Br. J. Pharmac. (1982), **75**, 587–598

KININS STIMULATE NET CHLORIDE SECRETION BY THE RAT COLON

ALAN W. CUTHBERT & HARRY S. MARGOLIUS

Department of Pharmacology, University of Cambridge, Cambridge CB2 2QD

1 Short circuit current (SCC), transepithelial conductance and ion fluxes were measured across the isolated descending colon of the rat in response to bradykinin or kallidin.

2 Kinins added to the serosal bath caused immediate increases in SCC but were ineffective when added to the mucosal bath. Increases in SCC were accompanied by significant increases in transepithelial conductance. Threshold kinin concentration was 0.5 nM and maximal increases were seen at 50–100 nM.

3 A rat glandular kallikrein (7 nM) or mellitin (2 μM) also increased SCC if added to the serosal bath.

4 Responses to kinins were unaffected by mucosal amiloride (100 μM) but attenuated or blocked by serosal frusemide (100 μM), indomethacin (1 μM) or mepacrine (50 μM).

5 Replacement of chloride ion in the serosal bath by gluconate and sulphate ions abolished responses to kinins which reappeared after chloride re-addition.

6 Measurement of ^{36}Cl, ^{22}Na and ^{86}Rb fluxes across the tissue showed that the kinin-induced increase in SCC resulted principally from increased net chloride secretion. Effects upon ^{22}Na or ^{86}Rb flux were minimal and made no contribution to the current responses observed in this tissue.

7 The results prove that kinins stimulate net chloride secretion in the rat colon, most probably via a prostaglandin-dependent pathway.

Introduction

The mammalian colon contains a kallikrein (E.C. 3.4.21.8) claimed to be similar to other glandular kallikreins (Zeitlin, Singh, Lambeck & Theiler, 1976; Zimmerman, Geiger & Kortmann, 1979) despite earlier evidence suggesting closer similarity to plasma kallikrein (Seki, Nakajima & Erdös, 1972). The activity of glandular kallikrein is increased by sodium-retaining steroid hormones (Geller, Margolius, Pisano & Keiser, 1972; Margolius, Horwitz, Geller, Alexander, Gill, Pisano & Keiser, 1974; Nasjletti, McGiff & Colina-Chourio, 1978; Nishimura, Alhenc-Gelas, White & Erdös, 1980). In addition, the mammalian glandular enzyme and a kallikrein-like enzymatic activity discovered in *Bufo marinus* bladder and skin are inhibited by amiloride (Margolius & Chao, 1980). This natriuretic and diuretic drug blocks sodium entry into a transport process in apical plasma membranes of tissues (renal distal nephron, salivary duct, colon) which contain high concentrations of kallikrein (for reviews, see Schachter, 1979; Cuthbert, Fanelli & Scriabine, 1979). Recently, it was shown that inhibitors of glandular kallikreins also inhibit sodium reabsorption across the tight epithelium of *Bufo marinus* bladder (Orce, Castillo & Margolius, 1980; 1981). This collection of evidence suggests that the kinin products of kallikreins might have significant effects upon ion transport, most conceivably sodium or potassium, across epithelial membranes.

Some indirect evidence relating to this notion has been gathered previously but understanding of the role of kinins in ion transport across any epithelial surface is limited. In the present work, we measured the effects of bradykinin or kallidin on some ion transporting properties of the rat descending colon, an accessible, aldosterone-sensitive, kallikrein-containing tissue. The results prove that, in this tissue, the principle effect of kinins is to promote net chloride secretion with concomitantly minimal effects upon sodium or potassium transport.

Methods

Male Sprague-Dawley rats (200–500 g) were used in all experiments. The descending colon was opened longitudinally along the mesenteric border, washed free of contents in oxygenated Krebs-Henseleit solution and pinned, mucosal side down, in a dissecting tray. The serosa and as much muscle as possible were

0007-1188/82/040587-12 $01.00

dissected away under a microscope to give a stripped epithelium, similar to that described previously for the rabbit colon (Frizzell, Koch & Schultz, 1976).

Short circuit current recording

Stripped epithelium was lightly clamped between the two halves of perspex Ussing-type chambers. Adjoining chamber halves were coated with yellow soft paraffin to minimize edge damage. Chambers with two different window sizes were used; 0.6 cm^2, circular and 4.24 cm^2, obloid. With the smaller chambers several preparations were obtainable from a single colon. The large chambers were used exclusively for flux studies and each preparation required a single colon. Transepithelial potential was recorded by means of polyethylene electrodes filled with Krebs-Henseleit solution, while current was passed across the epithelium via bridges filled with KCl-agar. Fine pins fixed to one half chamber served both to hold the tissue and locate the two half chambers together on assembly. The half-chambers were connected to water jacketed reservoirs maintained at 37°C. Bathing solutions were circulated through the chambers by using a 95% O_2:5% CO_2 mixture to operate an air lift. The volume of solution bathing each side of the epithelium was 10 or 20 ml. The tissues were voltage clamped (W-P Instruments Dual Voltage Clamp, New Haven, Conn., U.S.A.) at zero potential (short circuited) automatically with compensation for solution resistance between the potential electrodes and the tissue surface. To measure the transepithelial conductance the voltage was clamped automatically and periodically at 1 to 5 mV above and below zero. The current required to do this was displayed on a pen recorder, as was the short circuit current (SCC).

Flux measurements

Unidirectional fluxes of chloride, sodium and potassium were measured using ^{36}Cl, ^{22}Na and ^{86}Rb. Fluxes from the serosal to mucosal bathing solution (J_{SM}) and in the opposite direction (J_{MS}) were measured in separate experiments. These fluxes are referred to as outward fluxes or effluxes and inward fluxes or influxes respectively. The procedures for all three isotopes were similar and as follows. Preparations (4.24 cm^2) were allowed to equilibrate for 20 min after which the isotope under study was added ($\sim$10 μCi) either to the serosal or mucosal bathing solution. A further equilibration period was allowed for the isotope flux to become steady, 20 min for ^{36}Cl and ^{22}Na and 30 min for ^{86}Rb. The volume on either side of the tissue was 10 ml. Sixteen samples (0.1 ml) were taken at 2 min intervals, with fluid replacement, for a period 0–30 min from the side opposite to which the isotope was added. At 30 min, kallidin (1 μM) was added to the serosal solution and a further 16 samples taken during the period 32–62 min. Isotope concentration was measured in triplicate in the fluid to which it had been added at 0, 30 and 60 min and did not change during the experiments. Throughout the tissues were short-circuited. ^{36}Cl and ^{22}Na in the samples were measured by liquid scintillation spectrometry and ^{86}Rb was measured using Cerenkov radiation. The total amount of isotope moved across the epithelium in 30 min was calculated by applying linear regression analysis to cumulative totals for the 16 samples collected in either the 30 min control period or the 30 min period during which kallidin was present. In all instances regression coefficients were highly significant and the correlation coefficients close to 1, usually in excess of 0.97. The method developed here to measure fluxes was designed to detect small changes in unidirectional flux caused by kallidin against a background of a large leak flux characteristic of this tissue (Edmonds & Marriott, 1968). The values are the mean $\pm$ s.e. and significance of the results was evaluated using a paired one-tailed Student's *t* test.

