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## Perspective

# New Perspectives on Gut Peptides

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It is very well established that peptides can act as the circulating blood born messengers known as hormones. In addition, during the last decade, much attention has been focused upon peptides that act as neurotransmitters. Actually, the same peptide can often act both as a traditional hormone or a neurotransmitter relaying information over long or short distances. The gastrointestinal tract is the largest endocrine organ in the body. Peptide-containing endocrine cells are scattered along the gastrointestinal mucosa throughout the gut. Moreover, many of the neurones that constitute the intrinsic and extrinsic innervation of the gut utilize peptides as their neurotransmitters.

When considering the history of peptidergic messengers, the gut has played a central role. The first peptide hormone to be discovered was secretin in 1902 by Bayliss and Starling.[1] Gastrin and pancreozymin/cholecystokinin followed in subsequent decades.[2,3] It should also be noted that in spite of the enormous current interest in peptidergic neurotransmitters, the first member of this group, substance P, was detected in the gut in 1931 by Gaddum and Von Euler.[4] Some 20 peptides that possess endocrine or neurotransmitter functions in the gut have now been described. Several exciting areas of interest are now emerging. These include the following: (a) the identification of families of bioactive peptides, (b) interesting connections in the biosynthetic pathways of many of these peptides, (c) novel methods for the identification and purification of bioactive peptides, and (d) new sites of action for peptides in the gut and identification of multiple subtypes of receptors at which these peptides act. Obviously, it is not possible to cover all aspects of this subject adequately in a short article. However, in what follows, I shall describe some newer discoveries in this area and give examples that will illustrate the points I have alluded to.

## PHI

The discovery of the gut peptide PHI (*P*eptide having N-terminal *H*istidine and C-terminal *I*soleucine amide) is an excellent example of the success of "modern" approaches to peptide pharmacology. Traditionally, bioactive peptides have been isolated on the basis of their biological actions. Thus, back in 1902, secretin was discovered as a substance that stimulated pancreatic bicarbonate and fluid secretion.[1] More recently, enkephalin was discovered because of its opiate like effects.[5,6] A few years ago, however, Tatemoto and Mutt proposed a novel idea.[7] They noted that several peptide hormones had characteristic chemical features such as a C-terminal $\alpha$-amide structure. For example, gastrin, secretin, calcitonin, cholecystokinin, vasopressin, oxytocin, bombesin, vasoactive intestinal polypeptide (VIP), and substance P all possess this feature. They argued that rather than identifying a function and then looking for a peptide that produces it, why not just look for novel peptides with C-terminal $\alpha$-amides. The likelihood would be that such novel peptides would be bioactive. Using this "chemical" approach, Tatemoto and Mutt isolated PHI and a second peptide PYY (vide infra) from porcine intestinal tissue. PHI proved to have 27 amino acids.[8] Its sequence can be seen in Table I.[9] It is quite evident that PHI has many sequence homologies with other gut peptides in the secretin/glucagon family. In particular, the homology is greatest between PHI and VIP. Subsequent investigations have revealed further connections between these two peptides. For example, radioimmunoassay and immunohistochemical investigations in some 40 tissues showed that the two peptides had very closely related distributions.[10] Indeed they seemed to exist in the same cells. When such peptide colocalization has been observed previously it has often indicated that the peptides in question were related biosynthetically. The relationship between corticotropin (ACTH) and $\beta$-endorphin is an example of this phenomenon. This also proved to be the case for PHI and VIP. The DNA coding for human VIP has been cloned and the sequence of the protein precursor to VIP determined.[11]

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**Table I.** Comparison of the Amino Acid Sequence of PHI with Those of Other Members of the Glucagon-Secretin Family[a]

| | |
|---|---|
| PHI | *H A D G Y F T S D* F *S R L L G Q* L S *A K K Y L* E *S L* I* |
| VIP | *H* S *D A V F T* D N Y T *R L* R K *Q* M A V *K K Y L* N *S* I L N* |
| secretin | *H S D G T F T S* E L *S R L* R D S A R L O R L *L Q* G *L* V* |
| glucagon | *H* S Q *G* T *F T S D* Y *S* K Y *L* D S R R *A* Q D F V Q W *L* M N T |
| GIP | Y *A* E *G* T *F I S D* Y S I A M D K I R Q Q D F V N W *L* L A Q K G K K S D W K H N T Y Q |

[a] Identities are underlined. A. alanine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine, K, lycine; L, leucine; M, methionine; N, asparagine; Q, glutamine; R, arginine; S, serine, T, threonine; V, valine; W, tryptophan; Y, tryosine; GIP, gastric inhibitory peptide. (*) Amidated COOH terminus.

