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Mechanisms of carbachol-induced alterations in K^+ transport across the rat colon

Bernhard Heinke, Silke Hörger, Martin Diener *

Institut für Veterinär-Physiologie, Justus-Liebig-Universität Gießen, Frankfurter Str. 100, D-35392 Giessen, Germany

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Abstract

The effect of carbachol, an agonist of the Ca^{2+} pathway, on K^+ transport in rat proximal and distal colon was studied by measuring unidirectional fluxes, uptake, and efflux of Rb^+, a marker for K^+, in mucosa–submucosa preparations. Unidirectional ion flux measurements revealed that carbachol stimulated K^+ secretion in the proximal colon by a marked increase in the serosa-to-mucosa flux (J_{sm}^{Rb}) and a more moderate rise in the mucosa-to-serosa flux (J_{ms}^{Rb}). In the distal colon carbachol had no effect on J_{ms}^{Rb}, but J_{sm}^{Rb} was reduced after a transient increase finally resulting in an inhibition of K^+ secretion. Carbachol caused a stimulation of mucosal Rb^+ uptake in the distal colon, which was diminished in the presence of inhibitors of the apical H^+–K^+-ATPase, vanadate and ouabain. In contrast, in the proximal colon the serosal Rb^+ uptake was enhanced by carbachol, an effect, which could be prevented by bumetanide, an inhibitor of the basolateral Na^+–K^+–$2Cl^-$-cotransporter. Efflux experiments revealed that carbachol caused a transient increase of apical and basolateral Rb^+ permeability in both colonic segments. In the distal colon, stimulated K^+ efflux to the serosal side was reduced by quinine, efflux to the mucosal side was blocked by tetraethylammonium. In the proximal colon, carbachol-activated apical and basolateral K^+ efflux were inhibited by Ba^{2+}. In conclusion, these data suggest that in the distal colon carbachol stimulates the H^+–K^+-ATPase and the basolateral K^+ efflux through quinine-sensitive K^+ channels, whereas in the proximal colon carbachol induces K^+ secretion due to a stimulation of the basolateral Na^+–K^+–$2Cl^-$-cotransporter and an increased efflux to the luminal side via Ba^{2+}-sensitive apical K^+ channels.

Keywords: Ca^{2+}; Carbachol; Electrolyte transport; K^+ transport; K^+ channels; H^+–K^+-ATPase; Na^+–K^+–$2Cl^-$-cotransporter; Colon, rat

1. Introduction

Colonic K^+ transport is essential for K^+ homeostasis, because in contrast to other parts of the gastrointestinal tract the colonic epithelium is able to both actively absorb and to secrete this cation. The K^+ ions to be absorbed enter the epithelium via a an apical H^+–K^+-ATPase; their basolateral exit seems to be mediated by basolateral K^+ channels. K^+ secretion is driven by the intracellular accumulation of K^+ ions via the basolateral Na^+–K^+–$2Cl^-$-cotransporter and the Na^+–K^+-ATPase; they pass into the colonic lumen via apical K^+ channels (for review see Binder and Sandle, 1994).

K^+ transport is regulated by several intracellular second messenger systems. An increase in the intracellular concentration of cAMP or Ca^{2+} induces K^+ secretion and/or inhibits K^+ absorption in colonic tissues from different species (Foster et al., 1983; McCabe and Smith, 1985; DuVall and O'Grady, 1994). The mechanisms involved in the action of cAMP in the rat colon have been recently characterized in detail (Diener et al., 1996). Activation of the adenylate cyclase by forskolin stimulates the basolateral Na^+–K^+–$2Cl^-$-cotransporter and causes a redistribution of the cellular K^+ permeability from a predominant basolateral one to a near equal apical and basolateral K^+ permeability by reducing the basolateral K^+ conductance (Diener et al., 1996; Schultheiß and Diener, 1997). In contrast, carbachol, an agonist of the Ca^{2+} pathway, causes a transient activation of both an apical and a basolateral K^+ conductance as shown by experiments with epithelia, in which either the apical or the basolateral membrane were short-circuited by an ionophore or an increased K^+ concentration, respectively (Schultheiß and Diener, 1997). The effect of the opening of these K^+ channels on trans-

* Corresponding author. Tel.: +49-641-99-38161; Fax: +49-641-99-38179; E-mail: martin.diener@vetmed.uni-giessen.de

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epithelial K^+ fluxes and the putative effect of carbachol on other transporters involved in K^+ transport have not been investigated. Therefore, the aim of the present study was to elucidate the mechanisms of Ca^{2+}-induced alterations in K^+ transport by measuring unidirectional Rb^+ fluxes, Rb^+ uptake and Rb^+ efflux in the rat colon.

2. Materials and methods

2.1. Solutions

The standard buffer for the Ussing chamber experiments was a modified Parsons solution containing (mmol l^{-1}): NaCl 107, RbCl 4.5, $NaHCO_3$ 25, Na_2HPO_4 1.8, NaH_2PO_4 0.2, $CaCl_2$ 1.25, $MgSO_4$ 1 and glucose 12. The solution was gassed with carbogen (5% CO_2 in 95% O_2); pH was 7.4. A HCO_3^--free Tyrode solution was used in experiments performed with the K^+-channel blocker Ba^{2+}; it had the following composition (mmol l^{-1}): NaCl 140, RbCl 5.4, $CaCl_2$ 1.25, $MgCl_2$ 1, HEPES (*N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid) 10, glucose 12.2. This HCO_3^--free solution was gassed with O_2; pH was 7.4.

2.2. Tissue preparation

Wistar rats were used with a weight of 180–380 g. The animals had free access to water and a standard rat diet (diet no. C1000, Altromin, Lange, Germany) until the day of the experiment. Animals were killed by a blow on the head followed by exsanguination (approved by Regierungspräsidium Gießen, Gießen, Germany). The serosa and muscularis propria were stripped away to obtain mucosa–submucosa preparations of the proximal and distal colon. Briefly, the colon was placed on a small plastic rod with a diameter of 5 mm. A circular incision was made near the anal end with a blunt scalpel and the serosa together with the lamina propria were gently removed in a proximal direction. The appearance of palm-like striae was used to define the beginning of the proximal colon (Lindström et al., 1979).

