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Kinin effects on chloride secretion do not require eicosanoid synthesis

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1 The actions of bradykinin on colonic epithelia from essential fatty acid-deficient (EFAD) rats has been examined. Electrogenic chloride secretion as short circuit current (SCC) and release of immunoreactive prostaglandin E_2 (iPGE$_2$) and i 6-keto PGF$_{1\alpha}$ have been measured.

2 Resting release of prostanoids was significantly less in EFAD than in control tissues. Bradykinin, in a maximally effective concentration, produced no increase in prostanoid release in EFAD tissues in contrast to controls, while the SCC response was 55% of that in controls.

3 In EFAD tissues the SCC response to bradykinin was the same whether or not the cyclo-oxygenase inhibitor piroxicam was present.

4 EFAD tissues were not more sensitive to prostaglandins than control tissues.

5 We conclude that while prostaglandin release contributes to the totality of the response to bradykinin, the latter's effect on electrogenic chloride secretion does not require the obligatory production of arachidonic acid metabolites.

Introduction

In 1982 it was discovered that kinins, such as bradykinin and kallidin, are potent stimulants of electrogenic chloride secretion in epithelia lining the alimentary tract (Cuthbert & Margolius, 1982; Manning *et al.*, 1982). It soon became clear that the effects of kinins were, at least in part, indirect and involved the formation of eicosanoids. For example, indomethacin, a fatty acid cyclo-oxygenase inhibitor, attenuated responses to kinins and these peptides stimulated the release of several different eicosanoids in isolated epithelia. In addition prostaglandins of the E series are known to increase chloride secretion in the colon (Cuthbert & Margolius, 1982; Musch *et al.*, 1983; Cuthbert *et al.*, 1984b).

From studies of the dependence on extracellular calcium for the kinin effect it was concluded that a primary action was to increase calcium influx through the basolateral aspect of the epithelium. It was envisaged that the consequences of this are many, only one of which is to stimulate eicosanoid formation (Cuthbert *et al.*, 1984a,b). As raised intracellular calcium can itself stimulate chloride secretion (Frizzell, 1977), it is possible that eicosanoid formation is not obligatory for the kinin effect, although it can, undoubtedly, contribute to the totality of the response.

Previous studies of the dependency of the kinin response upon eicosanoid formation have relied, in part, upon inhibition of fatty acid cyclo-oxygenase by compounds like indomethacin (Cuthbert *et al.*, 1982; 1984b). These compounds have additional actions, in particular effects on calcium fluxes (Burch *et al.*, 1983) which confound the interpretation of the results. An alternative way to circumvent these problems is to use tissues deficient in the essential fatty acid precursors for eicosanoid formation. It might be expected that such tissues would be unable to produce eicosanoids in response to kinin. Here we show this is so; nevertheless the tissues show substantial chloride secretory responses on challenge with kinin, indicating that eicosanoid formation is not mandatory in their generation.

Methods

All experiments were carried out with epithelia dissected from the lining of the colon of female Long-Evans rats (250–300 g). These were mounted in Ussing chambers (window area 0.6 cm^2) and bathed in Krebs-Henseleit solution at 37°C gassed with 95% O_2–5% CO_2. The method for voltage clamping at zero potential (short circuiting) was as previously given (Cuthbert & Margolius, 1982).

