Review

Secretin, 100 years later

WILLIAM Y. CHEY and TA-MIN CHANG
Rochester Institute for Digestive Diseases and Sciences, 222 Alexander Street, Suite 3100, Rochester, NY 14607, USA

One hundred years have elapsed since the discovery of secretin by Bayliss and Starling in 1902. In the past century, the research on secretin has gone by many milestones including isolation, purification and structural determination, chemical synthesis, establishment of its hormonal status by radioimmunoassay and immuno- neutralization, identification of the specific receptor, cloning of secretin and its receptor, and identification of a secretin-releasing peptide. It has become clear that secretin is a hormone-regulating pancreatic exocrine secretion of fluid and bicarbonate, gastric acid secretion, and gastric motility. The release and actions of secretin are regulated by hormone-hormonal and neurotransmitter interactions. The vagus nerve, particularly its afferent pathway, plays an essential role in the physiological actions of secretin. Substantial information about the property of the secretin receptor has been accumulated, but a potent secretin receptor-specific antagonist remains to be formulated. The neural regulatory mechanisms of the release and action of secretin await further elucidation. The physiological role of secretin in intestinal secretions and motility and extragastrointestinal organs remains to be defined. The presence of secretin and its receptor in the central nervous system is well documented, but its function as a neuropeptide has been recognized gradually and requires extensive study in the future.

Key words: secretin, discovery, first gut hormone, secretin-releasing peptides

At the end of the nineteenth century, Pavlov observed in the dog that pancreatic exocrine secretion is controlled by a dual mechanism, one part by the vagus nerve and the other by a stimulus originating from the

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Correspondence to: W.Y. Chey

Minutes of secretin research

The research on secretin has gone through many major events of isolation, purification, structural determination, and verification. Establishment of its hormonal status, molecular cloning of secretin and its receptor, and mechanism of its release and action that can be considered milestones for secretin research (Fig. 1).

It took more than 60 years after its discovery before porcine secretin was purified by Jorgus and Mutt and its amino acid sequence was determined. Soon after the
sequence of porcine secretin was determined, it was chemically synthesized and its bioactivity confirmed by Bodansky and coworkers.13 The next major obstacle for secretin research was to establish its hormonal status in late 1970s. Using Bodansky’s synthetic secretin, we had successfully raised a high-titer and specific rabbit antiserum to develop a specific high sensitivity radioimmunoassay method for secretin, and we used it to demonstrate that plasma secretin level is elevated in the dog and human upon duodenal administration of dicyclic acid and, more importantly, after ingestion of a meal.14 Schaffalitzky de Muckadell and Fahrenkrug15 reported similar results at the same time.16 Subsequently, immunonneutralization studies in dogs16 definitively proved that secretin is a hormone that drives pancreatic secretion of fluid and bicarbonate. In that study, we showed that antiserum to secretin nearly abolished the postprandial pancreatic secretion (Fig 2). The next major event was the demonstration of secretin receptor in the pancreas by Gardner and Jensen17 that appeared to a prerequisite for its action in the pancreas. In the early 1990s, three major accomplishments were made, namely, the discovery of secretin-releasing peptide,18 cloning of the secretin gene,19 and cloning of the secretin receptor.20 Today, the research in secretin has become even more diversified, particularly with questions regarding its physiological roles in other organs, neurohormonal regulation of its release and action, and its function as a neurotransmitter, which remain to be elucidated.

Structure of secretin

Secretin has been isolated in several animal species including humans, pigs, dogs, rats, mouse, goats, rabbits, guinea pigs, and chickens. Aside from avian secretin, mammalian secretins (Fig 3) are highly homologous, with 1–3 amino acid residues differing from the sequence of porcine/bovine secretin (which are identical). The structure of secretin also has sequence homology with other subsequently isolated regulatory peptides forming a secretin/glucagon/cholecystokinin intes-
final polypeptide (VIP) superfamily of more than 10 peptides (Table 1). In addition, Mutt and coworkers have isolated several forms of prossecretins with either C- or N-terminal extensions that exhibited various extents of bioactivity. The structures of some of the prossecretins agree well with those deduced from the nucleotide sequence of secretin cDNA cloned by Kopin et al.13 To date, secretin mRNA has been cloned from the rat, mouse, pig, and human.15

