REPORT FROM MADRID

Enkephalinergic control of gastrointestinal motility: From "gut brain" to trimebutine

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NEURAL CONTROL OF THE GASTRO-intestinal tract is highly complex. There is a double innervation of the digestive tract: an intrinsic or intramural neuronal network forming a true nervous system within the plexuses of Auerbach and Meissner, and an extrinsic innervation including interconnections to neuronal centres located outside the gut (to brain, spinal cord, sympathetic ganglia). Both types of innervation involve afferent (sensory) and efferent components, and both excitatory and inhibitory activity (C. Roman). The presence of excitatory and inhibitory neurons within the intrinsic neural network provide a basis for local autonomous control of the digestive tract. The term "gut brain" is, therefore, an appropriate description.

The function of the excitatory and inhibitory neurons is mediated by a number of neurotransmitters including acetylcholine, serotonin and, as demonstrated more recently, opiate peptides such as enkephalins.

Enkephalins are found not only in

many neurons but also in endocrine cells of the digestive tract, and their release allows interaction with specific receptors. Trimebutine, originally thought to be an antispasmodic, in fact acts on enkephalinergic receptors. This explains, at least in part, its action on the small intestine and the colon.

EFFERENT NERVOUS SUPPLY

Efferent nervous supply arises from both the sympathetic and parasympathetic nervous systems. In both cases, the efferent pathway consists of two neurons placed in series and separated by a synaptic junction. This junction occurs in a ganglion situated between the central neural axis and the gut (sympathetic system) or within the intramural neural network (parasympathetic system).

Parasympathetic preganglionic efferent fibres: These originate either in the bulb or the sacral spinal cord. The former (bulbar) fibres travel in vagal nerves, the latter (sacral) in the pelvic nerves. Their stimulation produces two

types of effect on gastrointestinal motility: excitation and inhibition.

The excitatory effect involves cholinergic preganglionic fibres synapsing with intramural cholinergic postganglionic neurons the nerve endings of which stimulate gastrointestinal smooth muscle. There may also be excitatory neurons releasing substance P as neurotransmitter. The inhibitory effect is the result of excitation by preganglionic cholinergic fibres of noncholinergic nonadrenergic inhibitory neurons within the intramural plexus. The inhibitory neurotransmitter is not established but could be a purine nucleotide such as ATP or a neuropeptide such as vasoactive intestinal polypeptide (VIP).

Sympathetic efferent nervous supply:

Efferent sympathetic nervous supply consists of preganglionic cholinergic neurons with cell bodies in the thoracic or lumbar spinal cord. Their axons synapse with postganglionic noradrenergic neurons in the prevertebral or previsceral sympathetic ganglia (celiac, or superior and inferior mesenteric ganglia). The postganglionic noradrenergic axons have their nerve endings within the gastrointestinal wall; in the intramural plexus and to a lesser degree in the muscle layers.

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Sympathetic efferent neurons usually produce inhibitory effects on gastrointestinal smooth muscle, except within sphincters (cardia, ileocecal valve, anal sphincter) where the effect is excitatory. Sympathetic inhibition is the result of noradrenergic reduction of activity of cholinergic intramural neurons (postganglionic parasympathetic neurons), as well as through a less important direct action of norepinephrine on smooth muscle.

Intrinsic nervous system: The intrinsic nervous system is composed of groups of neurons located between the two muscle layers (myenteric plexus of Auerbach) and between the circular muscle layer and the submucosa (submucosal plexus of Meissner).

All ganglia are interconnected by non-myelinated nerve fibres to form a complex circuitry that is as yet poorly understood. It is clear that the myenteric plexuses contain both excitatory and inhibitory neurons (parasympathetic postganglionic efferent neurons). Not all intramural neurons are in direct contact with smooth muscle. Some act as interneurons connecting adjacent areas, while others are sensory neurons.

Presence of the various intramural neurons provides an important mechanism for local autonomous control of the digestive tract. Propulsive motor activity (peristalsis) is preserved in the absence of all extrinsic nerve supply. On the other hand, motor activity is reduced if intramural nervous activity is blocked pharmacologically.

Several types of neurons are involved in the intramural network. Among the "classical" mediators, acetylcholine is the best known but ATP, serotonin, dopamine and GABA may also be active. Among the neuropeptides, enkephalins, substance P, VIP, somatostatin and others likely play a role, as demonstrated by their immunohistochemical visualization within intramural neurons and nerve fibres.