This precursor is a protein of 170 amino acids with a molecular weight of about 20 000. Residues 125–152 correspond to VIP. In addition, residues 81–107 are virtually identical with porcine PHI. In fact there are two differences in that the human peptide contains a lysine instead of the arginine residue at position 12 of PHI and the C-terminal residue is a methionine rather than an isoleucine. Thus, the human peptide is designated "PHM" for consistency. Two important general points arise from this discovery. The first is that there are now several examples of gut peptides from the same family that share biosynthetic precursors. PHI and VIP are one example. However, this is also true for the opioid peptides and for substance P/substance K as will be discussed below. In addition, this discovery also illustrates the impact of molecular biological techniques in this area. In every instance where the biological precursor of a gut peptide has been identified, the sequence has been obtained by cloning and sequencing the gene that codes for it rather than by isolating and sequencing the protein per se.

PHI is localized in enteric neurones as is VIP.[12,13] As might be expected, PHI has several actions in the gastrointestinal system and associated tissues. An important question however, relates to the identification of the receptors on which PHI acts. Does PHI act on a unique population of receptors or does it exert its effects at receptors for secretin, VIP, or another member of this peptide family? It appears that PHI exerts its effects mainly at VIP receptors. For example, pancreatic acinar cells possess receptors for both VIP and/or secretin depending on the species in question. Stimulation of these receptors leads to an increase in amylase and fluid secretion. The effects of PHI on amylase secretion examined in various species fit the VIP profile rather than that for secretin.[14-16] It is not clear whether all PHI effects are exerted via VIP receptors or not. One possibility is that VIP receptors will ultimately prove to be heterogeneous as has been shown to be the case with substance P and opiate receptors (vide infra). In such a situation, it could turn out that PHI and VIP favored different receptor subclasses. VIP/(PHI) containing nerves are very well represented in the intestinal mucosa.[17,18] It is well known that mucosal cells in the small intestine possess VIP receptors[19] and that VIP is a powerful stimulator of electrolyte secretion by this tissue.[20] As expected, PHI shares this property of VIP.[21,22] Thus, both PHI and VIP secreted from enteric neurones may have an important physiological role in the control of intestinal electrolyte transport. This may also be important in pathological situations. Certain pancreatic tumors have been found to synthesize and hypersecrete VIP and PHI. This leads to an enormous and abnormal stimulation of electroyte secretion and a state known as the "watery diarrhea syndrome". Here again the case of PHI illustrates an important point. That is, the sensitivity of the intestinal mucosa to bioactive peptides and other agents. It is well known that intestinal smooth muscle is sensitive to many neurotransmitters. Indeed these responses have furnished generations of pharmacologists with many useful in vitro assay systems. The sensitivity of enteric neurones to bioactive peptides has also been evident. When considering the gastrointestinal effects of drugs such as opiates, actions on gut muscles and neurones have been the focus of attention. However, over the last few years, it has become evident that the interstinal mucosa also possesses receptors for virtually all the bioactive substances that act on gut smooth muscle and nerve. Activation of such mucosal receptors regulates electrolyte transport by the mucosal epithelium. This is probably important from the point of view of both normal and pathological gut function. It is known that many pathological diarrhea states result primarily from dysfunctions of mucosal electrolyte transport. Thus, the pharmacology of this process is clearly of great importance for the development and design of new drugs for treating such disorders. The effects of PHI/VIP on the mucosa have been mentioned above. Effects of other peptides will be discussed below. In addition to altering transport in the small intestine, VIP/PHI are also active in the gall bladder where they relax the bladder and inhibit fluid absorption.[23,24]

Thus, the case of PHI illustrates many of the current trends associated with the study of gut bioactive peptides. A combination of pharmacological, chemical, anatomical, immunological, and molecular biological techniques have been applied to the study of this peptide. The information obtained about PHI is in many respects "typical" of that known about many gut bioactive peptides.