2.3. Unidirectional flux measurements

The mucosa–submucosa preparations of the proximal and distal colon, respectively, were fixed in modified Ussing chambers, bathed with a volume of 3.5 ml on each side of the mucosa. The tissue was incubated at 37°C in Parsons solution and short-circuited by a computer-controlled voltage clamp (Ing. Büro für Meß- und Datentechnik K. Mußler, Aachen, FRG) with correction for solution resistance. The exposed surface of the tissue was 1 cm^2. Short-circuit current (I_{sc}) was continuously recorded and tissue conductance (G_t) was measured every minute.

Ion flux studies were performed as previously described (Diener et al., 1996). After an equilibration period of 60 min, $^{86}Rb^+$ (120 kBq) was added to one side (= labeled side) of the epithelium. After an additional 20 min to allow isotope fluxes to reach a steady state, Rb^+ accumulation on the unlabelled side was measured under control conditions in five sequential 3-min periods followed by five sequential 3-min periods starting 3 min after administration of $5 \cdot 10^{-5}$ mol l^{-1} carbachol at the serosal side. Transport rates for Rb^+ were calculated separately by linear regression of isotope accumulation on the unlabelled side during the control and the carbachol period. All aliquots from the labeled side were replaced by unlabelled buffer solution and appropriate correction for this replacement solution was performed. From the measured unidirectional fluxes net ion fluxes were calculated according to: $J_{net} = J_{ms} - J_{sm}$.

2.4. Uptake experiments

The measurement of Rb^+ uptake was started after an equilibration period of 60 min in Lucite chambers with a volume of 2.5 ml on each side of the tissue. When the uptake was measured in the presence of the agonist, carbachol was administered 1 min prior to the addition of $^{86}Rb^+$ (22 kBq) to either the mucosal or the serosal side of the chamber. Blocking drugs were added 30 min (bumetanide) or 15 min (vanadate, ouabain) prior to administration of carbachol in order to stabilize ion fluxes to a new plateau as indicated by a new stable I_{sc} in orientating experiments.

Five min after administration of $^{86}Rb^+$ standards were taken from the labeled side and the uptake was stopped by washing the chamber with 20 ml of fresh, unlabelled buffer solution on both sides. The tissue was removed from the chamber and blotted on filter paper. This procedure took 1–2 min. The tissue was solubilized in 1 ml 0.1 N HNO_3 for 20 h at 70°C (Venglarik et al., 1990). After neutralization with 0.1 ml 1 N NaOH, the radioactivity in the sample was determined in a liquid scintillation counter. Results were calculated as uptake per area mucosa (nmol cm^{-2}).

2.5. Efflux experiments

The tissue was loaded with $^{86}Rb^+$ (74 kBq at both sides of the chamber) for 90 min in 2.5 ml Parsons or Tyrode, respectively, on each side of the tissue. Then the serosal and the mucosal compartments were washed twice at 5 min intervals with 20 ml buffer. These solutions contained K^+ channel blockers, when they were tested. Release of $^{86}Rb^+$ into the mucosal and the serosal compartment was determined simultaneously by taking 2×0.5 ml aliquots at 6 min intervals. All aliquots were replaced by unlabelled buffer solution, containing agonists and blockers in appropriate concentrations. Correction for this replacement vol-

ume was performed. Carbachol was added 1 min after the third sample. At the end of the experiment, the resting amount of $^{86}Rb^+$ in the tissue was determined as described for the uptake experiments. Release was expressed as efflux of the actual amount of radioactivity in the tissue per minute (Mandel et al., 1986).

2.6. Drugs

Ouabain was dissolved in dimethylsulphoxide (DMSO; final concentration 0.25%, v/v). Bumetanide and quinine were dissolved in ethanol (final maximal concentration 0.25%, v/v). $BaCl_2$, carbachol, sodium orthovanadate (Calbiochem, Bad Soden, Germany), and tetraethylammonium chloride (TEA) were dissolved in aqueous stock solutions diluted in salt buffer just before use. If not indicated differently, drugs were from Sigma, Deisenhofen, Germany. Radioisotopes were obtained from NEN, Dreieich, FRG. The initial activity of ^{86}Rb amounted to 411 GBq g^{-1}.

2.7. Statistics

Values are given as means ± one standard error of the mean (S.E.M.). When the means of several groups had to be compared, first an analysis of variances was performed. If the analysis of variances indicated significant differences between the groups investigated, further comparison was carried out by a Student's *t*-test or by the *U*-test. An *F*-test decided which test method was to be used. Both paired and unpaired two-tailed Student's *t*-tests were applied as indicated. Regression lines were compared by analysis of co-variances (Kenakin, 1987). The quality of regressions was checked by the squared non-linear regression coefficient (r^2). $P < 0.05$ was considered to be statistically significant.

Standard errors for calculated values, i.e., net ion transport, were calculated according to the law of error propagation from the errors of J_{ms} and J_{sm} (Sachs, 1982). Statistical comparisons between net transports were performed by means of the test of Scheffé for comparison of linear contrasts (Sachs, 1982).

3. Results

3.1. Unidirectional Rb^+ fluxes

The effect of carbachol ($5 \cdot 10^{-5}$ mol l^{-1}) on unidirectional Rb^+ fluxes was measured using a short-time flux protocol under voltage-clamp conditions; transport rates for Rb^+ were calculated by linear regression of isotope accumulation on the unlabelled side. In the distal colon carbachol induced an increase in short-circuit current from 2.2 ± 0.2 μEq h^{-1} cm^{-2} under control conditions to a peak value of 8.2 ± 0.6 μEq h^{-1} cm^{-2} ($P < 0.05$, $n = 18$), which then declined slowly as described earlier (Strabel and Diener, 1995). In the proximal colon, the cholinergic agonist induced an increase in I_{sc} from 1.5 ± 0.1 μEq h^{-1} cm^{-2} to 11.2 ± 0.6 μEq h^{-1} cm^{-2} ($P < 0.05$, $n = 19$). This response was concomitant with an increase in G_t from 9.8 ± 0.6 mS cm^{-2} to 12.8 ± 0.8 mS cm^{-2} ($P < 0.05$, $n = 18$) in the distal and from 20.5 ± 2.5 mS cm^{-2} to 27.3 ± 2.6 mS cm^{-2} ($P < 0.05$, $n = 19$) in the proximal colon.

