Medical Hypotheses 81 (2013) 601–606

Contents lists available at SciVerse ScienceDirect

Medical Hypotheses

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

An alternative explanation for the occurrence of short circuit current increases in the small intestine following challenge by bacterial enterotoxins

M.L. Lucas *

Laboratory of Gastrointestinal Physiology, School of Life Sciences, College of Medical, Veterinary & Life Sciences, Glasgow University, Glasgow G12 8QQ, Scotland, United Kingdom

ARTICLE INFO

Article history:
Received 8 March 2013
Accepted 5 July 2013

ABSTRACT

Secretory diarrhoeal disease due to enterotoxins is thought to arise from the enhancement to pathologically high rates of normally occurring chloride ion and therefore fluid secretion from enterocytes. In support of this concept, many enterotoxins increase intestinal short-circuit current, regarded now as faithfully reflecting the increased chloride ion secretion. Contradicting this assumption, STa reduces absorption but does not cause secretion *in vivo* although short-circuit current is increased *in vitro*. There is therefore a mismatch between an assumed enterocyte mediated secretory event that should but does not cause net fluid secretion and an undoubtedly increased short-circuit current.

It is proposed here that short-circuit current increases are not themselves secretory events but result from interrupted fluid absorption. A noteworthy feature of compounds that inhibit the increase in short-circuit current is that the majority are vasoactive, neuroactive or both. In general, vasodilator substances increase current. An alternative hypothesis for the origin of short-circuit current increases is that these result from reflex induction of electrogenic fluid absorption. This reflex enhances a compensatory response that is also present at a cellular level. An intestinal reflex is therefore proposed by which decreases in interstitial and intravascular volume or pressure within the intestine initiate an electrogenic fluid absorption mechanism that compensates for the loss of electrically neutral fluid absorption.

This hypothesis would explain the apparently complex pharmacology of short-circuit current increases since many depressor substances have receptors in common with enterocytes and enteric nerves. The proposed alternative view of the origin of short-circuit current increases assumes that these *do not* represent chloride secretion from the enterocytes. This view may therefore aid the successful development of anti-diarrhoeal drugs to overcome a major cause of infant mortality worldwide, if short-circuit current data are being persistently misinterpreted. The putative but testable link between interstitial volume or pressure and fluid absorption also provides support for the alternative view of secretion; namely, that enhanced capillary and epithelial cell tight junctional permeability together with increased intracapillary pressure may cause secretion and not chloride exit from the enterocytes.

Introduction

Many intestinal enterotoxins such as heat stable (ST) and heat labile (LT) *Escherichia coli* toxin hinder fluid absorption in the small intestine. In the cases of cholera toxin, LT and many other toxins, there is additional secretion meaning that fluid enters the small intestine. The prevalent explanation is that these toxins cause enterocytes to work in reverse and secrete fluid into the lumen, often at rates that are life threatening. In order to achieve secretion, chloride ion must be extruded by the enterocytes into the lumen through channels in the mucosal membrane, with sodium ion perhaps passively drawn into the lumen. Chloride ion is assumed to enter the cells from the serosal surface by the serosally sited sodium: potassium: chloride co-transporter. The net result is that osmolarity at the mucosal surface is elevated, causing fluid to move into the lumen either by the paracellular or transcellular route, down this osmotic gradient. A deduction from this view is that secretory diarrhoeal disease is treatable by interrupting the enhanced enterocyte chloride secretion following enterotoxin challenge.

The hypothesis of chloride ion secretion assumes that all the required structural and biochemical elements for secretion are necessarily within the enterocytes. The case for chloride secretion being a 'doctrine untroubled by proof' has been made elsewhere [1]. Fluid secretion deriving energy from metabolic processes within cells is limited by the maximum work rate that cells can achieve. Fluid entry into the lumen after enterotoxin exposure

* Tel.: +44 141 339 4494; fax: +44 141 330 4100.
E-mail address: M.Lucas@bio.gla.ac.uk

0306-9877/$ - see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.mehy.2013.07.010

e.g. in cholera, is often so great that it likely can only be achieved by pressure energy generated by the heart. Although mean arterial blood pressure is adequate to achieve profuse fluid loss, it does not normally cause extrusion through the vasculature into the lumen at a rate that can overwhelm absorption. An alternative view of pathological secretory diarrhoeal disease is that secretion arises from vasodilatation of the intestinal vasculature causing increased intracapillary pressure, together with increased permeability of the vascular endothelium and the mucosal epithelium. During the period when the existence of permeability enhancing enterotoxins remained unknown, the view that cholera toxin acted solely by enhancing naturally arising chloride ion secretion was tenable, provided vasodilatation was regarded as a coincidental, minor occurrence that was not central to cholera pathology. However, the realisation that cholera not only causes widespread systemic vasodilatation but also secretes enterotoxins (zona occludens toxins) that make the tight junctions leakier, makes the hydrostatic model of secretion being a capillary exudate just as likely as the enterocyte chloride secretion model.

