Drugs of the Future 2004, 29(2): 149-161

CCC: 0377-8282/2004

**Review Article**

# Galanin receptors and actions

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**Abstract**

Galanin is a neuropeptide and putative neurotransmitter that is expressed by neurons in the brain, spinal cord and ganglia of the peripheral autonomic nervous system. Three cloned receptor subtypes mediate the actions of galanin. Galanin is generally inhibitory at the level of single neuronal elements in the nervous system. Binding of galanin to its receptors on neuronal cell bodies leads to elevated potassium conductance, membrane hyperpolarization and suppression of excitability. Activation of galaninergic receptors at transmitter release sites on axons inhibits the release of neurotransmitter and suppresses synaptic transmission. Emergent effects of the two inhibitory actions at the integrated circuit level of organization include alterations in cognition and memory in the brain, involvement in the processing of nociceptive information in the spinal cord and alterations of motility and secretory behavior in the gastrointestinal tract. The galanin receptor subtypes are logical drug targets for development of selective agonists and antagonists.

## Introduction

Galanin is a 29 amino acid neuropeptide (30 amino acids in human) that was originally isolated from porcine intestine by Tatemoto *et al.* (1). Immunohistochemistry, RT-PCR and Western blot analysis finds galanin and its receptor subtypes widely distributed in neurons of the brain, spinal cord and ganglia of the autonomic nervous system and in a broad cross-section of non-neural tissues and organs. Galanin is generally thought to be a neurotransmitter/neuromodulator that is released at neuronal synapses in the central and peripheral nervous system and at neuroeffector junctions (*e.g.*, smooth muscle, secretory glands and endocrine tissues).

Galanin expression in the brain and peripheral autonomic ganglia is co-localized with several "classical" neurotransmitters, including norepinephrine, serotonin and acetylcholine. Putative signaling functions of galanin in the brain include an influence on acetylcholine release in regions (*e.g.*, hippocampus) involved with memory and cognition where disordered signaling is related to Alzheimer's disease and actions in regions of the hypothalamus associated with appetite and food intake. Galanin signaling in the spinal cord occurs in spinal afferent pathways involved with the processing of nociceptive information and with pathophysiological changes in the spinal cord associated with peripheral inflammatory neuropathy. Actions of galanin on neural elements of sympathetic ganglia and the enteric nervous system are significant in both normal and disordered gastrointestinal motor and secretory functions. The primary focus of this review is on the actions of galanin on neurons and the receptors that mediate the galaninergic actions.

## Galanin receptors

Galanin produces its biological actions by interacting with high-affinity cell surface receptors. Pharmacological studies with peptide fragments of galanin or chimeric galanin peptides suggest the existence of multiple galanin receptor subtypes with variable expression in different tissues and cell types (2-6). Three galanin receptors referred to as GAL1, GAL2 and GAL3 have been cloned and characterized in human, rat and mouse (7-16). Each of these galanin receptor subtypes has

seven transmembrane domains and belongs to the G-protein-coupled receptor superfamily.

*GAL1 receptor*

GAL1 is the first galanin receptor to be cloned and characterized (7). The cloned GAL1 receptor consists of 349 amino acids in human, 346 amino acids in rat and 348 amino acids in mouse and displays 92-93% identity among species at the amino acid level (7, 9, 12). This receptor in humans shares 42% of the amino acid sequence homology with GAL2 and 38% with GAL3 (16). The GAL1 receptor is coupled through a pertussis toxin-sensitive $G_i/G_o$ type G-protein. Activation of GAL1 inhibits adenylate cyclase with subsequent lowering of intracellular cAMP concentration (7-9), opening of G-protein-coupled inwardly rectifying potassium channels (13) and stimulation of mitogenactivated protein (MAP) kinase activity (17). Opening of potassium channels hyperpolarizes the membrane potential of excitable cells and suppresses neuronal excitability. Northern blot analysis, the reverse transcriptase-polymerase chain reaction and *in situ* hybridization reveal mRNA for the GAL1 receptor in the brain, the spinal cord and in several peripheral tissues (8, 9, 18).

*GAL2 receptor*

Expression and homology screening strategies were used to clone a second galanin receptor subtype named GAL2. Messenger RNA transcripts for the GAL2 receptor are widely distributed in various areas of the brain and several peripheral tissues including dorsal root spinal ganglia, vas deferens, prostate, uterus, ovary, stomach and small and large intestine (10, 14, 15, 19, 20). The GAL2 receptor displays low homology with the rat and human GAL1 receptor. The cloned GAL2 receptor contains 372 amino acids in rat (10, 11, 13, 14), 387 amino acids in human (19, 21) and 370 amino acids in mouse (22). The rat GAL2 receptor shares 85% homology with the human GAL2 and 94% homology with the mouse GAL2 (19, 22).

Unlike GAL1, activation of GAL2 leads to the stimulation of multiple intracellular postreceptor transduction pathways. Most commonly observed is an increase in pertussis toxin-resistant inositol phospholipid turnover and mobilization of intracellular calcium, which suggests that the primary signal transduction cascade for GAL2 involves stimulation of phospholipase C via the $G_{q/11}$ type G-proteins (10, 13, 17). Stimulation of GAL2 receptors also evokes a weak pertussis toxin-sensitive reduction of forskolin-stimulated elevation of intracellular cAMP. Postreceptor coupling through $G_{i/o}$ type G-proteins mediates this action to suppress stimulation of adenylate cyclase (17, 23).

