Receptors and Transmission in the Brain-Gut Axis: Potential for Novel Therapies
IV. GABA\textsubscript{B} receptors in the brain-gastroesophageal axis

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GABA\textsubscript{B} receptors are inhibitory G protein-coupled receptors that are commonly associated with postsynaptic inhibition of transmitter release in the central nervous system. In the brain-gastroesophageal axis, a role has recently been demonstrated for GABA\textsubscript{B} receptors on extrinsic afferent endings within the stomach and esophagus, where they reduce mechanosensitivity. This action is compounded by inhibition of communication centrally from these afferents in the brain stem and within central circuits. There is a final peripheral action on the motor pathway where GABA\textsubscript{B} receptors reduce output of acetylcholine from vagal preganglionic motoneurons. These potent, multiple actions of GABA\textsubscript{B} receptors may have therapeutic benefit by reducing the triggering of transient lower esophageal relaxations, which are the major cause of gastroesophageal reflux. An important clinical application is therefore emerging for this recent discovery.

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GABA is the most prevalent inhibitory neurotransmitter in the central nervous system, for which there are three major classes of receptor: GABA\textsubscript{A}, GABA\textsubscript{B}, and GABA\textsubscript{C}. GABA\textsubscript{A} and GABA\textsubscript{C} receptors form ion channels that allow entry of chloride into the cell and mediate rapid hyperpolarization. The role of ionotropic GABA receptors in visceral reflex control is unclear and is beyond the scope of this article. GABA\textsubscript{B} receptors are coupled via G proteins, and they give rise to slower events. They have long been recognized as widespread, potent inhibitors of neuronal circuits in the central nervous system (CNS) acting both pre- and postsynaptically. They may be involved in processes as diverse as memory and blood pressure regulation. Their current major clinical application is the use of an agonist (baclofen) to inhibit involuntary skeletal muscle movement in neurological conditions, probably through an action in the spinal cord on the stretch reflex.

The recent cloning and expression of GABA\textsubscript{B} receptors (15) has led to intense interest in all aspects of their biology. Attention to their effects in the gastrointestinal system was focused in 1999 with the finding that they inhibit triggering of transient lower esophageal sphincter (LES) relaxations and therefore gastroesophageal reflux (6, 16, 17). The mechanism underlying this action is not yet fully understood and its clinical potential not yet accurately gauged, although it is clear that transient LES relaxations are triggered after maintained activation of gastric vagal mechanoreceptors by distension (20, 29). They are then coordinated by a central program generator that initiates vagal preganglionic outflow to inhibitory enteric neurons in the LES (Ref. 20; Fig. 1). This review deals with the molecular biology, structure, and coupling of GABA\textsubscript{B} receptors, their localization and actions at various points along the pathway of transient LES relaxations within the brain-gastroesophageal axis (Fig. 1), and the evidence supporting their potential therapeutic applications.

STRUCTURE AND COUPLING OF GABA\textsubscript{B} RECEPTORS

The first cloning of the GABA\textsubscript{B} receptor in 1997 confirmed the notion that it was a member of the seven-transmembrane-domain G protein-coupled receptor superfamily (15). The receptor was found to have a large extracellular domain that probably bound ligands in a “Venus flytrap” mechanism. It bears structural similarity to previously cloned metabotropic glutamate receptors, calcium-sensing receptor, and bacterial amino acid binding proteins. The expression of the cloned GABA\textsubscript{B} receptor was at the same time a triumph and a disappointment. With the use of expres-
may mediate signal transduction through regulating act directly with important transcription factors that GABAB(1) have since been identified and their distribution of a G protein-coupled receptor. Other variants of proteins must occur—the first example of heterodimerization is not yet established whether this applies to the dorsal vagal complex. Although both major subtypes are expressed in similar proportions in the CNS, GABAB(1) may be expressed in peripheral tissues without GABAB(2) (11). This may indicate that receptors exist as monomers or homodimers but, perhaps more likely, that other major subtypes of GABAB receptors exist that heterodimerize with GABAB(1). All of these features are encouraging from the point of view of drug design and targeting. However, data on molecular variants and their distribution have so far not been found to support reports of pharmacological subtypes (see below).