In some experiments samples of bathing fluid were

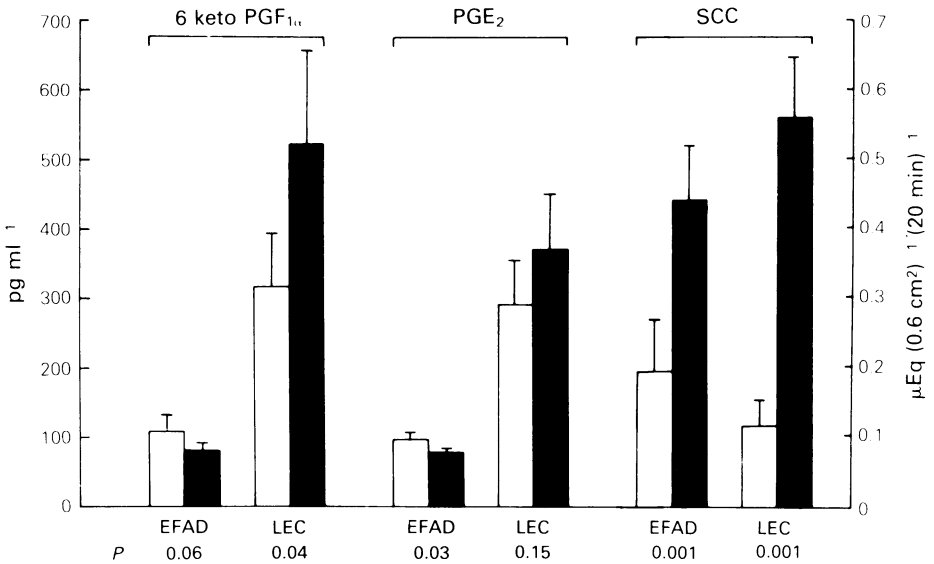

Figure 1 Data from experiments on isolated colonic epithelia from EFAD ($n = 8$) and LEC ($n = 7$) tissues. Tissues were short circuited and bathed on both surfaces with Krebs-Henseleit solution (20 ml). At time zero the bathing solution was changed and 20 min later a sample of the basolateral bathing fluid was taken and frozen immediately. The bathing solution was changed once more and bradykinin (5 μM) added to the fluid on the basolateral side. After a further 20 min another sample of the basolateral fluid was taken and frozen. The frozen samples were thawed and assayed for 6-ketoPGF$_{1\alpha}$ and PGE$_2$.

The area under the curve relating SCC with time was integrated and converted to μEq, using the Faraday relation.

The means are shown with s.e. indicated by vertical lines. Open columns give values for the period before kinin was added while closed columns show values for the period of treatment with kinin. Values obtained before and during kinin action were compared using a paired *t* test. The *P* values are shown below the pairs of columns. The total amounts of prostanoid released during 20 min can be obtained by multiplying the values given by 20. Epithelial area was 0.6 cm^2 in all experiments.

taken, frozen at −20°C and stored until assayed for 6-ketoPGF$_{1\alpha}$, a stable metabolite of prostacyclin, and PGE$_2$ by previously described methods (Burch *et al.*, 1983; Cuthbert *et al.*, 1984b).

Essential fatty acid deficient (EFAD) rats were bred according to protocols given before (Cook et al., 1979) and where necessary the responses of their tissues compared with those of tissues from weight-matched Long-Evans controls (LEC) fed a standard laboratory diet.

The Krebs-Henseleit solution used had 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 had a pH of 7.4 at 37°C when bubbled with 95% O_2 and 5% CO_2.

Results

Colonic epithelia from EFAD rats were compared with those from LEC controls in the experiment illustrated in Figure 1. The amounts of immunoreactive (i) 6-ketoPGF$_{1\alpha}$ and iPGE$_2$ released into the bathing solution before and during exposure to bradykinin (Bk) were measured together with the SCC responses in voltage clamped epithelia. The basal release of both eicosanoids was significantly less ($P < 0.01$, Student's *t* test) in EFAD tissues compared with those from LEC rats. Addition of Bk (5 μM) to the solution bathing the basolateral side of the tissue caused no increase in release of either eicosanoid in EFAD tissues, indeed the levels fell slightly during the second collection period, significantly so in the case of iPGE$_2$. By contrast, exposure of LEC tissues to Bk caused an increased release of both eicosanoids, which was significant for i6-ketoPGF$_{1\alpha}$. Thus there is a clear distinction between EFAD and LEC tissues. Nevertheless Bk caused a significant ($P < 0.001$) increase in SCC in both types of tissue. The increase in SCC due to a maximally effective concentration of Bk was 0.243 ± 0.055 μEq $(0.6\,cm^2)^{-1}\,(20\,min)^{-1}$ in EFAD tissues compared to 0.447 ± 0.099 μEq $(0.6\,cm^2)^{-1}\,(20\,min)^{-1}$ in LEC tissues. The value for LEC tissues was significantly greater ($P < 0.01$ Student's *t* test) than that for EFAD epithelia.

