The sigma ligand, igmesine, inhibits cholera toxin and *Escherichia coli* enterotoxin induced jejunal secretion in the rat

J L Turvill, P Kasapidis, M J G Farthing

Abstract

***Background*—Cholera toxin, and *Escherichia coli* heat labile (LT) and heat stable (STa) enterotoxins induce small intestinal secretion in part by activating enteric nerves. Igmesine is a novel sigma receptor ligand that inhibits neurally mediated secretion.**

***Aims*—To assess the antisecretory potential of igmesine in cholera toxin, LT, and STa induced water and electrolyte secretion using an in vivo rat model of jejunal perfusion.**

***Methods*—After pretreatment with igmesine, 0.03–10 mg/kg intravenously, jejunal segments of anaesthetised, adult male Wistar rats were incubated with cholera toxin (25 µg), LT (25 µg), or saline. Jejunal perfusion with a plasma electrolyte solution containing a non-absorbable marker was undertaken. In some cases 200 µg/l STa was added to the perfusate. After equilibration, net water and electrolyte movement was determined. In additional experiments rats received igmesine, intravenously or intrajejunally, after exposure to cholera toxin.**

***Results*—Cholera toxin induced net water secretion was inhibited by 1 mg/kg igmesine (median −120 versus −31 µl/min/g, $p<0.001$). LT and STa induced secretion were also inhibited by 1 mg/kg igmesine (−90 versus −56, $p<0.03$; and −76 versus −29, $p<0.01$, respectively). Igmesine reduced established cholera toxin induced secretion.**

***Conclusion*—The sigma ligand, igmesine, inhibits neurally mediated enterotoxigenic secretion. Its ability to inhibit established secretion makes it an agent with therapeutic potential.**

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Keywords: igmesine; cholera toxin; *Escherichia coli* enterotoxin; jejunal secretion

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The diarrhoeal diseases caused by infection with *Vibrio cholerae* and enterotoxigenic *Escherichia coli* are characterised by the production of highly potent enterotoxins.[1] Binding of these enterotoxins to the small intestinal epithelium results in the activation of cyclic nucleotide second messenger systems which initiate metabolic cascades characterised by the opening of apical chloride channels and the onset of small intestinal secretion. Cholera toxin and *E coli* heat labile enterotoxin (LT) stimulate the intracellular synthesis of cyclic AMP, while *E coli* heat stable enterotoxin (STa) increases cyclic GMP.[2–4] All three enterotoxins additionally promote secretion through activation of the enteric nervous system.[5]

Oral rehydration solutions have become the mainstay of therapy for such diseases, which continue to be major causes of morbidity and mortality worldwide. Although antibiotics such as tetracycline and the fluoroquinolones can reduce the duration and severity of these illnesses, there is not yet widely available a truly antisecretory agent that can pharmacologically inhibit enterotoxin induced secretion and so ameliorate the severity of the diarrhoeal illness.[6–8]

In 1976, Martin *et al* attributed one of the three major profiles of opioid associated effects in the dog (canine delirium) to a putative fourth opiate receptor designated sigma.[9] Later, because of its insensitivity to blockade by naloxone and its enantioselectivity, it became clear that the sigma receptor was not a type of opiate receptor. It was declassified and is now considered as a separate receptor class. Sigma receptors are found throughout the central nervous system, but their function remains unclear. It has been proposed that they attenuate the ability of acetylcholine to activate the phosphoinositide second messenger system, possibly by inducing the internalisation of cholinergic receptors.[10] In 1988, Roman *et al* presented evidence for the existence of sigma sites in guinea pig myenteric plexus.[11] Further autoradiographic studies have revealed the existence of a dense distribution of sigma binding sites in the mucosa and submucosa of guinea pig intestine.[12] Then, in 1989 a functional role for these receptors in modulating intestinal motility was suggested. Campbell *et al* showed that longitudinal muscle contraction induced by electrical stimulation of myenteric plexus was blocked by the prototypical sigma ligand, (+)-SKF 10,047.[13] This was accompanied by the inhibition of acetylcholine release. Since this initial observation, Pascaud *et al* and others have also proposed that sigma ligands influence intestinal water and electrolyte transport.[14–16]

