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Galanin and spinal pain mechanisms: Where do we stand in 2008?

X.-J. Xu[a], T. Hökfelt[b] and Z. Wiesenfeld-Hallin[a,*]

[a] Department of Clinical Neuroscience, Division of Clinical Neurophysiology, Karolinska University Hospital-Huddinge, 141 86 Huddinge (Sweden), Fax: +46 8 58587050,
e-mail: zsuzsanna.wiesenfeld-hallin@ki.se
[b] Department of Neuroscience, Division of Chemical Neurotransmission, Karolinska Institutet, Stockholm (Sweden)

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Abstract. Since the discovery of galanin in 1983, one of the most frequently mentioned possible physiological functions for this peptide is spinal pain modulation. This notion, initially based on the preferential presence of galanin in dorsal spinal cord, has been supported by results from a large number of morphological, molecular and functional studies in the last 25 years. It is generally agreed that spinally applied galanin produces a biphasic dose-dependent effect on spinal nociception through activation of GalR1 (inhibitory) or GalR2 (excitatory) receptors. Galanin also appears to have an inhibitory role endogenously, particularly after peripheral nerve injury when the synthesis of galanin is increased in sensory neurons. In recent years, small-molecule ligands of galanin receptors have been developed, raising the hope that drugs affecting galaninergic transmission may be used as analgesics. (Part of a Multi-author Review)

Keywords. Neuropathic pain, nerve injury, inflammation, spinal cord, opioids.

Galanin and galanin receptors in sensory neurons and in the spinal cord

Rökaeus et al. were the first to show that galanin-like immunoreactivity (-LI) could be observed in neurons and fiber networks in dorsal spinal cord [1]. This was quickly followed by several studies showing that galanin is expressed in a small number of small-sized dorsal root ganglion (DRG) neurons in rat and that the number of galanin-LI-containing fibers in the dorsal horn is markedly reduced following dorsal rhizotomy or neonatal capsaicin treatment, suggesting the occurrence of galanin in sensory neurons and their terminals [2, 3]. The galanin-positive sensory neurons in rodents also contain substance P (SP) and calcitonin gene-related peptide (CGRP) [4]. The number of neurons that express galanin under normal conditions in the DRG appears higher in monkeys [5]. In the dorsal horn, galanin-LI has been localized mainly in neurons in lamina II, where it coexists with GABA, enkephalin and neuropeptide Y (NPY) [6, 7]. Another population of galanin-immunoreactive (IR) neurons is in the area around the central canal (laminae VII and X) [8], and these neurons contain cholecystokinin (CCK) and project to contralateral, medial posterior, thalamic structures [8].

High-density galanin binding sites have been found in laminae, I, II and X of the normal rat and monkey spinal cord which are not affected by dorsal rhizotomy or neonatal capsaicin, suggesting that galanin receptors mainly are present on postsynaptic dorsal horn neurons [9–11]. Three subtypes of galanin receptors, GalR1, GalR2 and GalR3, have been identified [12]. *In situ* hybridization studies have shown that many intrinsic neurons express GalR1 receptor mRNA in the dorsal horn of the spinal cord [13–16], whereas only few dorsal horn neurons express detectable GalR2 receptor mRNA [15, 16, see also 17]. GalR3

* Corresponding author.

receptor mRNA has so far only been described in spinal cord using blot techniques [17].
Galanin receptors in DRG neurons have been analysed with *in situ* hybridization and with blot techniques, which show a high level of expression of GalR1 and -R2 and lower levels of GalR3 mRNAs in DRGs [15, 17–21]. The *in situ* studies have shown the presence of GalR1 mRNA mainly in medium-sized and large, often CGRP-positive neurons [15, 19, 21] and GalR2 mRNA in many, mostly small CGRP-positive neurons [20, 21].