AFFERENT (SENSORY) NERVE SUPPLY

Both intrinsic and extrinsic elements are involved in the afferent nerve supply. It transmits information primarily from pressure and chemoreceptors. Within the intramural plexuses sensory

neurons may also release substance P as a neurotransmitter. The extrinsic sensory fibres reach the central nervous system or sympathetic ganglia via either the sympathetic or parasympathetic nerves. The afferent fibres are two to three times more numerous than efferent fibres in these nerves.

Apart from their quantity, sensory messages from the digestive tract are characterized by their qualitative diversity, eg, chemoreceptors in the proximal small intestine specifically responsive to inorganic acids, carbohydrates, amino acids or lipids.

ORGANIZATION OF GASTROINTESTINAL NERVOUS CONTROL

Gastrointestinal nervous control can be schematically shown as a hierarchy of superimposed levels: intramural (intramural nervous plexuses); extramural (sympathetic ganglia); and central (brain and spinal cord). At all three levels, connections of variable complexity exist between afferent (sensory) and excitatory and/or inhibitory efferent neurons. The resulting integration of activity is responsible for: organization of peristaltic movements and of migrating motor activity, for example the migrating myoelectric complex (intramural level); functional interrelationships between different regions of the digestive tract, eg, enterogastric, intestinointestinal, colonocolonic, anorectal reflexes (intramural, extramural or central level); and integration of digestive tract motility with various somatic and vegetative functions such as eating behaviour or emotional behaviour (central level).

ENDOGENOUS OPIATES

Biosynthesis, metabolism and receptors: Opiate peptides, endogenous substances capable of interacting with opiate receptors, consist of three main subgroups defined by the structure of their precursor molecules, as well as by the chemical messengers (hormones or neurotransmitters) which are produced by enzymatic cleavage of the precursor molecules during biosynthesis (Schwarz). The complete sequence of the three precursors has been determined using molecular cloning techniques; proenkephalin

A, proenkephalin B (or prodynorphin) and pro-opiomelanocortin (POMC). Once formed from the precursors, the opiate peptides are released as result of maturation and exocytosis, processes which differ from one tissue to another.

The action of these peptides on target cells involves opiate receptors of three principal subtypes. These receptor subtypes have been characterized through the availability of more selective pharmacological agents. The selective agonist for μ receptors, the stimulation of which results in the morphine type of analgesic effects of opiates, is DAGO, while δ receptors are preferentially activated by Mosberg agonists and κ receptors by dynorphins.

The opiates are inactivated by a number of peptidases that have not yet been fully characterized.

Activity of enkephalins and other opiates on gastrointestinal tract motility: The physiological role of endogenous opiates is highly complex because of the presence of different opiates and different receptor subtypes and the potential for action at multiple sites within the central nervous system and at the periphery.

Activity on esophagus: Opiates act on esophageal muscle, as well as on esophageal nerves controlling the esophagus. However, the role of enkephalins and other endogenous opiates in the regulation of esophageal motility is still unknown. It appears that enkephalins are involved in modulation of adrenergic, cholinergic, and nonadrenergic noncholinergic (NANC) neural activity.

Activity in stomach and duodenum: Several types of μ , δ and κ opiate receptors have been characterized and isolated in the brain, stomach and duodenum. Administration of μ (morphine) and δ (met-enkephalin) agonists inhibits stomach motor activity (except for the pylorus) and decreases gastric emptying. The mechanisms for the decrease in emptying are not fully elucidated. Ketocyclazocine (κ agonist) also decreases evacuation of a liquid meal in a dose dependent manner, but tends to stimulate gastric motor activity.

Recent studies have shown that enkephalins may be involved in the production of stress ulcers and idiopathic dys-

pepsia (Dubois). Intravenous injection of metenkephalin induces phase III activity of the migrating motor complex in the duodenum. The effect is local and probably cholinergic in nature (Vantrappen).

Activity in the intestine: The small intestine is rich in enkephalins, mainly in the myenteric ganglia and in the smooth muscle layers. Submucosa ganglia contain a moderate number of enkephalinergic fibres. Enkephalins are present in the neurons and in intestinal endocrine cells. The endocrine cells are identical to the enterochromaffin cells containing 5-hydroxytryptamine and they produce proenkephalin A (Sundler). Enkephalin-containing myenteric neurons are probably a subpopulation of cholinergic neurons and they exert an excitatory effect on smooth muscle cells. Their activation induces slow depolarizations, excitatory junction potentials (EJP), in muscle cells.