Recently the highly selective sigma ligand, igmesine (JO 1784, (+)-cinnamyl-1-phenyl-1-*N*-methyl-*N*-cyclo-propylene) has been

Abbreviations used in this paper: CT, cholera toxin; 5-HT, 5-hydroxytryptamine; LT, *Escherichia coli* heat labile enterotoxin; NPY, neuropeptide Y; PEG, polyethylene glycol 4000; PES, plasma electrolyte solution; STa, *Escherichia coli* heat stable enterotoxin; VIP, vasoactive intestinal polypeptide.

described.[17] It is capable of displacing tritiated (+)-SKF 10,047 from brain sites in mice and is effective when administered parenterally or orally. Furthermore, it seems to have antisecretory properties. Igmesine reverses vasoactive intestinal polypeptide (VIP) induced increases in short circuit current in stripped ileal sheets mounted in Ussing chambers, and in vivo studies in mouse and man have showed that igmesine inhibits prostaglandin induced diarrhoea.[18 19]

As both VIP and prostaglandins have been implicated as secretagogues in the pathophysiology of enterotoxigenic small intestinal water and electrolyte secretion, we determined whether igmesine would inhibit cholera toxin, LT, or STa induced secretion.[20 21] An established in vivo rat model of small intestinal perfusion was used.[22]

Methods

BASIC EXPERIMENTAL DESIGN

Adult male Wistar rats (180–220 g body weight), fasted for 18 hours with free access to water, were anaesthetised with intraperitoneal sodium pentobarbitone (60 mg/kg) and maintained by interval injections (15–30 mg/kg) as necessary throughout the experiment. Body temperature was maintained at 37°C using a heat pad. The abdomen was opened through a midline incision and a 20 cm jejunal segment was created by the insertion of a proximal cannula into the small intestine 5 cm beyond the duodenojejunal flexure and a second cannula 20 cm more distally. Both cannulae were ligated as previously described.[22] The isolated segment was gently flushed with isotonic saline (37°C) and air to clear it of residual contents, the intestine was returned to the abdominal cavity, and the abdomen closed.

The jejunal segment was then perfused at a rate of 0.5 ml/min with plasma electrolyte solution (PES) containing Na 140 mmol/l, K 4 mmol/l, Cl 104 mmol/l, and HCO_3 40 mmol/l to which 5 g/l polyethylene glycol 4000 (PEG) and 4 μCi/l of ^{14}C-PEG had been added. Thirty minutes were allowed to elapse to ensure establishment of a steady state after which three consecutive 10 minute collections of the effluent were obtained from the distal cannula. The samples were stored at −50°C for up to 48 hours prior to analysis of ^{14}C-PEG and electrolyte concentration. At the end of the experiment rats were killed by an overdose of pentobarbitone; the perfused segment was removed, rinsed, blotted, and desiccated in an oven at 100°C for 18 hours to obtain the dry weight.

BASAL STATE EXPERIMENTS

Fifteen minutes before jejunal perfusion was undertaken, rats were intravenously pretreated with 10 mg/kg igmesine, dissolved in 0.25 ml saline, or saline alone.

CHOLERA TOXIN EXPERIMENTS

Three groups of experiments were carried out:

Intravenous administration of igmesine before cholera toxin exposure—Rats were intravenously pretreated with 0.03, 0.1, 0.3, 1, 3 or 10 mg/kg igmesine or saline alone. Fifteen minutes later 25 μg cholera toxin in 2 ml isotonic saline was instilled into the isolated segment and both cannulae were clamped. After two hours incubation, the clamps were removed and the jejunal perfusion commenced as described above.