Exposure to galanin also stimulates MAP kinase activity in Chinese hamster ovary (CHO) cells after stable transfection of the CHO cells with the rat GAL2 receptor (17). GAL2 receptor-mediated activation of MAP kinase is blocked by preincubation in pertussis toxin or the protein kinase C inhibitor bis[indolylmaleimide] or protein kinase C depletion, but not by expression of the β-adrenoceptor kinase C-terminal peptide, which specifically inhibits $G_{\beta\gamma}$ signaling (17). These findings are consistent with MAP kinase activation mediated through receptor coupling to $G_o$.

The evidence suggests that the GAL1 receptor is linked exclusively to the $G_i/G_o$ pathway and that GAL2 receptor linkage takes place through $G_q/G_{11}$, $G_i$ and $G_o$. The differential intracellular signaling mechanisms used by the GAL1 and GAL2 receptor may underlie the spectra of functional activity mediated by the galanin receptors at the tissue level and might explain the diversity of roles for galanin in physiological regulatory mechanisms.

*GAL3 receptor*

GAL3 represents the most recently cloned galanin receptor for the rat (15, 24) and human (20, 24). Rat GAL3 mRNA encodes a protein of 370 amino acids with 36% and 54% identity to rat GAL1 and GAL2 (15). Human GAL3 mRNA encodes a protein of 368 amino acids with 90% homology to rat GAL3 (24). Northern blot analysis found expression of GAL3 in heart, spleen and testis, but not in the brain (15). Smith *et al.* (24) used the more sensitive method of solution hybridization/RNase protection assays to find mRNA transcripts for GAL3 in the rat hypothalamus, pituitary and other brain areas and in peripheral tissues including the liver, kidney, testicles, adrenal cortex, adrenal medullar, lung, spleen, pancreas and stomach. Like GAL1, GAL3 postreceptor signal transduction involves receptor coupling to $G_i/G_o$ type G-proteins.

**GAL receptor pharmacology**

The pharmacology of GAL receptors has lagged by several years the molecular biology and cloning of the receptors. The literature is replete with reports of the effects of stimulating native GAL receptors by exogenous application of galanin in a diverse array of tissues and organ systems. Nevertheless, success in linking one or another of the cloned receptors to physiological events evoked by galanin has not been forthcoming.

Advances in understanding of the pharmacology of galanin receptors continues to be limited by the lack of selective agonists and antagonists for each of the three receptor subtypes.

A series of chimeric peptide analogs (*i.e.*, C7, M15, M32, M35 and M40) initially held promise as galanin receptor antagonists (25). However, none of the antagonists proved to be selective for any of the galanin receptor subtypes. Moreover, later reports suggest that the chimeric peptides act as galanin receptor agonists rather than antagonists in some systems and cell lines (26-29).

On the other hand, some compounds do have promise. Development of the peptide agonist AR-M961, which has high affinity and functional activity for both the GAL1 and GAL2 receptors, and the selective GAL2 receptor agonist AR-M1896 is promising in this respect (30). Likewise, the nonpeptide selective GAL1 receptor antagonists, Sch-202596 (31) and 2,3-dihydro-dithiin and -dithiepine-1,1,4,4-tetroxides (32) have promise as pharmacological tools for studying the physiological and pathophysiological roles of galanin and its receptor subtypes.

## Galanin receptors and actions in the brain

Galanin acts at both pre- and postsynaptic receptors to influence glutamatergic and cholinergic neurotransmission in the brain (28, 33, 34). It acts in hypothalamic brain slices to suppress the release of glutamate and AMPA receptor-mediated fast excitatory transmission.

The action of galanin at presynaptic inhibitory receptors on axonal terminals suppresses the release of acetylcholine in the neural circuitry of the hippocampus and its hyperpolarizing action at receptors on the cell bodies of hippocampal neurons offsets the excitatory postsynaptic depolarizing action of acetylcholine. Intraventricular injection of galanin in animals impairs choice accuracy in learning and memory paradigms (35). Suppression of cholinergic neurotransmission from projections in the septum and basal forebrain areas that terminate in the hippocampus and cortex probably underlies galanin-evoked suppression of memory functions in animal models (36-38).

### *Alzheimer's disease*

Alzheimer's disease is the prototypical neurodegenerative disease characterized by abnormalities in the brain that affect neurons in specific regions that include the cortex, hippocampus, amygdala and nucleus basalis of Meynert. In Alzheimer's disease, galanin is overexpressed in the nucleus basalis of Meynert where a high density of cholinergic innervation is found (37). Transgenic mice, which overexpress galanin, exhibit cognitive and neurochemical deficits reminiscent of Alzheimer's disease (39, 40). Although heterogenous populations of GAL receptors that bind galanin with both high and low affinity are present in the brain regions associated with Alzheimer's disease, identification of specific GAL receptor subtypes involved in cholinergic mechanisms of neurotransmission in these parts of the brain remains unclear for both humans and animals. This lack of information underscores the need for further investigation of mechanisms by which galanin actions are beneficial or deleterious for cholinergic cell survival and activity within the brain. Identification of the receptor subtypes is a prerequisite for development of pharmacological strategies for manipulation of the receptors that might be a key for treatment of the cognitive deficits in Alzheimer's disease (41).

### *Food intake*

Intracerebroventricular administration of galanin in rats increases food intake and a preference for fat that is mediated by binding to receptors in the hypothalamus and amygdala (42, 43). These central effects of galanin on feeding occur without change in grooming, resting or other behaviors. Application of chimeric peptide antagonists (*e.g.*, C7 and M40) offsets the changes in feeding behavior evoked by galanin (44). Nevertheless, the identification of the specific receptor subtype involved is equivocal due to lack of selectivity of the antagonists.