Effects of galanin on spinal nociception and its pharmacology under normal condition

The early behavioral studies examining the effect of intrathecal (i.t.) galanin on nociceptive responses generated conflicting results with analgesia in thermal nociceptive tests and hyperalgesia or analgesia in tests using mechanical stimulation [22–26]. By using a nociceptive flexor reflex model, we systematically analysed the effect of i.t. application of galanin on spinal nociception over a large dose range in the late 1980s [27, 28] (Fig. 1). Galanin produced a biphasic dose-dependent effect on spinal nociceptive excitability with facilitation at low doses and depression at high doses (Fig. 1). The depressive effect of galanin on the flexor reflex is not associated with an effect on the monosynaptic reflex, suggesting that the effect is not due to motor neuron inhibition [28]. Thus, at the time galanin was thought to be a unique sensory peptide in that other peptides, such as SP, CGRP, vasoactive intestinal peptide and somatostatin, produced purely excitatory effects. We have also shown that galanin may be particularly effective in reducing spinal hyperexcitability. Thus, i.t. galanin at doses which did not depress the baseline flexor reflex strongly blocked activity-dependent increase in reflex magnitude after repetitive activation of unmyelinated fibers innervating muscle and skin [28–30]. We and others have shown that this effect of galanin is at least in part due to a postsynaptic blockade of the excitatory effect of SP and CGRP, peptides known to be involved in the mediation of spinal hyperexcitability [29, 31]. Galanin may also reduce SP release in the spinal cord [31]. In addition to producing behavioral antinocieption, galanin also potentiated the antinociceptive effect of morphine and the NMDA antagonist AP-5 [24, 32]. Galanin receptor antagonists reduce the spinal effect of morphine and several other antinociceptive agents [33, 34].
Both the excitatory and inhibitory effects of galanin can be fully mimicked by the N-terminal fragment galanin-(1–16), but not by the C-terminal fragment galanin-(17–29) [35], agreeing with pharmacological studies showing that the N-terminal portion of galanin is essential for receptor recognition and agonist activity at all three subtypes of galanin receptors [13, 36]. Moreover, the spinal effect of galanin is antagonized by chimeric peptide antagonists, such as M-15 [galanin-(1–13)-SP-(5–11)], M-32 [galanin-(1–13)-neuropeptide Y-(25–36)] and M-35 [galanin-(1–13)-bradykinin-(2–9)] [37–39].
The inhibitory, but not excitatory, effect of i.t. galanin was reduced in rats treated with peptide nucleic acid (PNA) antisense reagents against GalR1 receptor [40], suggesting that GalR1 receptor is responsible for the inhibitory effect of galanin. Recent extensive work by Hua et al. have also confirmed the involvement of GalR1 receptor in mediating galanin antinociception and in its interaction with opioids and substance P [31, 32]. On the other hand, Liu et al. [36, 41] have shown that the hyperalgesic effect of low doses of galanin is mimicked by a selective GalR2 agonist. In contrast to GalR1 receptors, which are only coupled to Gi proteins and induce inhibition, activation of the GalR2 receptor may lead to the stimulation of multiple intracellular events, including inositol phosphate hydrolysis, mobilization of intracellular calcium and Ca^{2+} dependent Cl^{-} channel activation [12, 42].

The physiological role of galanin in nociception under normal condition and after nerve injury

Galanin is normally expressed only at low levels in an apparently limited number of sensory neurons in rodents. Spinally administered galanin receptor antagonist M-35 produced a minimal enhancement of C-fiber stimulation-induced spinal sensitization in normal rats [43]. Mice with null mutation of either galanin or GalR1 receptors showed moderately decreased response threshold in some, but not all, tests of heat nociception [44–46]. In agreement, several lines of mice overexpressing galanin in sensory neurons exhibited moderately increased nociceptive threshold [47–49]. Taken together, these results suggest that the role of endogenous galanin played in sensory neurons is that of inhibition of spinal nociception, although such a role is moderate [36, 50].
It was shown early on that sciatic nerve transection induced a marked increase in galanin expression in DRG neurons, with detection of galanin-IR in about 50% of DRG neurons of all sizes [51, 52] (Fig. 2). The increased expression of galanin is seen already 24 h after nerve injury, reaches a maximum within a week and is maintained, if nerve regeneration does not take place [52]. Galanin is also upregulated in rat DRG after partial sciatic nerve injury and/or nerve con-