Intravenous administration of igmesine after cholera toxin exposure—Rats were incubated with 25 μg cholera toxin as described above. After an initial 30 minute period of perfusion to confirm the onset of secretion, rats intravenously received 0.3 or 1 mg/kg igmesine in 0.25 ml saline or saline alone. The perfusion was then extended for a further 30 minutes.

Luminal administration of igmesine after cholera toxin exposure—Rats were incubated with 25 μg cholera toxin as described above. Igmesine 40 μg/ml was added to the plasma electrolyte perfusion fluid and was present throughout the experiment.

LT EXPERIMENTS

Isolated jejunal segments, prepared as described above, were intravenously pretreated with 1 mg/kg igmesine or saline alone 15 minutes prior to incubation with 25 μg LT in 2 ml saline. After two hours in situ jejunal perfusion was undertaken to determine net water and electrolyte movement.

STa EXPERIMENTS

Isolated jejunal segments were prepared as described above. Rats were intravenously pretreated with 1 mg/kg igmesine or saline. Fifteen minutes later in situ small intestinal perfusion was undertaken using PES into which 200 μg/l STa (equivalent to 50 000 mouse units) had been added. Net STa induced water and electrolyte movement was determined.

MATERIALS

Cholera toxin was obtained from the Swiss Serum and Vaccine Institute, Berne, and LT and STa from Sigma Chemical Company. Radiolabelled polyethylene glycol (^{14}C-PEG 4000) was obtained from Amersham International. Igmesine was kindly supplied by Institut de Recherche Jouveinal, Fresnes, France and all other chemicals were supplied by British Drug House.

ANALYTICAL METHODS

^{14}C-PEG concentrations in the effluent were measured in triplicate by liquid scintillation spectroscopy in an LKB Wallac Ultra-beta 1210 scintillation counter. Mean net fluid movement was calculated from three consecutive effluent samples and expressed as μl/min/g dry intestinal weight. Positive values denote net absorption and negative values net secretion. Chloride concentration was determined by a Corning 925 chloride analyser, and sodium and potassium by flame photometry (Instrumentation Laboratories 943). Net solute movement is expressed as μmol/min/g.

Steady state condition was confirmed by less than 5% variation in net water movement between consecutive 10 minute collections. Values were accepted only if ^{14}C-PEG recovery fell between 95 and 105%.[22 23]

Table 1 Effect of intravenous pretreatment with igmesine on cholera toxin induced jejunal net secretion of electrolytes

Igmesine (mg/kg)	*Net Cl^- flux (μmol/min/g)*	*Net Na^+ flux (μmol/min/g)*	*Net K^+ flux (μmol/min/g)*	*Number*
0.00	−24 (−29 to −17)	−27 (−33 to −18)	−2.0 (−2.0 to −1.4)	29
0.03	−19 (−21 to −17)	−24 (−31 to −17)	−1.7 (−1.7 to −1.5)	5
0.10	−17* (−23 to −11)	−17* (−27 to −13)	−1.5* (−2.0 to −1.2)	19
0.30	−18* (−20 to −15)	−14* (−16 to −13)	−1.5 (−1.7 to −1.4)	9
1.00	−18* (−20 to 12)	−12* (−19 to −10)	−1.4* (−1.6 to −1.1)	10
3.00	−16* (−16 to −14)	−13* (−17 to −8)	−0.9* (−1.0 to −0.6)	8
10.00	−11* (−13 to −9)	−16* (−17 to −16)	−1.3* (−1.5 to −1.0)	8

Data expressed as median (interquartile range).
*p<0.05 compared with cholera toxin (Kruskal-Wallis test).

STATISTICAL ANALYSIS

Results are expressed as median and interquartile range in each group of animals studied. Differences in net fluid and electrolyte movement were examined using the Mann-Whitney test or a non-parametric analysis of variance (Kruskal-Wallis test with intergroup analysis) whenever appropriate. Cuzick's test for trend was applied to appropriate data sets.

When assessing the efficacy of igmesine or placebo in established secretion, data are expressed as percentage change in net fluid movement between the first and second periods of collection. This corrects for any baseline differences between groups. Positive percentages reflect reversal of secretion.

