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Choline acetyltransferase and organic cation transporters are responsible for synthesis and propionate-induced release of acetylcholine in colon epithelium

Sandra Bader [a], Jochen Klein [b], Martin Diener [a,*]

[a] Institute for Veterinary Physiology and Biochemistry, Justus-Liebig-University Giessen, Giessen, 35392, Germany
[b] Institute of Pharmacology, Goethe-University Frankfurt, Frankfurt am Main, Germany

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ABSTRACT

Acetylcholine is not only a neurotransmitter, but is found in a variety of non-neuronal cells. For example, the enzyme choline acetyltransferase (ChAT), catalyzing acetylcholine synthesis, is expressed by the colonic epithelium of different species. These cells release acetylcholine across the basolateral membrane after luminal exposure to propionate, a short-chain fatty acid. The functional consequence is the induction of chloride secretion, measurable as increase in short-circuit current (I_{sc}) in Ussing chamber experiments. It is unclear how acetylcholine is produced and released by colonic epithelium. Therefore, the aim of the present study was the identification (on mRNA and protein level) and functional characterization (in Ussing chamber experiments combined with HPLC detection of acetylcholine) of transporters/enzymes in the cholinergic system of rat colonic epithelium. Immunohistochemical staining as well as RT-PCR revealed the expression of high-affinity choline transporter, ChAT, carnitine acetyltransferase (CarAT), vesicular acetylcholine transporter (VAChT), and organic cation transporters (OCT 1, 2, 3) in colonic epithelium. In contrast to blockade of ChAT with bromoacetylcholine, inhibition of CarAT with mildronate did not inhibit the propionate-induced increase in I_{sc}, suggesting a predominant synthesis of epithelial acetylcholine by ChAT. Although being expressed, blockade of VAChT with vesamicol was ineffective, whereas inhibition of OCTs with omeprazole and corticosterone inhibited propionate-induced I_{sc} and the release of acetylcholine into the basolateral compartment. In summary, OCTs seem to be involved in regulated acetylcholine release by colonic epithelium, which is assumed to be involved in chemosensing of luminal short-chain fatty acids by the intestinal epithelium.

1. Introduction

Acetylcholine is regarded as a classical neurotransmitter in the central and peripheral nervous system. However, a large number of non-neuronal cells have the ability to synthesize acetylcholine such as endothelial cells, immune cells (e.g. macrophages), and different epithelial cells e.g. in the airways, the urinary bladder, or the skin (Wessler and Kirkpatrick, 2008). Immunoreactivity for the acetylcholine synthesizing enzyme, choline acetyltransferase (ChAT), has been detected in the surface epithelium of the small and large human intestine (Klapproth et al., 1997). Although intestinal epithelial cells have the ability to synthesize acetylcholine, its functional significance is poorly understood.

In the human colon cancer cell line, H508, it was found that acetylcholine produced by these cells stimulates cell proliferation, i.e. acts as autocrine signal molecule (Cheng et al., 2008). Furthermore, there are findings indicating that epithelial acetylcholine improves the tightness of the colonic barrier by regulating epithelial permeability (Lesko et al., 2013).

Quite recently, Yajima et al. (2011a) observed that propionate evokes a release of epithelial acetylcholine from colonic mucosa. Short-chain fatty acids such as propionate are produced by bacterial fermentation of dietary fibers in the colonic lumen (Karasov and Douglas, 2013). Propionate, when applied to the apical side of mucosal preparations of rat distal colon in Ussing chambers, induces a release of acetylcholine into the basolateral compartment (Yajima et al., 2011a, 2011b). Acetylcholine in turn stimulates Cl^- secretion measurable as increase in short-circuit current (I_{sc}) in Ussing chamber experiments. Because this secretion was inhibited by the muscarinic antagonist atropine, but was resistant to the neurotoxin tetrodotoxin, which blocks neuronal

* Corresponding author. Tel.: +49 641 9938161; fax: +49 641 9938159.
E-mail address: Martin.Diener@vetmed.uni-giessen.de (M. Diener).

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voltage-dependent Na^+ channels and thus suppresses the propagation of action potentials, it was concluded that propionate induces a release of epithelial acetylcholine, an effect which seems to be mediated by G-protein coupled receptors (GPR) GPR41 und 43 (Yajima et al., 2011b).

The mechanisms for storage and release of acetylcholine are thought to differ strongly between neurons and non-neuronal cells with acetylcholine production (Kummer et al., 2008). For example, in neurons acetylcholine synthesized in the cytosol is translocated into synaptic vesicles via the vesicular acetylcholine transporter (VAChT). In non-neuronal tissues, VAChT has not been consistently found. Studies on human placenta indicate that in non-neuronal tissues, acetylcholine release may be mediated by organic cation transporters (OCT) (Wessler et al., 2001). As the enzymes and transporters involved in these steps for handling non-neuronal acetylcholine are unknown for intestinal epithelium, the expression of candidate proteins for acetylcholine handling discussed for other cells types was investigated in rat colonic epithelium using immunohistochemical and PCR techniques. Their functional significance was controlled by the use of pharmacological inhibitors of the respective enzymes/transporters using propionate-induced Cl^- secretion as functional read-out to study the colonic epithelial acetylcholine release, which, when necessary, was confirmed by high pressure liquid chromatography (HPLC) measurements of the amount of acetylcholine released by the colonic epithelium.

2. Material and methods

2.1. Animals

Female and male Wistar rats with a body mass of 180–200 g were used. The animals were bred and housed at the Institute for Veterinary Physiology and Biochemistry of the Justus-Liebig-University at an ambient temperature of 22.5 °C and air humidity of 50–55% on a 12 h: 12 h light–dark cycle with free access to water and food until the time of the experiment. Animals were stunned by a blow on the head and killed by exsanguination (approved by Regierungspräsidium Giessen, Germany).