Solutions and chemicals

Krebs-Henseleit solution of the following composition was used (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. To make chloride-free bathing solution, NaCl and KCl were replaced with Na and K gluconate and $CaCl_2$ replaced with $CaSO_4$. Solutions were equilibrated with 95% O_2:5% CO_2 to a pH of 7.3–7.4.

Amiloride hydrochloride and indomethacin (sodium salt) were gifts from the Merck Institute for Thereapeutic Research (West Point, Pa., U.S.A.). Bradykinin triacetate, lysyl-bradykinin, mellitin, frusemide and mepacrine hydrochloride were obtained from the usual suppliers. Radioisotopes were purchased from The Radiochemical Centre, Amersham. Rat urinary kallikrein B was purified to homogeneity as described previously (Chao & Margolius, 1979) and lyophylized. It was dissolved in sterile distilled water in a 100 μM stock solution before use.

Diets

In some experiments, colons were taken from rats fed for 10 days to 3 weeks on low sodium diet. The diet was formulated and supplied by BP Nutrition (Witham, Essex) and by calculated analysis contained 0.05% Na, whereas the identically formulated normal sodium diet contained 0.3% Na. Rats eating these diets drank distilled water *ad libitum*.

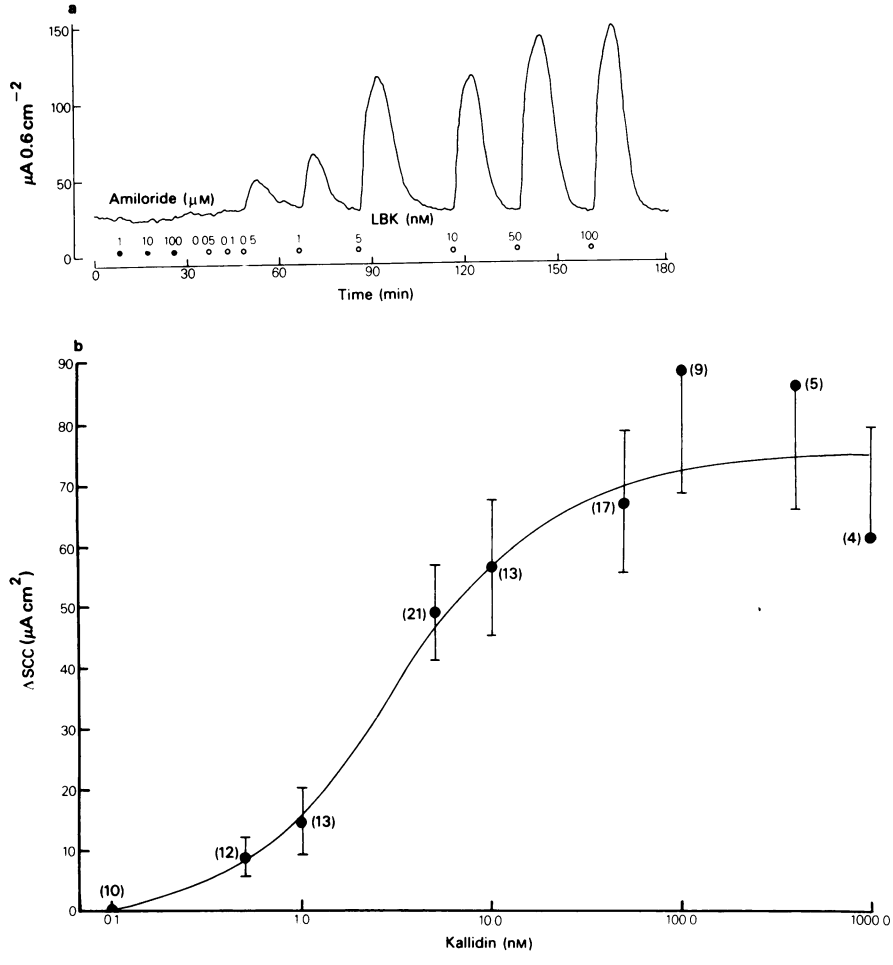

Figure 1 Effect of kallidin on rat colon short-circuit current (SCC). (a) Typical effects of kallidin (LBK), added cumulatively to serosal bath of an Ussing-type chamber, on SCC. Added amounts of amiloride (up to 111 μM cumulative added to the mucosa) did not affect basal SCC in this or 9 other experiments. The SCC response to kinins was generally transient for unknown reasons. (b) Dose-response relation of kallidin on SCC. Numbers in parentheses indicate number of separate tissues at each dose. Not more than 2 descending colon segments came from a single rat. The EC_{50} for kallidin on SCC in the colon is ~3 nM.

Results

Short circuit current responses to kinins, kallikrein and mellitin

Typical effects of kinins, kallikrein and mellitin are shown in Figures 1a and 2. Even in the presence of high concentrations of mucosal amiloride (100 μM), previously shown to have no effect on SCC in the normal rat colon (Will, Lebowitz & Hopfer, 1980), either bradykinin or kallidin caused a prompt and rapid increase in SCC when added to the serosal bath. Both bradykinin and kallidin were ineffective in changing SCC when added to the mucosal bath in concentrations up to 10 μM. The presence of mucosal amiloride 100 μM did not affect the short circuit current response to serosal kinin. Figure 1b shows that the threshold kinin concentration is about 500 pM and maximal responses were seen with 50–100 nM kinin. Kinin responses were transient for unknown reasons. We showed it was unnecessary to wash out the previous dose before a subsequent exposure to kinin was made.

A purified rat urinary kallikrein (Chao & Margolius, 1979) or mellitin, a bee venom polypeptide shown previously to be the most potent activator of

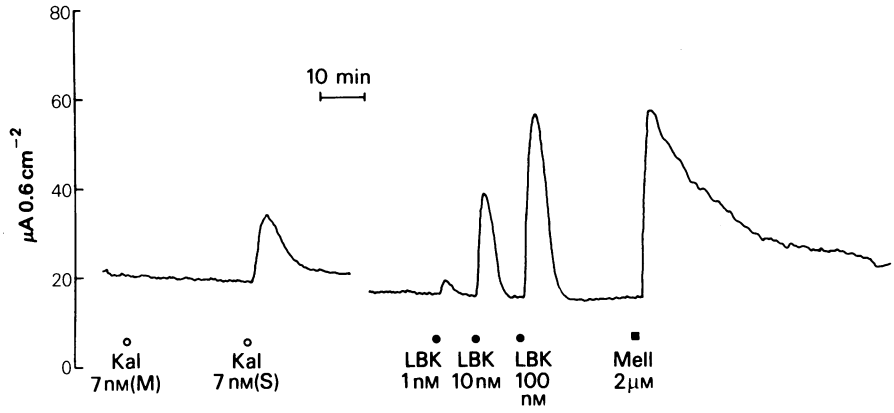

Figure 2 Effect of purified rat urinary kallikrein (Kal) (Chao & Margolius, 1979) or mellitin (Mell) on SCC. Responses relative to kallidin (LBK) in the same preparation are shown. Addition of kallikrein, mellitin or kallidin to the mucosal bath never affected SCC, although kallikrein concentrations tested never reached the μM range.

plasma membrane-bound kallikrein (Nishimura *et al.*, 1980), were also potent stimuli to SCC but only if added to the serosal bath (Figure 2).