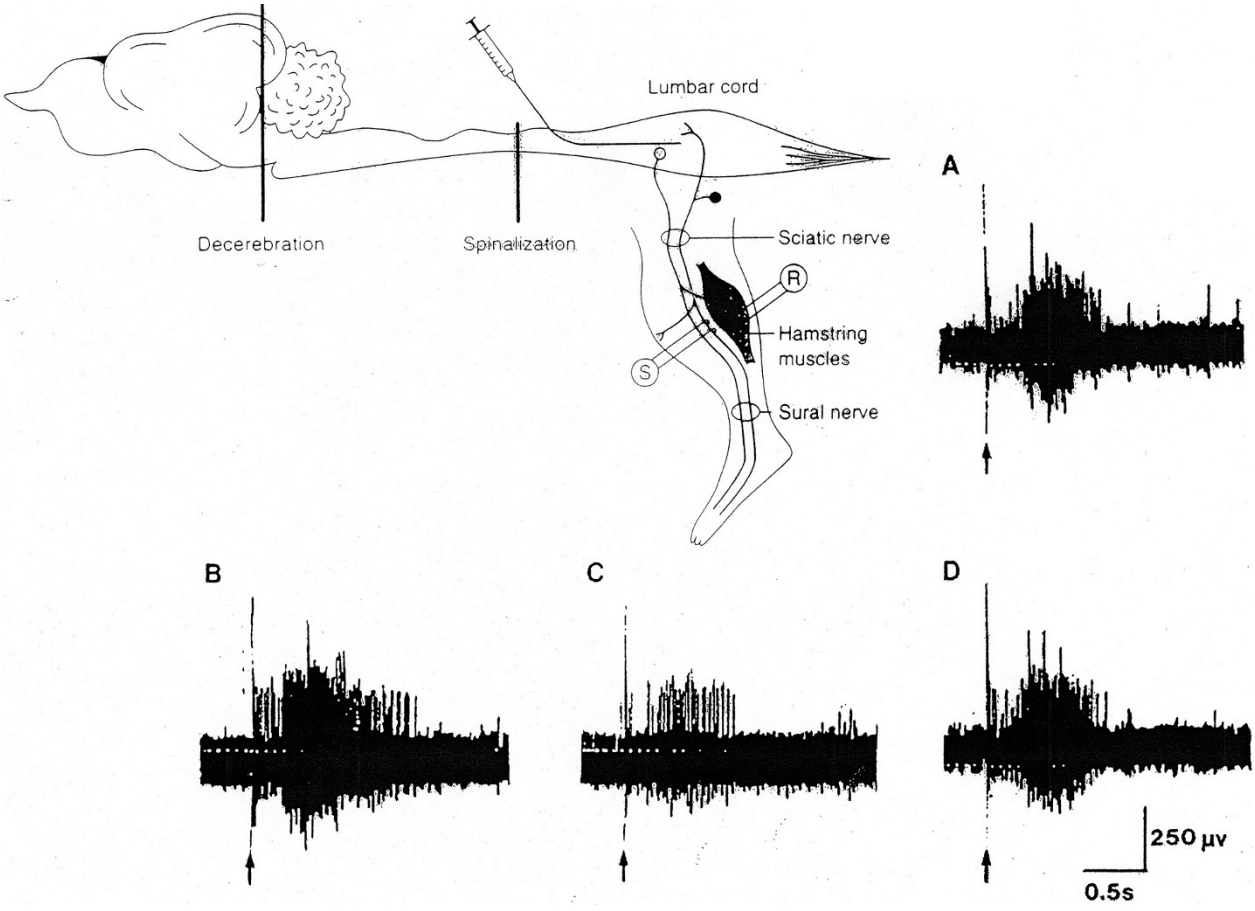

Figure 1. Schematic illustration of the flexor reflex preparation used to study the effect of i.t. galanin on spinal nociceptive excitability and illustration of the biphasic dose-dependent effect of galanin on reflex discharges. The flexor reflex is elicited by electrical stimulation of the sural nerve or its innervation area in the foot at a strength that activated C-fibers, and the reflex is recorded as EMG activity from the ipsilateral hamstring muscles. An i.t. catheter is placed so that peptides can be administered directly onto the spinal cord lumber enlargement. In *A-D*, the effect of galanin on reflex discharges to a single stimulation of the sural nerve activating C-fibers. (*A*) Control response. (*B*) Facilitatory effect of a low dose of 100 ng i.t. galanin 1 min after injection. (*C*) Inhibitory effect of 10 µg galanin 20 min after injection. (*D*) Recovery of reflex discharge 60 min after 10 µg i.t galanin.