In the distal colon, the serosa to mucosa flux of Rb^+ (J_{sm}), which amounted to 11.0 ± 0.59 nmol min^{-1} cm^{-2}, exceeded the corresponding mucosa to serosa flux (J_{ms}) of 6.9 ± 0.61 nmol min^{-1} cm^{-2} ($n = 9$), resulting in a net Rb^+ secretion of 4.1 ± 0.85 nmol min^{-1} cm^{-2} ($P < 0.05$ vs. zero, test of Scheffé; Fig. 1A) under control conditions. Carbachol caused an inhibition of J_{sm}^{Rb}, which decreased to 8.1 ± 0.75 nmol min^{-1} cm^{-2} ($P < 0.05$, $n = 9$), but had no significant effect on J_{ms}^{Rb}, abolishing net Rb^+ secretion to a value of 0.95 ± 1.05 nmol min^{-1} cm^{-2}, $n = 9$), which was not significantly different from zero (Fig. 1A).

Although the secretory transport rate for Rb^+ was finally reduced by carbachol, there seemed to be a tran-

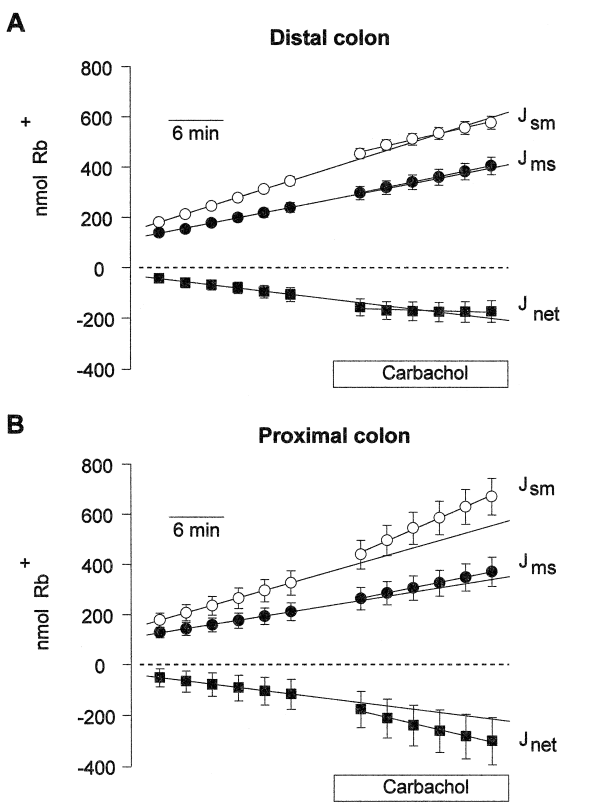

Fig. 1. Effect of carbachol on unidirectional $^{86}Rb^+$ fluxes in the distal (A) and proximal (B) rat colon: (○) J_{sm}^{Rb}, (●) J_{ms}^{Rb}, (■) J_{net}^{Rb}. Ion fluxes were measured in five sequential 3-min periods followed by five sequential 3-min periods starting 3 min after administration of $5 \cdot 10^{-5}$ mol l^{-1} carbachol to the serosal side. Values are means (symbols) ± S.E.M. (vertical bars), $n = 9$–10. The solid lines through the data are the best fit linear regressions, indicating the transport rates of Rb^+ before and after administration of the drug.

sient stimulation of J_{sm}^{Rb} in the early phase of the carbachol response. The amount of Rb^+ at the unlabelled side, measured 3 min after administration of the drug, which amounted to 454.0 ± 22.2 nmol, significantly exceeded the expected value of 432.4 ± 21.0 nmol, calculated from control J_{sm}^{Rb} by linear regression ($P < 0.05$, $n = 9$), suggesting a transient increase of J_{sm}^{Rb}, too fast to be registered by the experimental protocol.

In the proximal colon, basal transport rates for Rb^+ were similar (Fig. 1B). The serosa to mucosa flux of 9.9 ± 1.3 nmol min^{-1} cm^{-2} ($n = 9$) was higher than the movement of Rb^+ in the opposite direction (J_{ms}^{Rb} was 5.6 ± 1.0 nmol min^{-1} cm^{-2}, $n = 10$), resulting in a net Rb^+ secretion of 4.4 ± 1.6 nmol min^{-1} cm^{-2} ($P < 0.05$ vs. zero, test of Scheffé). Carbachol (5.10^{-5} mol l^{-1}) led to a marked increase of J_{sm}^{Rb} to 15.2 ± 1.1 nmol min^{-1} cm^{-2} ($P < 0.05$, $n = 9$), and a more moderate, but nevertheless significant increase of J_{ms}^{Rb} to 7.1 ± 0.9 nmol min^{-1} cm^{-2} ($P < 0.05$, $n = 10$). The consequence was a stimulation of net Rb^+ secretion to 8.2 ± 1.4 nmol min^{-1} cm^{-2} ($P < 0.05$ vs. control, $n = 9$–10, test of Scheffé; Fig. 1B).

3.2. Mucosal Rb^+ uptake

The changes of K^+ transport induced by carbachol might be due to a modification of apical and/or basolateral entry of K^+ ions. In order to distinguish between these possibilities, the uptake of Rb^+ from the mucosal and from the serosal compartment was measured. Carbachol (5.10^{-5} mol l^{-1}) had no significant effect in the proximal colon but doubled the mucosal uptake of Rb^+ from 19.4 ± 4.1 nmol cm^{-2} to 37.2 ± 4.5 nmol cm^{-2} ($P < 0.05$, $n = 7$) in the distal part (Fig. 2). This increase was completely abolished by prior addition of vanadate (10^{-4} mol l^{-1}) combined with ouabain (10^{-3} mol l^{-1}) to the mucosal solution, both well known inhibitors of the apical H^+–K^+-ATPase (Sweiry and Binder, 1990; Del Castillo et al., 1991; Tabuchi et al., 1992), indicating an activation of this pump by the cholinergic agonist.