PERIPHERAL GABA B RECEPTOR ACTIONS ON VAGAL AFFERENTS

GABA B receptors inhibit signaling from peripheral vagal afferent endings. Indirect evidence for this arose from inhibition of the cough reflex by peripherally restricted GABA B receptor agonists (7). Direct evidence was provided by in vitro electrophysiological studies of gastroesophageal vagal afferents. These showed that baclofen selectively reduced mechanosensitivity in a concentration-dependent way that was reversed by a GABA B receptor antagonist (22). Three types of gastroesophageal afferent endings were tested: mucosal receptors with fine tactile sensitivity that do not respond to tension; tension receptors in the muscularis externa that respond only to tension; and tension-mucosal receptors showing both properties. Mucosal receptors and tension receptors were all inhibited by baclofen, whereas only 60% of esophageal tension-mucosal receptors (and only tension sensitivity) were inhibited. These selective actions suggested selective expression or coupling in discrete populations of vagal afferents. Subsequent in vivo experiments corroborated the in vitro data on gastric tension receptors (illustrated in Fig. 1) but showed no action of baclofen on gastric mucosal receptors (24). However, the in vivo study evaluated only sensitivity of mucosal afferents to CCK, whereas the in vitro study evaluated only their mechanical sensitivity. Therefore, showed receptors concentrated in areas of the CNS associated with integration, memory, neuroendocrine, and motor and visceral functions (13). An important finding of these studies with regard to the brain-gastroesophageal axis was the loss of binding after unilateral nodose ganglionectomy, indicating the likely presence of GABAB receptors on vagal afferents. Functional studies support this, with the majority of patch-clamped nodose ganglion neurons being affected by baclofen (27). After the cloning of the receptor, antibodies and probes to major subtypes and variants became available. Localization with immunohistochemistry and in situ hybridization confirmed and extended earlier autoradiographic findings and emphasized the density of GABAB receptors in the vagal nuclei (18). There have been reports suggesting that different variants of GABAB may be expressed at pre- and postsynaptic sites in the cerebellum (3), but it is not yet established whether this applies to the dorsal vagal complex.
GABA\textsubscript{B} receptors either may have different actions in vitro and in vivo or may affect mechanosensitivity and chemosensitivity differently. Actions of GABA\textsubscript{B} receptor antagonists alone on primary afferents are lacking, so a role for endogenous GABA in modulation of vagal afferents is not yet apparent. Nonetheless, the actions of GABA\textsubscript{A} receptor agonists on naïve receptors may be sufficient to confer a rationale for clinical use (see below).