The pressure view of fluid secretion assumes that secretion cannot be fully explained by reference only to the enterocytes but must also take into consideration vascular physiology. In contrast, the view that secretion originates in the enterocytes alone justifies the use of cell and tissue preparations *in vitro* to study the mechanisms underlying secretion since all relevant factors are assumed still to be present when tissue is extracted or cells are cultivated and grown *ex-vivo*. It is worth emphasising again here that stopping the absorption mechanisms present in the enterocyte reveals only the ability of enterotoxins to inhibit fluid absorption and this is not a secretion event. Secretion is the ability of the intestine to secrete more fluid into the lumen than is initially present in the intestine. There may exist a secretory mechanism within the enterocytes as the prevailing dogma requires but it could also mean that secretion (not simply reduced absorption) is not an event occurring within the enterocyte but arises because of increased vascular permeability and transmucosal pressure.

Electrophysiological background

Given that all relevant factors for the secretory event to occur are judged to be present within the enterocyte, methods have evolved to study them *in vitro*, despite the fact that there are no convincing experiments showing secretion *in vitro* when this is defined as the mass transport of water. A proxy measurement has been the short-circuit current that can be measured across the mucosa as this is assumed to be caused by the chloride anion moving towards the lumen. The increase in current detected after challenge with enterotoxin is assumed to represent the expected increase in chloride secretion. This has been measured often and is so reliable an occurrence after intestinal derangement that it is now synonymous with fluid secretion, with the terms being used interchangeably.

Jejunal short-circuit current is known to increase after *E. coli* STa enterotoxin exposure [2,3]. A consequence of enterotoxin challenge *in vitro* is that the serosally positive short-circuit current increases in the positive direction, consistent with more sodium ion being electrogenically absorbed but is generally accepted as evidence for electrogenic chloride secretion towards the lumen. Bumetanide, which inhibits chloride ion secretion, reduces STa mediated elevations in short circuit current *in vitro* [4]. However, it does not restore the reduced net fluid absorption caused by STa *in vivo* [5]. There is therefore a contradiction between *in vivo* and the *in vitro* findings assumed to represent fluid secretion. In sodium ion free buffers, STa has no secretory action beyond that expected from inhibiting absorption. STa can be shown to stop absorption (consistent with interference with absorptive mechanisms) but there is no net secretion of fluid (inconsistent with the secretory view), yet STa still elevates short-circuit current. There is therefore a mismatch between secretion measured as transport of fluid and increases in short-circuit current.

If the enterocyte is not the origin of fluid secretion, then some other explanation must be found for the undoubted increases in short circuit current. *E. coli* STa enterotoxin increases short-circuit current *in vitro* but fails to cause net fluid secretion *in vivo*: instead it causes fluid absorption to come close to ceasing. Hence, while these *in vivo* experiments show that short-circuit current increases are not secretory events, they do not indicate what the increases are. It is important nevertheless to determine instead what other explanations for the increases in short-circuit current are possible as part of the process of examining the validity of the enterocyte secretion hypothesis. If the current increases do not reflect a secretory event, then they may reflect aspects of the *alternative* view that fluid secretion, as opposed to cessation of fluid absorption, is caused by changes in pressure gradients across the mucosa.

The proposed hypothesis

The failure over the decades since 1971, when enterocyte secretion was first proposed, to develop an effective anti-secretory drug based on the chloride secretion hypothesis, has nevertheless provided detailed knowledge of the pharmacology of the current that is assumed to reflect secretion. Mucosal structure, neuroactive agents and compounds causing vasomotion all affect the current increases. In many cases, where receptors for these compounds exist, they are present on smooth muscle and on the enterocytes, with overlap also to peripheral nerves. Vasoactive drugs affecting short-circuit current include sodium nitroprusside [5], nitric oxide synthase inhibitors [2], cholinergic and adrenergic substances and their antagonists [5,6], vasoactive intestinal polypeptide [6], histamine [7] and 5-HT [8]. With the compounds so far tested, vasodilatatory compounds increase and inhibitors of vasodilatation decrease short-circuit current.

The essence of the proposed hypothesis is therefore that short-circuit current increases do not reflect secretion after enterotoxin challenge; they represent compensatory absorption mechanisms that are initiated by a sudden fall in interstitial volume or pressure. All data to date can be accommodated within this novel hypothesis.

The relevance of the proposed hypothesis

If secretion is not an enterocyte phenomenon, then the search for anti-diarrhoeal drugs will be hindered. The body of work done *in vitro* to date within the enterocyte secretion paradigm has been extremely useful in determining the mechanisms by which bacterial enterotoxins prevent fluid absorption but it cannot assist with understanding secretion if the cause resides elsewhere. In industry, anti-secretory drugs are screened on the basis of current increases induced by enterotoxin being inhibited by candidate drugs. There may be merit in this approach since a candidate anti-secretory drug might be identified if reduced current equates to restored absorption. However, if short-circuit current increases only reflect challenge by bacterial enterotoxin, then a candidate anti-secretory compound may reduce the overt sign of enterotoxin mediated derangement without actually reversing any secretory event. It is noteworthy that there are no chloride secretion inhibitors in daily use today despite the enterocyte chloride secretion hypothesis having been proposed forty years ago.

Part of the problem in finding useful anti-diarrhoeal drugs may be that cessation of absorption is undoubtedly an event that occurs