Kallidin and transepithelial conductance

In nine descending colons, transepithelial conductance and SCC responses to a supramaximal serosal concentration (1 μM) of kallidin were measured (Table 1). In each preparation, conductance increased and the change was highly significant, although to only a fractional degree (+13.2%) of basal conductance in this leaky epithelium.

Table 1 Effect of kallidin on transepithelial conductance and short circuit current (SCC)

	SCC ($\mu A\ cm^{-2}$)	*Conductance* ($mmho\ cm^{-2}$)
Control	21.9± 5.3	12.1±2.7
Kallidin (1 μM)	62.3±14.0	13.6±2.8
Δ	40.4±10.3*	1.6±0.3**

Results (mean±s.e.) were obtained using nine separate descending colon preparations mounted in chambers in the usual fashion (see Methods) and allowed to equilibrate to 30 min–1 h before control measurements were made. During the period of stable control SCC, the height of 20 consecutive current pulses resulting from clamping the voltage across the tissue at from 1–5 mV above and below zero alternately every min, was measured. Then the peak SCC response to 1 μM kallidin was recorded and the height of the 20 subsequent and consecutive current pulses was also measured. Total conductance was measured as the ratio of the size of the current pulses to the size of the command voltages. In every instance mean transepithelial conductance increased after kallidin. *$P<0.005$; **$P<0.001$ using a paired one-tailed Student's *t* test.

Effects of chloride replacement and drugs on kallidin responses

When serosal medium chloride was substituted, kallidin did not increase SCC when added to the serosal bath (Figure 3). Subsequent replacement of chloride-containing medium was accompanied by a return of SCC responses to kallidin.

The possibility that kallidin was affecting chloride transport was increased by studies with frusemide. Serosal frusemide (100 μM) significantly attenuated the SCC response to kallidin. Basal SCC usually was decreased slightly by this dose of frusemide (Figure 4). In 9 preparations the response to kallidin (0.1 μM) was $58.9\pm15.1\ \mu A\ cm^{-2}$ before and $19.8\pm6.2\ \mu A\ cm^{-2}$ after frusemide (100 μM). The difference was significant at $P<0.05$.

Indomethacin (50 μM) or mepacrine (10 μM) added to the serosal bath abolished or attenuated SCC responses to kallidin (10 μM) (Figure 5). Subsequent increases in SCC in response to prostaglandin E_1 also could be attenuated by frusemide. This latter effect is similar to that described by Frizzell & Heintze (1979) using prostaglandin E_2 (PGE_2). Indomethacin (50 μM) usually reduced SCC to zero in these preparations (Figure 5a).

Kallidin and ion fluxes

The large leak fluxes present in colonic epithelia together with the possibility that the changes in ion flux caused by a kinin might be rather small required that the methodology used to measure ion fluxes be modified from that in preceding parts of this study. Ussing-type cells with an obloid window were used to

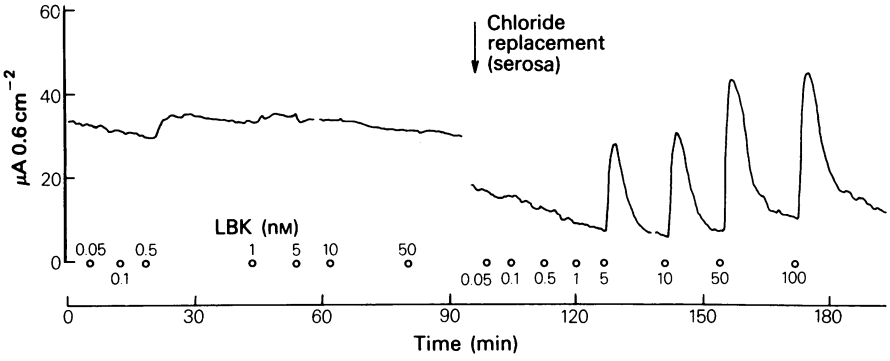

Figure 3 Effect of serosal kallidin on SCC in the absence (left) and after replacement (right) of serosal gluconate and sulphate with chloride. The result is typical of experiments in 3 animals. Throughout the mucosal solution was normal.

increase exposed tissue area to 4.24 cm^2. As a result, only one preparation was available from each animal. A supramaximal concentration (1 μM) of kallidin was used so that a maximal and sustained increase in current was obtained for the duration of the flux measurement. In every instance the change in ionic flux caused by kallidin was compared to the change in SCC in the same experiment.

(a) Effects on chloride fluxes The effects of kallidin on chloride fluxes are shown in Table 2. In the absence of kallidin there was net chloride movement from mucosa to serosa corresponding to 4.32 $\mu Eq\,cm^{-2}\,h^{-1}$. The control values of J_{SM} and J_{MS} for chloride were significantly different ($P<0.05$).

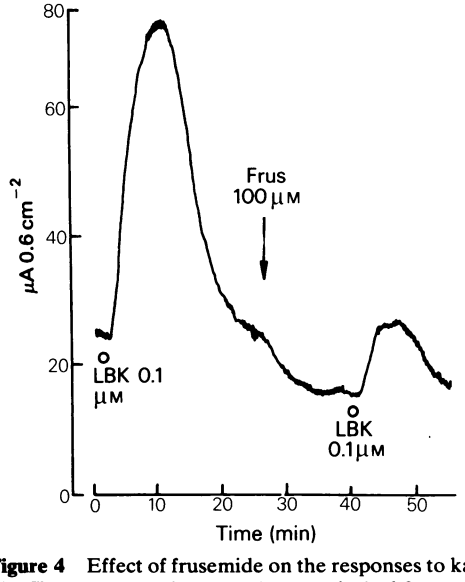

Figure 4 Effect of frusemide on the responses to kallidin. The responses illustrated are typical of 9 separate experiments. Note frusemide itself causes a reduction in SCC as well as inhibiting responses to kallidin.

The mean current in these tissues before kallidin was 25.2 ± 6.1 $\mu A\,cm^{-2}$ ($n=14$), which would correspond to a net outward, that is towards the lumen, chloride movement of 0.94 $\mu Eq\,cm^{-2}\,h^{-1}$. It is clear, therefore, that the resting current cannot be equated to chloride movement. It is probable that most, if not all, of the chloride absorption is electrically silent, probably absorbed in exchange for bicarbonate (Phillips & Schmalz, 1970). Kallidin (1 μM) reduces net chloride absorption to only 0.23 $\mu Eq\,cm^{-2}\,h^{-1}$, this value being not significantly different from zero. The change in chloride movement caused by the kinin amounts to 4.09 $\mu Eq\,cm^{-2}\,h^{-1}$, which is equivalent to ~ 137% of the current change caused by the kinin (or alternatively, if kinins influence only chloride movement through electrogenic pathways, the movement would be more than adequate to

Table 2 Effects of kallidin on chloride fluxes

	Kallidin		
J_{SM} (0–30)	J_{SM} (32–62)	ΔJ_{SM}	ΔSCC
16.95 ± 1.35	19.08 ± 1.57	2.13 ± 0.41	3.20 ± 0.40
$P<0.0025$			
	Kallidin		
J_{MS} (0–30)	J_{MS} (32–62)	ΔJ_{MS}	ΔSCC
21.27 ± 1.36	19.31 ± 1.64	−1.96 ± 0.57	2.73 ± 0.56
$P<0.05$			

All values are given as $\mu Eq\,cm^{-2}\,h^{-1}$. Each measurement is the mean value (± s.e.) for seven determinations. P values were obtained using a one-tailed paired t test. J_{SM} and J_{MS} refer, respectively, to the serosal to mucosal and to the mucosal to serosal unidirectional chloride flux. Kallidin (1 μM) was present in the serosal bathing solution during the period 32–62 min, while 0–30 min was the control period. The Δ values refer to the changes in flux or in SCC caused by kallidin.

