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Effect of Ganglionic Blocking Compounds on In-vivo Fluid Secretion in the Rat Small Intestine

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Abstract

It is well-known that enteric, secreto-motor nerves mediate cholera toxin-induced fluid secretion in the rat small intestine. This notion is, in part, derived from experiments on anaesthetized animals in which the response to cholera toxin was antagonized by the ganglionic nicotinic receptor antagonist, hexamethonium. In the current study, such anti-secretory action of ganglionic blocking compounds was analysed in an experiment designed to minimize any possible negative effect of general anaesthesia on intestinal secretion.

Rats were anaesthetized with ether for 5–10 min, during which time a jejunal loop (10–12 cm) was constructed. The loop was challenged with one of the secretagogues, cholera toxin, prostaglandin E_1 (PGE_1) or okadaic acid. Saline (control) or either of the ganglionic blockers, hexamethonium and chlorisondamine, was administered intravenously. The rats were killed 5 h (cholera toxin) or 1·5 h (PGE_1 and okadaic acid) after challenge, and the amount of fluid accumulated in the loops was determined. Cholera toxin-induced secretion was unchanged by hexamethonium but reduced by approximately 80% by chlorisondamine. The difference in effect between the two blockers might relate to the duration of ganglionic blockade. Chlorisondamine blocked secretion induced by either PGE_1 or okadaic acid by approximately 60%.

It is suggested that the anti-secretory effect of ganglionic blocking compounds might be a result of blockade of secreto-motor nerves but other mechanisms, for example interference with haemodynamic factors, cannot be ruled out.

There are two main hypotheses about the mechanism by which cholera toxin elicits excessive secretion of water and electrolytes by the mucosa of the small intestine. The traditional view (based mainly on in-vitro studies) is that the cholera toxin molecule binds to the enterocyte and causes secretion by a mechanism dependent on cyclic AMP (Field & Semrad 1993). At variance with this hypothesis of a direct mechanism of action, Lundgren and co-workers, in the early 1980s, (on the basis of in-vivo studies) proposed that the cholera toxin-induced secretion is the result of an intramural nervous reflex–the toxin molecule binds to endocrine cells in the villus epithelium, leading to the release of serotonin and neurotensin. These substances, in turn, activate enteric afferent nerves and thereby a reflex arc that causes the release of secretory neurotransmitters (mainly vasoactive intestinal peptide; see Jodal & Lundgren (1995) for references). This hypothesis of an indirect mechanism of action of cholera toxin is based principally on two lines of evidence. Firstly, whereas cholera toxin binds to the epithelium of the villus region, the resulting secretion emanates from the crypt cells, as noted in-vivo (Hallbäck et al 1982). Secondly, cholera toxin-induced secretion can be antagonized by the nerve blockers tetrodotoxin and lidocaine or by the ganglionic nicotinic cholinoceptor blocker hexamethonium as demonstrated in anaesthetized laboratory animals (see Jodal & Lundgren (1995) for references).

There are, however, recent reports (supporting previous observations by de Jonge (1975) and Weiser & Quill (1975)) that could refute the view that secretory processes in the gut are confined solely to the crypt region (Stewart & Turnberg 1989; Köckerling & Fromm 1993). Moreover, according to Coupar (1986), tetrodotoxin also appears to affect the epithelial cells directly (see, however, our comments on this reference in the Discussion section of this paper). Therefore, the results obtained by means of this pharmacological tool must be regarded with some caution. It should also be emphasized that various gastrointestinal transport processes might not function optimally when the animal under investigation is anaesthetized, because of a depressant effect (to a greater or a lesser extent) on absorption or secretion induced by the anaesthetic agent used (Larsen 1982; Coupar 1985; Uhing & Kimura 1995). This could imply that the complexity of the secretory process as initiated and maintained by cholera toxin cannot be fully appreciated in a model in which the experimental animal is subjected to continuous, general anaesthesia.