## PYY and NPY

Using their procedure of identifying novel amidated peptides, Tatemoto and Mutt isolated a second peptide

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**Table II.** Amino Acid Sequences of NPY and Related Pancreatic Polypeptides[a]

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NPY | Tyr | Pro | Ser | Lys | Pro | Asp | Asn | Pro | Gly | Glu | Asp | Ala | Pro | Ala | Glu | Asp | Leu | Ala |
| PYY | Tyr | Pro | Ala | Lys | Pro | Glu | Ala | Pro | Gly | Glx | Asx | Ala | Ser | Pro | Glx | Glx | Leu | Ser |
| APP | Gly | Pro | Ser | Gln | Pro | Thr | Tyr | Pro | Gly | Asp | Asp | Ala | Pro | Val | Glu | Asp | Leu | Ile |
| HPP | Ala | Pro | Leu | Glu | Pro | Val | Tyr | Pro | Gly | Asp | Asn | Ala | Thr | Pro | Glu | Gln | Met | Ala |
| PPP | Ala | Pro | Leu | Glu | Pro | Val | Tyr | Pro | Gly | Asp | Asn | Ala | Thr | Pro | Glu | Gln | Met | Ala |

| | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NPY | Arg | Tyr | Tyr | Ser | Ala | Leu | Arg | His | Tyr | Ile | Asn | Leu | Ile | Thr | Arg | Gln | Arg | Tyr-$NH_2$ |
| PPY | Arg | Tyr | Tyr | Ala | Ser | Leu | Arg | His | Tyr | Leu | Asn | Leu | Val | Thr | Arg | Gln | Arg | Tyr-$NH_2$ |
| APP | Arg | Phe | Tyr | Asp | Asn | Leu | Gln | Gln | Tyr | Leu | Asn | Val | Val | Thr | Arg | His | Arg | Tyr-$NH_2$ |
| HPP | Gln | Tyr | Ala | Ala | Asp | Leu | Arg | Arg | Tyr | Ile | Asn | Met | Leu | Thr | Arg | Pro | Arg | Tyr-$NH_2$ |
| PPP | Gln | Tyr | Ala | Ala | Glu | Leu | Arg | Arg | Tyr | Ile | Asn | Met | Leu | Thr | Arg | Pro | Arg | Tyr-$NH_2$ |

[a] Abbreviations: NPY = neuropeptide Y, PYY = peptide YY, APP = avian pancreatic polypeptide, HPP = human pancreatic polypeptide, PPP = porcine pancreatic polypeptide. Amino acids common to APP and NPY are underlined.

at the same time that they isolated PHI.[8] This peptide had 36 amino acids and possessed a tyrosine at both N and C termini. It was therefore named PYY.[25] Just as with PHI, it quickly became apparent that PYY fitted nicely into a preexisting family of bioactive peptides, the pancreatic polypeptides (Table II). There is, in fact, an approximately 50% homology between porcine PYY and pancreatic polypeptides from various sources. This group of peptides occurs in endocrine cells in the pancreas and also in the gastrointestinal mucosa.[26] PYY was also found to be localized in gut endocrine cells.[27] These cells were absent from the stomach and duodenum but present in large numbers in the distal small intestine, colon, and rectum. The distribution of PYY-containing cells was clearly different from those containing pancreatic polypeptide.[26] The effects of PYY on the gut have not yet been extensively investigated; however, some actions are already clear or implied. PYY has a vasoconstrictor action[28,29] and inhibits jejunal and colonic motility.[27] It can also inhibit secretin- and cholecystokinin-stimulated pancreatic secretion in the anesthetized cat[25] and has an inhibitory effect on hormone secretion from the endocrine pancreas.[30] It should be noted, however, that in some cases the physiologically significant peptide may be NPY (vide infra) rather than PYY. Ultrastructural examination of PYY-containing mucosal cells shows that they emit cytoplasmic processes that impinge upon neighboring goblet cells. Thus, PYY may also have a local hormonal (paracrine) role in the control of mucous secretion.[26]