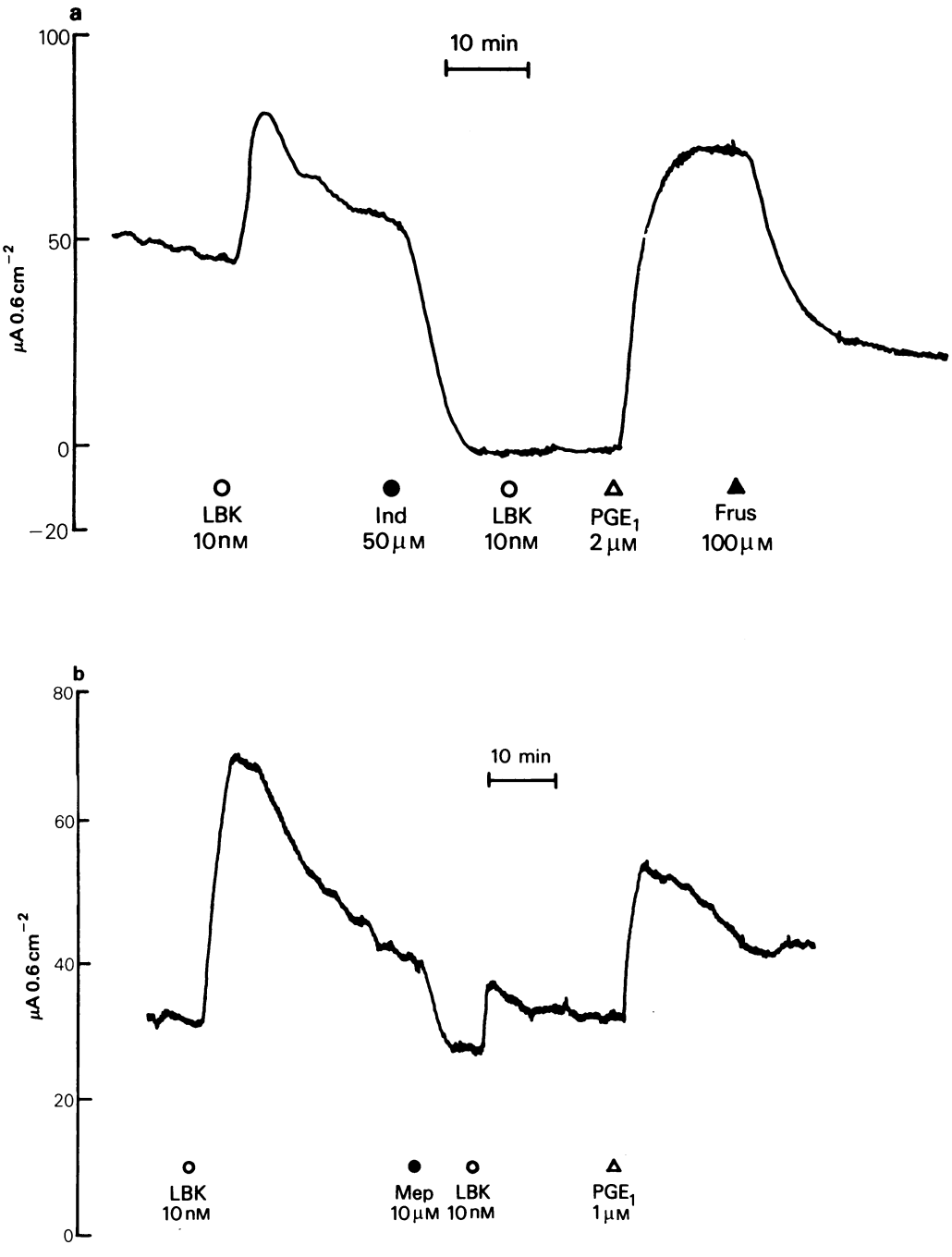

Figure 5 Effect of prostaglandin synthesis inhibition on kinin-induced SCC responses. (a) Indomethacin (Ind), 50 μM, abolishes the response to kallidin (LBK), 10 nM, as well as reducing basal SCC to zero. The subsequent response to prostaglandin E (PGE_1), 2 μM, is attenuated by frusemide (Frus), 100 μM. Tracing is typical of 4 preparations from 4 rats. (b) Mepacrine (Mep), 10 μM, reduces SCC and attenuates the SCC response to kallidin (LBK), 10 nM. PGE_1 produces the expected increase in SCC. Typical of 3 preparations from 3 rats.

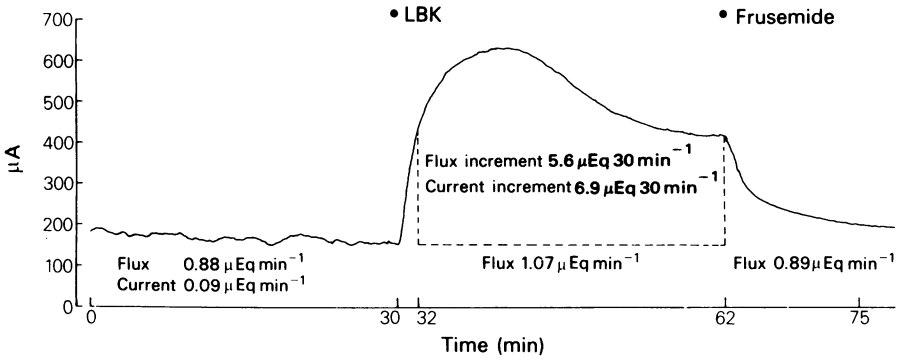

Figure 6 Results of a single chloride flux experiment measuring J_{SM}. The current record is shown. The actual outward fluxes of chloride in the serosal to mucosal direction in μEq min^{-1} are shown below the current record. The area enclosed by the dotted line shows the increment in current due to the addition of kallidin to the serosal bath. The coulombic equivalent of this area was 6.9 μEq in 30 min The increased outward flux was equivalent to 5.6 μEq in 30 min. This value was calculated from the increase in unidirectional flux (1.07–0.88) μEq min^{-1} multiplied by the duration of the response (30 min).

account for the current changes). The effects of kallidin are both to reduce chloride movement inwards from the lumen and to increase chloride efflux to similar extents.

