Antisecretory factor suppresses intestinal inflammation and hypersecretion

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Abstract

Background—Antisecretory factor (AF) is a recently identified regulatory protein which inhibits the intestinal fluid secretion induced by cholera toxin.

Aims—To test the effect of AF on: (a) inflammation and hypersecretion induced by toxin A from Clostridium difficile; and (b) morphological changes and hypersecretion induced by okadaic acid (the blue mussel toxin) in rat intestinal mucosa.

Methods—Morphological changes and fluid accumulation were observed in intestinal loops challenged with 1 µg of toxin A or 3 µg of okadaic acid administered before or after injection of 0.1 µg of recombinant AF (rAF).

Results—The cytotoxic and inflammatory reaction caused by toxin A was abolished after treatment with rAF given either intravenously or intraluminally prior to the toxin or one hour after the toxin. The intestinal fluid response induced by toxin A and okadaic acid was reduced 55–80% by rAF. However, the characteristic increase in goblet cells at the tips of villi in the okadaic acid treated mucosa was not inhibited by rAF.

Conclusion—Results suggest that AF might be involved in protection against inflammation and in counteracting dehydration caused by enterotoxins. Both effects are probably mediated via the enteric nervous system.

Keywords: okadaic acid; Clostridium difficile toxin A; diarrhoea; neuropeptide; SSa; rat

Bacterial enterotoxins induce fluid secretion and inflammation in the intestine. A fatal form of intestinal toxinoisis is caused by toxin A from Clostridium difficile, a bacterium which frequently colonises the gut after treatment with antibiotics.1 Toxin A causes fluid secretion, inflammation, and mucosal damage in the small intestine of hamsters, rabbits, and rats2,3 and is probably the main cause of pseudomembranous colitis in man.1 After an initial attachment to oligosaccharide receptors,1,3 the toxin seems to penetrate into the mucosal epithelium and acting as an enzyme it transfers glucose residues to the G-protein rho causing rearrangement of the cytoskeleton.4–7 The initial cytopathic effect of toxin A on the intestinal mucosa is followed by an inflammatory response, probably via a nerve mediated effect on intestinal mast cells and neutrophils.8–8

Diarrhoeal shellfish poisoning appears in man after consumption of shellfish which have been contaminated with marine phytoplankton. The causative agent, okadaic acid, is produced by the plankton and induces diarrhoea, nausea, and vomiting without any inflammatory reaction.10,11 In the rat the toxin induces rapid fluid secretion12 and a concomitant shedding of epithelial cells in the intestinal mucosa without inflammation or any apparent disruption of the epithelial barrier.13 The cellular action of okadaic acid is to inhibit cellular phosphatases types 1 and 2A, an action which has been demonstrated in epithelial cells, nerve cells and neutrophils.14–15

The mucosal barrier protects against the action of bacterial toxoids by means of both physiological and immunological defence mechanisms. We have previously shown that antisecretory factor (AF), a protein produced in the pituitary gland and in the intestinal mucosa, reverses intestinal hypersecretion induced by bacterial enterotoxins. Thus, a small amount of porcine AF was able to inhibit the action of a variety of enterotoxins in the pig16 and in the rat,17,18 including the heat labile enterotoxins from Vibrio cholerae and Escherichia coli and toxin A. With the help of antibodies raised against AF purified from porcine blood we were able to clone complementary DNA (cDNA) and express the human AF in E coli.19 Less than one picomole of homogeneous human recombinant AF (rAF) was shown to inhibit cholera toxin induced fluid secretion in rat intestinal loops. In the present paper we describe the action of rAF on fluid secretion induced by toxin A and okadaic acid in rat small intestine. The effect of rAF on toxin A induced cytotoxicity and inflammation in rat intestinal mucosa is also described.

Methods

RECOMBINANT ANTISECRETORY FACTOR

Human rAF was expressed in E coli and purified as previously described.19 In brief, the full length cDNA was subcloned into the glutathione S-transferase (GST) expression vector pGEX-17T (Pharmacia, Uppsala, Sweden), and the expressed fusion protein was purified on a glutathione-Sepharose column (Pharmacia) and cleaved with thrombin to elute pure rAF protein. The purity of the protein was evaluated by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis using Coomassie brilliant blue staining.10

ENTEROTOXINS

C difficile toxin A was produced and purified as previously described10 and checked for
homogeneity by SDS polyacrylamide gel electrophoresis. Okadaic acid was a gift from Professor Lars Edebo, Gothenburg, Sweden.

RAT INTESTINAL LOOP TEST
The study design was approved by the Ethics Committee of the University of Gothenburg. Male Sprague-Dawley rats (300±10 g), housed in a controlled environment, were fasted for 20 hours before experimentation but given free access to water. Intestinal secretion was investigated with the “ligated loop” method as described previously. Under ether anaesthesia one loop, about 10 cm in length, was ligated in the jejunum. The loop was challenged with 1.5 ml of phosphate buffered saline (PBS; 0.15 M NaCl, 0.05 M sodium phosphate, pH 7.5) in the control group, and in the test groups with 3 µg of okadaic acid or 0.5 µg of C. difficile toxin A, dissolved in the same volume of PBS. Two millilitres of 0.1 µg rAF (experimental groups) or PBS (controls) were administered intravenously via the dorsal vein of the penis. The animals were then allowed to wake up. The duration of challenge was five hours for toxin A and 90 minutes for okadaic acid, intervals based on findings in earlier kinetic studies of fluid accumulation induced by the respective toxins. At the end of the observation period, the rats were sacrificed by cervical spine dislocation, the abdomen was opened and the loops dissected out. The net fluid secretion (mg/cm) was estimated by subtracting the weight of a control loop from that of the experimental loop. Specimens for histological examination (see later) were taken from each loop.