## Galanin receptors and actions in the spinal cord

Neuronal expression of galanin, in spinal ganglia and the dorsal horns of the spinal cord, reflects mechanisms that regulate transmission and interpretation of pain information by interneuronal integrative circuits in the spinal cord. Galanin immunoreactivity and mRNA transcripts for galanin are normally found in a small population of small diameter dorsal root ganglion cells (DRG) in rodents and primates (45-49). Crushing injury to peripheral nerves or inflammation surrounding the nerves (*e.g.*, ligation of the sciatic nerve with chromic suture material) leads to major upregulation of galanin expression in DRG neurons of all sizes (50, 51) and release of elevated amounts of galanin in the dorsal horn of the spinal cord (52). These changes in galanin expression and release in response to inflammatory stimuli are similar to the upregulation of other neuropeptides implicated in the generation of inflammatory pain states (*e.g.*, substance P, calcitonin gene-related peptide and endogenous opioid peptides). The evidence suggests that upregulation of galanin in primary afferent pathways is associated with development of the hyperalgesia and neuropathic pain that follows peripheral nerve injury (52-54).

DRG neurons express all three of the galanin receptor subtypes (*i.e.*, GALl, GAL2 and GAL3). GAL1 and GAL2 receptors are extensively expressed in the DRG with about one-fifth of the neurons expressing both receptor types (55). Following synthesis, the receptor proteins are transported to terminations of DRG sensory fibers in the periphery, in the dorsal horn or in both places. Galanin receptors associated with primary afferents are co-localized with chemical codes for primary sensory nerve fibers (*e.g.*, calcitonin gene-related peptide and substance P). Transection of the sciatic nerve in rats is followed by a long-lasting drop in the numbers of the galanin receptor types in lumbar DRGs (56).

Immunohistochemical studies find galanin immunoreactivity localized to small neurons in lamina II of the spinal cord dorsal horn where it is co-expressed with GABA, enkephalin and neuropeptide Y (49). A dense plexus of

galanin immunoreactive fibers is present in the superficial laminae of the dorsal horn. Either dorsal rhizotomy or capsaicin treatment ablates the immunoreactivity and suggests that C- and A-delta sensory fibers and their terminals in the dorsal horn contain galanin (46). This is consistent with reports that immunoreactivity for galanin and calcitonin gene-related peptide is coexpressed in primary afferent terminals and varicosities in the grey matter of the dorsal horn (49, 57). The distribution of galanin immunoreactivity in the dorsal horn increases following peripheral nerve injury and this probably reflects increased expression in the terminals of DRG afferents (49). A high density of binding sites for labeled galanin is found in laminae I, II and IX of the dorsal horn in both rodents and primates (49, 58).

These binding sites in the dorsal horn are interpreted as postsynaptic galanin receptors that are expressed mainly by interneurons because they do not change after cutting the dorsal spinal roots or after capsaicin treatment.

Inflammation in the peripheral environment of sensory afferent fibers evokes changes in the expression of galanin and its receptors in the DRG and spinal cord that differ from the changes found when peripheral nerve damage has occurred. Whereas upregulation of galanin immunoreactivity occurs in DRG neurons following peripheral nerve injury by crushing or ligation, a decrease in the number of DRG neurons expressing galanin immunoreactivity occurs within a few days after the induction of inflammation by insults such as local injection of carrageenan in the footpad of rats (59, 60).

Unlike in DRG neurons, mRNA transcripts for galanin in the dorsal horn become elevated when inflammation is present in the environment of peripheral nerves. Changes in expression of two of the galanin receptor types in DRG neurons takes place in parallel with the observed increases in the numbers of dorsal horn neurons expressing galanin mRNA. GAL1 and GAL2 receptors in the DRG change in opposite directions when inflammation is present in the region of the peripheral sensory nerve fibers. Expression of the mRNA transcript for the GAL1 receptor in DRG neurons is downregulated during inflammation while the GAL2 receptor is upregulated (61, 56).

The presence of galanin and galanin receptors in DRG neurons and in dorsal horn interneurons is evidence that galanin might be a transmitter or neuromodulator in spinal pain transmission pathways. Suppression of spinal nociceptive responses by intrathecal injection of galanin in animals is evidence for such a role in the transmission of pain signals (61, 63-66). Potentiation of the spinal analgesic effect of morphine by intrathecal injection of galanin is additional evidence for an antinociceptive action (67). Nevertheless, evidence obtained from targeted knockout of the galanin gene in mice does not fully support an antinociceptive action for endogenously released galanin (see below).

Two possible mechanisms of action might explain the antinociceptive effects of intrathecally injected galanin. One possibility is a presynaptic inhibitory action to suppress release of neurotransmitter (*e.g.*, substance P and calcitonin gene-related peptide) from primary afferent endings in the dorsal horn and the second is a hyperpolarizing action and suppression of excitability of second-order interneurons in the dorsal horn. In effect, either presynaptic inhibition of transmitter release or membrane hyperpolarization in postsynaptic neurons would suppress transmission of sensory information from primary afferents to second-order neurons in the dorsal horn. Exposure to galanin hyperpolarizes neurons in the dorsal horn and this can account for suppression of the amplitude of ventral root electrical potentials in studies where the ventral root potentials are evoked by either electrical stimulation of C-fibers in the dorsal roots or by application of capsaicin during exposure to galanin (62, 68).

Development and availability of a mouse model with targeted knockout of the galanin gene opened new avenues for understanding the role of galanin in nociceptive signaling alterations in pain sensitivity associated with injury to primary afferent C-fibers. Mice lacking a functional galanin gene are viable, grow normally and can reproduce (69). The mutation reduces the numbers of small DRG neurons by less than 15% and therefore the numbers of nonmyelinated C-fibers in the peripheral sensory nerves (70). Results obtained from galanin null mutant mice do not agree fully with earlier suggestions by Wiesenfeld-Hallin *et al.* that galanin acts as an antinociceptive mediator (67). Galanin null mutants differ from normal wild-type mice in that threshold responses to noxious stimulation in the null mutants are significantly higher than in the normal wild-type mice. Kerr *et al.* (71, 72) investigated thermal hyperalgesia evoked after induction of inflammation by injection of carrageenan or formalin into the animal's footpad and found that thermal hypersensitivity was attenuated in the null mutants relative to normal wild-type mice. This was interpreted as evidence that promotion of pain is associated with conditions in which galanin is released.