There is still another dimension to this particular family of peptides. As many gut bioactive peptides are also found in the central nervous system, it was natural to explore the possibility that pancreatic polypeptide existed in the brain. Early experiments using antisera against avian pancreatic polypeptide (APP) revealed an abundance of immunoreactive neurones.[31-33] However, biochemical analysis of brain extracts revealed precious little authentic peptide. Thus, it was hypothesized that the peptide responsible for the APP immunoreactivity seen histochemically might be a closely related peptide rather than APP itself. PYY appeared to be an excellent candidate, and attempts were made to purify PYY from the brain. However, the brain peptide was found to differ slightly in sequence from PYY and was therefore named neuropeptide Y (NPY)[34] (Table II). NPY-containing nerves are found throughout the central and peripheral nervous systems, including the enteric nervous system.[18,35-40] Indeed in spite of the close structural homologies between PYY and NPY, the latter peptide seems to have an exclusively neuronal localization and the former peptide seems to be found exclusively in endocrine cells. The gut possesses a large number of NPY-containing neurones.[18,37] Cell bodies containing the peptide are found in both the myenteric plexus and the submucous plexus. Some NPY-containing fibers also seem to have an extrinsic origin as they can be removed by an abdominal sympathectomy. It seems possible that, in some cases, NPY and norepinephrine are colocalized in sympathetic neurones.[38,39] In addition, it has recently been demonstrated that some intrinsic NPY-containing fibers in the submucous plexus also contain choline acetyltransferase, indicating that these neurones are also cholinergic (ref 18 and J. Furness, personal communication). NPY-containing fibers are found in all layers of the gut wall. This suggests that NPY might modulate several gut

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**Table III.** Structures of the Tachykinins

| | |
|---|---|
| Molluscan tachykinins | |
| Pyr-Pro-Ser-Lys-Asp-Ala-Phe-Ile-Gly-Leu-Met-$NH_2$ | Eledoisin |
| Amphibian tachykinins | |
| Pyr-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Gly-Leu-Met-$NH_2$ | Physalaemin |
| Pyr-Ala-Asp-Pro-Lys-Thr-Phe-Tyr-Gly-Leu-Met-$NH_2$ | [$Lys^5$,$Thr^6$]Physalaemin |
| Pyr-Pro-Asp-Pro-Asn-Ala-Phe-Tyr-Gly-Leu-Met-$NH_2$ | Uperolein |
| Pyr-——-Asn-Pro-Asn-Arg-Phe-Ile-Gly-Leu-Met-$NH_2$ | Phyllomedusin |
| Asp-Val-Pro-Lys-Ser-Asp-Gln-Phe-Val-Gly-Leu-Met-$NH_2$ | Kassinin |
| Asp-Glu-Pro-Lys-Pro-Asp-Gln-Phe-Val-Gly-Leu-Met-$NH_2$ | [$Glu^2$,$Pro^5$]Kassinin |
| Asp-Pro-Pro-Asp-Pro-Asp-Arg-Phe-Tyr-Gly-Met-Met-$NH_2$ | Hylambatin |
| Mammalian tachykinins | |
| Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-$NH_2$ | Substance P |
| Arg-His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-$NH_2$ | Substance K |

functions, including the firing of gut neurones, gut smooth muscle tone, epithelial electrolyte transport, and local blood flow. Indeed, potent vasoconstrictor effects of NPY have already been reported in several instances.[28] Recently, we have found that NPY is an extremely potent inhibitor of ileal electrolyte transport (unpublished observations). This is particularly interesting as NPY is clearly potentially one of the major neurotransmitters in the mucosa. It is also interesting that, in the mouse vas deferens, both NPY and PYY block electrically induced contractions of the tissue by inhibiting the electrically stimulated release of norepinephrine from sympathetic nerves.[41] As NPY and norepinephrine are often colocalized in sympathetic nerves, this observed inhibition may indicate a role for NPY in a local negative feedback regulation of norepinephrine release. Obviously the list of potent actions of both NPY and PYY is rapidly growing. Like PHI, these are novel gut peptides with a large number of possible physiological functions.

