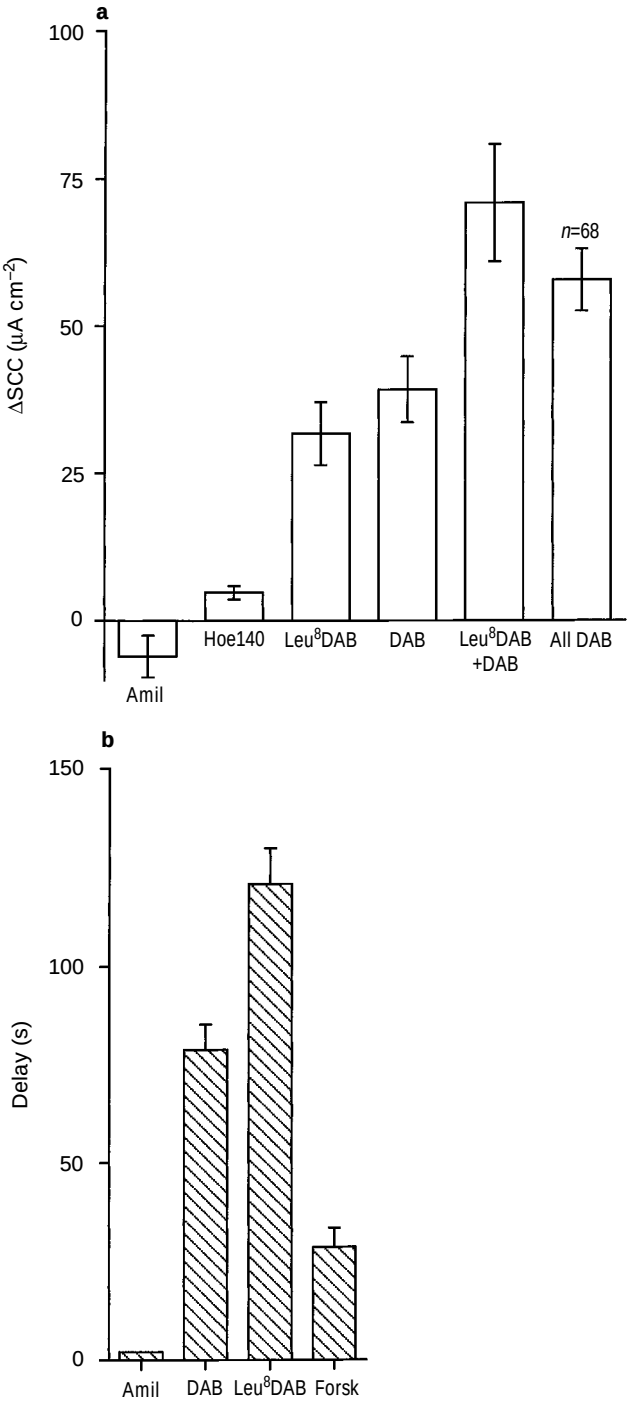

Figure 7 Effect of Leu^8-des-Arg^{9}bradykinin (Leu^8-DAB) on SCC responses. (a) Results with 8 preparations were exposed sequentially to amiloride (100 μM), Hoe140 (1 μM), Leu^8-DAB (1 μM), DAB (1 μM) and forskolin (10 μM). Means±s.e.mean (except for forskolin) are shown together with the values for Leu^8-DAB plus DAB and the cumulative value for DAB (All DAB) from Figure 5. The additive response to Leu^8-DAB plus DAB was not significantly different from the responses to DAB alone. (b) Delay, in seconds, between the addition of compounds and the time at which the SCC began to change, $n=8$. Responses to DAB, Leu^8-DAB and forskolin were significantly slower than the response to amiloride ($P<0.001$). Responses to DAB were significantly faster than those to Leu^8-DAB ($P<0.002$) and significantly slower than those to forskolin ($P<0.001$) (Student's t test).

tissues (see Marceau, 1995). However, it is noted that the responses to forskolin were somewhat smaller, although not significantly so, after treatment with either cycloheximide or actinomycin D. This suggests that these agents may have some non-specific effects on secretion. However, the effects on DAB were significantly depressed, suggesting an absolute requirement for protein synthesis.