Results

BASAL STATE EXPERIMENTS

Basal net jejunal water movement (192.5 μl/min/g (149.5–237.8), n=14) was unaffected by igmesine (172.9 μl/min/g (132.2–239.9), n=6). Net jejunal electrolyte movement paralleled net jejunal water movement (control: Cl^-, 1.1 μmol/min/g (−2.2 to 2.3); Na^+, 16.5 (12.3 to 18.5); K^+, 0.1 (0.0 to 0.1); igmesine: Cl^-, 0.8 (−2.5 to 1.1); Na^+, 17.7 (13.6 to 19.0); K^+, 0.1 (−0.1 to 0.1)).

CHOLERA TOXIN EXPERIMENTS

Intravenous administration of igmesine before cholera toxin exposure

Cholera toxin produced net water secretion in all rats. Igmesine had an antisecretory effect at doses of 0.1, 0.3, 1, 3 and 10 mg/kg (p=0.007; fig 1). Although Cuzick's test for trend was positive (p<0.0001), intergroup analysis revealed no significant difference in antisecretory effect above a dose of 0.1 mg (p>0.08). Net electrolyte movement was comparable with net water movement (table 1).

Intravenous administration of igmesine after exposure to cholera toxin

In keeping with a steady state, jejunal net water secretion did not change during the 30 minute collection periods pre- and post-saline (median reduction in secretion 17.6% (interquartile range −2.8% to 38.1%), n=32; p>0.05, Mann-Whitney). An intravenous bolus of 0.3 mg/kg igmesine similarly failed to modify the steady state (20.9% reduction (−8.8% to 73.3%), n=10). By contrast however, 1.0 mg/kg igmesine significantly reduced established cholera toxin induced jejunal net water secretion (42.4% reduction in secretion (23.7% to 67.4%), n=12; p=0.04; fig 2).

Luminal administration of igmesine after exposure to cholera toxin

A highly significant reduction in net water secretion (57.4% (41.6% to 66.7%), n=8; p=0.002) occurred after the intrajejunal delivery of 6.0 mg/kg igmesine via the perfusate (fig 3).

LT AND STa EXPERIMENTS

Both LT and STa induced jejunal net water and electrolyte secretion in all rats. Pretreatment with 1 mg/kg igmesine significantly inhibited both LT and STa induced jejunal net secretion of water and electrolytes (fig 4, table 2).

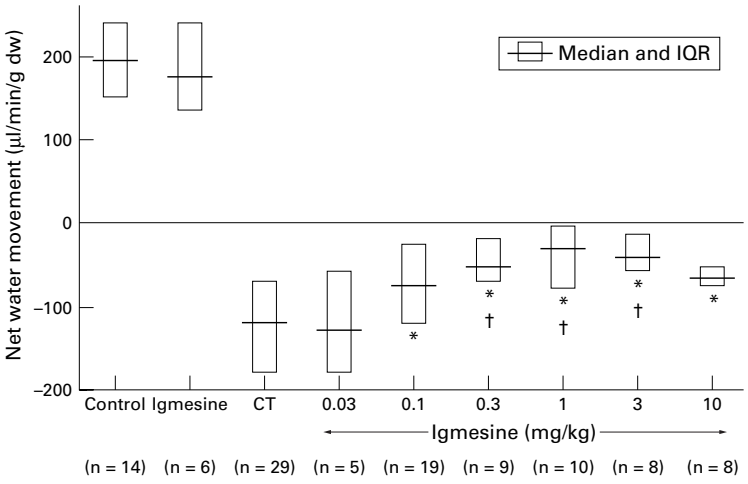

*Figure 1 Effect of pretreatment with igmesine on cholera toxin (CT) induced jejunal net water secretion. Data expressed as median and interquartile range. *p<0.04 compared with CT; †p<0.02 compared with CT + 0.03 mg/kg igmesine (Kruskal-Wallis).*

