Segmental differences along the crypt axis in the response of cell volume to secretagogues or hypotonic medium in the rat colon

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Abstract. VIP caused a decrease in the diameter of rat colonic crypts. This decrease was followed by a volume increase at the middle and the upper third of the crypt, which finally led to an increase of crypt diameter above the initial control values. At the fundus only a cell shrinkage was observed. The volume increase at the upper parts of the crypt was suppressed by the inhibitor of the Na^+-K^+-Cl^--cotransporter, furosemide. When crypts were exposed to a hypertonic medium, cell shrinkage was followed by a regulatory volume increase at the middle and the upper third of the crypt but not at the fundus region. These results suggest a gradient in the distribution of the Na^+-K^+-Cl^--cotransporter along the crypt axis.

Key words: Volume regulation - Cl^- secretion - vasoactive intestinal peptide - carbachol - rat colon

Introduction

Exposure of isolated crypts from the small intestine of the guinea-pig to hypertonic media causes a cell shrinkage followed by a regulatory volume increase (RVI) [5]. This RVI is caused by the activation of a basolateral Na^+-K^+-Cl^--cotransporter. A cell shrinkage is also observed after stimulation of Cl^- secretion by secretagogues like vasoactive intestinal peptide (VIP) or carbachol [5]. The present study was conducted in order to compare the behaviour of crypts from the colon with that reported for the small intestine. Because the colonic crypts show several gradients, e.g. in cell membrane potential [1,6] or in the distribution of the Na^+/H^+ exchanger [2], the effect of a hypertonic medium and secretagogues was studied at three different positions of the crypts: the fundus, the middle and the upper third.

Materials and methods

Crypts from the rat distal colon were isolated by Ca^{2+} chelation [1]. Changes in cell volume were registered by measuring the outer crypt diameter [2,3]. Whole-cell recordings were performed with nystatin-permeabilized patches [1]. The pipette solution consisted of ($mmol \cdot l^{-1}$): K gluconate 100, KCl 30, NaCl 10, $MgCl_2$ 2, EGTA 0.1, TRIS 10, ATP 5; pH was 7.2. The perfusion medium contained ($mmol \cdot l^{-1}$): NaCl 107, KCl 4.5, $NaHCO_3$ 25, Na_2HPO_4 1.8, NaH_2PO_4 0.2, $CaCl_2$ 1.25, $MgSO_4$ 1, glucose 12. The solution was gassed with 5 % CO_2 in 95 % O_2; pH was 7.4. For the administration of VIP, the solution contained in addition bovine serum albumin (0.1 %; w/v) and antifoam B (0.005 %; v/v) in order to prevent adsorption of the peptide to the perfusion system and foaming of the gassed solution, respectively. Furosemide was dissolved in ethanol, final concentration 0.25 % (v/v). Experiments were carried out at room temperature. Significances of differences were tested by analysis of variances and, if indicated, by paired or unpaired two-tailed Student's t-test or an U-test, respectively.

Results

Exposure of the crypts to VIP (10^{-8} $mol \cdot l^{-1}$) caused a decrease in the crypt diameter (Fig. 1, Table 1). At the fundus cell shrinkage was persistent in the presence of VIP similar as it was reported for the small intestine [5]. However, at the middle or the upper third of the crypt, the response to VIP was biphasic: an initial decrease in diameter was followed by an increase, i.e. cell shrinkage was followed by cell swelling. Final crypt diameter rose to a value even higher than that observed under control conditions ($P<0.05$). In the presence of the inhibitor of Na^+-K^+-Cl^--cotransporter, furosemide (10^{-4} $mol \cdot l^{-1}$), the unexpected swelling at the upper parts of the crypts was prevented and only a cell shrinkage was observed.