Spinal reflex "wind-up" is attenuated in galanin deficient mutant mice (54, 71, 72). Wind-up is a phenomenon that is observed when repetitive stimulation of nonmyelinated C-fibers in the sciatic nerve leads to a progressive increase in the strength of the flexor reflex for the same leg. Wind-up reflects facilitation of spinal reflex excitability. Inflammation of the paw, induced by injection of carrageenan, is accompanied by enhancement of the wind-up phenomenon in normal mice. Wind-up in the mutants occurs with essentially the same characteristics as in normal mice without inflammation. However, the facilitation of spinal reflex excitability that occurs during the inflammatory state in wild-type mice does not happen in the mutants.

Kerr *et al.* (54, 72) suggested that the elevated thresholds to various forms of noxious stimulation in the galanin-deficient mice reflect diminished release of galanin in the spinal cord of the null mutants relative to normal mice. They concluded that galanin is normally pronociceptive in persistent pain states related to inflammation and that the site of action is at second and/or

higher order neurons in intraspinal sensory pathways. The results for galanin null mutants, which suggest that galanin release in the spinal cord is involved in the mechanisms that lead to spinal excitability and central sensitization, are inconsistent with an earlier conclusion that endogenous release of galanin suppressed spinal reflex excitability (64, 65). The earlier conclusion was based on results obtained with intrathecal injection of galanin and observations that the inhibitory effects of exogenously injected galanin were enhanced after nerve injury when levels of endogenous galanin are greatly elevated. Kerr *et al.* (71, 72) suggested that galanin has a concentration-dependent biphasic action in the spinal cord. At lower concentrations, it has a pronociceptive effect; at higher concentrations (*e.g.*, intrathecal injection or during nerve injury) it is inhibitory for spinal reflexes.

Galanin will bind to one or more of the GAL1, GAL2 or GAL3 receptor subtypes when released from DRG neuronal terminals in the spinal cord, when released from higher order neurons in the spinal cord or when applied by intrathecal injection. Large diameter DRG neurons predominantly express GAL1 receptors. Small diameter DRG neurons are nociceptive and predominately express GAL2 receptors (73). Consequently, GAL2 receptors are transported from the cell bodies to the terminals of the nociceptive DRG neurons where the terminals form synapses that deliver sensory information to second-order neurons in laminae I and II of the dorsal horns. GAL1 receptors are likely to be located on terminals of large diameter DRG neurons that form synapses with second-order neurons in deeper layers of the spinal cord and in pools of motor neurons in ventral and intermediolateral horns. Both receptor types are also expressed by interneurons in the spinal cord (73). GAL1 receptors predominate on second and higher order interneurons in the superficial laminae of the dorsal horns and GAL2 receptors are expressed mainly by neurons in the ventral horns. Expression of the receptors at DRG neuronal terminals and by spinal interneurons suggests that galanin is released from both DRG sensory endings and from spinal interneurons and once released acts at presynaptic receptors on DRG terminals and at postsynaptic receptors expressed by interneurons. Less is known about expression of GAL3 receptors relative to the other receptor subtypes in the spinal cord. The differential locations of GAL1 and GAL2 receptors on nervous elements within the spinal cord and the fact that the two receptor types use different postreceptor signal transduction cascades probably accounts for the variability in results with intrathecal administration of galanin relative to the findings in galanin knockout mice.

Results obtained with GAL1 knockout mice and a selective GAL2 receptor agonist suggest that galanin binding to GAL1 receptors in intraspinal sensory pathways is involved in the antinociceptive action observed when galanin is elevated to high levels either by intrathecal injection or by peripheral nerve injury. Liu *et al.* (30) reported that high doses of a dual GAL1/GAL2 receptor agonist increased the threshold for behavioral responses to noxious stimulation in rats, while in the same study a selective GAL2 receptor agonist did not alter the threshold for responses to noxious stimulation. These findings were interpreted as evidence for involvement of the GAL1 receptor in the mechanism responsible for the antinociceptive action of high concentrations of galanin in the spinal cord. Noxious stimulation (*i.e.*, hot plate test and cold stimulation) evoked exaggerated responses in GAL1 knockout mice, which is consistent with involvement of the GAL1 receptor in antinociceptive activity in the rat model (74). Nevertheless, the wind-up phenomenon for spinal reflex excitability was unaffected in the GAL1 receptor knockouts, suggesting that galanin receptors other than GAL1 are also involved in inhibition in the spinal cord (75, 76).

## Galanin receptors and actions in the enteric nervous system

Galanin-immunoreactive neurons in the guinea pig enteric nervous system are present in the myenteric and submucosal plexuses of the small and large intestine and the myenteric plexus of the stomach (47, 77, 78). Galanin-immunoreactive nerve fibers are present in all layers of the gut wall and at all levels of the gastrointestinal tract (47, 77, 79). These galaninergic fibers are primarily intrinsic to the enteric nervous system (77, 79).