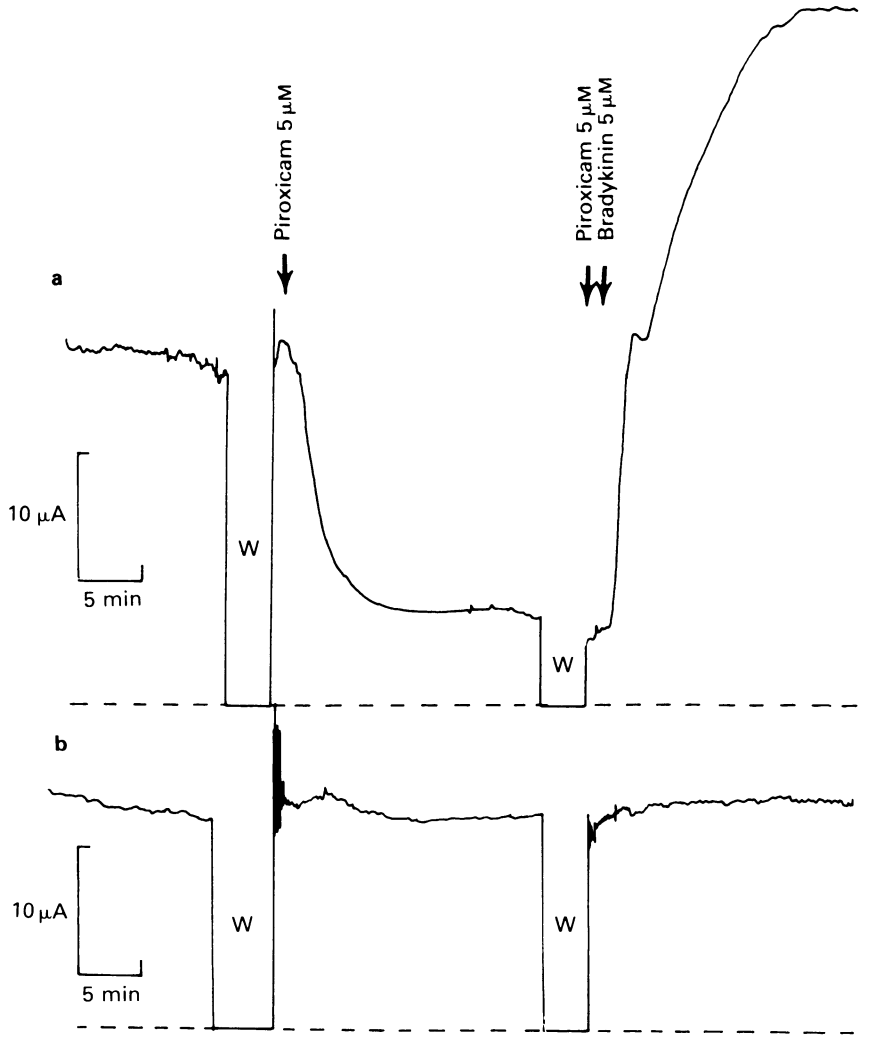

Figure 2 Sample SCC records made using colonic epithelia (0.6 cm^2) from EFAD animals. In (a) piroxicam, 5 μM, was added after the first wash and piroxicam, 5 μM and bradykinin, 5 μM added after the second wash. In (b) the solution bathing the tissue was simply changed at each wash interval. All drugs were added on the basolateral side of the tissue. Samples of the fluid bathing the basolateral surface of the tissue were taken at the end of each 20 min period. They were immediately frozen and subsequently used for immunoassay for eicosanoids. Each preparation was bathed in 20 ml solution on each side. Areas under the SCC curves were measured by planimetry.

Although no apparent increase in prostaglandin release was demonstrable in EFAD tissues we did a further series of experiments in the presence of an inhibitor of cyclo-oxygenase, piroxicam. The experimental protocol can be appreciated by reference to Figure 2. All tissues were from EFAD animals. Bathing fluids were changed at 20 min intervals and samples preserved for prostaglandin assays. One set of tissues received no other treatment and were used to see if the prostaglandin levels fell in consecutive collection periods. The other set were exposed to piroxicam at the material time and to Bk during the second collection period.