Investigations with B_1-kinin receptor antagonists indicated that the receptors induced in the rat colon did not have the expected specificity. Two antagonists, Leu^8-DAB and des-Arg^{10} Hoe 140 had agonist activity, which was not due to the activation of B_2-kinin receptors, at least with Leu^8-DAB. Indeed Leu^8-DAB together with DAB gave the same total response as a supramaximal concentration of DAB alone. Des-Arg^{10} Hoe 140 had less agonist activity than the Leu^8 compound and was able to inhibit DAB responses which were readily surmounted. We did not attempt to determine affinity constants for antagonists as the quality of concentration-response curves to DAB in colonic epithelia was not high and we needed recourse to 'percentage of maximum' to establish the EC_{50}, because of inherent variations. However, the new B_1-kinin receptor antagonist, R-715, gave the clearest indication that the receptors in rat colon are B_1. R-715 has a pA_2 of 9 in rabbit and guinea-pig tissues, but only 7 in rodents (Regoli, personal communication). Taking a pD_2 of 2×10^7 M^{-1} for DAB and a pA_2 of 7 for R-715, the conditions depicted in Figure 9b and applying mass action law the mean inhibition of DAB responses should be 91%, and indeed 94% was found. Increasing the DAB to 10 μM should restore the responses to those where no inhibitor was present, again as was found. It can be concluded therefore that the receptors in rat colon epithelium are of a non-classical B_1-type, but non-classical simply means less well investigated. There is molecular evi-

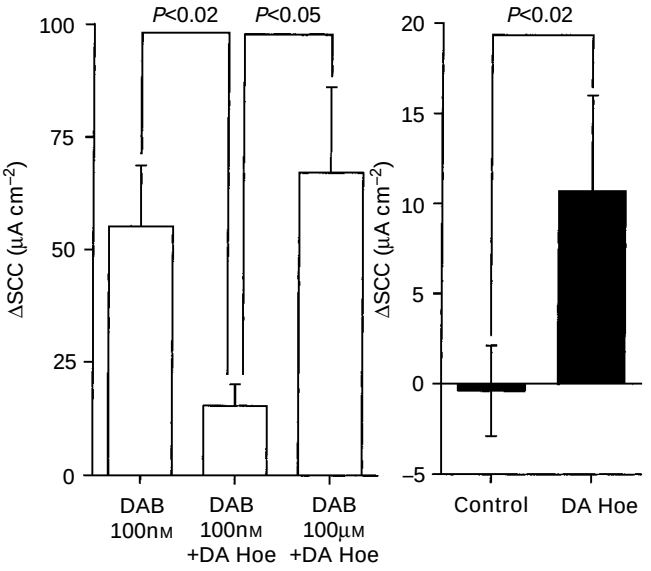

Figure 8 Effects of des-Arg Hoe 140 on the responses to des-Arg^9 bradykinin (DAB). Paired preparations were used to examine the effects of des-Arg Hoe 140 (2 μM, DA Hoe) on responses to DAB, 100 nM. Ten minutes after DAB had been added in the presence of DAB Hoe the DAB concentration was increased to 10 μM. Means $\pm$ s.e. are given for 6 paired preparations, statistical comparisons were made by a paired, two-tailed Student's t test. Also shown (solid columns) are the changes in SCC 10 min after DA Hoe was added compared to control preparations during the same period.

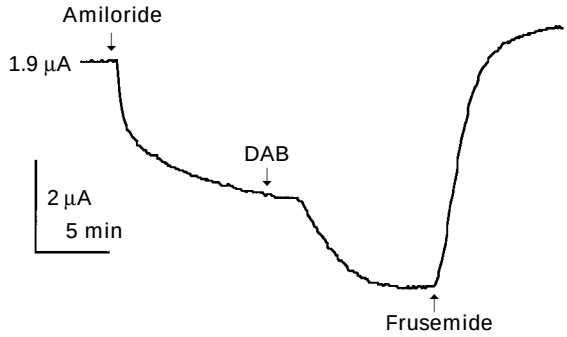

Figure 10 SCC recording from a cystic fibrosis (CF) mouse CF colonic epithelium (20 mm^2). Amiloride (100 μM), des-Arg^9 bradykinin (DAB), (1 μM) and frusemide (1 mM) were added sequentially. The recording was made three hours after mounting.