An alternative arrangement for studying cholera toxin-induced secretion, in which the duration of general anaesthesia is kept to a minimum, was described by Lange (1982; see below). This model was used in the current study with the primary aim of investigating whether or not blockade of nicotinic ganglionic receptors (by hexamethonium) abolishes cholera toxin-induced secretion in the awake animal also. Because we found that there is a discrepancy between the results obtained in anaesthetized and awake animals, we proceeded to investigate the effect of chlorisondamine on some secretagogues, using the same experimental arrangement. Chlorisondamine is a non-competitively acting ganglionic blocking compound (Trendelenburg 1961) which seems to be more potent than hexamethonium (Alaranta et al 1990).

Materials and Methods

General

The study design was approved by the Ethics Committee of Göteborg University. The experiments were conducted with

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male Sprague-Dawley rats, 230–380 g, purchased from B&K Universal AB, Sollentuna, Sweden (n = 79). The animals were housed in an environmental room at 22°C and 58–65% relative humidity, with a controlled 12-h light-dark cycle; they were allowed free access to a standard pellet diet and water. For at least seven days before the experiments the rats were kept in cages containing up to five animals.

Chemicals

The drugs used were α-D(+)-glucochloralose (E. Merck, Darmstadt, Germany), chlorisondamine chloride (Ciba-Geigy, Basel, Switzerland), cholera toxin (List Chemical, Vandell, CA), hexamethonium chloride, prostaglandin E_1 (Sigma, St Louis, MO), okadaic acid (a generous gift from Professor L. Edebo) and pentobarbital sodium (pentobarbitalnatrium; Apoteksbolaget, Umeå, Sweden). All solid drugs were dissolved in isotonic saline.

Effect of ganglionic blockers on intestinal fluid transport

Surgical procedures. The rats, average weight 340 g (range 330–350 g), were deprived of food for 24 h before the experiment (water was still freely available). Intestinal secretion was investigated by the 'ligated loop' method. Surgical procedures were performed during a 5–10-min period of ether anaesthesia, as described elsewhere (Lange 1982). Briefly, in each rat a 10–12-cm jejunal loop (oral end situated some 10 cm distal to the ligament of Treitz) was tied off with silk ligatures. The loop was challenged with 1·5 mL of one of various secretagogues (see Experimental protocol, below) in phosphate-buffered saline (PBS), injected intraluminally by means of a 2-mL syringe with a needle of 0·4 mm o.d. The loop became moderately distended after injection of this volume. The abdominal wall was closed by sutures. The systemic administration of drugs (see below) was undertaken (during ether anaesthesia) via the dorsal vein of the penis. On completion of surgery the rats were left to wake up. After 2–3 min recovery, they moved about freely in the cage, apparently without any discomfort for the whole of the observation period (up to 5 h). The rats were thereafter killed by cervical spine dislocation and the loops were dissected for subsequent analysis of water transport (see below).

Experimental protocol. In the first series of experiments the rats were divided into four groups (1–4; n = 6 or 7 for all groups). All animals received cholera toxin (3 μg) intraluminally in the jejunal loop. Immediately before cholera toxin challenge, PBS (1 mL kg^{-1}; group 1 (control)) or hexamethonium (25 mg kg^{-1}; group 2) was administered intravenously. In groups 3 and 4, no injection was performed until 75 min after cholera toxin challenge. These animals were then re-anaesthetized with ether and PBS (group 3 (control)) or hexamethonium (group 4, doses as above) were administered. The duration of cholera toxin challenge was 5 h. Fluid accumulation in the loop was expressed as the intestinal weight increase per length intestine (mg cm^{-1}) attributable to challenge.

In the second series of experiments, the animals received as a luminal challenge (volumes as above) PGE_1 (150 μg; groups 5 and 6), cholera toxin (dose as above, groups 7 and 8) or okadaic acid (3 μg; groups 9 and 10); n = 5–8 for all groups. Immediately before toxin challenge, all rats received an intravenous injection of either PBS (1 mL kg^{-1}; groups 5, 7 and 9 (control)) or chlorisondamine (5 mg kg^{-1}; groups 6, 8 and 10).