striction, although the magnitude of such upregulation varies, possibly due to differences at the site of the lesion and the extent of injury [53–55]. Disruption of axonal transport by local application of vinblastine can also induce galanin upregulation, indicating that factors synthesized in peripheral target tissues may inhibit galanin production [56]. Nerve growth factor (NGF) seems to be one of the factors, since it has been shown to partly counteract axotomy-induced galanin upregulation both *in vivo* [57] and *in vitro* [58]. However, leukemia inhibitory factor (LIF) has turned out to be a key molecule in controlling galanin expression after axotomy [59–61]. After nerve injury, there is also a moderate increase in the number of galanin-IR primary afferent terminals in laminae I and II, with a limited expansion of galanin-IR into lamina III of the spinal cord [62]. No change in galanin expression in dorsal horn interneurons can be detected in rats and monkeys [62]. Moreover, there is also no change in the expression of GalR1 and R2 in dorsal horn neurons [62]. The number of DRG neurons expressing GalR1 and R2 is moderately reduced in axotomized rats [19, 20].

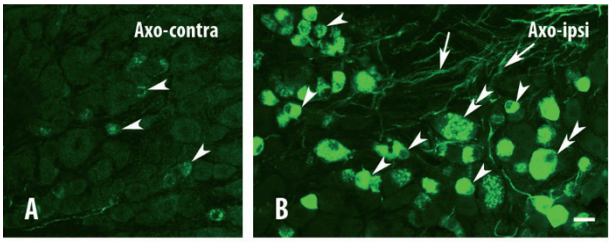

Figure 2. Immunohistochemical demonstration of galanin upregulation in DRG neurons after unilateral nerve transection. (*A*) Shows a small number of galanin-positive neurons (arrows) on the contralateral side, and (*B*) the dramatic, ipsilateral increase in intensity and number of galanin-immunoreactive cell bodies and axons. Bar indicates 25 µm. Micrographs by Dr. Tiejun Shi.

The increased synthesis, central transport and terminal storage of galanin after complete or partial nerve injury suggest that galanin may be released in increasing amounts from damaged sensory neurons. Indeed, using the antibody microprobe technique, Duggan's group has shown an increased unstimulated galanin release in rats following peripheral nerve injury and that such release can be further increased by electrical stimulation of injured peripheral nerves at intensities that activate C-fibers [63, 64].

Functional studies have shown that the physiological inhibitory role of galanin becomes more important after nerve injury. Thus, we and others have shown that the inhibitory effect of i.t. galanin on reflex and neuronal excitability is increased after nerve injury [65, 66]. The enhancement of C-fiber-mediated spinal sensitization by i.t. M-35 is also markedly increased in axotomized rats [43] (Fig. 3). In behavioral studies, reducing the action of galanin by either chronic i.t. infusion of M-35 [67] or by galanin antisense [68] leads to increased neuropathic pain-like behaviors. Conversely, adding galanin or galanin-secreting cells reduced neuropathic pain in animal models [69–71]. Mice overexpressing galanin also showed diminished neuropathic pain-like behaviors after nerve injury [47, 48]. Thus, it may be suggested that galanin is tonically active in suppressing painful input from injured sensory fibers. Consequently, insufficient galanergic control may contribute to the development of neuropathic pain. Indeed, in rats subjected to the Bennett model of nerve injuy, the extent of galanin upregulation has been shown to be inversely correlated to the severity of pain-like behavior among individual rats subjected to the same type of injury [55], and i.t. M-35 triggers a significant pain-like state in rats that did not develop neuropathic pain [72].