## Substance P/Substance K

In spite of the fact that substance P was discovered as long ago as 1931,[4] research on this peptide only reached its now ferocious pace in the last few years. The wide distribution of substance P in both the peripheral and central nervous systems,[42] including the enteric nervous system,[17,18] and its role in mediating nociceptive and other responses have made it a prime target for research. Recently, several areas of research on substance P have coalesced to form a most satisfying story. One of the threads in this story concerns the pharmacology of responses to substance P in various tissues.[43] Substance P is one of a group of bioactive peptides known as the tachykinins (Table III). Apart from substance P, these other tachykinins are generally thought to exist only in nonmammalian species[44] (physalaemin may be an exception). All members of the group are structurally related, particularly with respect to their C-terminal 5 amino acids, which are extremely similar in each case. These peptides can therefore be considered naturally occurring analogues of substance P. In addition, however, many synthetic substance P analogues have been produced. Interestingly, some of these peptides appear to be partial agonists or competitive antagonists of the effects of substance P.[43,45] In particular, [D-$Pro^2$,$Phe^7$,D-$Trp^9$]- and [D-$Pro^2$,D-$Trp^{7,9}$]-substance P appear to possess considerable antagonist properties although in some situations they are clearly not pure antagonists. This is one of the few examples of antagonists to the effects of peptide neurotransmitters reported to date. Comparison of the pharmacological profiles of natural and synthetic substance P analogues in different tissues has yielded strikingly different potency series.[43,45] In general, two types of sensitivity can be distinguished, suggesting the existence of two subclasses of substance P receptors. Eledoisin and kassinin have potent actions at both types of receptor. However, whereas substance P and physalaemin act in the nanamolar range at one receptor subtype, 3 orders of magnitude higher concentrations are required to activate the second receptor type. In the guinea pig ileum, for example, a potency series physalaemin > substance P > eledoisin > kassinin is found for the contraction of the smooth muscle. This receptor has been designated "SP-P" as physalaemin is the most potent peptide. Similar receptors are found in the guinea pig vas deferens and the rat bladder. In the rat vas deferens, however, the same four tachykinins show a potency series kassinin > eledoisin $\gg$ substance P $\simeq$ physalaemin. This receptor has been designated "SP-E" and is also found in the hamster bladder and rat duodenum. Some synthetic analogues of substance P show a greater receptor selectivity than the parent molecule. This is true for example with the C-terminal methyl ester analogue of substance P which exhibits increased selectivity for the SP-P subtype.[43,45] Of the synthetic substance P antagonists, there is also some data suggesting that [D-$Pro^2$,D-$Trp^{7,9}$]-substance P may be SP-E receptor selective.[50]

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The finding of a kassinin-selective tachykinin receptor has recently taken an added significance. Studies on the biosynthesis of substance P revealed that in bovine brain two substance P precursors could be found.[51] Both of these protein precursors contained the sequence of substance P. However, in addition, one of these molecules also contained a second tachykinin that had a sequence that was extremely similar to that of kassinin. This mammalian equivalent of kassinin was named substance K. The peptide has now been actually detected in porcine spinal cord. The group that detected it named it neurokinin $\alpha$.[52] However, the name substance K will probably be the one that sticks. The interesting thing about substance K is that, when analyzed in a series of pharmacological assays, it behaves in a very similar fashion to kassinin rather than to substance P,[53] i.e., it has potent effects on both SP-E and SP-P receptors. Clearly, therefore, substance K and substance P could be looked upon as the endogenous ligands for the SP-E and SP-P receptors, respectively. However, such a simplistic scheme is unlikely to be wholly correct.

The ability of substance P to contract gut smooth muscle is well established. Moreover, as both SP-P and SP-E receptors appear to occur in the gut, it is likely that both substance P and substance K play a physiological role in the control of gastrointestinal motility.[42,57,58] Both peptides probably also act on intestinal nerves.[54-56] In addition, both substance P and kassinin (and therefore presumably also substance K) are potent stimulators of intestinal electrolyte transport.[59] This observation takes on particular significance with respect to the actions of the opiate antidiarrheal drug loperamide. It has been recently shown that low concentrations of loperamide ($<10^{-6}$ M) completely inhibit substance P induced electrolyte secretion.[60] The effects of other secretogogues such as carbachol, $PGE_2$, or VIP are not blocked. At a molecular level, tachykinins appear to function in the mucosa by increasing the influx of $Ca^{2+}$ into mucosal cells. This can be demonstrated by observing an increase in the fluorescence of the $Ca^{2+}$ sensing dye, quin-2, loaded into enterocytes. Substance P induced $Ca^{2+}$ uptake can also be completely blocked by loperamide.[60] Interestingly, all these actions of loperamide are unaffected by opiate antagonists such as naloxone and diprenorphine. Thus, loperamide may have some ability to block calcium channels linked to substance P receptors in addition to being a potent opiate agonist. Such ancillary nonopiate effects may explain the unusual effectiveness of this drug as an antidiarrheal agent. Indeed, drugs such as verapamil, which can block various kinds of calcium channels, are known to produce constipation as one of their major side effects. Other workers have also detected blockade of voltage-sensitive calcium channels by loperamide in smooth muscle.[61] It will be interesting to see whether loperamide can also antagonize the effects of subnstance P in tissues apart from the ileal mucosa.