Distribution of secretin
Secretin-containing cells are distributed mainly in the upper small intestine (duodenum and jejunum).16 However, secretin has also been shown, either through immunohistochemical or molecular biological methods, to exist in other organs including heart, lung, kidney, ileum, colon, stomach, and brain of various species. For example, secretin cells that are found in the atrial and oxyntic mucosa of rat stomach are distinguished from gastrin and somatostatin cells, respectively. Moreover, secretin mRNA is in the same molecular size as that found in the duodenum is found in both gastric mucosa either by reverse transcriptase-polymerase chain reaction (RT-PCR) or Southern blot after RT-PCR.

The mechanism of secretin release
Secretin is released mainly by gastric acid delivered into the duodenal lumen. In addition, secretin is also released by digested products of fat and protein, bile acid, and herbal extracts.17 The stimulants of secretin release are listed in Table 2. The importance of gastric acid for postprandial release of secretin is demonstrated by the observation that suppression of gastric acid secretion with a histamine H₂ blocker, cimetidine, resulted in a complete suppression of secretin release after ingestion of a meal in dogs (Fig. 4).18 Like cholecystokinin, the release of secretin along with pancreatic exocrine secretion is controlled through a feedback regulatory mechanism, as first proposed by Green and Lyman,19 that is mediated by pancreatic proteases. Thus, diversion of pancreatic juice from the duodenum in dogs augmented postprandial pancreatic secretion and the release of secretin that was suppressed by duodenal infusion of pancreatic juice or trepina but not by infusion of bicarbonate.20 This feedback effect was shown to involve suppression of secretin release in both fasting and postprandial states in both humans and rats.21 In anesthetized rats, diversion of pancreatic juice from the duodenum results in a time-dependent increase in secretin release and pancreatic secretion. The increased secretin release and pancreatic exocrine secretion was suppressed by duodenal infusion of either freshly collected or precollected pancreatic juice.21 As presented, the effect of proteases or pancreatic juice is believed to result from degradation of a luminal secretin-releasing peptide (SRP).

Table 1. Peptides of secretin/glicentin/vasoactive intestinal polypeptide superfamily

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Discovered or isolated by</th>
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<tbody>
<tr>
<td>Secretin</td>
<td>Baylin and Starling 1962</td>
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<tr>
<td>GIP</td>
<td>Kimball and Mattin 1925</td>
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<tr>
<td>VIP</td>
<td>Brown et al. 1970</td>
</tr>
<tr>
<td>Oxytocin (prologagnist)</td>
<td>Wood et al. 1976</td>
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<tr>
<td>Oxytomodulin (glucagon-37)</td>
<td>Bataille et al. 1987</td>
</tr>
<tr>
<td>PHI (PHM)</td>
<td>Tassone and Mun 1981</td>
</tr>
<tr>
<td>GLP-1, GLP-2</td>
<td>(Itoh et al. 1985)</td>
</tr>
<tr>
<td>GRP</td>
<td>Lund et al. 1982</td>
</tr>
<tr>
<td>PACAP</td>
<td>Guillemot et al. 1982</td>
</tr>
<tr>
<td>Hypocretin (orexin)</td>
<td>De Lecea et al. 1998</td>
</tr>
<tr>
<td>H-10dermin</td>
<td>Hidaka et al. 1984</td>
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<tr>
<td>Helospermin</td>
<td>Parker et al. 1984</td>
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<tr>
<td>Extin-3, -4</td>
<td>Eng et al. 1990, 1992</td>
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Table 2. Stimulants of secretin release

<table>
<thead>
<tr>
<th>Exocrine secretions</th>
<th>Digestive food</th>
<th>Herbal extracts</th>
<th>Chemicals</th>
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<tbody>
<tr>
<td>Gastric acid</td>
<td>Long-chain fatty acids</td>
<td>Licorice extract</td>
<td>Camomile</td>
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<tr>
<td>Bile salts</td>
<td>Sodium oleate</td>
<td>1-Pyrroline</td>
<td>Terpenoids</td>
</tr>
<tr>
<td>Pancreatic juice (SRP)</td>
<td>Peptone</td>
<td>2-Pyrroline</td>
<td>Plasminogen</td>
</tr>
<tr>
<td>Intestinal secretion (SRP)</td>
<td>Oligopeptides</td>
<td>4-Pyrroline</td>
<td>MCT-727</td>
</tr>
</tbody>
</table>

*Terpenoids (geranyl geranyl acetone); plasminogen, and MCT-727 are utilized agents used in Japan.