Numerous reports suggest roles for galanin in the enteric neural control and coordination of gastrointestinal motility and secretion. Galanin alters contractile behavior of the gastrointestinal musculature in a variable and species-dependent manner. Exposure to galanin suppresses spontaneous contractions of the circular muscle both *in vivo* and *in vitro* in canine small intestine and the gastric pylorus (80-82). Contractions of the intestinal circular muscle coat and the release of acetylcholine evoked by transmural electrical field stimulation are suppressed with galanin present in the bathing solution in organ bath studies (83, 84). Application of galanin in studies of electrogenic chloride secretion in Ussing chamber experiments suppresses secretion in porcine jejunal and rabbit ileal mucosa (85, 86). The presence of galanin in the Ussing chamber attenuates secretory responses to electrical field stimulation of intramural neurons in preparations of guinea pig intestinal mucosa (87). These actions of galanin at the organ level reflect suppression of neuronal excitability in the enteric nervous system.

### *Neuronal cell bodies*

There are two actions of galanin in the enteric nervous system, both of which are inhibitory and reminiscent of the inhibitory actions in the central nervous system. One action is hyperpolarization of the membrane potential and suppression of excitability at the level of the neuronal cell bodies and the second is presynaptic inhibition of the release of neurotransmitters (Figs. 1, 2).

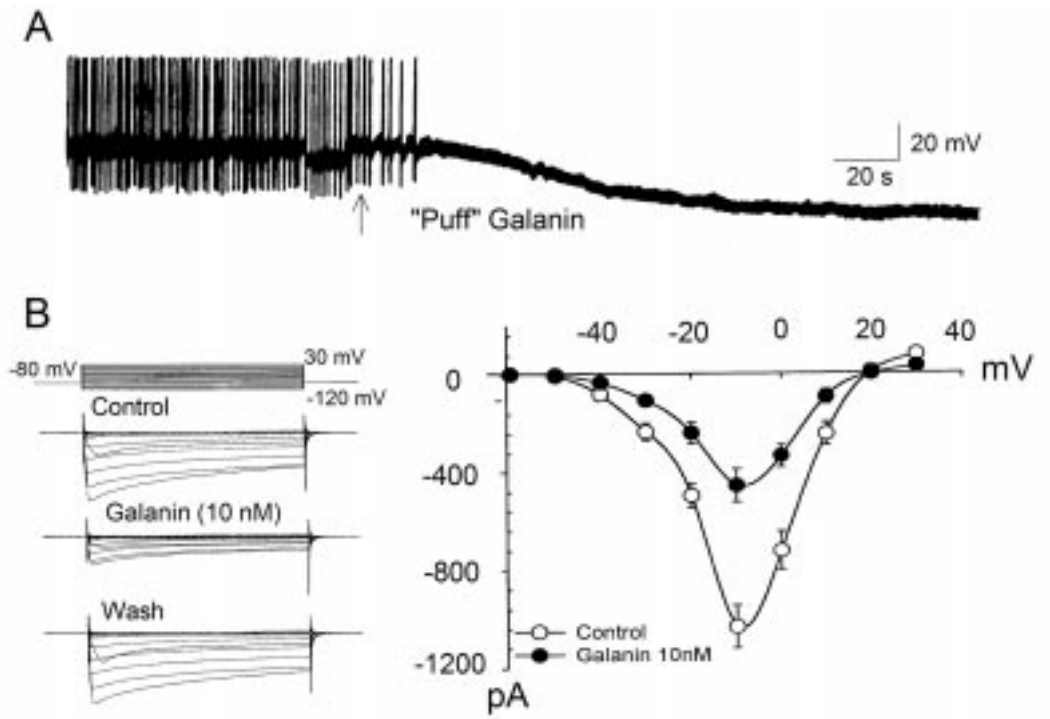


Fig. 1. Inhibitory action of galanin on neuronal excitability illustrated for the enteric nervous system. (A) Micropressure application of a 20 μM, 20 ms puff of galanin during an ongoing stimulus-evoked slow EPSP inhibited the discharge of action potentials and hyperpolarized the membrane potential of an AH-type enteric neuron in the guinea pig small intestinal myenteric plexus. (B) Application of 10 nM galanin reversibly suppressed inwardly directed, voltage-activated calcium current in an AH-type enteric neuron cultured from the myenteric plexus of guinea pig small intestine in a voltage-clamp study. The voltage-clamp protocol, in which the membrane potential was held at –80 mV and depolarized or hyperpolarized in successive steps to 30 mV and –120 mV, respectively, is shown on the left. Current-voltage relations in the presence and absence of 10 nM galanin are shown in the right panel.

The inhibitory action of galanin on neuronal cell bodies is restricted to enteric neurons with AH-type electrophysiological behavior and Dogiel type II multipolar morphology in the myenteric and submucosal plexuses. The resting membrane potential and excitability of enteric neurons with S-type electrophysiological behavior and Dogiel type I uniaxonal morphology are unaffected by exposure to exogenous galanin (28).

AH-type enteric neurons are distinguished by characteristics of their electrophysiological behavior which include: 1) higher resting membrane potential and lower input resistance than S-type neurons; 2) no spike discharge to depolarizing current injection or discharge of one or two spikes only at the onset of intraneuronal injection of long duration depolarizing current pulses; 3) absence of anodal break excitation at the offset of hyperpolarizing current pulses; 4) prolonged postspike hyperpolarizing potentials of several seconds duration; 5) calcium contribution to the inward current of the action potential; 6) tetrodotoxin-resistant action potentials; 7) exposure to multivalent cationic calcium entry blockers (*e.g.*, manganese, lanthanum or cadmium) depolarizes the membrane potential, increases input resistance and augments excitability; 8) activation of adenylate cyclase and elevation of intracellular cAMP depolarizes the membrane potential, increases input resistance and augments excitability. Aside from their electrophysiological properties, most AH neurons (*i.e.*, ≈ 80%), but not S-neurons, express immunoreactivity for the calcium-binding protein, calbindin (88). AH neurons comprise the largest proportion of neurons in the intestinal myenteric plexus (*i.e.*, ≈ 70%) and the smallest proportion of submucosal plexus neurons (*i.e.*, < 10%).