## The Opioid Peptides

Perhaps the most intensively investigated of all the peptidergic families found in the gut are the endogenous opioid peptides. The effects of opiate drugs on the gastrointestinal system have been known about and studied for many years. Indeed the use of morphine in the treatment of gastrointestinal disorders preceded its use of as an analgesic.[62] The sensitivity of various isolated gastrointestinal preparations (particularly the guinea pig ileum) to the effects of opiates are also well known, and such preparations have been important in the assay of opiate agonist activity. We now realize, of course, that opiate effects on the gut are a reflection of the endogenous opioid system that presumably normally operates in this tissue and elsewhere. Research on this system has revealed it to be rather complex. We are now aware of the existence of several related families of endogenous opioid peptides and at least five types of receptors on which they can potentially exert their effects. Briefly, there appear to be three opiate peptide families that are the products of three separate genes[63-66] (Figure 1). The individual opioid peptides are released from their large precursors by proteolytic processing enzymes. $\beta$-Endorphin is derived from a precursor named proopiomelanocortin along with the nonopioid peptide hormone corticotropin (ACTH). The enkephalins and two other related short opioid peptides are derived from proenkephalin A. The dynorphins and $\alpha$ and $\beta$-neoendorphins are derived from proenkephalin B. There is some suggestion that proenkephalin B might also give rise to [Leu$^5$]enkephalin, but this is still speculative.[67] Actually, although these are the major opioid peotides, variations in processing could potentially give rise to several others as well. Thus the final word on exactly how many endogenous opioid peptides exist and what the functional significance of many of them might be is yet to come. The five major opiate receptors are named $\mu$, $\delta$, $\kappa$, $\sigma$, and $\epsilon$.[68] Again further categories have been suggested. The first four on the list have been identified in the gut in at least one species. Indeed, in some tissues (e.g., the opposum lower esophageal sphincter[62,69]) up to four receptor subtypes may coexist and all mediate different effects. In general, the enkephalins show a preference for $\delta$-receptors, $\beta$-endorphin for $\epsilon$-receptors, and the products