The average values given in Table 2 obscure the variability which existed amongst different tissues. This is illustrated in extreme examples shown in Figures 6 and 7. In the experiment illustrated in Figure 6 the unidirectional chloride flux was measured in the serosal to mucosal (outward) direction. During the control period $J_{SM}Cl$ was almost 10 times the current equivalent. $J_{SM}Cl$ increased ~22% in the presence of kallidin (1 μM) and returned to control values when frusemide (100 μM) was added to the serosal bath. The extra current caused by kallidin (6.9 μEq 30 min^{-1}) was somewhat larger than the increment in $J_{SM}Cl$ (5.6 μEq 30 min^{-1}). Thus, 81% of the current increase in this experiment might be explained on the basis of an augmented serosal to mucosal chloride flux. Further, the augmented $J_{SM}Cl$ and SCC induced by kallidin were returned to control levels by frusemide, 100 μM. Figure 7 illustrates the opposite extreme and shows that all of the increment in current caused by kallidin can result from a decrease in $J_{MS}Cl$ (from −1.81 to −1.67 μEq min^{-1}).

(b) Effects on sodium fluxes The results are shown in Table 3. In unstimulated colons, there was a difference amounting to 2.67 μEq cm^{-2} h^{-1} between $J_{SM}Na$ and $J_{MS}Na$ suggesting a small net inward flux. However, the values of J_{SM} and J_{MS} were not significantly different. The serosal positive nature of the transepithelial potential suggests that in the unstimulated state there is net transfer of positive charge inwards across the tissue. In the 10 preparations used in this study the resting SCC was 38.9 ± 15.1 μA cm^{-2} corresponding to a transfer of univalent positive ions inwards at a rate of 1.45 μEq cm^{-2} h^{-1}.

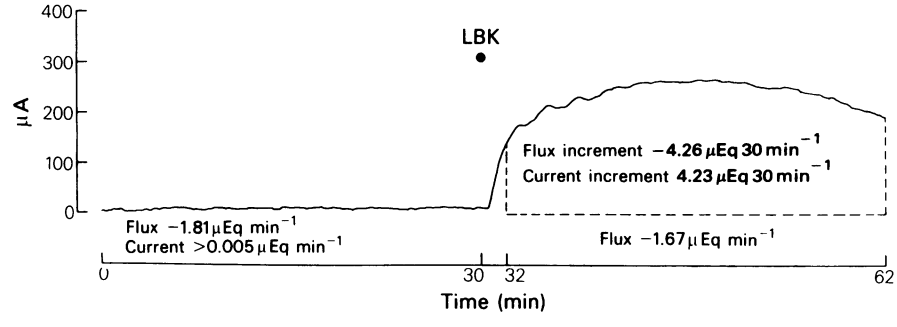

Figure 7 Results of a single chloride flux experiment measuring J_{MS}. The actual current record is shown. The actual inward fluxes of chloride from mucosal to serosal baths are given as μEq min^{-1} below the record and are given as negative values (see Figure 6). The area enclosed by the dotted line shows the increment in current due to the addition of kallidin to the serosal side. The coulombic equivalent of this area was −4.23 μEq in 30 min. The reduced inward flux was calculated from the decrease in unidirectional flux −(1.81–1.67) μEq min^{-1} multiplied by the duration of the response (30 min).

Table 3 Effects of kallidin on sodium fluxes

	Kallidin		
J_{SM} (0–30)	J_{SM} (32–62)	ΔJ_{SM}	ΔSCC
13.70 ± 1.58	12.55 ± 1.32	−1.13 ± 0.50	5.74 ± 1.50
$P < 0.05$			
	Kallidin		
J_{MS} (0–30)	J_{MS} (32–62)	ΔJ_{MS}	ΔSCC
16.37 ± 0.53	14.64 ± 0.74	−1.70 ± 0.53	5.40 ± 1.20
$P < 0.025$			

All values are given as μEq cm^{-2} h^{-1}. Each measurement is the mean value (± s.e.) for five determinations. *P* values were obtained using a one-tailed paired *t* test. J_{MS} and J_{SM} have the same meaning as in Table 2. Kallidin (1 μM) was present in the serosal solution during the 32–62 min period. The Δ values refer to the changes in flux or SCC caused by kallidin.

Table 4 Effect of kallidin on rubidium fluxes

	Kallidin		
J_{SM} (0–30)	J_{SM} (32–62)	ΔJ_{SM}	ΔSCC
0.48 ± 0.06	0.58 ± 0.04	0.10 ± 0.02	8.14 ± 2.00
$P < 0.0125$			
	Kallidin		
J_{MS} (0–30)	J_{MS} (32–62)	ΔJ_{MS}	ΔSCC
0.50 ± 0.06	0.54 ± 0.08	0.04 ± 0.06	7.22 ± 1.08

All values are given as μEq cm^{-2} h^{-1}. Each measurement is the mean value (± s.e.) for five determinations. *P* value was obtained using a single tailed paired *t* test. J_{MS} and J_{SM} have the same meaning as in Table 2. Kallidin (1 μM) was present in the serosal bathing solution during the 32–62 min period. The Δ values refer to the changes in flux or SCC caused by kallidin.

Kallidin caused a small but significant reduction in both J_{SM}Na and J_{MS}Na. Thus, at most, kallidin reduces net sodium influx by only 0.57 μEq cm^{-2} h^{-1}. Clearly, changes in sodium transport can have little importance for the effects of kallidin on SCC, which in this series of experiments produced a charge transfer equivalent to 5.57 μEq cm^{-2} h^{-1}. The direction of the changes would reduce by ~10% the discrepancy between net chloride flux and SCC.

(c) Effects of rubidium (potassium) fluxes Fluxes of rubidium through colonic epithelium were very small and approximately equal in the M→S and S→M direction (Table 4). In this series the colons were particularly sensitive to kallidin with increments in current equivalent to a mean value of 7.70 μEq cm^{-2} h^{-1} of monovalent ion. It is clear that potassium cannot contribute to this current significantly. Kallidin did cause a significant, although small, increase in flux in the S→M direction The possibility cannot be excluded that this flux represents shedding of a few epithelial cells loaded with rubidium into the mucosal bathing fluid under the influence of kallidin.

Amiloride and kallidin effects in rats eating low sodium diet

In each of 8 experiments in normal rat colons, mucosal amiloride 50 nM to 100 μM, was completely ineffective in reducing basal SCC (see Figure 1a as typical). In contrast, Figure 8 shows that a colon removed from a rat eating low sodium diet for 14

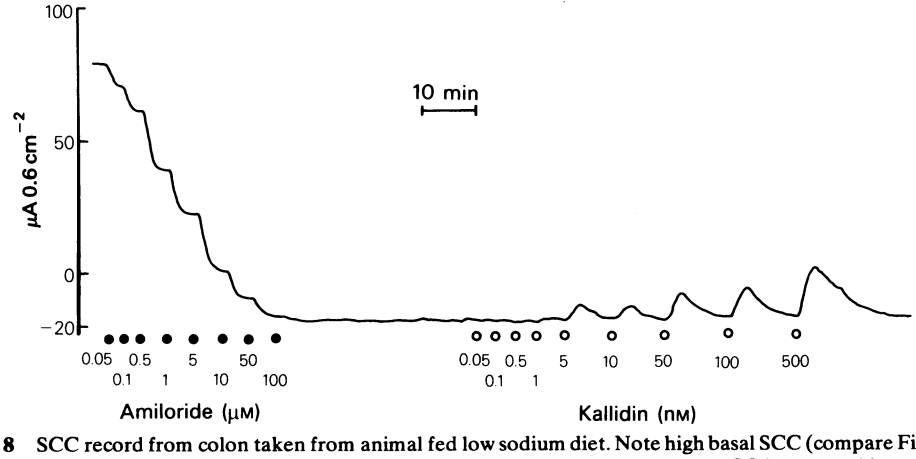

Figure 8 SCC record from colon taken from animal fed low sodium diet. Note high basal SCC (compare Figure 1a) and sensitivity to amiloride (added to the mucosal bath). At high concentration the SCC is reversed by amiloride Note too the responses to kallidin are smaller than with control tissues (Figure 1a).