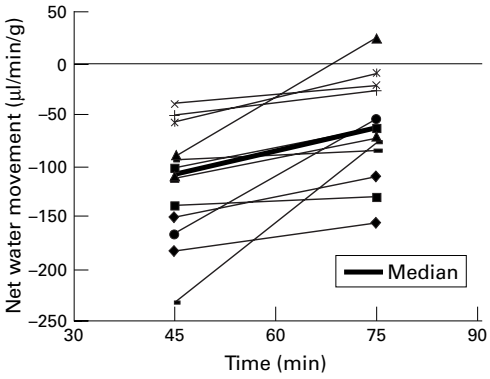

Figure 2 Effect of igmesine (1 mg/kg intravenously) on established cholera toxin induced jejunal net water secretion. Net water secretion in individual rats (n=12) is represented at 45 minutes (pre-igmesine) and at 75 minutes (post-igmesine). p<0.04 compared with cholera toxin alone (Mann-Whitney test).

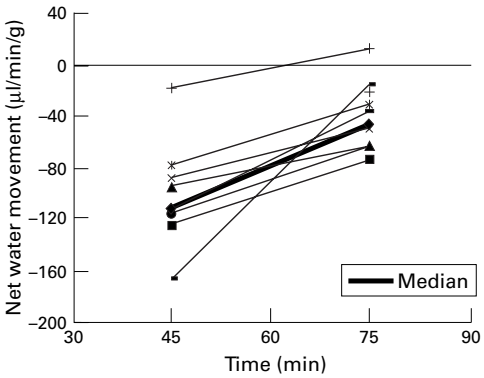

Figure 3 Effect of the addition of igmesine (40 µg/ml) to the perfusate, on established cholera toxin induced jejunal net water secretion. Net water secretion in individual rats (n=8) is represented on equilibration at 45 minutes and after receiving 6 mg/kg igmesine, intrajejunally, at 75 minutes. p<0.002 compared with cholera toxin perfused with standard plasma electrolyte solution (Mann-Whitney test).

Discussion

The high affinity sigma ligand, igmesine, dose dependently inhibits cholera toxin induced jejunal net water and electrolyte secretion and reduces established cholera toxin induced secretion when given either as an intravenous bolus or intraluminally, in the perfusate. The maximal antisecretory dose was 1 mg/kg, given intravenously. Igmesine also inhibits LT and STa induced secretion at that dose, but does not have any effect on basal water and electrolyte transport. These findings are in keeping with those of Carlisi *et al*,[24] who showed the antisecretory effect of sigma ligands, and of Rao and colleagues[18] and Roze and colleagues[19] who found that igmesine inhibits both VIP induced secretion in vitro and prostaglandin induced secretion in humans, respectively. Riviere *et al* have also found that igmesine is proabsorptive in unstimulated mouse jejunum, in vitro.[16]

In a study of rat and mouse brain sigma sites, igmesine was compared with 1,3-di-*o*-tolyguanidine and haloperidol, the two compounds previously found to have a high affinity for the sigma site. Igmesine was more potent than 1,3-di-*o*-tolyguanidine and similar in affinity to haloperidol at inhibiting (+)-SKF 10,047 binding to sigma sites. Igmesine has no significant affinity for a range of opioid, muscarinic, adrenergic, dopaminergic, serotoninergic, histaminergic, and GABAergic binding sites.[17] It displaces tritiated (+)-SKF 10,047 from sigma sites in rat and mouse brain with ID_{50} values in the mouse of 1.2 mg/kg after intraperitoneal administration and 3.5 mg/kg when given orally. The displacement by igmesine is long lasting, with at least 60% inhibition of (+)-SKF 10,047 binding at four hours. The ID_{50} values are comparable with the intravenous and intraluminal doses of igmesine required in our study to inhibit enterotoxin induced secretion.