The action of galanin on AH neurons, as recorded with intracellular “sharp” microelectrodes, consists of hyperpolarization of the membrane potential, decreased input resistance (*i.e.*, increased membrane conductance) and suppression of excitability (88). The reversal potential for the hyperpolarizing action of galanin on AH neurons is near the potassium equilibrium potential. Application of tetraethylammonium (TEA) to AH neurons broadens the action potential and enhances the long-lasting hyperpolarizing afterpotentials. Addition of galanin or depletion of calcium in the bathing solution offsets the effects of TEA to broaden the action potential and to enhance the AH. Galanin or reduced calcium has the same effect when both TEA and tetrodotoxin are present in the bathing solution. Suppression of delayed rectifier and A-type potassium currents by simultaneous exposure to TEA and 4-aminopyridine evokes spontaneous spike

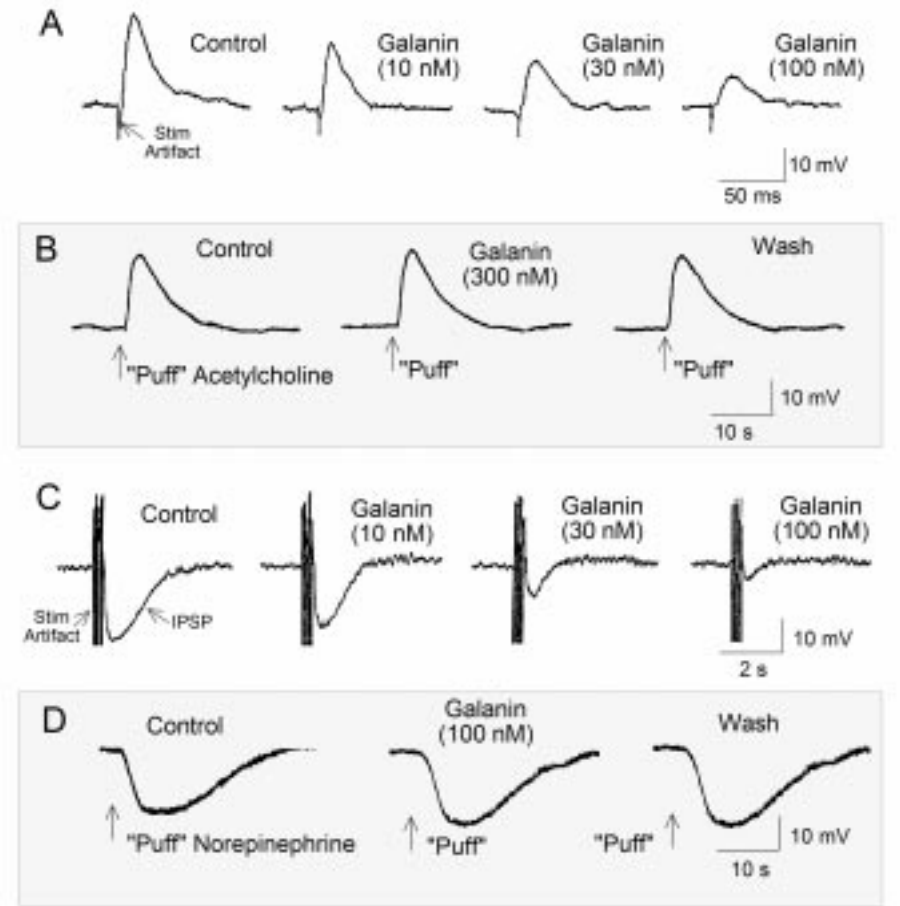


Fig. 2. Inhibitory action of galanin on neurotransmission illustrated for the enteric nervous system. (A) Nicotinic fast EPSPs were suppressed in a concentration-dependent manner by progressively increasing concentrations of galanin ranging from 10-100 nM. (B) Galanin did not suppress depolarizing responses to acetylcholine in the same neuron as A. Acetylcholine (1 μM) was applied by pressure microejection ("puffs') from fine-tipped micropipettes. (C) Noradrenergic slow IPSPs were suppressed in a concentration-dependent manner by progressively increasing concentrations of galanin from 10-100 nM. (D) Galanin did not suppress hyperpolarizing responses to norepinephrine in the same neuron as C. Norepinephrine (10 μM) was applied by pressure microejection (puffs) from fine-tipped micropipettes. The records were obtained with "sharp" intracellular microelectrodes from neurons with S-type electrophysiological behavior and uniaxonal morphology in the submucosal plexus of guinea pig small intestine. Suppression of the synaptic events by galanin and lack of any effects on the postsynaptic mimicry of the nicotinic fast EPSPs by puffs of acetylcholine or the postsynaptic mimicry of the noradrenergic IPSPs by puffs of norepinephrine satisfy criteria for a presynaptic inhibitory action of galanin.

discharge with broadened spikes and enhanced AH. Application of galanin suppresses this activity. Intraneuronal injection of cesium in the presence of tetrodotoxin suppresses all potassium conductances in AH neurons and leaves pure calcium-dependent action potentials in response to depolarizing current pulses. Galanin abolishes the calcium spikes. These observations suggest two primary mechanisms of action for galanin at the level of the neuronal cell bodies. One is to open potassium channels, increase input resistance and hyperpolarize the membrane potential toward the potassium equilibrium potential (Fig. 1A). The second is suppression of conductance in voltage-gated calcium channels and suppression of the AH by indirect prevention of opening of calcium-gated potassium channels (Fig. 1B).