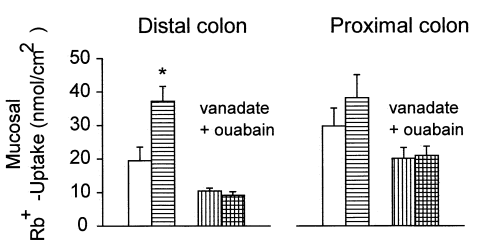

Fig. 2. Effect of carbachol ($5 \cdot 10^{-5}$ mol l^{-1} at the serosal side) on mucosal Rb^+ uptake in the distal (left side) and in the proximal colon (right side). Uptake was measured in the absence of any drugs (white bars), in the presence of carbachol (horizontally dashed bars), in the presence of vanadate (10^{-4} mol l^{-1} at the mucosal side) and ouabain (10^{-3} mol l^{-1} at the mucosal side; vertically dashed bars), and in the combined presence of carbachol, vanadate and ouabain (crossed bars). Values are means (bars) + S.E.M. (vertical bars), $n = 6$–7. $*$ $P < 0.05$ vs. control (unpaired t-test).

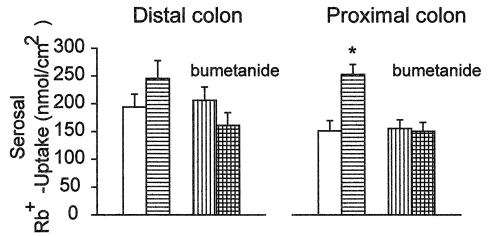

Fig. 3. Effect of carbachol ($5 \cdot 10^{-5}$ mol l^{-1} at the serosal side on serosal Rb^+ uptake in the distal (left side) and in the proximal colon (right side). Uptake was measured in the absence of any drugs (white bars), in the presence of carbachol (horizontally dashed bars), in the presence of bumetanide (10^{-4} mol l^{-1} at the serosal side; vertically dashed bars), and in the combined presence of carbachol and bumetanide (crossed bars). Values are means (bars) + S.E.M. (vertical bars), $n = 6$–7. $*$ $P <$ 0.05 vs. control (unpaired t-test).

3.3. Serosal Rb^+ uptake

When the serosal uptake of Rb^+ was measured, carbachol ($5 \cdot 10^{-5}$ mol l^{-1}) caused a significant increase from 151.0 ± 18.5 nmol cm^{-2} to 252.7 ± 18 nmol cm^{-2} ($P <$ 0.05, $n = 6$) in the proximal colon, whereas the increase of the uptake in the distal segment from 194.2 ± 23.0 nmol cm^{-2} to 245.6 ± 31.8 nmol cm^{-2} was insignificant ($n =$ 6). Although bumetanide (10^{-4} mol l^{-1}), an inhibitor of the Na^+–K^+–$2Cl^-$-cotransporter, had no significant effect on basal serosal Rb^+ uptake, it prevented completely the stimulation of serosal Rb^+ uptake by carbachol (Fig. 3). Consequently, carbachol activates the basolateral Na^+–K^+–$2Cl^-$-cotransporter, an effect, which will contribute to the induction of K^+ secretion in the proximal colon by carbachol observed in the unidirectional flux measurements (Fig. 1B).

3.4. Rb^+ efflux

In another series of experiments the effect of carbachol on the efflux of Rb^+ in tissues preloaded with this tracer was investigated. In the distal colon under control conditions, efflux from the tissue to the serosal side (3.1 ± 0.17% min^{-1}, $n = 12$) exceeded that to the mucosal side (0.74 ± 0.06% min^{-1}, $n = 12$) about four times as already observed previously (Diener et al., 1996). Basal Rb^+ efflux from the proximal colon showed a similar ratio and amounted to 1.0 ± 0.07% min^{-1} ($n = 12$) to the mucosal and 2.5 ± 0.15% min^{-1} ($n = 12$) to the serosal compartment.

Carbachol ($5 \cdot 10^{-5}$ mol l^{-1}) induced a transient rise in the efflux to the serosal compartment from 2.8 ± 0.25% min^{-1} to 4.0 ± 0.41% min^{-1} in the distal colon ($P < 0.05$, $n = 6$) and from 2.2 ± 0.14% min^{-1} to 2.9 ± 0.19% min^{-1} ($P < 0.05$, $n = 6$) in the proximal colon (Fig. 4A). Serosal Rb^+ efflux fell within 12 min to the former control levels in the distal colon or even below initial values in the proximal part.