2.2. Solutions

Ussing chamber experiments were carried out in a bathing solution containing (in mmol/l): 107 NaCl, 4.5 KCl, 25 $NaHCO_3$, 1.8 Na_2HPO_4, 0.2 NaH_2PO_4, 1.25 $CaCl_2$, 1 $MgSO_4$, 12.2 glucose. The solution was gassed with 5% (v/v) CO_2 and 95% (v/v) O_2at 37 °C and had a pH of 7.4 (adjusted by $NaHCO_3$/HCl).

For the immunohistochemical experiments, a 100 mmol/l phosphate buffer was used containing (in mmol/l): 80 Na_2HPO_4 and 20 NaH_2PO_4; pH was 7.4 (adjusted by NaOH/HCl). For crypt isolation, a Ca^{2+}- and Mg^{2+}-free Hanks balanced salt solution containing 10 mmol/l ethylenediaminotetraacetic acid (EDTA) was used. The pH was adjusted to 7.4 by tris(hydroxymethyl)-aminomethane. The isolated crypts were stored in a high potassium Tyrode solution consisting of (in mmol/l): 100 K gluconate, 30 KCl, 20 NaCl, 1.25 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 12.2 glucose, 5 Na pyruvate, and 1 g/l bovine serum albumin (BSA); pH was 7.4 (adjusted by KOH).

2.3. Tissue preparation

The distal colon was quickly removed and placed in ice-cold Ussing chamber bathing solution. The colon was carefully flushed before it was mounted on a thin plastic rod. A circular incision was made near the distal end with a blunt scalpel. The serosa and muscularis propria were stripped away by hand in order to obtain a mucosa–submucosa preparation. This preparation was opened along the mesenteric border and placed onto a glass plate with the mucosal surface upwards. The proximal end of the tissue was clamped with a clip between a microscope slide and the upper end of the glass plate. The distal end of the colon was fixed with a blunt object slide. With a fresh and sharp glass slide the mucosa was carefully separated from the submucosal layer in order to obtain a mucosa preparation.

2.4. Ussing chamber experiments

The mucosa preparation was fixed in a modified Ussing chamber, bathed with a volume of 3.5 ml on each side of the mucosa. The tissue was incubated at 37 °C and short-circuited by a computer-controlled voltage-clamp device (Ingenieur Büro für Mess- und Datentechnik Mussler, Aachen, Germany) with correction for solution resistance. Tissue conductance (G_t) was measured every minute by the voltage deviation induced by a current pulse ($\pm$ 50 μA, duration 200 ms) under open-circuit conditions. Short-circuit current (I_{sc}) was continuously recorded. I_{sc} is expressed as μEq/h cm^2, i.e. the flux of a monovalent ion per time and area, with 1 μEq/h cm^2 = 26.9 μA/cm^2. Substances were administered after an equilibration period of about 60 min. The maximal increase in I_{sc} evoked by mucosal administration of propionate is given as difference to the baseline just prior administration of the drug. At the end of each experiment, the activator of adenylate cyclases(s), forskolin (5×10^{-6} mol/l at the mucosal and the serosal side), was administered in order to exclude a direct inhibition of Cl^- secretion by the inhibitors used (data not shown, as no inhibitory action of the blockers applied was observed).

2.5. Measurement of acetylcholine release

Mucosa preparations were mounted in Ussing chambers. After a stabilization phase of about 60 min, tetrodotoxin (10^{-6} mol/l) and eserine (an inhibitor of acetylcholine esterase; 5×10^{-5} mol/l) were administered at the basolateral side 15 min prior to sample collection. At the time points t=0, 5 and 10 min, basolateral buffer samples (250 μl) were collected and replaced by the same volume of fresh buffer. Immediately after the third sampling, propionate (2 mmol/l) was administered to the mucosal compartment and two further samples were taken from the basolateral medium at t=15 and t=20 min. The amount of acetylcholine in those samples was determined by microbore high-performance liquid chromatography as described previously (Mohr et al., 2013). Briefly, we used an Eicom HTEC-500 system (Kyoto, Japan) that included an enzyme reactor carrying immobilised AChE and choline oxidase, and an electrochemical detector with a platinum electrode operating at 500 mV. The mobile phase consisted of $KHCO_3$ 50 mmol/l (Merck, Darmstadt, Germany), EDTA-2Na 134.3 μmol/l (BDH, Poole, UK), and sodium decane-1-sulfonate 1.64 mmol/l (Alfa Aesar, Karlsruhe, Germany) in RotisolV® HPLC gradient grade water (Sigma-Aldrich, Munich, Germany), brought to pH 8.4. The flow rate was 150 μl/min. At an injection volume of 5 μl, the detection limit of this system was 1 fmol/injection. Data acquisition was performed using EPC-500 PowerChrom software.

2.6. Immunohistochemical experiments

The tissue was fixed with 40 g/l paraformaldehyde in phosphate buffer at 4 °C overnight. After washing in phosphate buffer (3 times for 60 min), the tissue was embedded in gelatin (gelatin type A from porcine skin; 100 g/l) and cryofixed with N_2-cooled isopentane. Sections (about 4 μm thick) were cut and mounted on glass slides coated with gelatin containing chrome alaun (chromium(III) potassium sulfate; 0.5 g/l). For immunofluorescence staining, after rehydration in phosphate buffer, the sections were incubated for 2 h in phosphate buffer containing 2 ml/l triton-X-100, 30 g/l BSA and