In the control EFAD tissues, SCC remained constant throughout the experiment as did the release of prostaglandins (Table 1), the latter being comparable in amount to those from EFAD tissues of the first series. Piroxicam produced a fall in SCC in spite of the significantly reduced eicosanoid synthesis in EFAD tissues. In 9 experiments the SCC was 12.1 ± 2.5 μA immediately before piroxicam was added and 4.7 ± 0.8 μA 10 min after its addition

Table 1 SCC responses and prostaglandin release in EFAD tissues in response to bradykinin in the presence of piroxicam

	Control			*Test*		
	Period 1	*Period 2*		*Period 1*		*Period 2*
SCC (μEq)	0.132 ± 0.028 (6)	0.117 ± 0.019 (6)		0.082 ± 0.013 (9)		0.310 ± 0.040 (9)
		NS			$P < 0.001$	
$iPGE_2$ (pg ml^{-1})	44.8 ± 5.4 (6)	50.8 ± 6.6 (5)		48.0 ± 4.0 (9)		37.0 ± 2.2 (8)
		NS			$P < 0.05$	
i6-ketoPGF$_{1\alpha}$ (pg ml^{-1})	41.5 ± 6.2 (6)	32.3 ± 3.0 (6)		31.0 ± 2.7 (9)		23.1 ± 1.7 (9)
		NS			$P < 0.01$*	

All experiments were carried out on EFAD tissues. Periods 1 and 2 were each of 20 min. In test tissues piroxicam (5 μM) was present throughout periods 1 and 2 and bradykinin (5 μM) was present during period 2. A paired *t* test was required to establish a significant difference for the asterisked value.

($P < 0.01$, paired *t* test). In 6 controls the currents immediately before and 10 min after changing the bathing solution, but without piroxicam, were 9.8 ± 2.4 μA and 8.8 ± 2.0 μA, values not significantly different from each other. In the presence of piroxicam, Bk (5 μM) produced a significant increase in SCC without any increase in prostaglandin formation (Table 1). Indeed there was a significant fall in $iPGE_2$ and i6-ketoPGF$_{1\alpha}$ following Bk.

Thus no evidence for prostaglandin release in re-

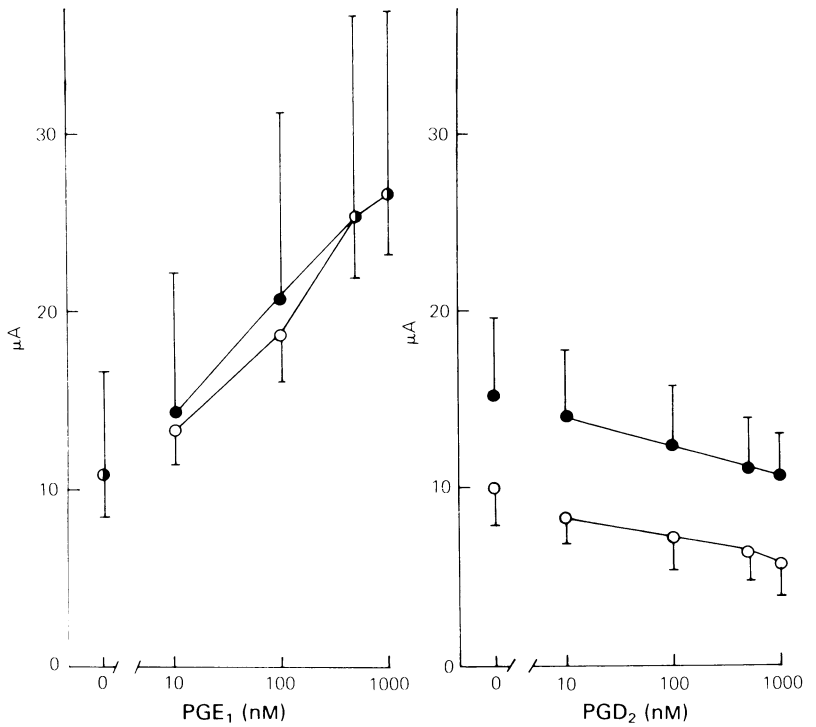

Figure 3 Concentration-response curves for prostaglandin E_1 (PGE_1) and PGD_2 in colonic epithelia from EFAD and LEC animals. Responses were obtained cumulatively and represent the SCC measured in epithelia of 0.6 cm^2. The basal SCC values are also shown. Open circles (○) show results for EFAD tissues while closed circles (●) represent the values for LEC tissues. Each value is the mean for either 6 (EFAD) or 5 (LEC) measurements with s.e.mean indicated by vertical lines.