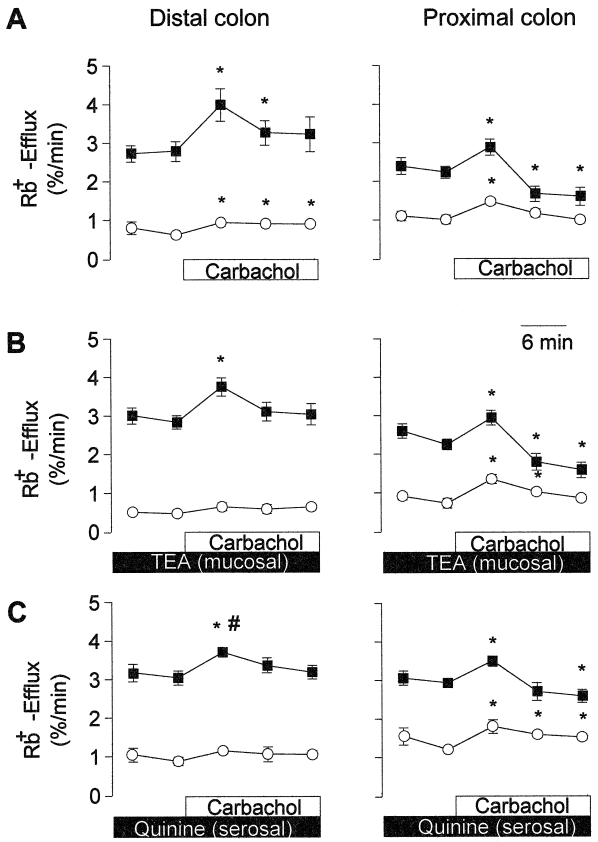

Fig. 4. Effect of carbachol ($5 \cdot 10^{-5}$ mol l^{-1} at the serosal side) on the efflux of Rb^+ in the distal (left side) and in the proximal colon (right side): (○) efflux on the mucosal side, (■) efflux on the serosal side. (A) Effect of carbachol under control conditions, (B) effect after pretreatment with $5 \cdot 10^{-3}$ mol l^{-1} tetraethylammonium (TEA) at the mucosal side, (C) effect after pretreatment with 10^{-3} mol l^{-1} quinine at the serosal side. Values are means ± S.E.M. (vertical bars), $n = 6$. $*$ $P < 0.05$ vs. last control period (paired t-test). $^{\#}P < 0.05$ vs. carbachol control (A, unpaired t-test).

The rate of Rb^+ efflux into the mucosal bath also increased after the addition of carbachol, although the magnitude of increase was quite small. In the proximal colon, mucosal Rb^+ efflux showed a similar behavior as the serosal one, rising transiently from $1.0 \pm 0.11\%$ min^{-1} to $1.5 \pm 0.09\%$ min^{-1} ($P < 0.05$, $n = 6$) and reaching control level again after 12 min (Fig. 4A). In the distal colon, there was a more prolonged increase in the efflux of Rb^+ to the mucosal compartment from $0.62 \pm 0.06\%$ min^{-1} to $0.94 \pm 0.04\%$ min^{-1} ($P < 0.05$, $n = 6$).

In order to characterize the efflux pathway activated by carbachol, the tissue was pretreated with different K^+ channel blockers. Mucosal administration of tetraethylammonium ($5 \cdot 10^{-3}$ mol l^{-1}), a blocker of apical K^+ channels in the rat colon (Sweiry and Binder, 1989), completely prevented the carbachol-induced increase of mucosal Rb^+ efflux in the distal segment ($n = 6$; Fig. 4B). In addition, tetraethylammonium decreased the basal apical efflux from $0.73 \pm 0.04\%$ min^{-1} under control conditions ($n = 12$) to $0.38 \pm 0.03\%$ min^{-1} ($P < 0.05$, $n = 6$). In the proximal colon, however, tetraethylammonium was ineffective (Fig. 4B), even when the concentration of the blocker was elevated to $2 \cdot 10^{-2}$ mol l^{-1} ($n = 6$; data not shown).

Serosal administration of quinine (10^{-3} mol l^{-1}) significantly diminished the carbachol-induced increase of serosal efflux in the distal segment ($P < 0.05$, $n = 6$), but was ineffective in the proximal colon (Fig. 4C). Consequently carbachol stimulates a tetraethylammonium-sensitive K^+ conductance in the apical membrane and a quinine-sensitive K^+ conductance in the basolateral membrane of the enterocytes in the distal part of the rat colon.

Since only a part of the carbachol-stimulated Rb^+ efflux could be inhibited by tetraethylammonium and quinine, another K^+ channel blocker, Ba^{2+}, was tested. This particular series of experiments was performed in HCO_3^--free buffer in order to avoid precipitation of Ba^{2+} as $BaCO_3$. In control experiments, no fundamental difference

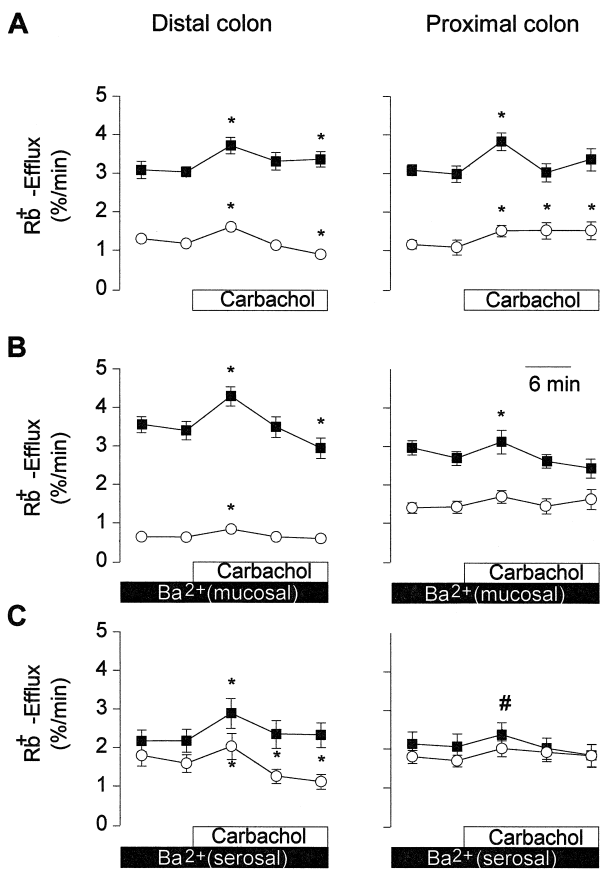

Fig. 5. Effect of carbachol ($5 \cdot 10^{-5}$ mol l^{-1} at the serosal side) on the efflux of Rb^+ in the distal (left side) and in the proximal colon (right side): (○) efflux on the mucosal side, (■) efflux on the serosal side. Efflux was measured in tissues bathed in HCO_3^--free Tyrode solution in the absence of any inhibitors (A), after pretreatment with Ba^{2+} (10^{-2} mol l^{-1}) at the mucosal side (B), and after pretreatment with Ba^{2+} (10^{-2} mol l^{-1}) at the serosal side (C). Values are means ± S.E.M. (vertical bars), $n = 6$–8. $*$ $P < 0.05$ vs. last control period (paired t-test). $^{\#}P < 0.05$ vs. carbachol control (A, unpaired t-test).

could be detected in the effect of carbachol ($5 \cdot 10^{-5}$ mol l^{-1}) on Rb^+ efflux measured in HCO_3^--buffered Parsons or HCO_3^--free Tyrode solution (cf. Fig. 4A, Fig. 5A). Preincubation of the tissue with Ba^{2+} (10^{-2} mol l^{-1}), applied either on the serosal or the mucosal side, had no effect on Rb^+ efflux in the distal colon (Fig. 5A,B) except that — paradoxically — stimulation of basolateral efflux by carbachol was less constant in the presence of mucosal Ba^{2+} (Fig. 5B). In contrast, Ba^{2+} was effective in the proximal colon: in the presence of mucosal Ba^{2+} carbachol no longer stimulated mucosal Rb^+ efflux (Fig. 5B) and in the presence of serosal Ba^{2+} the drug did not significantly enhance serosal Rb^+ efflux (Fig. 5C).