Cholera toxin, LT, and STa are thought to induce small intestinal secretion by different mechanisms of action. Cholera toxin and LT share a remarkable structural homology. Both are composed of a pentameric binding subunit and an enzymic subunit. The former attaches to an enterocyte ganglioside (GM_1) receptor which permits the latter to activate irreversibly a stimulatory G protein on the basolateral membrane of the enterocyte.[1 25] This results in the activation of adenylate cyclase and an increase in intracellular concentrations of cyclic AMP.[2 3] Cyclic AMP is thought to initiate an intracellular signalling cascade resulting in phosphorylation of apical anion channels and efflux of chloride ions.[26 27] Differences between the two toxins have been recognised however.[28–30] As well as the GM_1 receptor, LT binds to a glycoprotein receptor which also activates adenylate cyclase.[31] In addition, we have recently identified differences between the two in the recruitment of the secretagogue and neurotransmitter, 5-hydroxytryptamine (5-HT). Cholera toxin is known to induce the release of 5-HT from enterochromaffin cells, stimulating secretion via adjacent mucosal $5\text{-}HT_2$ receptors and $5\text{-}HT_3$ receptors located on sensory dendrites.[32 33] By contrast, LT does not induce 5-HT release and is not inhibitable by $5\text{-}HT_2$ or $5\text{-}HT_3$ antagonists.[34 35] STa is a smaller toxin, which binds to and activates two membrane bound cyclic GMP dependent kinases on the cell surface. It initiates a secretory signalling cascade which is much more rapid in onset than cholera toxin and LT and is characterised by a rise in intracellular concentrations of cyclic GMP.[4]

Despite these differences, the antisecretory efficacy of igmesine implies a common pathway for these enterotoxins, on which the sigma ligand acts. This pathway is likely to involve components of the enteric nervous system. The role

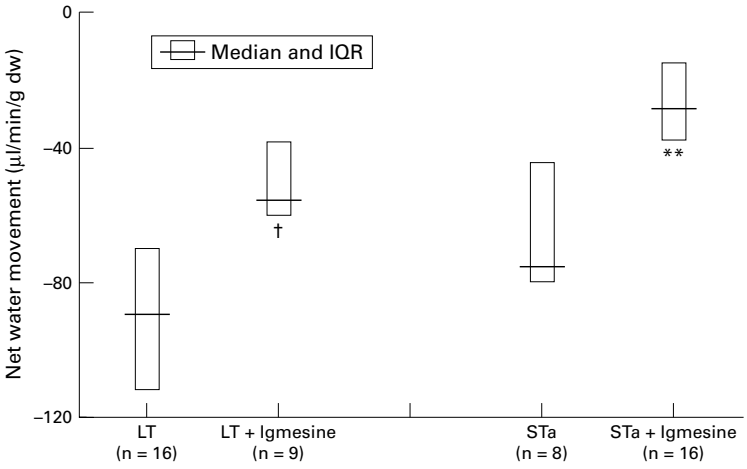

*Figure 4 Effect of pretreatment with igmesine (1 mg/kg intravenously) on heat labile (LT) and heat stable (STa) enterotoxin induced jejunal net water secretion. Data expressed as median and interquartile range. †p<0.03 compared with LT; **p<0.01 compared with STa (Mann-Whitney test).*

Table 2 Effect of igmesine (1 mg/kg intravenously) on Escherichia coli heat labile (LT) and heat stable (STa) enterotoxin induced jejunal net secretion of electrolytes

	Net Cl^- flux (µmol/min/g)	*Net Na^+ flux (µmol/min/g)*	*Net K^+ flux (µmol/min/g)*	*Number*
LT	−19 (−28 to −17)	−20 (−24 to −18)	−1.1 (−1.4 to −0.8)	16
LT + igmesine	−15* (−18 to −13)	−10* (−13 to −5)	−0.6* (−0.8 to −0.5)	9
STa	−11 (−14 to −10)	−18 (−20 to −15)	−1.3 (−1.5 to −1.1)	8
STa + igmesine	−5.3† (−6 to −5)	−7.6† (−9 to −6)	−0.6† (−0.6 to −0.6)	5