Both pancreatic juice and intestinal secretin contain a secretin-releasing peptide (SRP) to stimulate secretin release.
Discovery of secretin-releasing peptide

Li et al.\(^{12}\) reported that the acid-stimulated release of secretin is mediated by a SRP. SRP was discovered in the intestinal acid perfusate collected from donor rats. The rat was anesthetized, a duodenal cannula was inserted through the stomach, and the pylorus was ligated. Diluted HCl or saline was then infused and the intestinal perfusate was collected from a distal cannula placed in the jejunum. After neutralization, the concentrated acid perfusate (CAP) and saline perfusate (CSP) were prepared and administered intraduodenally to recipient rats and their effect on pancreatic secretion and secretin release were compared. As shown in Fig. 5,\(^{12}\) CAP caused an increase in pancreatic bicarbonate output whereas CSP did not. Plasma secretin level increased to 6.5 PM after infusion of CAP, whereas no increase was found with CSP, indicating the presence of an SRP in CAP. The bioactivity in CAP was inactivated by trypsin but not by boiling, indicating that it is a heat-stable peptide. The result of an ultrafiltration study indicated that SRP in CAP had an apparent MW less than 10\,000. It was therefore concluded that an SRP mediates the release of secretin and that the activity of SRP was subject to feedback regulation by pancreatic proteases.\(^{12}\)

Canine pancreatic juice also contains a secretin-releasing factor because the concentrate of pancreatic juice stimulated secretin release and pancreatic bicarbonate secretion when it was infused into the duodenum of both dogs and rats.\(^{12,22}\) An active fraction of canine pancreatic juice with an apparent MW < 4,000 (Fr. 3) was found to stimulate pancreatic juice volume and bicarbonate outputs as well as elevation of plasma secretin concentration in recipient rats. These effects of the fraction were abolished by i.v. infusion of a specific antiserum.\(^{23}\) Thus, a factor in pancreatic juice that mediates a positive feedback regulation for secretin release and pancreatic secretion was found. Subsequently, two SRPs of 14 kDa, SRP-1 and SRP-2, were purified from canine pancreatic juice.\(^{24}\) The N-terminal sequiMotor of SRP-1 was identical to canine pancreatic phospholipase A\(_2\) (PL\(_{A2}\)), whereas SRP-2 had 71% homology with the enzyme. Both canine SRPs\(^{25}\) and porcine pancreatic PL\(_{A2}\) stimulated secretin release from secretin-producing cells in vitro through activation of calcium influx and protein kinase C.\(^{26}\) In addition, acid in the duodenum released pancreatic PL\(_{A2}\)-like immunoreactivity. Moreover, pretreatment of CAP with a specific anti-PL\(_{A2}\) serum abolished its stimulatory effects on secretin release and pancreatic exocrine secretion (the SRP activity) in recipient rats (Fig. 6).\(^{27}\) These observations suggested that PL\(_{A2}\) acts as an SRP. It should be noted, however, that intraduodenal infusion of purified porcine pancreatic PL\(_{A2}\) is unable to elicit secretin release in rats, suggesting that an additional factor is required for its action. The unidentified factor may either function as a co-stimulating factor for S-cells or provide transport of PL\(_{A2}\) through the mucous layer.