sponse to Bk was obtained in either set of experiments. We were, however, concerned that minute amounts of these substances might still be formed in close proximity to the epithelial cells and furthermore, EFAD tissues may have been supersensitive to them. Another concern was that other prostaglandins, perhaps PGD_2, which had not been measured might be involved in the kinin response. To meet these concerns SCC responses to PGE_1 and PGD_2 were compared in tissues from EFAD and LEC animals. Neither type of tissue showed any response to PGD_2. Furthermore there was no indication of supersensitivity to PGE_1 in EFAD tissues (Figure 3).

Discussion

We have demonstrated that colonic epithelia from animals made deficient in essential fatty acids show substantial SCC responses to kinin without any increase in the release of $iPGE_2$ or i6-keto$PGF_{1\alpha}$. Furthermore, responses to kinin in EFAD tissues were comparable whether or not a potent inhibitor of cyclo-oxygenase, piroxicam, was present (0.23 μEq $(0.6\,cm^2)^{-1}(20\,min)^{-1}$ in the presence and 0.25 μEq $(0.6\,cm^2)^{-1}(20\,min)^{-1}$ in the absence of piroxicam, from Figure 1 and Table 1). The IC_{50} for piroxicam as a cyclo-oxygenase inhibitor is 0.35 μM (Burch *et al.*, 1983), 14 times less than the concentrations used in these experiments, so that virtually no significant quantities of other prostaglandins we have not measured could have been formed in our experiments.

Although it has been reported that supersensitivity can occur to exogenous prostaglandins after indomethacin (Bukhave & Rask-Madsen, 1980), we have no evidence for supersensitivity in these EFAD tissues.

In colonic epithelia from Sprague-Dawley rats we have shown that kinin increases the release of $iPGE_2$, $iTXA_2$ and i6-keto $PGF_{1\alpha}$ (Cuthbert *et al.*, 1984b). In this more limited study a significant increase in the release of i6-keto $PGF_{1\alpha}$ was recorded. As some prostaglandins increase SCC in colons, including those of Long-Evans rats (Figure 3), it is likely that prostaglandin release contributes to the totality of the response. Indeed we actually show that responses in LEC tissues are significantly greater than those from EFAD animals.

Spontaneous release of $iPGE_2$ and i6-keto$PGF_{1\alpha}$ was significantly less in EFAD tissues compared to controls. Following kinin there was, in some instances, a further reduction in release, even in the presence of piroxicam (Table 1). Clearly the SCC responses to kinin in EFAD tissues cannot be due to PGE_2 or prostacyclin formation and it is unlikely that other prostanoids, that we have not measured, are involved. In any event PGD_2 is without activity in the colon (Figure 3).

One other possibility is that arachidonic acid is diverted down the lipoxygenase pathway by kinin, as it has been shown that 5-hydroperoxyeicosatetraenoic acid and 5-hydroxyeicosatetraenoic acid cause chloride secretion in rabbit colon (Musch *et al.*, 1982). Also it might be expected that addition of piroxicam would favour the conversion of arachidonic acid via the lipoxygenase pathway, yet this agent causes the SCC to fall for, as yet, unexplained reasons.

Thus tissues deprived of essential fatty acids are able to maintain SCC responses to kinin larger than anticipated if all the actions are dependent upon arachidonic acid metabolites. These results support the notion that kinins trigger a number of events on the basolateral aspect of the tissue of which eicosanoid formation is only one (Cuthbert *et al.*, 1984b) and, furthermore, that the latter is not essential for the expression of the response.

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