Results obtained with whole-cell patch clamp recording methods in AH-neurons generally confirm the conclusions derived from results obtained with intracellular recording with sharp microelectrodes in current-clamp mode (90). Exposure to galanin suppresses the voltage-dependent inward calcium current carried by N-type calcium channels and activates and an inwardly rectifying potassium current in the AH neurons. Suppression of the N-type calcium current and activation of the inwardly rectifying potassium current by galanin are concentration-dependent with $EC_{50}$ values of 1.4 nM and 55 nM, respectively (90). Preincubation of the neurons with pertussis toxin abolishes both actions of galanin, suggesting that both galanin inhibition of N-type calcium current and activation of inwardly rectifying potassium current are

mediated by activation of $G_i/G_o$ proteins. The inhibitory actions of galanin are unaffected by preincubation in cholera toxin, suggesting that its actions do not involve receptor coupling to $G_s$ proteins. A similar conclusion was reached for the action of galanin to suppress voltage-activated calcium current in mudpuppy parasympathetic neurons (91).

Both suppression of calcium and activation of $K^+$ conductance by galanin are mimicked by the N-terminal fragment of galanin – galanin(1-16) – suggesting that the first 16 amino acids of the peptide are sufficient for both actions as is the case for neurons in the central nervous system (16). The chimeric peptide receptor antagonist, galantide, suppresses galanin-evoked activation of inwardly rectifying potassium conductance with an $EC_{50}$ of 16 AM. Nevertheless, galantide behaves like a galanin receptor agonist with respect to suppression of calcium conductance in enteric neurons.

Galanin and galanin(1-16) are essentially equipotent in their hyperpolarizing actions on AH-type neurons, while GALP is less potent. GALP is a 60-amino acid peptide that displays high affinity for the GAL2 receptor over GAL1 and GAL3 receptors. The acknowledged GAL2-selective agonist, [D-Trp$^2$]galanin, is without effect on AH neurons. Absence of any action for [D-Trp$^2$]galanin and the low potency of GALP is evidence against the GAL2 receptor as the mediator of the inhibitory action of galanin on AH neurons. There is little evidence for or against involvement of the GAL3 receptor in the hyperpolarizing action on AH-type neurons.

*Presynaptic inhibition*

Criteria for presynaptic inhibition in the enteric nervous system are firmly established (92, 93). In order to satisfy the criteria for presynaptic inhibition, the mediator in question must suppress stimulus-evoked synaptic potentials and at the same time not alter mimicry of the synaptic potential by application of the neurotransmitter responsible for the synaptic potential in the same neuron. Galanin meets the criteria at multiple kinds of synapses in the enteric nervous system (Fig. 2).

Focal electrical stimulation of presynaptic axons in both the myenteric and submucosal divisions of the enteric nervous system evokes fast < 50 ms excitatory postsynaptic potentials (EPSPs) that are mediated by the release of acetylcholine and its binding to nicotinic postsynaptic receptors. Tamura *et al.* (93) first reported that the presence of galanin in the bathing medium suppressed the stimulus-evoked fast nicotinic EPSPs. Micropressure ejection of acetylcholine from fine-tipped pipettes (*i.e.*, "puffs" of acetylcholine) mimicked the fast EPSPs. The fast EPSP-like responses to puffs of acetylcholine were unaffected by galanin while stimulus-evoked fast EPSPs were suppressed (29, 93). Galanin has no local anesthetic action on the neurites or axons of enteric neurons and does not alter threshold or conduction velocity in these nerve fibers (93).

The functional significance of the braking action of galanin on fast nicotinic transmission in the integrative microcircuits of the enteric nervous system is not fully understood. Nevertheless, suppression of transmission at the multitude of fast nicotinic synapses that make up the enteric neural networks would be expected to deenergize the network and thereby alter its output to the intestinal effector systems (*i.e.*, secretory glands, blood vasculature and the musculature).

Apart from suppression of fast EPSPs, galanin suppresses slow inhibitory postsynaptic potentials (IPSPs) in enteric neurons (29). Slow IPSPs are slowly activating hyperpolarizing synaptic potentials associated with increased potassium conductance and suppressed excitability in both myenteric and submucosal ganglion cell somas. Slow IPSPs evoked by release of norepinephrine from sympathetic postganglionic axons in the submucous plexus are a unique property of secretomotor neurons that display S-type electrophysiological behavior and uniaxonal morphology (94). Firing of secretomotor neurons releases vasoactive intestinal peptide and/or acetylcholine, both of which act on epithelial cells of the crypts of Lieberkühn to stimulate mucosal secretion (95). Activation of sympathetic input to the bowel during physical exercise shunts blood from the splanchnic to systemic circulation during which time intestinal secretion is not advantageous. Noradrenergic inhibitory input to secretomotor neurons is a compensatory mechanism that suppresses secretion in concert with reduction in intestinal blood flow.

Galanin suppresses stimulus-evoked slow IPSPs in submucosal neurons and the mechanism satisfies the criteria for presynaptic inhibition. The presence of galanin in the bathing solution does not change the amplitude of hyperpolarizing responses to puffs of norepinephrine at the same time that stimulus-evoked slow IPSPs are suppressed (28). Presynaptic inhibition of norepinephrine release from sympathetic postganglionic fibers in the submucosal plexus removes sympathetic braking action from the secretomotor neurons.