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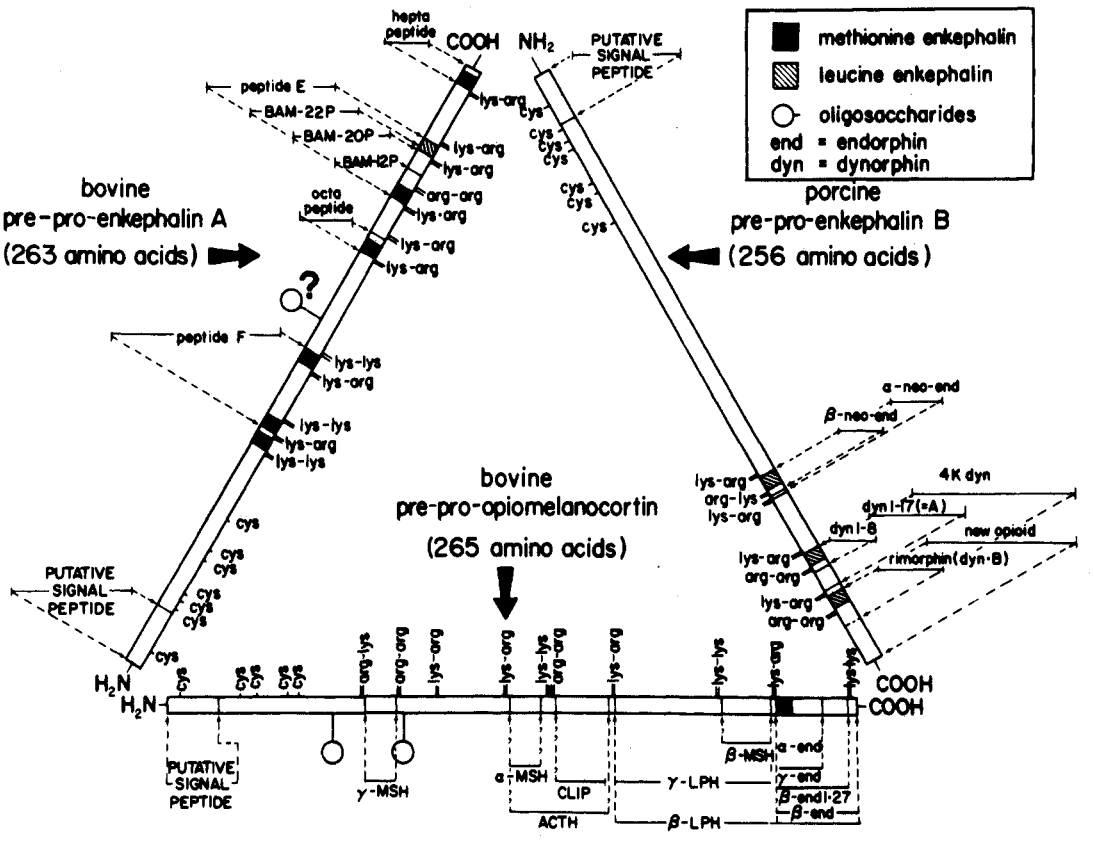


**Figure 1.** Precursors for the three families of opioid peptides and their possible biosynthetic products.[63]

of proenkephalin B for κ-receptors. Morphine and many of the opiates used as antidiarrheal agents such as diphenoxylate and loperamide (vide supra) act preferentially at μ-receptors.[62,68]

It is likely that endogenous opioids play several significant roles in the regulation of gastrointestinal function. The localization of these peptides within the gut is quite well established. The enkephalins are clearly widely distributed, and some recent data also suggests that dynorphin is also present.[17,18,70,71] β-Endorphin has not been consistently reported to occur in the gut. Interestingly β-endorphin-specific ε-receptors have also not been reported in this tissue. Enkephalin-containing nerve cell bodies are localized mainly in the myenteric plexus of many parts of the gut.[70] In the guinea pig ileum, for example, these perikarya give rise to processes that innervate the myenteric plexus, the circular muscle, and submucosa. Enkephalin-containing fibers in the mucosa are not frequently found however. The distribution of dynorphin-containing fibers has been reported to be similar to that of enkephalin-containing fibers although the former are usually rather less well represented.[71] Thus, neurones containing endogenous opioids are in a position to influence most types of gastrointestinal functions. Indeed, opiate drugs and endogenous opioid peptides can alter intestinal motility,[72] epithelial electrolyte transport,[73,74] neurotransmission in enteric ganglia,[75] and hormone secretion from intestinal endocrine cells.[76] It is well known that the overall effect of opiates on the gut is a constipating one.[79] It is not really clear which of the many actions of opiates is the most "important" in producing this overall effect. This particularly is true as the precise actions of opiates in different species differ considerably. Thus in some species opiates slow contractions of the intesine while in others these contractions are enhanced.[72] However opiates produce constipating effects in all species.

The enkaphalins are thought to act as neurotransmitters within the enteric ganglia.[75,77] Electrophysiological effects of opioid peptides within the myenteric plexus are readily demonstrated.[77] Enkephalins hyperpolarize myenteric neurones and increase the duration of the after-hyperpolarization that follows an action potential. Such effects serve to impede the progess of action potentials into the nerve terminal and hence the release of neurotransmitters. It is thought that these opioid effects result from an increase in a specific $Ca^{2+}$-activated $K^+$ conductance in the nerve cell.[78,79] This type of action seems to be linked to opiates acting at μ- and δ-receptors.[80-82] Another opiate receptor linked action appears to be a direct modulation (inhibition) of voltage-sensitive calcium channels by agents acting at κ-receptors.[83] However, the net result of both types of action is the same with respect to neurotransmitter