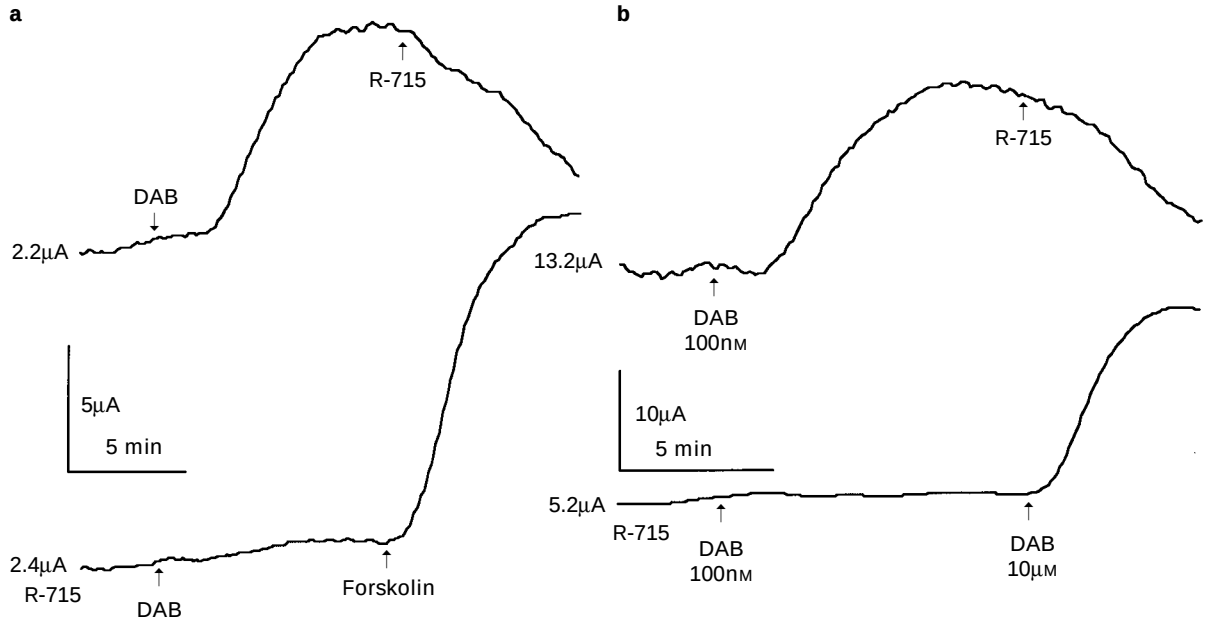

Figure 9 Effects of R-715 on the responses to des-Arg^9 bradykinin (DAB). (a) Illustrates a paired preparation in which the effects of DAB (1 μM) were examined in the presence and absence of R-715 (2 μM). Subsequent additions of R-715 and forskolin are indicated. (b) Shows antagonism of DAB (100 nM) by R-715 (2 μM) in two preparations. Subsequent increase in the DAB concentration to 10 μM overcame the effect of R-715.

dence to support the notion that rodent B_1-receptors are different from those in man and rabbit. Human and rabbit B_1-receptors show a distinct agonist preference for des-Arg^9 kallidin, this peptide being several orders of magnitude more potent than des-Arg^9 bradykinin, while for the mouse the sensitivity to des-Arg^9 bradykinin is 2–3 fold greater than for the lysyl derivative (Menke *et al*., 1994; Hess *et al*., 1996). The homology between the mouse B_1-receptor protein and the rabbit and human proteins is 73% and 72%, respectively (Hess *et al*., 1996). Major differences are that the mouse protein has an 8-amino acid insertion in the first intracellular loop and the C-terminus is truncated. Mouse and rat kininogen genes code for a sequence Arg-bradykinin (Hess *et al*., 1996), and hence DAB can be formed in the rat from bradykinin carboxypeptidases. Thus there is circumstantial evidence that in the rat DAB is a potent, endogenous ligand which activates atypical B_1-type kinin receptors.

It is not yet known if the B_1-receptors are on the epithelial cell themselves or elements in the submucosa, although no evidence was found that the receptors might be on remaining neuronal elements, so causing epithelial secretory effects indirectly by release of neurotransmitters. Activation of B_2-kinin receptors in this tissue also leads to a chloride secretory effect via an increase in intracellular Ca^{2+}, and following the formation of prostaglandins and the activation of adenylate cyclase, an increase of intracellular cyclic cAMP (Cuthbert *et al*., 1984). However, indomethacin did not inhibit the responses to DAB, as it does following the activation of B_2 receptors (Cuthbert & Margolius, 1982). The transduction mechanisms involved in the DAB effect on the colonic epithelium have yet to be investigated. There are two further ways in which the actions of kinins at B_1- and B_2-receptors differ. First, there is a considerable delay before the response to DAB becomes apparent, and this is true also of Leu^8-DAB, whereas effects at B_2-receptors are rapidly evoked (Cuthbert *et al*., 1996). Secondly, responses to DAB are well sustained; on some occasions responses have been recorded for over 30 min, even increasing slowly after 15 min or so, but by contrast, B_2-receptor effects are not well maintained due to receptor internalization (Roscher *et al*., 1984). These differences may throw light on the possible functional significance of B_1-receptors. B_1 receptor formation is generally triggered by damage or traumatic event, of which removal from the animal and separation of the muscle layers is an example. Clearly if the activation of B_1-receptors is to be effective in signalling the effects of damage, responses will be need to be maintained. Abnormal kallikrein levels have been associated with disorders of intestinal function (Zeitlin & Smith, 1973) and it is possible that induction of B_1-kinin receptors is involved in conditions such as colitis and inflammatory bowel disease.

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