4. Discussion

Under basal conditions there was a net K^+ secretion in both colonic segments in these series of experiments (Fig. 1). This differs from most (but not all; see Tannen et al., 1986) reports, in which the distal colon exhibited a net K^+ absorption (see e.g. Sweiry and Binder, 1990). The reason for these discrepancies in basal K^+ transport, which have been observed and discussed intensively also for other tissues such as the rabbit colon (Halm and Frizzell, 1986), is probably the fact that the present experiments were performed in the absence of indomethacin, as prostaglandins, which are produced spontaneously in the colonic preparations, are well known to stimulate K^+ secretion (see e.g. Rechkemmer et al., 1996).

Carbachol, an agonist of the Ca^{2+} pathway, inducing an increase in the cytosolic Ca^{2+} concentration in colonic epithelium (Dharmsathaphorn and Pandol, 1986; Diener et al., 1991), has segment-specific effects on Rb^+ transport in the rat colon. Unidirectional flux measurements showed that the acetylcholine receptor agonist inhibited the secretory, i.e., serosa to mucosa flux of Rb^+, but had no effect on J_{ms}^{Rb} in the distal colon (Fig. 1). In contrast, carbachol caused an increase of both J_{ms}^{Rb} and J_{sm}^{Rb} in the proximal colon, leading to a stimulation of Rb^+ secretion because of a relatively stronger rise in J_{sm}^{Rb}.

Different ion transporters in the basolateral and the apical membrane are involved in the action of the cholinoceptor agonist. One of the key enzymes involved in K^+ absorption, whose activity is affected by carbachol, is the H^+–K^+-ATPase in the apical membrane, which is thought to be responsible for the active entry of K^+ into the colonic cell during K^+ absorption (Binder and Sandle, 1994). Our data confirm the presence of a vanadate- and/or ouabain-sensitive K^+ entry process, consistent with the activity of an H^+–K^+-ATPase, across the apical membrane of the rat distal colon (Fig. 2), which has already been demonstrated by Sweiry and Binder (1990). Similar transport processes are observed in other colonic tissues such as rabbit (Kaunitz and Sachs, 1986) and guinea pig colon (Watanabe et al., 1990; Del Castillo et al., 1994). Both drugs were used because considerable evidence exists for the existence of two H^+–K^+-ATPases: one that is ouabain-sensitive and the other that is ouabain-resistant but vanadate-sensitive, as it is typical for P-type ATPases (Sweiry and Binder, 1990; Del Castillo et al., 1991; Tabuchi et al., 1992; Lee et al., 1995; Sangan et al., 1997). The lack of a significant vanadate- or ouabain-sensitive K^+ entry across the apical membrane of the proximal colon (Fig. 2) is consistent with other findings in rat (Binder and Sandle, 1994; Lee et al., 1995), rabbit (Sullivan and Smith, 1986) and guinea pig (Watanabe et al., 1990; Del Castillo et al., 1994). Very little is known about the control of apical K^+ uptake, i.e., the regulation of the H^+–K^+-ATPase. In contrast to its profound effect on Rb^+ efflux, forskolin does not influence mucosal Rb^+ uptake in the rat colon, indicating no direct regulation of the K^+-pump by the cAMP pathway (Diener et al., 1996). The present results reveal an increase of apical K^+ entry in the presence of carbachol (Fig. 2) suggesting that the activity of the H^+–K^+-ATPase is regulated by the cholinergic agonist in the rat distal colon. However, the stimulation of the H^+–K^+-pump does not lead to a stimulation of the transepithelial absorptive K^+ flux (J_{ms}^{Rb}; Fig. 1A). This might indicate that carbachol causes a 'recycling' of K^+ ions, absorbed by the H^+–K^+-ATPase, across the apical membrane back to the lumen leaving the cell through activated tetraethylammonium-sensitive K^+ channels (Fig. 4B).

The second transport system controlled by the intracellular Ca^{2+} signaling pathway is the Na^+–K^+–$2Cl^-$-cotransporter in the basolateral membrane, which is stimulated by carbachol in the proximal but not in the distal colon as indicated by the increase in the bumetanide-sensitive basolateral Rb^+ uptake (Fig. 3). A rise in intracellular Ca^{2+} has been demonstrated to be implicated in the control of Na^+–K^+–$2Cl^-$-cotransporter activity in other epithelia such as T_{84} cells (McRoberts et al., 1985), distal colon of the guinea pig (Del Castillo and Sepúlveda, 1995), intestine of the winter flounder (Suvitayavat et al., 1994), or avian salt gland (Torchia et al., 1994). The missing effect of carbachol on serosal Rb^+ uptake in the distal colon suggests a segmental heterogeneity in the regulation of this transporter. The missing stimulation of the Na^+–K^+–$2Cl^-$-cotransporter is probably responsible for the fact that carbachol does not stimulate transepithelial K^+ secretion (but rather reduces J_{sm}^{Rb}; cf. Fig. 1A) in the distal colon despite the observed increase in apical K^+ permeability measured in the efflux experiments (Fig. 4A, Fig. 5A).