ANIMAL GROUPS
Six groups of rats (I–VI) were studied, each group containing at least five animals. Rats serving as controls were subjected to 1.5 ml of intraluminal PBS challenge in combination with intravenous (n=6) or intraluminal (n=5) PBS injection. Groups I and V received 2 ml PBS by intravenous injection some 10–20 seconds before toxin challenge into the loop, while groups II and VI were injected with rAF in the same volume and by the same route. Group III was injected with rAF 60 minutes after challenge with toxin A, while group IV was injected with rAF intraluminally just before challenge with toxin A.

HISTOLOGICAL EXAMINATION
A histological investigation of the gut wall was undertaken in all experimental groups (I–VI and controls without toxin). Without cut-

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Toxin challenge</th>
<th>AF/PBS treatment</th>
<th>Fluid response (mg/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>7</td>
<td>Toxin A</td>
<td>PBS (iv 0')</td>
<td>484 (12)</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>Toxin A</td>
<td>AF (iv 0')</td>
<td>203 (6)*</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>Toxin A</td>
<td>AF (iv 60')</td>
<td>156 (15)*</td>
</tr>
<tr>
<td>IV</td>
<td>10</td>
<td>Toxin A</td>
<td>AF (ii 0')</td>
<td>210 (41)*</td>
</tr>
<tr>
<td>V</td>
<td>5</td>
<td>Okadaic acid</td>
<td>PBS (iv 0')</td>
<td>338 (5)</td>
</tr>
<tr>
<td>VI</td>
<td>5</td>
<td>Okadaic acid</td>
<td>AF (iv 0')</td>
<td>151 (3)*</td>
</tr>
</tbody>
</table>

*Significantly different from controls, p<0.05.
given either intravenously or intraluminally prior to or one hour after toxin challenge. Okadaic toxin does not cause inflammation in rat intestine but induces a characteristic redistribution of epithelial and goblet cells.13 These changes were not prevented by treatment with rAF, the morphology being similar in groups V and VI (not shown).

Discussion

We have previously reported the inhibitory effect of rAF on cholera toxin induced intestinal fluid secretion. The results of the present study suggest that AF from human pituitary gland also inhibits intestinal fluid secretion induced by toxin A or okadaic acid in the rat. These three toxins differ from each other in their biochemical mechanism of action. Toxin A binds to receptors resembling the glycolipid B5 or X2 whereas cholera toxin binds to receptors resembling GM1 ganglioside.22 Their intracellular target structures and mechanisms of action also differ, toxin A and cholera toxin being invasive enzymes transferring glucose residues to rho and rac proteins1 and ADP ribosyl groups to adenylate cyclase, respectively; okadaic acid is a specific inhibitor of the serine/threonine specific protein phosphatases 1 and 2A.14 It should be emphasised that, despite our detailed knowledge of the biochemical action of the three toxins, it is still unclear how these activities result in fluid secretion and diarrhoea.

Treatment with rAF counteracted the cytotoxic and inflammatory activity of toxin A, further emphasising the close relation between these morphological effects and the induced hypersecretion.5 6 The abolition of the inflammatory reaction and the reduction in hypersecretion suggest that rAF or a peptide derivative of AF might be used as a drug against intestinal inflammation and hypersecretion. rAF did not inhibit the morphological effects induced by okadaic acid, whereas it inhibited the hypersecretion induced by the toxin. The toxin induced increase in goblet cells at the tips of the villi13 thus seems to occur independently of the induced hypersecretion.

The in vitro effects of AF on nerve cells suggest that it modulates postsynaptic signals.24 25 Most of the in vivo effects exerted by AF might be explained by actions on nerve reflexes, since both intestinal fluid secretion and inflammation seem to be regulated by the enteric nerve system. Thus cholera toxin induced fluid secretion is inhibited by neuromodulators such as neuropeptide Y, somatostatin, and neurotrophic drugs such as neuroleptic agents, tranquillisers, and anaesthetics.26–29 Neuronal involvement in the intestinal effects of toxin A was recently suggested by Castagliuolo et al.8 Neurotropic drugs such as lignocaine and capsaicin have been shown to reverse toxin A mediated secretion and inflammation. Whereas cholera toxin induced hypersecretion seems to be transmitted by 5-hydroxytryptamine,26 toxin A induced secretion is probably mediated by substance P and nitric oxide synthase.89 Thus, the afferent sensory signals triggered by cholera toxin and toxin A are probably different. AF should therefore act on the efferent neuromotor neurones which innervate the epithelium and probably control fluid transport across the mucosa. Okadaic acid has been studied less than the other toxins. The symptoms it causes during shellfish poisoning in humans—diarrhoea, nausea, vomiting, and abdominal pain11—suggest a neurogenic component. Moreover, the toxin has been shown to inhibit...
the regeneration of axons in the frog sciatic sensory nerve. 20

The cellular receptor for AF and the possible intracellular signal transduction triggered by this receptor-agonist interaction is unknown. However, AF like sequences seem to contain binding sites for polyubiquitin 21 which has been shown to cause lethal diarrhoea in calves when transfected into the intestinal mucosa by means of a virus vector. 22 Ubiquitin and its polymer are heat shock proteins known to be induced during various neurological 23 and inflammatory bowel 24 diseases. The expression of these proteins in the enteric nerve cells might lead to the release of substance P or 5-hydroxytryptamine involved in enterotoxigenic diarrhoea. The counteraction of intestinal hypersecretion and inflammation by AF might thus result from its interaction with polyubiquitin in enteric nerve cells.