Effect of ganglionic blockers on arterial blood pressure

Surgical procedures. Fourteen rats, average weight 275 g (range 230–380 g), were anaesthetized with pentobarbital (60 mg kg^{-1} i.p.). After tracheotomy, a cannula was inserted to maintain free airways. The left carotid artery and the right jugular vein were catheterized. Anaesthesia was then continued with α-chloralose (a bolus dose of 12·5 mg kg^{-1} intra-arterially (i.a.), followed by an infusion of 25 mg kg^{-1} h^{-1}, i.a.).

Mean arterial pressure (MAP) was continuously monitored in the carotid artery by means of a Statham P23 AC transducer, on a Grass polygraph.

Experimental protocol. Upon completion of surgery, a 5-min period of stable MAP was monitored (control), whereupon hexamethonium (25 mg kg^{-1}; n = 7) or chlorisondamine (5 mg kg^{-1}, n = 7) was administered intravenously. MAP was sampled 5 and 115 min after drug injection for a period of 5 min at each time.

Statistical analysis

Results are presented as means ± s.e.m. Non-parametric statistical methods were used. Friedman two-way analysis of variance, then Wilcoxon's signed rank test with Bonferroni's correction for repeated comparisons, were performed for analyses within the groups. For analyses between the groups, either the Mann-Whitney *U*-test (comparison between two groups) or Kruskall-Wallis one-way analysis of variance, followed by the Mann-Whitney *U*-test with Bonferroni's correction for repeated comparisons (> 2 groups) were utilized. Blood pressure results were compared within the groups as absolute values, and between the groups as percent of control. $P < 0.05$ was considered indicative of statistical significance.

Results

Effect of ganglionic blockers on intestinal fluid transport

In the first series of experiments, one rat died upon administration of hexamethonium (presumably owing to cardiovascular collapse) and was, therefore, omitted from the study. All other animals withstood the treatment well.

The results in Table 1 demonstrate that the secretory response to cholera toxin was remarkably similar for all groups. Hexamethonium, administered either immediately before or 75 min after toxin challenge, had no significant effect on cholera toxin-induced secretion when compared with PBS (group 2 compared with group 1, $P > 0.05$; group 4 compared with group 3, $P > 0.05$).

In the second series of experiments, it was found that in contrast with hexamethonium, chlorisondamine markedly inhibited the effect of the three different secretagogues studied (Table 2).

Effect of ganglionic blockers on arterial blood pressure

The ganglionic blockers hexamethonium and chlorisondamine induced an immediate reduction in MAP to approximately the same extent (63 ± 1·9 and 59 ± 2·5% of control, respectively;

Table 1. The effect of phosphate-buffered saline (PBS) or hexamethonium on cholera toxin (3 μg)-induced net fluid secretion of rat small intestine, in-vivo.

	Net secretion (mg cm^{-1})	
	Drug given before challenge	Drug given after challenge
PBS	392 ± 5 (group 1, n = 6)	414 ± 7 (group 3, n = 6)
Hexamethonium	396 ± 4 (group 2, n = 7)	428 ± 6 (group 4, n = 6)

PBS (1 mL kg^{-1}, i.v.; control) or hexamethonium (25 mg kg^{-1}, i.v.) was administered either immediately before or 75 min after toxin challenge of the gut. Data presented as means ± s.e.m.

$P < 0.05$ for both compounds). Whereas the effect of hexamethonium was no longer apparent 2 h after administration (97 ± 9.3% of control; $P > 0.05$), that of chlorisondamine was unchanged (59 ± 2.7% of control; $P < 0.05$). Moreover, MAP after 2 h was significantly lower after injection of chlorisondamine than with after injection of hexamethonium ($P < 0.002$).

