European Journal of Pharmacology 764 (2015) 157–163

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Pulmonary, gastrointestinal and urogenital pharmacology

The effects of polyamines on human colonic mucosal function

Ailín C. Rogers [a,b,*], Frank D. McDermott [a,b], Helen M. Mohan [a,b], P. Ronan O'Connell [a], Desmond C. Winter [a], Alan W. Baird [b]

[a] Department of Surgery, St Vincent's University Hospital, Elm Park, Dublin 4, Ireland
[b] School of Veterinary Medicine and Conway Institute of Biomolecular & Biomedical Science, University College Dublin, Dublin 4, Ireland

ARTICLE INFO

Article history:
Received 4 April 2015
Received in revised form
30 June 2015
Accepted 1 July 2015
Available online 2 July 2015

Keywords:
Polyamines
Colon
Calcium-sensing receptor
Ussing chambers

ABSTRACT

Electrogenic ion transport in human colon is a surrogate marker for colonic mucosal function, and may be manipulated by a variety of hormonal, neural, immune and paracrine mediators. Polyamines are present in vast quantities in the colonic lumen and appear to be integral to cellular function. This study explores some of the mechanisms of polyamine action on colonic tissue through study of their effects on differential secretory pathways, as well as examining their actions on intracellular cAMP and Ca^{2+} accumulation.

Human colonic mucosa was mounted in Ussing chambers and treated with polyamines (spermine, spermidine and putrescine) with changes in ion transport recorded. In separate experiments colonic crypts were treated with polyamines and intracellular cAMP levels determined by ELISA and intracellular calcium concentrations were quantified by fluorescent imaging.

Polyamines at physiological concentrations (1 mM) exert no effects on basal mucosal chloride secretion or transepithelial electrical resistance. Polyamines inhibit electrogenic ion secretion as stimulated by forskolin (cAMP-mediated), but not carbachol (Ach-mediated). All the polyamines used in this study inhibited intracellular cAMP accumulation, according to potency (spermine > spermidine > putrescine). Spermine increased intracellular Ca^{2+} in a PKC-dependent manner, likely due to its effects on the extracellular calcium-sensing receptor (CaSR).

Polyamines act to prevent cAMP-mediated Cl^- hypersecretion in the colon, acting through CaSR to inhibit PKC-mediated $[Ca^{2+}]_i$ release from intracellular stores.

1. Introduction

Polyamines are low molecular weight aliphatic nitrogenous bases which are derivatives of protein digestion (Ladenburg, 1888; Leeuwenhoek and Observationes, 1678) and synthesized by almost all cells in varying quantities. They are essential for cell growth (Nitta et al., 2002; Thomas and Thomas, 2001), differentiation and proliferation. Polyamines are usually found in miniscule concentrations in extracellular fluid, however the intestinal epithelia are bathed in an environment with up to millimolar concentrations (Osborne and Seidel, 1990; Pegg and McCann, 1982). The rapid turnover of intestinal epithelium mandates an extrinsic source of polyamines, in the colon these are largely provided from the microbiota in the gut lumen (Bardocz et al., 1998; Hessels et al., 1989; Loser et al., 1999; Satink et al., 1989). It is not surprising then that polyamines are present in high concentrations in human milk (Dorhout et al., 1996; Loser, 2000), and contribute to maturation of the gut in the neonatal period (Capano et al., 1994; Dufour et al., 1988; ter Steege et al., 1997; Wild et al., 1993).

Structurally, polyamines comprise of a carbon backbone with two or more amino groups. Five occur naturally – with two (putrescine, agmatine and cadaverine), three (spermidine) or four (spermine) amino groups. Polyamines are protonated at body pH (Seiler et al., 1996) and their cationic amino groups confer most of their functionality. Through these amine groups they electrostatically bind to negatively charged molecules. This ability to bind to intracellular negative charges via their amine groups protects anionic molecules such as nucleic acids and proteins from oxidative damage (Wang, 2007; Zou et al., 2010). Spermine is the most functionally potent of the naturally occurring polyamines (Yuan et al., 2001), while the di-amines are more stable, they are relatively inert in comparison.

Polyamines have diverse effects on cellular function, however it is their role in modulation of cell proliferation and differentiation that has been most studied with regards to the intestinal mucosa (Seiler and Raul, 2007). After tissue injury, polyamines promote mucosal growth and regeneration (Jonas et al., 1991; Kummerlen

* Corresponding author at: Department of Surgery, St Vincent's University Hospital, Elm Park, Dublin 4, Ireland.
E-mail address: ailinrogers@gmail.com (A.C. Rogers).

http://dx.doi.org/10.1016/j.ejphar.2015.07.006
0014-2999/

et al., 1994; Luk and Yang, 1988; Sakamoto et al., 1996; Seidel et al., 1984; Wang and Johnson, 1992; Wang et al., 1991). Polyamines exert anti-inflammatory effects (Hasko et al., 2000; ter Steege et al., 1999; Van den Bossche et al., 2012; Zhang et al., 1997) and may be protective in colitis (Hong et al., 2010). They are especially active in cancer cells (Becciolini et al., 1991), however their exact role in carcinogenesis is unclear (Barry et al., 2011; Erdman et al., 1999; Ignatenko et al., 2004; Iwata et al., 1999).

Polyamines are known to activate the extracellular calcium-sensing receptor (CaSR), a 7 transmembrane spanning G-protein coupled receptor, to stimulate intracellular events (Cheng et al., 2004). Signaling following CaSR activation is not well described but likely follows a similar path to CaSR activation by other receptor analogs, primarily stimulating a Gαq-mediated mechanism leading to activation of Phospholipase C (PLC) and increases in intracellular calcium (Brown et al., 1987). Despite the distribution of polyamines throughout the gastrointestinal tract (GIT), their effects in human colon remain relatively unexplored. Previous studies are limited to animal models and cultured epithelial cell lines and have often focussed on their downstream translational effects rather than on immediate effects on mucosal function and physiology.