**CENTRAL GABA\textsubscript{B} RECEPTOR ACTIONS ON VAGAL AFFERENT INTEGRATION**

Interest in central GABA pharmacology of cardiovascular and respiratory reflexes has been focused for many years, and it is recognized that GABA\textsubscript{A} and GABA\textsubscript{B} receptors play an important role in ongoing regulation in these systems. GABA in the brain-gut axis has received sparse attention by comparison. Early studies demonstrated modulation of gastric acid secretion or motility, but these have not been pursued. Studies by a cardiovascular research group on brain stem slices determined that there were pre- and postsynaptic actions of baclofen on transmission from vagal afferent terminals in the nucleus tractus solitarii (NTS) (10). Although these actions were interpreted in terms of their relevance to the baroreceptor reflex, they may also be applicable to gastrointestinal vagal reflexes. Our group was prompted to investigate central GABA\textsubscript{B} receptor actions on vagal reflexes to the gut in the light of our findings on peripheral actions on vagal afferent sensitivity described earlier. We found (24) that central administration of baclofen did not affect spontaneous vagal outflow but inhibited communication between gastric vagal afferents and vagal preganglionic neurons. This was shown by electrophysiological studies of firing patterns in vagal preganglionic efferent fibers and in interneurons of the NTS. Our data indicated that the action of baclofen centrally was probably mediated at two stages, that of transmitter release from primary afferents (probably glutamate or acetylcholine; Ref. 23) and that of interneuronal integration via inhibitory mechanisms (probably both GABA\textsubscript{A} and GABA\textsubscript{B} receptor mediated) or excitatory mechanisms (via multiple candidate transmitters). Interestingly, it appeared that actions on interneurons along two different central pathways differed in their pharmacology. Stimulation of gastric tension receptors by distension evoked vagal efferent neuronal responses showing excitation or inhibition. Both types of response were reduced by baclofen, and the effect of baclofen was reversible by either of two GABA\textsubscript{B} receptor antagonists, CGP-35348 or CGP-62349. Stimulation of mucosal afferents with peripheral CCK gave rise to excitation of vagal efferents that was unaffected by baclofen. Inhibition of efferent firing after peripheral CCK occurred in some fibers, which was, in contrast, reduced by baclofen. This effect was reversible only by CGP-62349 (24). The indication in this case is of two pharmacological subtypes of GABA\textsubscript{B} receptor. The CGP-35348-insensitive receptor is probably local-ized specifically to interneurons along inhibitory central pathways activated by mucosal receptor input. These are not shown in Fig. 1 for reasons of simplicity. The antagonist CGP-35348 has also revealed evidence for pharmacological subtypes of GABA\textsubscript{B} receptors in other models, including inhibition of transmitter release from cortical synaptosomes (8), pre- and postsynaptic GABA\textsubscript{B} receptor actions in the septum (30), vagal motor pathways to the LES (5, 28), and inhibition of transient LES relaxations (6) (see below). Attempts to correlate pharmacological and molecular variants of the GABA\textsubscript{B} receptor have so far shown no links, but work continues in this area.

**GABA\textsubscript{B} RECEPTOR ACTIONS ON VAGAL MOTOR OUTFLOW**

Early studies indicated that baclofen increased gastric motility by reducing activity in vagal pathways connected to inhibitory enteric motoneurons (1). Baclofen was considered likely to have a central site of action, although a peripheral action may have occurred in addition. A peripheral action was confirmed by studies of LES responses to vagal motor stimulation, in which the sphincter response was reduced by baclofen (5). Baclofen had no effect on responses of LES strips to electrical field stimulation or neurotransmitter agonists, indicating a presynaptic action on transmitter release from vagal preganglionic neurons. This was confirmed by in vitro studies on a vagus-LES preparation that indicated that GABA\textsubscript{B} receptors inhibit acetylcholine release from vagal endings (28). A GABA\textsubscript{A} receptor agonist was without influence in this preparation. The pharmacology of the GABA\textsubscript{B} receptors involved in these peripheral actions was identical to that of the central interneuronal pathways discussed above, adding weight to the theory that pharmacological subtypes of GABA\textsubscript{B} receptor exist along the brain-gastroesophageal axis. Studies of GABA\textsubscript{B} receptor actions on swallow-induced and transient LES relaxation clearly showed that GABA\textsubscript{B} receptor agonists do not inhibit the depth of physiological LES relaxation (6, 16, 17), presumably because inhibition is overwhelmed by the physiological response. The direct functional implications of data on vagal stimulation-induced LES relaxation are not therefore immediately apparent, but these data do exemplify the importance of studying pharmacological heterogeneity.

**GABA\textsubscript{B} RECEPTOR ACTIONS ON TRANSIENT LES RELAXATIONS AND GASTROESOPHAGEAL REFUX**

Provocation of transient LES relaxations by gastric distension or meals is the major cause of gastroesophageal reflux in conscious dogs, ferrets, healthy humans, and patients with gastroesophageal reflux disease (20). As such, this mechanism provides an important clinical target that is preferable to indirect approaches such as inhibition of gastric acid secretion. Evidence from the effects of vagotomy, sympathectomy, sleep, and anesthesia on triggering of transient LES relaxations suggests that they are coordinated by