In the colon, enkephalin-containing neurons connected with parasympathetic preganglionic neurons of the sacral spinal cord exert an inhibitory effect on smooth muscle. Their activation hyperpolarizes smooth fibres with the production of inhibitory junction potentials (IJP). The mediator involved has not been identified.

Experimentally, enkephalins diminish the amplitude of either EJPs or IJPs. These alterations are reflected in changes of muscle cell action potentials and concomitant muscle cell contractions. Inhibition of these effects by naloxone indicates that they are due to specific opiate activity at receptors on intramural neural elements (Gonella). Therefore, opiates have both inhibitory and stimulatory effects on the intestine. Furthermore, enkephalins and other endogenous opiates seem to play an important role in the regulation of normal gastrointestinal motility at all levels of the digestive tract.

The ability to separate the central and peripheral actions of the opiates permits the search for and study of synthetic compounds which can act selectively at the periphery in the "gut brain". These compounds could have important effects on motility without undesirable central opiate effects, analgesia and risk of dependency and addiction (Roze). Studies with

trimebutine were conducted with these factors in mind.

ENKEPHALINS AND TRIMEBUTINE

Trimebutine was originally considered an antispasmodic because it inhibited spasms induced by different pharmacological agents. However, subsequent research into its effect on the digestive tract showed that the compound had different effects depending on the relative contractile state of the organ, that is, whether the muscle was actively contracting or was relaxed. This capability to modulate motility was difficult to explain and stimulated significant subsequent research.

The first results indicating involvement of enkephalinergic and other endogenous opiate receptors in the mechanism of action of trimebutine were experiments showing that the stimulatory effects induced in dogs were inhibited by naloxone, a μ receptor antagonist (Fioramonti and Bueno, Toulouse, France). Subsequently, these workers showed that the stimulatory effects of trimebutine, expressed as appearance of ectopic phase III activity, were not inhibited by intracerebroventricular injection of naloxone, while intravenous administration of the quaternary derivative of the antagonist (methylbromide naloxone) caused an inhibition. These results indicated that the drug's effect on gastrointestinal motility is mediated by peripheral and not central opiate receptors.

Complimentary pharmacological studies confirmed the peripheral action of the compound and, together with kinetic studies, helped to clarify its activity profile. In fact, trimebutine does not have any classical central opiate effects in man or in animals. Studies with radiolabelled drug showed a very strong concentration in the digestive tract and a very limited crossing of the blood-brain barrier.

Interaction of trimebutine with systems regulated by endogenous enkephalins was shown by several groups. Daniel (Hamilton, Ontario) noted that trimebutine stimulated or inhibited contractions in dog duodenum *in vivo*, depending on the contractile state of the organ. For example, enkephalins stimulated resting intestine (δ receptors), while dynorphin

(κ receptors) inhibited preparations induced to contract with electrical field stimulation. These results indicate non-selective affinity of trimebutine for these two types of receptors, which could allow activation of one or the other, depending on the physiological state of the organ. In fact, recent studies measuring specific radiolabelled ligand displacement *in vitro* or in isolated organ preparations, confirmed interaction of the drug with μ , κ and δ receptors with a similar degree of affinity (Daniel).

Poitras and Itoh (Montreal, Quebec) have also shown the involvement of opiate receptors in trimebutine-induced stimulation of motility in the dog. The effect was similar in the fasting and post prandial states. This stimulation was accompanied by a rise in plasma levels of motilin, a hormone which itself can induce propagated phase III activity along the upper gastrointestinal tract (Poitras). The stimulatory effects were reproduced in man by Couturier and by Collins, (Hamilton, Ontario) the latter finding that antroduodenal stimulation was inhibited by naloxone.

CONCLUSION

In contrast to antispasmodics, trimebutine acts not only on the colon but also on the small intestine. It is, therefore, potentially useful not only in functional colon disease but also in disorders that affect motility in the upper gut. Trimebutine does not act by inhibiting all gastrointestinal motility. The drug can act to alter both hyper- and hypomotile states, tending to return abnormal motility to physiological levels.

The "gut brain" within the intrinsic innervation of the intestinal wall is capable of independently regulating digestive tract motility. Newer understanding of the role of certain enkephalinergic neuromediators in the functioning of the "gut brain", as well as evidence of binding of trimebutine to enkephalinergic receptors, has served to elucidate the mechanisms by which trimebutine could exert its regulatory effect solely through a peripheral site of action. This mechanism of action puts trimebutine in a unique therapeutic class among drugs used for the treatment of functional gastrointestinal disorders.