The third action site of carbachol is the basolateral K^+ conductance, which is transiently stimulated by the cholinoceptor agonist (Fig. 4A, Fig. 5A). Carbachol induces an increase in the Rb^+ efflux to the serosal side in both the proximal and the distal colon, which can be explained by an opening of Ba^{2+} and/or quinine-sensitive Ca^{2+}-dependent K^+ channels (Strabel and Diener, 1995;

Schultheiß and Diener, 1997). The effect of carbachol on Rb^+ efflux is only transient as already described in T_{84} monolayers (Dharmsathaphorn and Pandol, 1986; Venglarik et al., 1990). This correlates well with the time course of the stimulation of a basolateral K^+ conductance in nystatin-permeabilized rat colon (Schultheiß and Diener, 1997). Different K^+ conductances seem to underlie the carbachol response in the two colonic segments. In the distal colon, this effect can at least partly be blocked by quinine (Fig. 4C) but not by Ba^{2+}, whereas in the proximal colon serosal Ba^{2+} abolished this increase (Fig. 5C) and quinine was ineffective suggesting that there are different K^+ channels present in the two colonic segments.

The missing effect of serosal Ba^{2+} on carbachol-stimulated efflux in the distal colon is at first glance surprising, because a 16 pS Ca^{2+}-dependent, Ba^{2+}-sensitive K^+ channel, which is activated by carbachol, has been observed in the basolateral membrane of rat distal colonic epithelium (Bleich et al., 1996). However, serosal Ba^{2+} does not (or only marginally) reduce the peak response in I_{sc} and G_t induced by carbachol, but only reduces the plateau phase (Strabel and Diener, 1995). Consequently, there must be other, Ba^{2+}-insensitive pathways in the basolateral membrane stimulated by the cholinergic agonist. Serosal Ba^{2+}, however, reduced the basolateral efflux in unstimulated proximal and distal colonocytes indicating the inhibition of a basal K^+ conductance. In addition, serosal Ba^{2+} caused an increase in the mucosal Rb^+ efflux (Fig. 5C). This—at first glance paradox—stimulation can be explained by an increase in the driving force for apical K^+ exit due to the basolateral depolarization induced by Ba^{2+}.

The last site of action of carbachol is the apical K^+ conductance. The efflux of Rb^+ ions to the mucosal side of the rat colonic epithelium can be stimulated by carbachol in both the distal and the proximal segment (Fig. 4A, Fig. 5A), but this response is small compared to the stimulation of efflux into the serosal compartment. It only leads to an increase of the transepithelial secretory flux (J_{sm}^{Rb}) in the proximal segment (Fig. 1B), i.e., in this colonic segment, where also the basolateral Na^+–K^+–$2Cl^-$-cotransporter is activated by carbachol (Fig. 3). In the distal colon, where this activation of the basolateral uptake mechanism is missing, J_{sm}^{Rb} even decreases after a supposed stimulation in the first few minutes (Fig. 1A), an effect, which is probably caused by the hyperpolarization due to the opening of basolateral K^+ channels (as indicated by the efflux experiments; Fig. 4A, Fig. 5A) reducing the driving force for apical K^+ exit. The ability of carbachol to open an apical K^+ conductance seems to be independent from the parallel induction of Cl^- secretion, which normally dominates the electrical response to the cholinergic agonist, because in mice lacking the cystic fibrosis transmembrane regulator (CFTR) Cl^- channel, the I_{sc} response to carbachol is often reversed into a transient decrease in I_{sc} reflecting the K^+ secretion induced by the drug (Cuthbert et al., 1994).

Segmental differences were also observed when basolateral K^+ permeability was investigated. In the distal colon, mucosal tetraethylammonium abolishes the carbachol-induced stimulation of Rb^+ efflux, whereas in the proximal colon the drug was completely without effect (Fig. 4C). This is in accordance with recent findings that Ca^{2+}-activated apical K^+ channels can be blocked by mucosal tetraethylammonium in the distal colon of mouse (Cuthbert et al., 1994) and rat (Butterfield et al., 1997; Schultheiß and Diener, 1997). In contrast to the distal colon, in the proximal segment the carbachol-induced stimulation of apical Rb^+ efflux was sensitive to mucosal Ba^{2+} (Fig. 5B).

In conclusion, these data suggest that—beside the well known importance of cAMP—the Ca^{2+} pathway plays a substantial role in the regulation of K^+ transport across the rat distal and proximal colonic epithelium. The effects of the cholinergic agonist carbachol on the different transporters involved in K^+ transport reveal a segmental heterogeneity. In the distal part carbachol increases the apical K^+ uptake by activating the H^+–K^+-ATPase and stimulates K^+ efflux to the serosal compartment through quinine-sensitive K^+ channels. In the proximal colon carbachol induces K^+ secretion concomitant with a stimulation of the basolateral uptake of K^+ by the Na^+–K^+–$2Cl^-$-cotransporter and an increased efflux to the luminal side by activated Ba^{2+}-sensitive K^+ channels in the apical membrane.

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References

Binder, H.J., Sandle, G.J., 1994. Electrolyte transport in the mammalian colon. In: Johnson, L.R. (Ed.), Physiology of the Gastrointestinal Tract, 3rd edn. Raven Press, New York, NY, pp. 2133–2171.

Bleich, M., Riedmann, N., Warth, R., Kerstan, D., Leipziger, J., Hor, M., van Driessche, W., Greger, R., 1996. Ca^{2+} regulated K^+ and non-selective cation channels in the basolateral membrane of rat colonic crypt base cells. Pflüg. Arch. 432, 1011–1022.

Butterfield, I., Warhurst, G., Jones, M.N., Sandle, G.I., 1997. Characterization of apical potassium channels induced in rat distal colon during potassium adaptation. J. Physiol. 501, 537–547.

Cuthbert, A.W., Macvinish, L.J., Hickman, M.E., Ratcliff, R., Colledge, W.H., Evans, M.J., 1994. Ion-transporting activity in the murine colonic epithelium of normal animals and animals with cystic fibrosis. Pflüg. Arch. 428, 508–515.

Del Castillo, J.R., Sepúlveda, F.V., 1995. Activation of an $Na^+/K^+/2Cl^-$ cotransport system by phosphorylation in crypt cells isolated from guinea pig distal colon. Gastroenterology 109, 387–396.