Lack of availability of effective pharmacological tools prevents unequivocal identification of the galanin receptor subtype responsible for presynaptic inhibition of the release of acetylcholine or norepinephrine. One or more of the three cloned galanin receptors (*i.e.*, GAL1, GAL2 and GAL3) could possibly be expressed at the presynaptic terminals. Unfortunately, all available antagonists for the galanin receptor are peptide derivatives of galanin itself and not type-selective. Nevertheless, data on the relative potency of galanin and its analogues can offer clues to the identity of the galanin receptors that might be involved. Galanin itself has high binding affinity for all three receptor subtypes, whereas galanin(1-16) has high affinity for galanin GAL1 and GAL receptors and low affinity for galanin GAL3 receptors (11, 16, 96). The modified galanin peptide [D-Trp$^2$]galanin is reported to have significant selectivity for galanin GAL2 over GAL1 and GAL3 receptors (13, 16). GALP, which is an isolated 60-amino acid galanin-like peptide, also displays high

affinity for the galanin GAL2 receptor over the galanin GAL1 receptor (97). Binding and pharmacological analysis reported by Ohtaki *et al.* (97) found high-affinity binding of galanin at the GAL1 receptor with binding of GALP being 44-fold less potent. GALP and galanin both have high affinity for the galanin GAL2 receptor (97). Galanin and galanin(1-16) inhibits slow noradrenergic IPSPs and fast nicotinic EPSPs with similar $IC_{50}$ values, while [D-Trp$^2$]galanin is inactive (28). GALP mimics the inhibitory actions of galanin and galanin(1-16), however, with significantly lower potency relative to that of galanin and galanin(1-16). Two observations support the galanin GAL1 rather than the galanin GAL2 receptor subtype as the mediator for presynaptic inhibition at sympathetic and cholinergic nerve terminals in the submucosal plexus. One is the absence of action of [D-Trp$^2$]galanin, which is an acknowledged galanin GAL2 receptor agonist. The second is that the rank order of potency galanin = galanin(1-16) > GALP for presynaptic inhibitory activity. On the other hand, expression of the galanin GAL3 receptor by noradrenergic and cholinergic axons in the enteric nervous system cannot be excluded. Availability of effective pharmacologic tools in the form of selective galanin GAL3 receptor agonists and antagonists will be necessary for resolution of this question.

The chimeric peptides C7, M15, M32, M35 and M40 held promise as effective tools for identification of the galanin receptor types involved in the presynaptic actions of galanin in the enteric nervous system based on reports that they were effective antagonists at galanin receptors in other systems (2, 43, 44, 98, 99). Nevertheless, experience with chimeric peptide fragments as galanin receptor antagonists was similar to an earlier experience in which peptide fragments of substance P were reported to be selective antagonists at substance P receptors on enteric neurons. In neither case did the peptide fragments behave as selective antagonists. They behaved instead as agonists (100, 101). The agonist activity of the chimeric peptides to suppress synaptic transmission in the enteric nervous system is notably weaker than galanin and this is similar to reports for other systems (26, 27).

In addition to fast EPSPs and slow IPSPs, focal electrical stimulation evokes slow EPSPs in both AH- and S-type neurons in the myenteric and submucosal plexuses. Slowly activating membrane depolarization continuing for several seconds to minutes after termination of release of the neurotransmitter from the presynaptic terminal underlies slow EPSPs. Enhanced excitability reflected by long-lasting trains of action potentials is the hallmark of the event. AH neurons, which fire only a single spike at the beginning of a depolarizing current pulse in the inactivated state, will fire repetitively in response to depolarizing pulses when the slow EPSP is in effect. Postspike hyperpolarization in AH neurons is suppressed during slow EPSPs. Suppression of the afterhyperpolarization is part of the mechanism that permits repetitive spike discharge at increased frequencies during the enhanced state of excitability. Several messenger substances mimic slow EPSPs when applied experimentally to enteric neurons and receptors for more than one of the messenger substances may be present on the same neuron. Substance P, serotonin and muscarinic action of acetylcholine fulfill criteria for function as neurotransmitters for enteric slow EPSPs.

The presence of galanin in the bathing medium suppresses both stimulus-evoked slow EPSPs and the slow EPSP-like actions of putative neurotransmitters. Action of galanin to open inwardly rectifying potassium channels in the postsynaptic membrane of AH neurons clamps the membrane potential near the potassium equilibrium potential of ≈ 90 mV and underlies part of the inhibitory action on the slow EPSP. Elevated potassium conductance offsets the decreased potassium conductance that accounts for the depolarizing phase of the slow EPSP. Whether galanin acts to prevent the release of the neurotransmitter(s) for the slow EPSPs, as is the case for fast EPSPs and slow IPSPs in enteric neurons, has not been determined. The evidence that the GAL1 receptor mediates the inhibitory action of galanin on slow EPSPs is essentially the same as cited above for the hyperpolarizing action of galanin at the cell bodies of AH-type enteric neurons and its presynaptic inhibitory actions.

## Conclusions

Galanin is a signal peptide that is expressed by neurons in the brain, spinal cord, dorsal root spinal ganglia and ganglia of the autonomic nervous system. The primary action of galanin in the nervous system is inhibitory. Interaction of galanin with its receptors on the cell bodies of neurons leads to opening of potassium conductance channels, suppression of calcium conductance, membrane hyperpolarization and suppression of excitability. Binding of galanin to its receptors on presynaptic terminals inhibits the release of neurotransmitters. These two actions of galanin at the single neuron level account for the emergent effects at the integrated circuit level of neural organization that include alterations in cognition and memory functions in the brain, sensory processing of nociceptive information in the spinal cord and integrative function of the enteric nervous system in control of gastrointestinal motility and secretory behavior. Three galanin receptors have been cloned and present tempting targets for pharmacological intervention for disorders including central deficits of cognition, neuropathic pain and a variety of functional gastrointestinal disorders. Lack of availability of selective nonpeptide antagonists for each of the three receptor types is presently a barrier to advancement of understanding of function at the basic science level and development of therapeutic strategies at the clinical level.

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