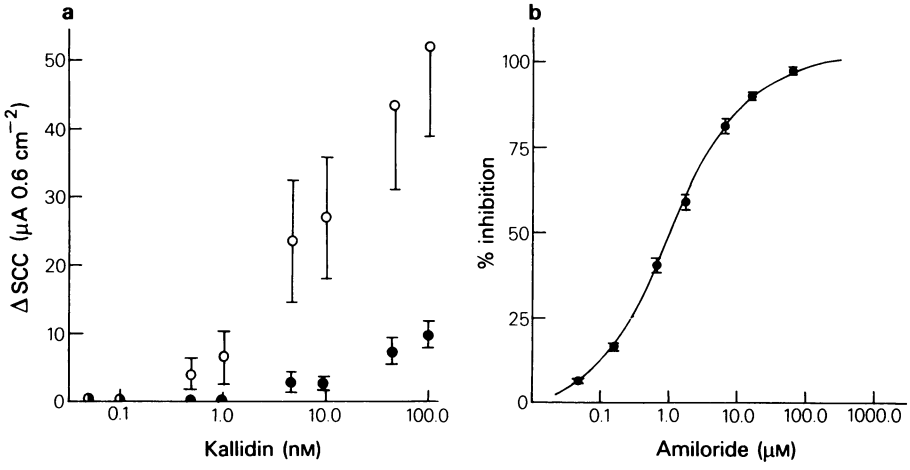

Figure 9(a) Concentration-response curves to kallidin in eight colons from low sodium animals (●) and eight from normal rats (○). Mean values are given; vertical lines show s.e.mean. (b) Concentration-response curve for amiloride in low sodium colons. 100% inhibition corresponds to total amiloride sensitive SCC (170 μM amiloride). Mean values ($n = 7$) are given; vertical lines show s.e.mean. Mean initial SCC was 88 ± 19 μA 0.6 cm^{-2}. Maximal inhibition by amiloride was 112 ± 20 μA 0.6 cm^{-2}.

days was highly sensitive to mucosal amiloride, confirming the observation of Will *et al.* (1980).

Colons from rats eating low sodium diet for 14 to 40 days were compared with a group eating normal diet for the same period. A composite concentration-response curve for amiloride derived from results with seven preparations of low sodium colons is shown in Figure 9b. The concentration of amiloride causing 50% inhibition of the inhibitor sensitive current is approximately 1 μM. The responses of both low sodium and normal colons to kallidin in the presence of amiloride (10^{-4} M) are compared in Figure 9a. In low sodium colons the response curve is shifted to the right and down with a threshold of 5–10 nM and with the SCC response reduced to 30% of normal at 100–500 nM kallidin. Immediately after setting up these preparations the SCC was 243.8 ± 39.6 μA 0.6 cm^{-2} and the open circuit potential was 11.21 ± 1.51 mV (serosal positive) ($n = 8$) in low sodium colons compared to values of 75.8 ± 19.2 μA 0.6 cm^{-2} and 1.95 ± 0.31 mV ($n = 8$) for normal colons. Both current and potential values were significantly different ($P < 0.005$) for the two groups. It is clear that the insensitivity to kallidin in low sodium colons is not due to failure of transporting capacity in these tissues.

Discussion

The results prove that kallidin stimulates net chloride secretion in the isolated descending colon of the rat. This conclusion is supported by the following findings. First, the tissue is extremely sensitive in responding to kinins with large increases in SCC. This response is consistent with the previously observed kinin-induced increase in serosal-positive transepithelial potential difference (Hardcastle, Hardcastle, Flower & Sanford, 1978) and, as they noted, could be due to either an increased J_{MS} for cation, J_{SM} for anion or both. The insensitivity of the normal rat colon to amiloride (the present results, Will *et al.*, 1980) and the lack of effect of this agent on the kinin-induced increases in SCC suggests that the SCC change does not occur as a result of increased $J_{MS}Na$ through amiloride-sensitive sodium channels. Nevertheless, the consistent increase in transepithelial conductance seen with the kinin shows that there is a significant increase in tissue ionic permeability. Second, the lack of kinin effect on SCC when serosal chloride is replaced with other anions, and attenuation of the kinin effect on SCC by serosal frusemide are consistent with the possibility that augmented chloride secretion is responsible, at least in part, for the observed changes in SCC and conductance. Third, the attenuation or blockade of the SCC response to kinin by indomethacin or mepacrine also suggests chloride secretion is being stimulated. Hardcastle *et al.* (1978) also noted that indomethacin reduced the effects of bradykinin to increase transmural potential differences in jejunum or colon. Indomethacin or mepacrine inhibit prostaglandin synthesis subsequent to cyclooxygenase or phospholipase inhibition at many sites including the gastrointestinal mucosa (Kimberg, Field, Gershon & Henderson, 1974). Prostaglandins of the E series are

known to increase SCC across rat colon, an effect secondary to electrogenic chloride secretion and blocked by frusemide (Frizzell & Heintze, 1979). Fourth and finally, the direct measurements of ion flux across the stripped rat colon in response to kallidin establish without doubt that the principal event responsible for the observed changes in SCC is net chloride secretion. This results from a combination of significantly reduced $J_{MS}Cl$ and increased $J_{SM}Cl$.

The notion that an endogenous glandular or gastrointestinal kallikrein-kinin system has a role in solute and water transporting events is not new (Schacter, 1979). Some studies of this possibility have assessed the effects of heterologous kallikreins upon the concentrations of hexoses, amino acids or ions on either side of small or large intestinal segments. Results have been conflicting. Moriwaki, Moriya, Yamaguchi, Kizuki & Fujimori (1972), Moriwaki & Fujimori (1981) and Meng & Haberland (1972) suggested that hog pancreatic kallikrein increases valine or glucose absorption but Caspary & Creutzfeldt (1972) showed that the same kallikrein had no effect on the transport of either these or other substances including calcium, a bile salt and other amino acids and sugars. Dennhardt & Haberich (1972) measured the effects of a hog pancreatic kallikrein perfused through the colonic lumen *in situ* of normal rats and showed inhibited water and sodium reabsorption. However, the enzyme stimulated reabsorption in rats with pancreatic ducts ligated. Reasonably comprehensive studies of the effects of homologous kallikreins upon intestinal ion transport are not available. Our preliminary results (Figure 2) show that a pure rat glandular kallikrein, added to the serosal bath, increases SCC and that mellitin, a 26-amino acid basic polypeptide from bee venom, does likewise. The latter effect is of interest since it was shown that mellitin is the most potent activator of membrane-bound kallikrein (Nishimura *et al*, 1980). The similarity of effect and potency of kallikrein and mellitin on SCC might signify interesting relations amongst an inactive gastrointestinal membrane-bound kallikrein and ion transport. On the other hand, mellitin is well known to perturb many membrane-bound enzyme systems (Habermann, 1972) and its effects upon SCC, while of interest, may not be related to kallikrein.