Physiological actions of secretin

Physiologic actions of secretin include stimulation of pancreatic exocrine secretion of water and bicarbonate and inhibition of gastric acid secretion and emptying. As shown in Fig. 7, i.v. administration of an antisecretin serum augmented postprandial gastric release and acid output in dogs\(^{28}\) suggesting that secretin is an enter-
Pancreatic exocrine secretion after a meal is mainly stimulated by sympathetic interaction between secretin and cholecystokinin (CCK), so that an adequate amount of pancreatic juice is produced for proper digestion. For example, pancreatic secretions of bicarbonate and protein in dogs stimulated by a combination of secretin and CCK-8 in physiological doses are abolished by the CCK antagonist proglumide. Secretin also potentiates with neurexinin to stimulate pancreatic enzyme secretion. Another example of hormone-hormonal interaction is that insulin plays a permissive role on the action of secretin and CCK. When the circulating insulin was neutralized with a specific antiserum in rats, there was a profound inhibition of pancreatic secretion stimulated with secretin in combination with CCK (Fig. 8) or a meal, in both isolated and perfused rat stomach and the intact animals, it has been clearly shown that inhibition of gastric acid secretion by secretin is mediated by somatostatin and prostaglandin.

Except in the rat, the action of secretin on exocrine pancreas is a physiological dose is highly sensitive to atropine, indicating an important mediation by cholinergic neurons. For example, pancreatic bicarbonate secretion in humans stimulated by secretin in graded doses (Fig. 9), and its potentiation with CCK was profoundly inhibited by administration of atropine.

The physiological action of secretin is highly dependent on the vagalafferent pathway. Thus, chemical ablation of vagal afferent fibers by perivagal application of capsazepine in rats resulted in a profound inhibition of the pancreatic secretion stimulated by a physiological, but not by a pharmacological, dose of secretin (Fig. 10). The release and actions of SRP are also neurally mediated and depend on the vagal afferent pathway. Thus, CAP prepared from donor rats treated with tetraethylammonium chloride or capsazepine was unable to stimulate pancreatic exocrine secretion or release of secretin in recipient rats, indicating substantial reduction in SRP activity. In addition, CAP prepared from untreated donor rats was also unable to stimulate secretin release.
release from the recipient rats pretreated with tetrodotoxin (TTX), vagotomy, or perivagal application of capsaicin (Fig. 11). Similarly, perivagal capsaicin treatment and vagotomy in conscious rats blocked the inhibition of pentagastrin-stimulated gastric acid secretion by secretin. Lu and Ouyang also demonstrated that the vagal afferent pathway mediates inhibition of gastric motility by a physiological dose of secretin, confirming a previous observation made by Raybould and Holzer. Electrical stimulation of medial amygdaloid in the rat augmented pancreatic bicarbonate and fluid secretion in response to duodenal acidification and a low dose of secretin. This effect of medial amygdaloid stimulation was abolished by bilateral truncal vagotomy, suggesting that stimulation of medial amygdaloid elicited a stimulatory signal transmitted through the vagal nerve to potentiate the action of secretin. We have also observed in rats that vagotomy, vagal ligation, or perivagal colchicine, but not perivagal capsaicin treatment, decreased the number of high-affinity secretin-binding sites in rat fundus and reduced secretin-elicted relaxations of rat fundus muscle strips. This observation suggested that the vagal efferent pathway also regulates secretin action through modulation of secretin receptor in the rat fundus muscle.

Some neuropeptides and neurotransmitters may modulate or mediate the release and action of secretin. Both Met-enkephalin (MkE) and somatostatin inhibit the release and action of secretin on the celiac nerve. Recently, we have observed that MEK also inhibited the release of SRF and its action on secretin release through the release of somatostatin. Thus, as shown in

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**Fig. 8.** Effect of an antisecretin serum on normal rabbit serum (NRS) on pancreatic exocrine secretion stimulated by a combination of physiological doses of secretin and CCK-8 in rats. The antisecretin serum abolished stimulation of pancreatic secretion by secretin and CCK-8. (From Lee et al., 15 with permission)

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**Fig. 9.** Inhibition of secretin-stimulated pancreatic secretion by atropine in humans. Atropine profoundly inhibited pancreatic secretion stimulated by secretin at various doses. (From You et al., 14 with permission.) **P < 0.05, 0.01; secretin alone vs secretin + atropine

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Fig. 10. Effect of perivagal treatment with capsaicin on secretin-stimulated pancreatic secretion in rats. Perivagal treatment with capsaicin significantly inhibited PES stimulated by secretin in physiological doses (2.5 and 5μmol/kg/h) but not at a pharmacological dose (10pμmol/kg/h). (Li et al., unpublished results; confirming data shown in reference 22.)