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release. As a result of such actions on neurotransmitter release in the gut, endogenous opioids are in a position to alter the contraction of gut smooth muscle. However, in addition, opiate receptors seem to be found on some gut smooth muscle cels, and so some direct effects of opioid peptides may also occur.[84] Opioid peptides also modulate intestinal electrolyte transport.[73,74,85,86] It has been demonstrated in vitro that stable enkephalin analogues reduce electrogenic anion transport across isolated ileal mucosa. This effect is produced by agents that interact with δ-opiate receptors such as the enkephalins. However μ-specific agents are rather ineffective. It is not entirely clear whether the δ-receptors that mediate these effects are actually localized on epithalial cells or on neurones of the submucous plexus that innervate the mucosa.[73] Clearly, therefore, there are local effects of opioid peptides on both motility (mostly mediated by μ-receptors) and electrolyte transport (mostly mediated by δ-receptors) that may be important in the control of overall gastrointestinal function. These effects might be exploited pharmacologically, particularly as it seems as though they exhibit differential pharmacological specificity.

Another interesting new development in this area is the realization that, as well as operating at the local level, opioid peptides can also regulate gut motility and electrolyte transport via central mechanisms. Opiates, particularly those with a μ-selectivity can inhibit intestinal transit following direct injection into the brain.[87] On the other hand, central administration of δ-specific opioid peptides but not μ-specific agents produces an inhibition of cholera toxin induced fluid secretion in the small intestine.[88] Further investigation of this latter effect showed that it was mediated by the release of norepinephrine from sympathetic nerves in the small intestine.[89] Thus, it seems that there is a hierarchy of sites at which opioid peptides can exert a control over gastrointestinal functions. This may also turn out to be case for other gut peptides as well.

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# *Articles*

## Heterocyclic Analogues of Chlorcyclizine with Potent Hypolipidemic Activity

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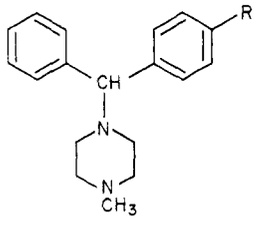
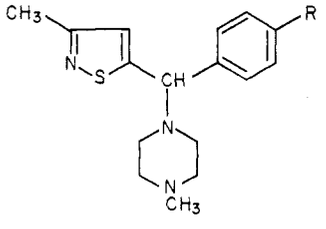
A series of [α-(heterocyclyl)benzyl]piperazines was synthesized and their effect of reducing serum cholesterol and triglyceride levels in the rat was evaluated. A systematic exploration of the structure–activity relationships led to the synthesis of (*R,S*)-(3,5-dimethylisoxazol-4-yl)[4-(1-methylethyl)phenyl](4-methylpiperazin-1-yl)methane dihydrochloride (M&B 31 426), which had potent activity in lowering serum lipid levels at a daily oral dose of 2 mg/kg and was 100 times more potent than clofibrate.

Several related (diphenylalkyl)piperazines, including the



1, R = H (cyclizine)
2, R = Cl (chlorcyclizine)



3, R = H
4, R = Cl

antihistaminics cyclizine (**1**) and chlorcyclizine (**2**), reduce serum cholesterol levels in the mouse, rat, and dog.[1-4] It was discovered during our search for novel hypolipidemic agents that the isothiazole cyclizine analogues **3** and **4** significantly reduced the concentration of serum cholesterol and triglycerides in the serum of rats. However, compounds **3** and **4** were considered to lack sufficient potency to warrant further investigation. We subsequently prepared and evaluated a number of analogues of these compounds in order to discover compounds with greater potency in lowering these serum lipids. A systematic investigation of structure–activity relationships in the series led to the synthesis and selection of (*R,S*)-(3,5-dimethylisoxazol-4-yl)[4-(1-methylethyl)phenyl](4-methylpiperazin-1-yl)methane dihydrochloride (**75**, M&B 31 426) for detailed biological evaluation.[5]

**Chemistry.** The heterocyclic analogues of chlorcyclizine (**2**) (**3–66, 74–82**; Tables I–III) were synthesized by the methods depicted in Scheme I. The carbinols were pre-

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