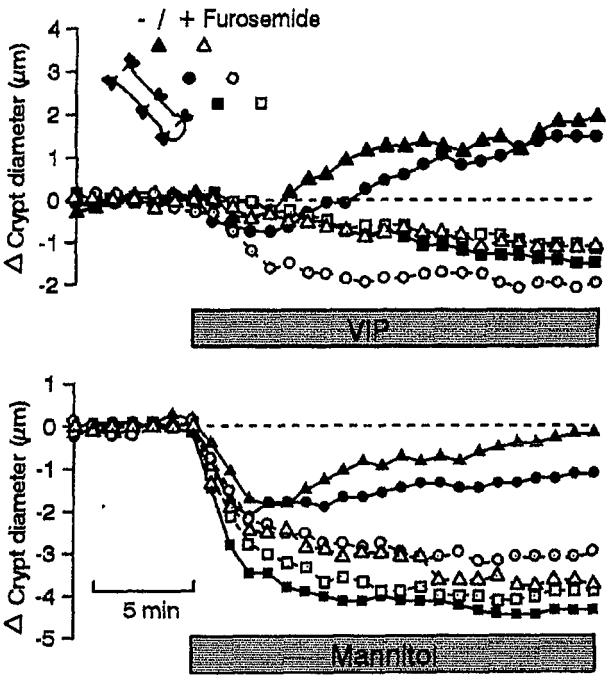

Fig. 1. Effect of VIP (10^{-8} mol·l^{-1}; top) and a hypertonic medium (100 mmol·l^{-1} mannitol; bottom) on the diameter of isolated crypts. Crypt diameter was measured at the upper third (triangles), the middle (circles) and the fundus (squares) in the absence (filled symbols) and presence (open symbols) of furosemide (10^{-4} mol·l^{-1}; added 3 min before addition of VIP or mannitol). Values are expressed as difference to the crypt diameter in the control period (Δ diameter). Means of 6 experiments. For statistics, see Table 1.

Condition		Maximal decrease (Δ μm)	Final change
VIP	Fundus	-1.5 ± 0.2$^{\#}$	-1.5 ± 0.2$^{\#}$
	Middle	-1.0 ± 0.1	+1.5 ± 0.4
	Upper th.	-0.7 ± 0.3	+1.9 ± 0.7
VIP	Fundus	-1.3 ± 0.3	-1.2 ± 0.3
+ Furo.	Midlde	-2.3 ± 0.2$^{*\#}$	-2.2 ± 0.3$^{*\#}$
	Upper th.	-1.3 ± 0.2	-1.1 ± 0.1*
Carbachol	Fundus	-1.9 ± 0.3	-1.9 ± 0.3$^{\#}$
	Middle	-	+0.5 ± 0.3
	Upper th.	-	+0.4 ± 0.5
Carbachol	Fundus	-2.4 ± 0.4	-2.2 ± 0.4$^{\#}$
+ Furo.	Middle	-	-0.3 ± 0.4
	Upper th.	-	+0.5 ± 0.5
Mannitol	Fundus	-4.4 ± 0.4$^{\#}$	-4.2 ± 0.5$^{\#}$
	Middle	-2.4 ± 0.3	-1.2 ± 0.4
	Upper th.	-2.0 ± 0.2	-0.2 ± 0.3
Mannitol	Fundus	-4.2 ± 0.3	-3.9 ± 0.4
+ Furo.	Middle	-3.3 ± 0.3*	-3.0 ± 0.4*
	Upper th.	-3.9 ± 0.7*	-3.5 ± 0.8*

Table 1. Effect of VIP (10^{-8} mol·l^{-1}), carbachol ($5x10^{-5}$ mol·l^{-1}) and mannitol (100 mmol·l^{-1}) on outer crypt diameter in the absence and presence of furosemide (10^{-4} mol·l^{-1}). Maximal decrease and final change in crypt diameter are given. * $p < 0.05$ versus same localization under control conditions, # $p < 0.05$ versus crypt upper third. The maximal changes in crypt diameter are greater than depicted in Fig. 1, because for each crypt the diameter was measured at its individual peak, which was reached at different times. Means ± SEM, n=6.

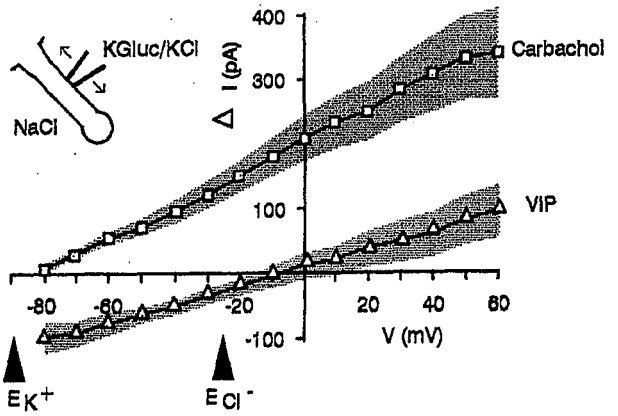

Fig. 2. Carbachol- ($5x10^{-5}$ mol·l^{-1}; squares) or VIP- (10^{-8} mol·l^{-1}; triangles) induced currents. The Δ currents were calculated by subtraction of the membrane current under control conditions from that observed in the presence of the agonist. For orientation, the equilibrium potentials of K^+ (E_{K^+}) and Cl^- (E_{Cl^-}) are given. Means ± SEM, n=6-7.