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a central program generator in response to increasing input from gastric vagal mechanoreceptors and are brought about by abrupt, intense activation of vagal preganglionic neurons that connect with inhibitory enteric neurons in the LES (20, 29). From the actions of GABA<sub>B</sub> receptors at several points along the vagal pathway described above, it follows that GABA<sub>B</sub> receptor agonists are likely to interfere with transient LES relaxations. However, it was actually before recent fundamental investigations were begun on the effects of GABA<sub>B</sub> receptor agonists on the brain-gastroesophageal axis that their actions on transient LES relaxations were being pursued. Experiments on dogs revealed a potent inhibition of transient LES relaxations and reflux by baclofen (16). This was confirmed first in ferrets (6) and later in humans (17). The effects of baclofen in animal models were dose dependent, mimicked by other GABA<sub>B</sub> receptor agonists, and reversible with certain GABA<sub>B</sub> receptor antagonists. Effects of GABA<sub>B</sub> receptor antagonists on the actions of baclofen closely matched those on central interneurons and vagal preganglionic neurons described above, further indicating relevance to pharmacological subtypes. GABA<sub>B</sub> receptor agonists were not associated with large changes in basal LES pressure or in behavioral parameters, indicating a selective action on triggering of transient LES relaxations by gastric distension. The GABA<sub>B</sub> receptor agonist muscimol was without effect on transient LES relaxations unless sedation occurred (6). More refined studies in dogs showed that at least part of the effect of baclofen was probably mediated in the CNS (4), but additional peripheral actions were considered likely. These are particularly relevant when one takes into account the potent inhibition of gastric mechanoreceptor sensitivity by baclofen described above.

These studies on GABA<sub>B</sub> receptors and reflux were the latest in a series from several groups investigating pharmacological manipulation of transient LES relaxations and consequent gastroesophageal reflux. Boulant et al. (9) reported an inhibitory action of a CCK receptor (CCK<sub>1</sub>) antagonist on triggering of transient LES relaxations in dogs. This was subsequently confirmed in humans by two groups. The background for these studies was that CCK is released by the nutrient content of a meal and that this may be responsible for a potentiation of the trigger signal for transient LES relaxations. This is, however, unlikely to explain the fact that transient LES relaxations are inhibited by the CCK antagonist after nonnutrient gastric distension in fasted animals, suggesting that central CCK receptors were responsible. However, centrally delivered CCK<sub>1</sub> antagonist was not effective in reducing transient LES relaxations (9), leaving the possibilities open. The study of Boulant et al. (9) and other more recent studies have shown that nitric oxide synthase (NOS) inhibitors are effective in reducing gastric distension-induced transient LES relaxations. The rationale behind these investigations was that nitric oxide is the final common mediator of LES relaxation in response to a range of stimuli, being released from myenteric inhibitory motoneurons that directly innervate the LES. Inhibition of NOS affected neither the depth of transient LES relaxation nor the duration, but only the frequency, suggesting an action on the central program generator rather than the LES. A later study in dogs investigated the role of 5-hydroxytryptamine (5-HT) receptors (5-HT<sub>3</sub>) in transient LES relaxations (26). This may be relevant to the fact that 5-HT shares a role with CCK in the postprandial signaling of luminal content. Only a modest effect was found, and this has not yet been followed up in humans. Atropine inhibited transient LES relaxations in humans despite reducing basal LES pressure and esophageal peristaltic amplitude (19). A later investigation revealed that this action was likely to be in the CNS, because a selective peripheral antimuscarinic agent (hyoscine butyl bromide) was ineffective (12). Morphine had a dramatic inhibitory effect on the number of transient LES relaxations in reflux disease patients but not in healthy subjects (25). These effects of morphine are most likely mediated within the CNS. The findings with CCK<sub>1</sub> and 5-HT<sub>3</sub> antagonists, NOS inhibitors, antimuscarinics, and opioids are intriguing and encouraging, but these types of drug are unlikely to become established treatments for reflux disease because of probable side effects. From this point of view it appears that the GABA<sub>B</sub> receptor is the most promising target to date for clinical intervention with gastroesophageal reflux via inhibition of transient LES relaxations.

In conclusion, GABA<sub>B</sub> receptors are densely distributed along afferent, integrative, and efferent components of the brain-gastroesophageal axis. Their commonality to all three components may place them in a unique position for strategic inhibition of central events triggered by gastric distension, of which transient LES relaxations present the best example. All of these factors combine to highlight the potential for use of GABA<sub>B</sub> receptor agonists as treatments for reflux disease.

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