Focus on: “G protein-dependent activation of smooth muscle eNOS via natriuretic peptide clearance receptor”

JAMES J. GALLIGAN
Department of Pharmacology and Toxicology and The Neuroscience Program, Michigan State University, East Lansing, Michigan 48824

COORDINATED CONTRACTION AND RELAXATION of gut smooth muscle are essential for normal swallowing, gastric emptying, intestinal propulsion, and defecation. Relaxation of gastrointestinal smooth muscle is brought about by the action of inhibitory substances released by enteric nerves and other cells including interstitial cells of Cajal and smooth muscle (3, 5, 6). Inhibitory neurotransmitters include ATP, nitric oxide (NO), vasoactive intestinal peptide (VIP), and pituitary adenylate cyclase-activating peptide (PACAP) (4, 6, 8). The relative role of each of these substances in causing smooth muscle relaxation varies across species and gastrointestinal tissues. Neurotransmitter-induced relaxation of gastrointestinal smooth muscle is associated with increases in intracellular cAMP and cGMP and activation of apamin-sensitive, Ca2+-activated K+ channels (4, 6, 9, 12). The relative role of these multiple postjunctional mechanisms of relaxation also varies across species and tissues and is a matter of intense debate. One hypothesis proposed to account for the action of multiple transmitters and mechanisms of relaxation in gastric smooth muscle states that VIP and NO are coreleased by inhibitory nerves; NO acts prejunctionally to facilitate further VIP release while VIP has two postjunctional actions (5, 11, 12). First, VIP acts at VIP2/PACAP3 receptors on gastric smooth muscle cells to increase intracellular cAMP via activation of the pertussis toxin (PTx)-insensitive, G protein (9). Responses mediated at the VIP2/PACAP3 receptor readily desensitize, and peptide histidine isoleucine is an agonist at this receptor (9). VIP also acts at a second receptor coupled via the PTx-sensitive G proteins, G1–2, to an increase in intracellular Ca2+ and activation of a constitutive, membrane-bound form of NO synthase (eNOS). The second VIP receptor desensitizes slowly and is peptide histidine isoleucine insensitive. The increase in intracellular Ca2+ occurs following activation of nifedipine-sensitive Ca2+ channels. Ca2+ activates eNOS in a calmodulin-dependent manner, and NO stimulates soluble guanylate cyclase to produce cGMP (9, 11, 12). Both cGMP and cAMP cause smooth muscle relaxation. Support for this hypothesis is also based on the localization of eNOS in smooth muscle cells using RT-PCR and Northern blot analysis (14). One issue that remained unresolved was the identity of the receptor at which VIP acts to stimulate eNOS. The article in focus by Murthy et al. (Ref. 10, see p. C1408 in this issue) provides strong evidence that VIP acts at the natriuretic peptide clearance receptor (NPR-C) to activate nifedipine-sensitive, voltage-operated Ca2+ channels with subsequent stimulation of eNOS.

The NPR-C is a single-transmembrane domain receptor that lacks the guanylate cyclase activity of other atrial natriuretic peptide (ANP) receptors (7). The NPR-C was originally identified as a receptor responsible for sequestration and degradation of circulating ANP (7). However, the NPR-C can couple to G1 and inhibition of adenylate cyclase (2). In guinea pig cecal smooth muscle cells, ANP and VIP bind to a common receptor to cause relaxation, indicating that VIP is an agonist at ANP receptors in gastrointestinal smooth muscle (1). Murthy and co-workers (10) have established the specific ANP receptor subtype at which VIP and ANP interact to cause smooth muscle relaxation. Using rabbit dispersed gastric smooth muscle cells, they showed that VIP, ANP, and the selective NPR-C agonist, cANP-(4–23), cause relaxation through a signaling pathway involving G1–2, activation of nifedipine-sensitive Ca2+ channels, an increase in intracellular Ca2+, NOS activation, and increases in cGMP. These responses were inhibited by VIP and ANP receptor antagonists. Furthermore, the same pattern of responses was produced in cells in which all receptors but the NPR-C had been inactivated using a selective receptor-protection protocol. Finally, the authors reconstituted the signaling pathway in COS-1 cells cotransfected with NPR-C and eNOS, proteins not normally expressed by these cells.

Murthy and colleagues have identified a novel pathway for activation of eNOS in smooth muscle cells. However, these authors have left some interesting questions unanswered about NPR-C-mediated regulation of gastrointestinal smooth muscle. Relaxation caused by NPR-C activation is dependent on activation of voltage-gated Ca2+ channels. It is unclear whether the NPR-C mediates a cellular response leading to smooth muscle depolarization and activation of Ca2+ channels or whether the NPR-C activates Ca2+ channels directly through G1–2. It will also be important to determine whether there is a functional relationship between the NPR-C, eNOS, and cGMP mechanism of smooth muscle inhibition and other established mechanisms of neurogenic relaxation in intact tissues.
Murthy et al. (10) have proposed a scheme in which VIP activates two signaling mechanisms to cause smooth muscle relaxation. One pathway involves activation of adenylyl cyclase and increases in intracellular cAMP, whereas the second involves activation of eNOS and increases in intracellular cGMP. These two pathways interact to facilitate relaxation and to ensure that there are adequate mechanisms in place to provide for efficient, nerve-mediated relaxation of smooth muscle. Identifying the diversity of neurotransmitters producing gastrointestinal smooth muscle relaxation has been a continuing focus of research by enteric neurobiologists. However, Murthy et al. have shown that the diversity of mechanisms for controlling smooth muscle contractility is also expanded when a single transmitter (VIP) acts at several postjunctional receptors that couple to multiple intracellular signaling pathways. This adds to the array of mechanisms by which enteric nerves and other cell types in the gastrointestinal tract control smooth muscle function.

REFERENCES