*P < 0.05 vs vehicle (n = 6 in each group)

Fig. 11. Effect of various neural blockade, vagotomy, or perivagal capsaicin treatment in recipient rats on CAP-stimulated secretin release. CSP, concentration of saline perfusate (from upper small intestine); CAP, concentration of acid perfusate (from upper small intestine); TTX, tetrodotoxin; VT, bilateral vagotomy; CS, perivagal capsaicin; AT, atropine; HX, hexamethonium; Prop, propofol. CAP-stimulated secretin release was blocked by TTX, VT, and CP but not by AT, Hx, or Prop, suggesting that the action of SRP in CAP is mediated by a noncholinergic and nonadrenergic vagal afferent pathway. (From Li et al., with permission.)

*P < 0.05 vs CAP

Fig. 12. CAP collected from donor rats pretreated with MEK had reduced SRP activity partially reversed by cotreatment of the donors with an antisomatostatin serum. Photolytic adenylate cyclase-activating polypeptide (PACAP) stimulated pancreatic exocrine secretion through the release of both secretin and CCK is an anesthetized rat, demonstrated by the inhibition of PACAP-stimulated pancreatic exocrine secretion by iv. infusion of an antiserum against the CCK-A receptor antagonist, loxoribine. PACAP also stimulated the release of secretin in vitro. In conscious rats, secretin-stimulated pancreatic exocrine secretion was inhibited by a NO synthase inhibitor, N-nitro-L-arginine, and the inhibition was reversed by the substrate of the enzyme arginine, suggesting that NO mediates the action of secretin. In anesthetized rats both the serotonin (5-hydroxytryptamine, 5-HT₃) antagonist ketanserin and the 5-HT₃ antagonist ondansetron dose-dependently inhibited pancreatic volume and bicarbonate secretion and secretin release elicited by duodenal acidification. Moreover, both 5-HT antagonist inhibited pancreatic secretion stimulated by physiological doses of secretin. These observations suggest that 5-HT mediates the release and action of secretin through the two 5-HT receptors subtypes. In isolated and perfused rat pancreas, electrical field stimulation enhanced stimulation of pancreatic secretion by secretin. The enhanced secretion was reduced by atropine or a specific anti-GRP serum and abolished by the combination of atropine and the antisera, suggesting that the enhancement of the effect of secretin was mediated by acetylcholine and GRP released from intrapancreatic neurons. The physiological stimulant of these intrapancreatic neurons is unknown at present. In isolated rat pancreatic ducts, secretin-stimulated fluid secretion was potentiated by acetylcholine. An observation also suggesting a possible interaction between the two stimulants.
The secretin receptor

Biochemical studies on the secretin receptor were carried out in the 1960s by the laboratories of Gardner and Jensen, Robberecht and Christophe, and Rossellini. These investigators found that secretin receptor is a glycoprotein receptor coupled to adenylyl cyclase through an oligomeric G protein and is widely distributed in many organs. The rat secretin receptor was first cloned by Tahiliani et al. Subsequently, human and rabbit secretin receptors were also cloned. Human secretin receptor is a 7-transmembrane G protein-coupled receptor with a long N-terminal extracellular tail, three extracellular and three intracellular loops, and a short hydrophilic cytoplasmic C-terminal chain. The extensive studies carried out by L. Miller's and P. Robberecht's groups have indicated that the N-terminal extracellular tail of the receptor is involved in binding secretin. The putative N-glycosylation site at position 72N is also crucial for ligand binding, whereas extracellular loops are also essential, probably for maintaining the active conformation of the receptor's extracellular binding domain. The cytoplasmic C-terminal tail is involved in desensitization of the receptor through phosphorylation by G protein-coupled receptor kinase. Secretin receptor is expressed in the pancreas, stomach, liver, kidney, colon, heart, lung, ovary, and brain of various species. In the pancreas, secretin receptor is present in both the ductal and acinar cells.