Data expressed as median (interquartile range).
*p<0.02 compared with LT, †p<0.02 compared with STa (Mann-Whitney test).

of the enteric nervous system in mediating more than 50% of cholera toxin and STa induced secretion was established by Lundgren *et al* who showed the inhibition of secretion by tetrodotoxin, lidocaine, and hexamethonium.[5] LT has been studied in considerably less depth but we have confirmed that LT induced secretion is also inhibitable by neuronal blockade.[35] It has been proposed that enterotoxins activate a neuronal secretory reflex composed of at least three enteric neurones. The first, a sensory neurone, has dendrites extending from the mucosa and relays information to an interneurone in the submucosal and myenteric plexuses. The interneurone then projects to a secretomotor efferent. The interneurone permits additional enteric and extrinsic neuronal inputs to modulate the reflex arc. Acetylcholine and VIP have been implicated as the secretory neurotransmitters of the secretomotor efferent for both cholera toxin and STa induced secretion.[5] As sigma ligands inhibit VIP induced secretion and attenuate the actions of acetylcholine, modulation of this limb of the neuronal arc may represent the common pathway by which igmesine acts. Evidence for this comes, firstly, from the fact that sigma receptors are located in the myenteric plexus, the mucosa, and the submucosa of guinea pig small intestine.[11 12] Secondly, the proabsorptive effects of sigma ligands are neurally mediated. Riviere *et al* have showed that sigma ligands, including igmesine, induce a sustained decrease in short circuit current in mouse jejunum, in vitro, without effecting tissue conductance.[16] This is prevented by the neural toxin, tetrodotoxin and the ganglionic blocker, chlorisondamine, implying the involvement of a preganglionic neurone in the sigma ligand effect.

The identification of the inhibitory neurotransmitter, neuropeptide Y (NPY), in efferent neurones further supports the suggestion of a common secretomotor pathway for cholera toxin, LT, and STa. NPY exhibits similar antisecretory properties to sigma ligands.[16] It is a potent inhibitor of VIP induced secretion and, like igmesine, inhibits both cholera toxin and STa induced secretion.[36 37] An identical pharmacological profile for NPY and sigma ligand stimulated duodenal alkaline secretion in the rat has been described. As a result it has been proposed that the endogenous peptides, NPY and PYY act through neuronal sigma binding sites.[14]

While the endogenous ligand for the sigma receptor has yet to be identified, Roman *et al* have reported that NPY and the related peptide YY have a high affinity for rat brain sigma receptors and that the distribution of NPY corresponds well with the distribution of sigma receptors.[38] Tam *et al* have been unable to confirm this observation however.[39] Furthermore, when Rao *et al* assessed the efficacy of igmesine and NPY in reversing VIP induced mouse ileal ion transport it was found that the addition of VIP antagonists significantly reduced the effects of VIP and igmesine but not of NPY.[18] It was concluded that although sigma receptors suppress the action of released VIP, igmesine and NPY acted on different receptors. Thus, it now seems untenable that NPY and peptide YY act directly by binding to sigma sites. However, the situation in vivo is such that sigma agonists and NPY/peptide YY display very similar effects. They seem to use some common neural pathway in such a way that sigma sites, possibly through an endogenous ligand, are permissive to the NPY/peptide YY effect. A similar model already exists as α_2 antagonists block the peptide YY mediated inhibition of the effect of VIP in rat jejunum.[40]

In conclusion, the high affinity sigma ligand, igmesine, is an effective antisecretory agent against both cholera toxin and the two enterotoxins responsible for the majority of cases of travellers' diarrhoea. Igmesine probably acts via a secretory pathway common to all three enterotoxins, which is likely to be neuronal, involving NPY related mechanisms. The demonstration by Roze *et al* that igmesine strongly inhibits prostaglandin E_2 induced secretion in human volunteers confirms the tolerability and efficacy of this agent in a clinical setting.[19] The broad antisecretory effect of igmesine and its ability to reduce established secretion by over 50% make it a drug with the promise of therapeutic potential.

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