A similar difference between the fundus cells and the cells at the middle or the upper third of the crypt was observed, when the crypts were exposed to a hypertonic medium (Fig. 1, Table 1). Addition of 100 mmol·l^{-1} mannitol to the buffer caused only a cell shrinkage at the fundus, whereas at the upper parts of the crypt shrinkage was followed by a RVI. This RVI was suppressed in the presence of furosemide (10^{-4} mol·l^{-1}). In contrast to VIP, carbachol ($5x10^{-5}$ mol·l^{-1}) had no significant action on the cell volume at the upper third or the middle of the crypt, but caused a sustained decrease in cell volume at the fundus, which was not followed by a RVI (Table 1). This pattern was not altered in the presence of furosemide.

Whole-cell patch-clamp recordings revealed that VIP (10^{-8} mol·l^{-1}) caused a membrane depolarization of 17.3 ± 3.5 mV (n=15, P<0.05). In general, the strongest responses to VIP were observed in the middle region of the crypts. In contrast, carbachol induced a hyperpolarization, which amounted to -15.9 ± 3.2 mV (n=7, P<0.05) and which was not dependent on the localization along the crypt axis as

already reported [1]. The VIP-induced depolarization was accompanied by an increase in membrane inward and outward current, whereas carbachol induced only an increase in membrane outward current in the voltage range examined (-80 to +60 mV; Fig. 2). Extrapolated reversal potential for the carbachol-induced current was close to the K^+ equilibrium potential, indicating that carbachol mainly increases cellular K^+ conductance [1].

Discussion

Exposure of isolated colonic crypts to VIP, a secretagogue acting via intracellular cAMP, caused an initial decrease in crypt diameter, i.e. a cell shrinkage. This shrinkage has already been observed in the small intestine [5] and has to be expected, if VIP initiates a loss of Cl^- ions by opening of apical Cl^- channels. In addition, substances increasing intracellular cAMP are reported to induce the opening of nonspecific basolateral cation channels [6]. Although not studied in detail here, this is supported by the observation that the VIP-induced current had a reversal potential more positive than the Cl^- equilibrium potential (Fig. 2). No studies were performed at the very basal part of the crypt, where agents increasing the intracellular cAMP concentration do not induce an increase in Cl^- conductance [1, 6].

Surprisingly, the VIP-induced cell shrinkage was only transient at upper parts of the crypt and was followed by a volume increase. This paradox volume increase, which is not present in the small intestine [5], was suppressed by furosemide. A secondary volume increase was not observed at the fundus region. These results support the finding that an increase in intracellular cAMP not only activates apical Cl^- channels but in addition activates the basolateral Na^+-K^+-Cl^--cotransporter [7]. This activation, which causes the uptake of solutes into the cell, seems to overbalance the effect of the activation of the Cl^- channels on cell volume. Interestingly, a paradox increase in the intracellular Cl^- concentration instead of the expected decrease was already observed, when cultured human colonic tumor cells were stimulated with the activator of the adenylate cyclase, forskolin [4].

A similar difference between the cells at the fundus and those at upper parts of the crypt was observed, when cell shrinkage was induced by an increase of the extracellular osmolarity. In the more differentiated cells in the upper parts of the crypt, the initial cell shrinkage induced by a hypertonic medium was followed by a RVI which was not observed at the fundus region (Fig. 1). The RVI was completely suppressed by furosemide, indicating that it is caused by the activation of the Na^+-K^+-Cl^--cotransporter. In contrast to the situation in the presence of VIP, the volume regulation only tended to counterbalance the effect of the hypertonic medium but did not induce a cell swelling above the control values. In difference to VIP, carbachol, a secretagogue acting via intracellular Ca^{2+}, did not affect cell volume at the middle or the upper third, but caused a cell shrinkage at the fundus (Table 1) similar as that reported for the small intestine [5]. Consequently, activation of cellular K^+ conductance by carbachol (Fig. 2), which is uniformly found along the crypt axis [1], is per se not sufficient at least in the middle or the upper third of the crypt to induce a loss of osmolytes in the colon. In conclusion, these results suggest a gradient in the distribution of the Na^+-K^+-Cl^--cotransporter along the crypt axis. Such a gradient has already been described for the rabbit distal colon from binding studies with the furosemide derivative, bumetanide [8]. This Na^+-K^+-Cl^--cotransporter seems to be activated by cell shrinkage and by intracellular cAMP.

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