Discussion

This study has investigated the potential effect of ganglionic blockade on cholera toxin-induced intestinal secretion (Cassuto et al 1982; Field & Semrad 1993; Jodal & Lundgren 1995). Experiments were conducted with rats, utilizing the 'ligated loop' method (Lange 1982). By this procedure, the animals were subjected to general anaesthesia for a minimum amount of time, thereby avoiding undue effects of anaesthesia on secretion (Larsen 1982; Coupar 1985). In our model, however, we were unable to verify the common observation in anaesthetized rats that hexamethonium blocks cholera toxin-induced fluid secretion (Cassuto et al 1982; Jodal & Lundgren 1995). As a tentative explanation of this discrepancy, we suggest that the duration of ganglionic blockade induced by a bolus injection of hexamethonium (25 mg kg^{-1} i.v.) might have been too short to influence cholera toxin-induced fluid secretion markedly, when collected during 5 h as in our study. Conversely, Cassuto et al (1982) found that the duration of the hexamethonium-induced (20 mg kg^{-1} i.v.) inhibition of the secretory response to cholera toxin was 60 min, and net fluid secretion had returned to normal approximately 105 min after hexamethonium injection. It does not seem unlikely that anaesthesia, because of its potentially depressant effect on intestinal secretion (Larsen 1982; Coupar 1985) could contribute to an experimental condition in which hexamethonium might block cholera toxin-induced secretion far longer than in unanaesthetized animals.

In contrast with our negative finding with hexamethonium, chlorisondamine, which blocks nicotinic ganglionic transmission by another mechanism (Trendelenburg 1961), antagonized the secretory effect of cholera toxin by about 80%. At the doses used hexamethonium and chlorisondamine were approximately equally efficient at reducing MAP by ganglionic blockade, although chlorisondamine seemed to cause a far more long-lasting blockade, as investigated in anaesthetized animals. Therefore, it is tempting to conclude that our findings for cholera toxin in principle confirm those of Cassuto et al (1982); when adequate duration of ganglionic blockade had been achieved, impressive inhibition of cholera toxin-induced fluid secretion could be demonstrated in our experimental arrangement also. The exact mechanism of the anti-secretory effect of ganglionic blockade cannot be determined, however, either from our results or from those of Cassuto et al (1982). A direct effect of cholera toxin on the enterocytes might be suggested by an in-vitro study performed with the Ussing chamber technique on rabbit ileum that had previously been subjected to challenge with cholera toxin, in-vivo, to elicit secretion—it was noted that cholera toxin-induced electrogenic Cl^- secretion was unaffected by the administration of tetrodotoxin or hexamethonium to the Ussing chamber (Ben Mansour et al 1991). Other inconsistencies in the anti-secretory effect of hexamethonium were recently pointed out by Delbro (1995).

In the current study we also found that chlorisondamine significantly antagonized (by approximately 60%) the secretion caused by PGE_1 or okadaic acid. Such results could indicate that either secretagogue acts both directly on the epithelial cells and indirectly via the enteric nervous system. Previous studies support this view of dual action of PGE_1

Table 2. The effect of phosphate-buffered saline (PBS) or chlorisondamine on net fluid secretion of rat small intestine, in-vivo, induced by prostaglandin E_1, cholera toxin or okadaic acid.

	Net secretion (mg cm^{-1})	
	PBS	Chlorisondamine
Prostaglandin E_1	293 ± 7 (group 5, n = 8)	120 ± 12 (group 6, n = 8)*
Cholera toxin	433 ± 23 (group 7, n = 6)	80 ± 5 (group 8, n = 5)†
Okadaic acid	305 ± 15 (group 9, n = 6)	128 ± 8 (group 10, n = 6)‡

PBS (1 mL kg^{-1}, i.v.; control) or chlorisondamine (5 mg kg^{-1}, i.v.) was administered immediately before challenge of the gut with prostaglandin E_1 (150 μg), cholera toxin (3 μg) or okadaic acid (3 μg) injected intraluminally. Data presented as means ± s.e.m. *$P < 0.001$, significant compared with group 5; †$P < 0.005$, significant compared with group 7; ‡$P < 0.005$, significant compared with group 9.