Active transport in colonic epithelium accounts for over 90% of cellular energy expenditure, thus ion secretion is often used as a surrogate marker for intestinal cell function. This study explores the role of polyamines in modulation of mucosal physiology in a number of models, firstly at tissue level in Ussing chambers, and then at organelle level through the study of isolated colonic crypts.

2. Materials and methods

2.1. Tissue source and ethical approval

Human colon was obtained from resected specimens of patients undergoing gastrointestinal surgery for benign and malignant diseases at St. Vincent's University Hospital from July 2010 to May 2013. The normal histologic appearance of tissue was confirmed with routine pathological examination of the specimens obtained during dissection. Tissues were transferred to the laboratory in pre-oxygenated Krebs–Henseleit (KH) solution (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, and glucose 11.1, pH 7.4). Ethical approval (including informed patient consent) was obtained from the institutional ethical approval board in May 2010.

2.2. Ussing chambers

The Ussing chamber is a system in which live tissue segments can be maintained in a temperature-controlled gassed solution over a period of time (Ussing and Zerahn, 1951) (Fig. 1). Tissue was examined in Ussing chambers as previously described (Rogers et al., 2013). The transepithelial potential difference (PD) generated by the epithelium was short circuited with a voltage clamp by passing current across the tissue. Chloride secretion was then quantified as changes in short circuit current (SCC) per cm^2, which was continuously monitored and recorded by computer. Using Ohm's law the transepithelial electrical resistance (TEER) was also calculated.

Electrogenic ion transport in the colon was stimulated through two reagents acting via differing pathways – carbachol (carbamylcholine, CCh), a cholinergic agonist at muscarinic acetylcholine receptors in the GIT (Bachmann et al., 2006) and forskolin (Fsk), which raises intracellular cAMP through activation of the enzyme adenylate cyclase (Reymann et al., 1985; Seamon and Daly, 1981). Cch increases anion secretion through Ca^{2+} activated chloride channels (CaCCs) in a Ca^{2+}- and PKC-dependent manner (Bachmann et al., 2006). Fsk leads to intracellular cAMP accumulation which opens apical ion channels (CFTR) and causes subsequent Cl^- secretion. Both of these secretory stimuli lead to ion secretion from the apical membrane, followed by fluid secretion.

Tissues were exposed to secretagogues (CCh and Fsk) and polyamines putrescine, spermidine and spermine. Drugs were added to solutions bathing the basolateral side of the preparation.

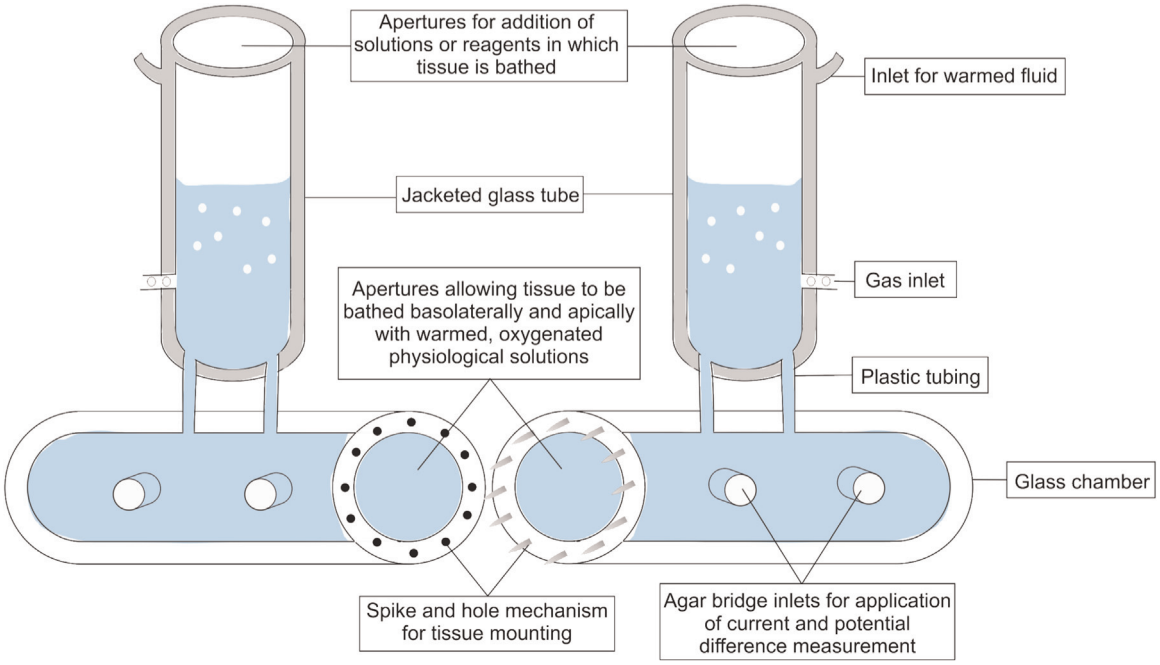

Fig. 1. Schematic representation of Ussing chamber set-up. Tissue of adequate size is mounted in glass chambers using a spike-and-hole mechanism to secure in place. Physiological solutions ± reagents are added to bathe apical and basolateral tissue surfaces. Tissues are temperature controlled with a jacketed mechanism and oxygenated via a gas inlet. Agar bridges allow application of current and measurement of potential difference through an external circuit. Bathing fluid can be sampled to assess drug/electrolyte transport.