Perhaps one of the more interesting aspects of bradykinin or kallidin-induced net chloride secretion is the potency of the peptides. Amongst the agents thought to stimulate this process, kinins appear to be more potent than PGE_2 (Frizzell & Heintze, 1979) or PGE_1 (Kimberg *et al.*, 1974), 5-hydroxytryptamine (Dharmsathaphorn, Racusen & Dobbins, 1980), acetylcholine (Hardcastle & Eggenton, 1973), the calcium ionophore A23187 or cyclic 3′,5′ AMP (Frizzell *et al.*, 1976; Frizzell, 1977), bile salts (Binder & Rawlins, 1973) and long-chain and hydroxy fatty acids (Ammon & Phillips, 1973; Bright-Asare & Binder, 1973).

As noted above, previous workers (Hardcastle *et al*, 1978) have predicted the possibility of kinin-induced intestinal secretion based upon their studies of kinin effects upon transintestinal potential difference. However, earlier experiments by Crocker & Willavoys (1975), who used everted sacs of rat jejunum and measured sodium and water leaving the surrounding nutrient media, showed that bradykinin might either stimulate or inhibit transfer, depending upon basal transfer rates. Stimulation of transfer out of the surrounding mucosal medium occurred when control transfer rates were low and inhibition when rates were high. Although it is difficult to understand the possible relations between their findings and ours, the potency of kinins in both studies are comparable and along with the known high intestinal concentration of enzyme(s) responsible for their production provides some plausible rationale for further study.

There are other issues which if understood might also provide clues to the role of the kallikrein-kinin system in membrane transport events. Previous histochemical evidence has led to the suggestion that glandular kallikrein, at least in the kidney and some salivary glands, might be principally localized to lumenal plasma membranes (Ørstavik Nustad, Brandtzaeg & Pierce, 1976; Simson, Spicer, Chao, Grimm & Margolius, 1979). This conclusion now seems premature, as pointed out by Nishimura *et al.* (1980), and since the concentration of bradykinin required to produce even a slight effect upon recovery of ^{22}Na from the lumen of microinjected rat distal nephron segments is 500,000 times higher (Kauker, 1980) than that required upon an antelumenal surface in our studies to affect an ion flux at another site of system localization. It seems more likely that receptors for kinins will be found at basolateral or perhaps intracellular sites than at apical ones Even this suggestion is confounded by data which show that amiloride is an effective inhibitor of mammalian kallikreins and an amphibian kallikrein-like enzyme (Margolius & Chao, 1980) and that glandular kallikrein inhibitors decrease Na reabsorption by an action at apical plasma membrane sites in the amphibian urinary bladder (Orce *et al.*, 1980; 1981). Thus, kallikrein inhibition is associated with decreased Na reabsorption at some sites, but the kinin peptide products stimulate chloride secretion at another.

It seems possible that important information might be obtained by further study of kinin effects on colonic ion transport in rats eating a low Na^+ diet. In the present study, we have confirmed the striking findings of Will *et al.* (1980) who demonstrated that

the normal rat colons' complete amiloride insensitivity and low basal SCC are converted to extreme sensitivity and high basal SCC by 10 days of low sodium diet. In addition, we have shown that SCC responses to serosal kinin are reduced by this dietary manoeuvre. It is known that low sodium intake consistently increases kallikrein activity and quantity in the kidney (Crosswell, Shimamoto, Chao, Westbury, Powell & Margolius, 1979). Frankish & Zeitlin (1980) have shown that after rats are fed for 60 h with an isotonic glucose solution alone, distal colon kininogenase activity doubles while proximal colonic levels remain unchanged. Because amiloride can inhibit kallikrein enzymatic activity, as well as SCC via a reduced Na transport at apical membranes, it becomes pertinent to ask whether the conversion of a leaky, amiloride-insensitive colonic epithelium to a tight, amiloride-sensitive one by a low sodium diet involves alterations in the activity of the epithelium's endogenous kallikrein-kinin system or ion flux responses to exogenous kinins.

These questions, as well as others including the implied role of prostaglandins in the maintenance of basal SCC (Figure 5), remains to be answered. Nevertheless, the possibility that the glandular kallikrein-kinin system is involved intimately in membrane ion transport events seems strengthened by proving that a kinin can stimulate net chloride secretion, most likely via a prostaglandin-dependent mechanism.

We are grateful to Margaret Hickman for excellent technical assistance and Dr Julie Chao for the purified rat urinary kallikrein. Supported by NIH grants No. HL 17705 and GM 20387 Harry S. Margolius was supported by the Burroughs Wellcome Fund as a Scholar in Clinical Pharmacology and the Medical University of South Carolina during a sabbatical leave at the University of Cambridge. A.W.C. is grateful for support from the M.R.C. and Wellcome Trust.

References

AMMON, H.V. & PHILLIPS, S.F. (1973). Inhibition of colonic water and electrolyte absorption by fatty acids in man. *Gastroenterology*, **65,** 744–749.

BINDER, H.J. & RAWLINS, C.L. (1973). Effect of conjugated dihydroxy bile salts on electrolyte transport in rat colon. *J. clin. Invest.*, **52,** 1460–1466.

BRIGHT-ASARE, P. & BINDER, H.J. (1973). Stimulation of colonic secretion of water and electrolytes by hydroxy fatty acids. *Gastroenterology*, **64,** 81–88.

CASPARY, W.F. & CRUETZFELDT, W. (1972). The influence of kallikrein on absorption of sugars and amino acids in rat small intestine *in vitro*. In: *Kininogenases. Kallikrein*, ed. Haberland, G.L. & Rohen, J.W. pp. 67–73. Stuttgart: Schattauer.

CHAO, J. & MARGOLIUS, H.S. (1979). Isozymes of rat urinary kallikrein. *Biochem. Pharmac.*, **28,** 2071–2079

CROCKER, A.D. & WILLAVOYS, S.P. (1975). Effect of bradykinin on transepithelial transfer of sodium and water *in vitro*. *J. Physiol.*, **253,** 401–410.

CROSSWELL, A., SHIMAMOTO, K., CHAO, J., WESTBURY, M., POWELL, B. & MARGOLIUS, H.S. (1979). Increased kallikrein in the renal cortex of rats fed a low sodium diet. *Fedn. Proc.*, **38,** 685.

CUTHBERT, A.W., FANELLI, G.M., Jr., & SCRIABINE, A. (1979). *Amiloride and Epithelial Sodium Transport.* Baltimore-Munich: Urban & Schwarzenberg.