Secretin as a neuropeptide

Secretin and its mRNA are detected in the brain. Secretin receptor is also present in the brain as demonstrated by specific binding. Stimulation of cAMP production in brain slices and presence of the receptor transcript in the brain. Recent studies have indicated that secretin indeed may function as a neuropeptide. For example, secretin specifically stimulated adenylate cyclase in hypothalamic and hippocampal slices.[1][2][3][4] Secretin was found to cross the blood-brain barrier and entered every brain region, with fastest uptake found in hypothalamus and hippocampus.[5] Intravenous infusion was found to stimulate activation of c-Fos expression in the central amygdala of rats.[6] In isolated rat superior cervical ganglia, secretin dose-dependently stimulated tyrosine hydroxylase activity, an effect potentiated by carbachol that was abolished by atropins but not by hexamethonium.[7] Moreover, secretin is known to inhibit the release of somatostatin from enriched rat enteric synaptosomes.[8] Intracerebroventricular injection of secretin stimulated pancreatic exocrine secretion in rats.[9] Secretin is also found in the brainstem, including a subpopulation of neurons in the primary sensory ganglia.[10] Secretin was reported to stimulate γ-aminobutyric acid (GABA) transmission from Purkinje cells in rat cerebellar slices.[11]

Clinical aspects of secretin

Diagnosis

Secretin has been widely used for pancreatic function tests for diseases involving the pancreas, particularly chronic pancreatitis. In recent years, secretin has been used to collect pancreatic juice for analysis of molecular biological markers to diagnose pancreatic cancer. Secretin is also used to enhance endoluminal magnetic resonance cholangiopancreatography. A secretin provocation test is useful for detecting gastrinomas in the pancreas or extrapancreatic region, whereas a selective arterial secretin injection was found to be useful for detecting gastrinomas in the duodenal submucosa.[12]

Pathophysiology of secretin

Hyposcretinemia is observed in two pathological states, namely, in patients with achlorhydria and adult celiac sprue. In achlorhydria patients, the content of secretin cells in the intestinal mucosa is normal and hyposcretinemia can be corrected by providing acidic drinks such as orange juice. In adult celiac sprue, mucosal atrophy in upper small intestine leads to loss of secretin cells, and hyposcretinemia can be corrected only after mucosal regeneration with a gluten-free diet. In a recent report, secretin and gastric inhibitory polypeptide coexist in the duodenal bulb were reduced and correlated well with malabsorption in patients with familial amyloid polyneuropathy. Hypersecretinemia is found in patients with Zollinger-Ellison syndrome, duodenal ulcer with hypersecretion of gastrin, and renal failure.[13] In a patient with a secretin-producing endocrine tumor in the pancreas causing hypersecretion of pancreatic juice and watery diarrhea.[14] Secretin-producing cells were also found in the tumor of a patient with esophageal small cell carcinoma.[15] It is possible that secretin-producing endocrine tumors may occur more frequently than one realizes but are overlooked unless causing hypersecretinemia and watery diarrhea.

Therapeutic use of secretin

Secretin has been reported to improve the behavior of autistic children.[16] This effect of secretin has been related by several groups of investigators.[17] We are aware of the observation that secretin upsets[18] and its...
Summary and future prospects

More than 100 years have elapsed since the discovery of serotonin by Baillie and Starling. Substantial accomplishments in serotonin research have been made with respect to purification, structure determination, establishment of hormonal status, cloning of serotonin and its receptor, and neural and hormonal regulation of its release and action during the past 10 decades. Although not mentioned in this article, a considerable amount of knowledge pertaining to the cellular action mechanism of serotonin has also been acquired. It is hoped that future studies will focus on identification of neuronal pathways through which the vagus nerve and pancreatic neurons participate in regulation of release and/or action of serotonin, particularly with respect to the key neurotransmitter(s) and/or neuropeptide(s) involved and their point of action in the neural pathways. It is our hope that the extensive molecular studies in serotonin receptor will lead to formulation of a potent and specific serotonin receptor antagonist to facilitate future study.

The physiological roles of serotonin in enteric-nerve motility and secretion and in the brain and other nongastrointestinal organs remain to be determined. It will be also interesting to determine the physiological roles of various forms of proserotonins.

References