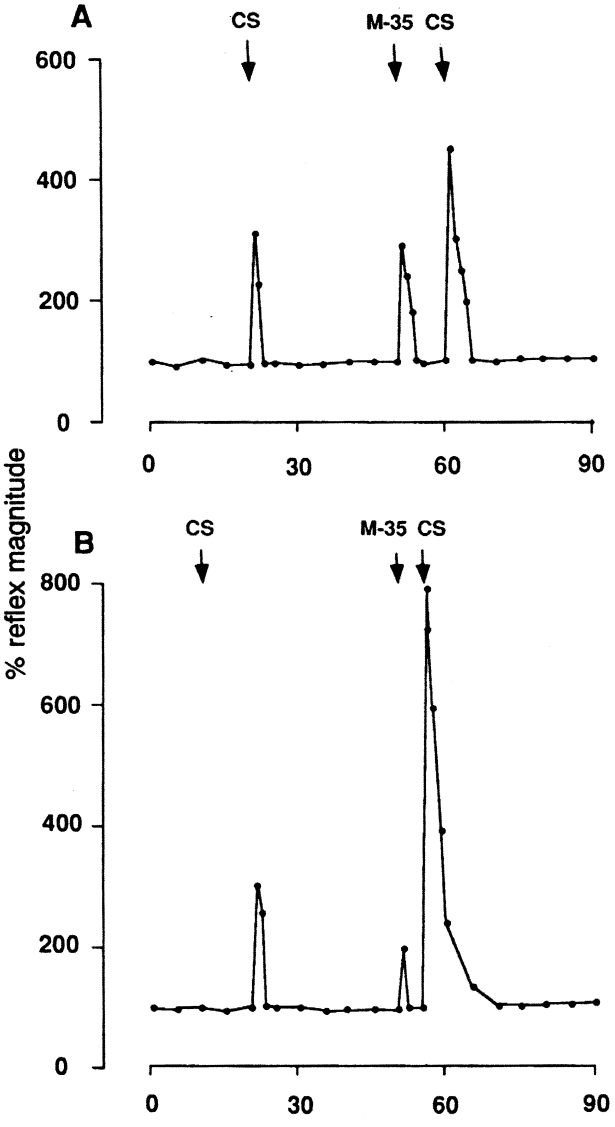

Figure 3. Illustration of the increased endogenous inhibitory control by galanin on flexor reflex excitability after nerve injury. The effect of i.t. M-35 on flexor reflex excitability and C-fiber CS-induced reflex facilitation is shown in normal rat (*A*) or rats after sciatic nerve section (*B*). Note the markedly increased effect by M-35 on C-fiber CS-induced facilitation of the flexor reflex in axotomized rats suggesting an increased galanergic inhibitory control after nerve injury in correlation with increased availability of galanin in the DRG neurons shown in Figure 2.

The potential of galaninergic drugs as analgesics

As mentioned above, the antinociceptive effect of galanin in normal rodents can only be seen at high doses and is moderate. Thus, it is unlikely that drugs that act on galanin receptors may be useful to treat acute and/or nociceptive pain. Peripheral nerve injury in humans frequently leads to the development of chronic neuropathic pain, which is difficult to treat [73]. Experimental studies have demonstrated that nerve injury induces complex plasticity in sensory neurons, spinal cord and supraspinal structures [62, 74], indicating that treatments based on our understanding of normal mechanisms may not be adequate for the treatment of neuropathic pain. Therefore, alternative treatments targeting mechanisms of neuropathic pain are needed. In this regard, galanin may be uniquely positioned as it plays an important inhibitory role after nerve injury and insufficient galaninergic control may be responsible for the development of neuropathic pain in some cases. Thus, drugs that target galanin receptors, possibly of the R1 subtype, deserve to be further evaluated for their analgesic potential in neuropathic pain.

Two low molecular weight ligands of galanin receptor have been developed in recent years that can be administered systemically [75–77]. We have shown that one of these compounds, galnon, reduces hyper-

algesia to heat stimulation in rats with partial sciatic nerve injury [76]. Another compound, galmic, has also been shown to exhibit an antinociceptive effect upon systemic administration, but in a model of inflammatory pain [77]. No data are available on the effect of galmic on neuropathic pain. In addition to being analgesics per se, drugs that act on the galanin receptor may also be useful clinically as adjuvant analgesics to opioids. It is interesting to note in this context that galanin and its agonists are also able to reduce opioid withdrawal [78].

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