DENNHARDT, R. & HABERICH, F.J. (1972). Effect of kallikrein on the absorption of water, electrolytes, and hexoses in the intestine of rats. In: *Kininogenases. Kallikrein.* ed. Haberland, G.L. & Rohen, J.W. pp. 81–88. Stuttgart: Schattauer.

DHARMSATHAPHORN, K., RACUSEN, L. & DOBBINS, J.W. (1980). Effect of somatostatin on ion transport in the rat colon. *J. clin. Invest.*, **66,** 813–820.

EDMONDS, C.J. & MARRIOTT, J. (1968). Electrical potential and short circuit current of an *in vitro* preparation of rat colon mucosa. *J. Physiol.*, **194,** 479–494.

FRANKISH, N.H. & ZEITLIN, I.J. (1980). The effect of diet on levels of kinin-forming enzyme in blood free rat gastro-intestinal tract. *J. Physiol.*, **298,** 361–370.

FRIZZELL, R.A. (1977). Active chloride secretion by rabbit colon: calcium-dependent stimulation by ionophore A23187. *J. membr. Biol.*, **35,** 175–187.

FRIZZELL, R.A. & HEINTZE, K. (1979). Electrogenic chloride secretion by mammalian colon. In: *Mechanisms of Intestinal Secretion*, ed. Binder, H.J. pp. 101–110. New York: Liss.

FRIZZELL, R.A., KOCH, M.J. & SCHULTZ, S.G. (1976). Ion transport by rabbit colon. I. Active and passive components. *J. membr. Biol.*, **27,** 297–316.

GELLER, R.G., MARGOLIUS, H.S., PISANO, J.J. & KEISER, H.R. (1972). Effects of mineralocorticoids, altered sodium intake and adrenalectomy on urinary kallikrein in rats. *Circulation Res.*, **31,** 857–861.

HABERMANN, E. (1972). Bee and wasp venoms. *Science*, **177,** 314–322.

HARDCASTLE, J., HARDCASTLE, P.T., FLOWER, R.J. & SANFORD, P.A. (1978). The effect of bradykinin on the electrical activity of rat jejunum. *Experientia*, **34,** 617–618.

HARDCASTLE, P.T. & EGGENTON, J. (1973). The effect of acetylcholine on the electrical activity of intestinal epithelial cells. *Biochim. biophys. Acta*, **298,** 95–100.

KAUKER, M.L. (1980). Bradykinin action on the efflux of luminal ^{22}Na in the rat nephron. *J. Pharmac. exp. Ther.*, **214,** 119–123.

KIMBERG, D.V., FIELD, M., GERSHON, E. & HENDERSON, A. (1974). Effects of prostaglandins and cholera enterotoxin on intestinal mucosal cyclic AMP accumulation. *J. clin. Invest.*, **53,** 941–949.

MARGOLIUS, H.S. & CHAO, J. (1980). Amiloride inhibits mammalian renal kallikrein and a kallikrein-like enzyme from toad bladder and skin. *J. clin. Invest.*, **65,** 1343–1350.

MARGOLIUS, H.S., HORWITZ, D., GELLER, R.G., ALEXANDER, R.W., Jr., GILL, J.R., Jr., PISANO, J.J. & KEISER, H.R. (1974). Urinary kallikrein in normal subjects: relationships to sodium intake and sodium-retaining steroids. *Circulation Res.*, **35,** 812–819.

MENG, K. & HABERLAND, G.L. (1972). Influence of kallikrein on glucose transport in the isolated rat intestine. In: *Kininogenases. Kallikrein.* ed. Haberland, G.L. & Rohen, J.W. pp. 75–80. Stuttgart: Schattauer.

MORIWAKI, C. & FUJIMORI, H. (1981). Studies on Kallikreins. VI. Effect of pancreatic kallikrein and kinin on the transport of amino acids and glucose across the rat small intestine. *Chem. Pharm. Bull.*, **29,** 804–809.

MORIWAKI, C., MORIYA, H., YAMAGUCHI, K., KIZUKI, K. & FUJIMORI, H. (1972). Intestinal absorption of pancreatic kallikrein and some aspects of its physiological role. In: *Kininogenases. Kallikrein.* ed. Haberland, G.L. & Rohen, J.W. pp. 57–66. Stuttgart: Schattauer.

NASJLETTI, A., McGIFF, J.C. & COLINA-CHOURIO, J. (1978). Interrelations of the renal kallikrein-kinin system and renal prostaglandins in the conscious rat. Influence of mineralocorticoids. *Circulation Res.*, **43,** 799–807.

NISHIMURA, K., ALHENC-GELAS, F., WHITE, A. & ERDÖS, E.G. (1980). Activation of membrane-bound kallikrein and renin in the kidney. *Proc. natn. Acad. Sci. U.S.A*, **77,** 4975–4978.

ORCE, G.G., CASTILLO, G.A. & MARGOLIUS, H.S. (1980). Inhibition of short-circuit current in toad urinary bladder by inhibitors of glandular kallikrein. *Am. J. Physiol.*, **239,** F459–F465.

ORCE, G.G. CASTILLO, G.A. & MARGOLIUS, H.S. (1981). Kallikrein inhibitors decrease short-circuit current by inhibiting sodium uptake. *Hypertension* (in press).

ØRSTAVIK, T.B., NUSTAD, K., BRANDTZAEG, P. & PIERCE, J.V. (1976). Cellular origin of urinary kallikreins. *J. Histochem. Cytochem.*, **24,** 1037–1039.

PHILLIPS, S.R. & SCHMALZ, P.F. (1970). Bicarbonate secretion by the rat colon: effect of intraluminal chloride and acetazolamide. *Proc. Soc. exp. Biol. Med.*, **135,** 116–122.

SCHACHTER, M. (1979). Kallikreins (kininogenases) – a group of serine proteases with bioregulatory actions. *Pharmac. Rev.*, **31,** 1–17.

SEKI, T., NAKAJIMA, T. & ERDÖS, E.G. (1972). Colon kallikrein, its relation to the plasma enzyme. *Biochem. Pharmac.*, **21,** 1227–1235.

SIMSON, J.A.V., SPICER, S.S., CHAO, J., GRIMM, L. & MARGOLIUS, H.S. (1979). Kallikrein localization in rodent salivary glands and kidney with the immunoperoxidase bridge procedure. *J. Histochem. Cytochem.*, **27,** 1567–1576.

WILL, P.C., LEBOWITZ, J.L. & HOPFER, U. (1980). Induction of amiloride-sensitive sodium transport in the rat colon by mineralocorticoids. *Am. J. Physiol.*, **238,** F261–F268.

ZEITLIN, I.J., SINGH, Y.N., LEMBECK, F. & THEILER, M. (1976). The molecular weights of plasma and intestinal kallikreins in rats. *Naunyn Schmiedebergs Arch. Pharmac.*, **293,** 159–161.

ZIMMERMAN, A., GEIGER, R. & KORTMANN, H. (1979). Similarity between a kininogenase (kallikrein) from human large intestine and human urinary kallikrein. *Hoppe-Seyler's Z. Physiol. Chem.*, **360,** 1767–1773.

(Received September 25, 1981.)