Del Castillo, J.R., Rajendran, V.M., Binder, H.J., 1991. Apical membrane localization of ouabain-sensitive K^+-activated ATPase activities in rat distal colon. Am. J. Physiol. 261, G1005–G1011.

Del Castillo, J.R., Súlbaran-Carrasco, M.C., Burguillos, L., 1994. K^+ transport in isolated guinea pig colonocytes: evidence for Na^+-independent ouabain-sensitive K^+ pump. Am. J. Physiol. 266, G1083–G1089.

Dharmsathaphorn, K., Pandol, S.J., 1986. Mechanism of chloride secretion induced by carbachol in a colonic epithelial cell line. J. Clin. Invest. 77, 348–354.

Diener, M., Eglème, C., Rummel, W., 1991. Phospholipase C-induced anion secretion and its interaction with carbachol in the rat colonic mucosa. Eur. J. Pharmacol. 200, 267–276.

Diener, M., Hug, F., Strabel, D., Scharrer, E., 1996. Cyclic AMP-dependent regulation of K^+ transport in the rat distal colon. Br. J. Pharmacol. 118, 1477–1487.

DuVall, M.D., O'Grady, S.M., 1994. cGMP and Ca^{2+} regulation of ion transport across the isolated porcine distal colon epithelium. Am. J. Physiol. 267, R1026–R1033.

Foster, E.S., Sandle, G.I., Hayslett, J.P., Binder, H.J., 1983. Cyclic adenosine monophosphate stimulates active potassium secretion in the rat colon. Gastroenterology 84, 324–330.

Halm, D.R., Frizzell, R.A., 1986. Active K transport across rabbit distal colon: relation to Na absorption and Cl secretion. Am. J. Physiol. 251, C252–C267.

Kaunitz, J.D., Sachs, G., 1986. Identification of a vanadate-sensitive potassium-dependent proton pump from rabbit colon. J. Biol. Chem. 261, 14005–14010.

Kenakin, T.P., 1987. Pharmacologic Analysis of Drug–Receptor Interaction. Raven Press, New York, pp. 129–162.

Lee, J., Rajendran, V.M., Mann, A.S., Kashgarian, M., Binder, H.J., 1995. Functional expression and segmental localization of rat colonic K-adenosine triphosphatase. J. Clin. Invest. 96, 2002–2008.

Lindström, C.G., Rosengren, J.-E., Fork, F.-T., 1979. Colon of the rat. An anatomic, histologic and radiographic investigation. Acta Radiol. Diagn. 20, 523–536.

Mandel, K.G., McRoberts, J.A., Beuerlein, G., Foster, E.S., Dharmsathaphorn, K., 1986. Ba^{2+} inhibition of VIP- and A23187-stimulated Cl^- secretion by T_{84} cell monolayers. Am. J. Physiol. 250, C486–C494.

McCabe, R.D., Smith, P.L., 1985. Colonic potassium and chloride secretion: role of cAMP and calcium. Am. J. Physiol. 248, C103–C109.

McRoberts, J.A., Beuerlein, G., Dharmsathaphorn, K., 1985. Cyclic AMP and Ca^{2+}-activated K^+ transport in a human colonic epithelial cell line. J. Biol. Chem. 260, 14163–14172.

Rechkemmer, G., Frizzell, R.A., Halm, D.R., 1996. Active potassium transport across guinea pig distal colon: action of secretagogues. J. Physiol. 493, 485–502.

Sachs, L., 1982. Applied Statistics. A Handbook of Techniques, Springer, New York.

Sangan, P., Rajendran, V.M., Mann, A.S., Kashgarian, M., Binder, H.J., 1997. Regulation of colonic H–K-ATPase in large intestine and kidney by dietary Na depletion and dietary K depletion. Am. J. Physiol. 272, C685–C696.

Schultheiß, G., Diener, M., 1997. Regulation of apical and basolateral K^+ conductances in the rat colon. Br. J. Pharmacol. 122, 87–94.

Strabel, D., Diener, M., 1995. Evidence against direct activation of chloride secretion by carbachol in the rat distal colon. Eur. J. Pharmacol. 274, 181–191.

Sullivan, S.K., Smith, P.L., 1986. Active potassium secretion by rabbit proximal colon. Am. J. Physiol. 250, G475–G483.

Suvitayavat, W., Dunham, P.B., Haas, M., Rao, M.C., 1994. Characterization of the proteins of the intestinal Na^+–K^+–$2Cl^-$ cotransporter. Am. J. Physiol. 267, C375–C384.

Sweiry, J.H., Binder, H.J., 1989. Characterization of aldosterone-induced potassium secretion in rat distal colon. J. Clin. Invest. 83, 844–851.

Sweiry, J.H., Binder, H.J., 1990. Active potassium absorption in rat distal colon. J. Physiol. 423, 155–170.

Tabuchi, Y., Takeguchi, M., Asano, S., Takeguchi, N., 1992. Ouabain-insensitive, vanadate-sensitive K^+-ATPase of rat distal colon is partly similar to gastric H^+–K^+-ATPase. Jpn. J. Physiol. 42, 577–589.

Tannen, R.L., Marino, R., Dawson, D.C., 1986. K^+ transport by rat colon: adaptation to a low potassium diet. Am. J. Physiol. 250, F483–F487.

Torchia, J., Yi, Q., Sen, A.K., 1994. Carbachol-stimulated phosphorylation of the Na–K–Cl cotransporter of avian salt gland. Requirement for Ca^{2+} and PKC activation. J. Biol. Chem. 269, 29778–29784.

Venglarik, C.J., Bridges, R.J., Frizzell, R.A., 1990. A simple assay for agonist-regulated Cl and K conductances in salt-secreting epithelial cells. Am. J. Physiol. 259, C358–C364.

Watanabe, T., Suzuki, T., Suzuki, Y., 1990. Ouabain-sensitive K^+-ATPase in epithelial cells from guinea pig distal colon. Am. J. Physiol. 258, G506–G511.