(Gaginella 1990); the mechanism of the secretory effect of okadaic acid is, however, obscure (Edebo et al 1988). The difficulties of analysing secretagogue action in-vivo are evident from some reports. For instance, Brunsson et al (1987) found that in the anaesthetized rat jejunal secretion induced by intraluminal instillation of either prostaglandin E_2 (PGE_2) or its precursor, arachidonic acid, was abolished (and in fact turned into absorption) by hexamethonium. Surprisingly, the secretion caused by arachidonic acid was unchanged by indomethacin (10 mg kg^{-1}, i.v.). This finding makes it highly unlikely that the secretory effect of arachidonic acid is mediated by the generation of, e.g., prostaglandins. Such a conclusion might, moreover, put in doubt the physiological relevance of the secretory effect induced by luminal challenge with PGE_2. In the same paper Brunsson et al (1987) reported that the intra-arterial administration of PGE_1 close to the jejunal segment under investigation also elicited secretion. It is difficult to explain their observation that hexamethonium was more or less totally ineffective in some experiments whereas in others secretion was markedly inhibited. Such inconsistent anti-secretory action of hexamethonium might not be related to blockade of nicotinic receptors in an enteric, secreto-motor neuronal pathway. Instead, hexamethonium might interfere with another variable which might influence secretion to a lesser or greater extent.

The same conclusion might be drawn from the results of Smedfors et al (1994). Thus, duodenal bicarbonate secretion was monitored in awake, chronically instrumented rats. The secretory response to the systemic administration of vasoactive intestinal peptide was markedly inhibited by hexamethonium. Vasoactive intestinal peptide can elicit secretion by a direct effect on the enterocytes (Cassuto et al 1982; Barrett & Dharmsathaphorn 1990; Cooke & Carey 1990; Mailman 1995), which suggests that hexamethonium interferes with some other factor that interacts with the secretory function of the gut mucosa. Speculatively, such a variable could be haemodynamic. Although vasoactive agents could modulate secretory processes of the gut (Strombeck 1971; Mailman 1995), it appears that ganglionic blockers, despite causing hypotension, might not affect intestinal blood flow (see, e.g., Nylander et al 1993). Moreover, cholera toxin-induced secretion seemingly withstood drastic reductions in mesenteric arterial pressure (Carpenter et al 1969). In our view, however, secretory processes in the gut could be secondarily affected (possibly as a part of homeostatic, compensatory adjustments) by a profound and long-lasting reduction of mean systemic pressure. Such a hypothesis might be supported by findings presented by Coupar (1986). Thus, in anaesthetized rat the jejunal secretory response to intravenous infusion of either VIP or PGE_1 was markedly inhibited or even abolished by intravenous injection of tetrodotoxin, which also caused a reduction of mean arterial pressure to about 32% of control (from approximately 125 to 40 mm Hg). This observation raises the question of whether active transport processes of the gut mucosa might remain unimpaired during circulatory shock. In fact, in a recent study, undertaken with chronically instrumented, unanaesthetized and unrestrained rats active absorption of glucose was found to be far greater than in corresponding investigations of anaesthetized animals. It was suggested that decreases in microvascular blood flow, because of shunting of flow through larger vessels during surgery and anaesthesia, resulted in an hypoxic environment for the mucosa, which in turn led to impairment of active transport mechanisms (Uhing & Kimura 1995). Thus, there is seemingly a need to challenge some established concepts of gut physiology with experimental arrangements that are truly physiological.

To conclude, in this study we demonstrate that the ganglionic blocker chlorisondamine significantly inhibited jejunal secretion in response to luminal challenge with cholera toxin, PGE_1 or okadaic acid. The mechanism of this anti-secretory effect cannot, in our view, be related unequivocally to interference with enteric secreto-motor nerves.

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