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Review

Neuropeptides in neuropathic and inflammatory pain with special emphasis on cholecystokinin and galanin

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Abstract

Neuropeptides present in primary afferents and the dorsal horn of the spinal cord have an important role in the mediation of nociceptive input under normal conditions. Under pathological conditions, such as chronic inflammation or following peripheral nerve injury, the production of peptides and peptide receptors is dramatically altered, leading to a number of functional consequences. In this review, the role of two neuropeptides that undergo such altered expression under pathological conditions, cholecystokinin (CKK) and galanin, is reviewed.

Keywords: Neuropeptide; Galanin; Cholecystokinin

1. Background

Neuropeptides are present in a subpopulation of primary afferents. Under normal circumstances, the most common neuropeptides found in primary afferents are substance P and calcitonin gene-related peptide (see Hökfelt et al., 1994, 1997 for review). Under pathological conditions, such as nerve injury or peripheral inflammation, peptide and peptide receptor content of primary afferents is dramatically altered. For example, substance P and calcitonin gene-related peptide levels are increased during inflammation, but dramatically decreased in dorsal root ganglion cells following axotomy (see Hökfelt et al., 1994, 1997 for review). Other peptides that are usually present in small quantities in normal DRG neurons, such as galanin and cholecystokinin (CKK), can be downregulated or unchanged during inflammation and upregulated following axotomy (see Hökfelt et al., 1994, 1997 for review). Furthermore, there are also changes in peptide and peptide receptor levels in the dorsal horn under these pathological conditions. In this review, the possible role of galanin and cholecystokinin in neuropathic and inflammatory pain will be presented.

2. Cholecystokinin–opioid interactions

2.1. The antiopioid effect of cholecystokinin

The concept of endogenous antiopioid peptides has been researched extensively (see Cesselin, 1995; Wiesenfeld-Hallin and Xu, 1999 for review). These peptides include cholecystokinin, angiotensin II and melanocyte inhibiting factor-related peptides (Faris et al., 1983; Kastin et al., 1984; Yang et al., 1985). Among these, cholecystokinin has been best studied and the development of specific nonpeptide antagonists of cholecystokinin receptors has enabled the elaboration of the function of cholecystokinin in opioid analgesia.

Cholecystokinin belongs to the gastrin family of peptides. In the nervous system, it is mainly present in the form of the carboxy-terminal octapeptide CCK-8 (Rehfeld, 1978) and cholecystokinin-like immunoreactivity and cholecystokinin mRNA is widely distributed in the central nervous system (Schiffmann and Vanderhaeghen, 1991; Lindefors et al., 1993). There is considerable overlap in the anatomical distribution of cholecystokinin, endogenous opioids and their receptors in the spinal cord and brain, which may form the basis for the documented interaction between the two systems.

Two subtypes of cholecystokinin receptors have been identified, those predominantly in the periphery (CCK-A

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or CCK_1) vs. those in the central nervous system (CCK-B or CCK_2) (Moran et al., 1986). However, the "peripheral" type CCK_1 receptor is also present in the central nervous system, particularly in primates (Hill et al., 1990). Receptor binding sites for cholecystokinin are located throughout the spinal dorsal horn with highest density in the superficial laminae. The receptors are predominantly type 2 in rat, whereas in primate, the majority of receptors are type 1 (Hill et al., 1990; Ghilardi et al., 1992). In normal rats, only a small number of DRG neurons express cholecystokinin receptor mRNA (Zhang et al., 1993).

Itoh et al. (1982) and Faris et al. (1983) were the first to demonstrate that cholecystokinin attenuated antinociception induced by β-endorphin or morphine. Many studies since then has shown that cholecystokinin reduces the effect of exogenous opioids upon systemic, intrathecal (i.t.) or intracerebral injection as tested with behavioral and electrophysiological techniques (see Cesselin, 1995; Wiesenfeld-Hallin and Xu, 1996, 1999 for review). Cholecystokinin also blocks the antinociceptive effect of endogenous opioids following electroacupuncture or electric shocks (Watkins et al., 1985; Han et al., 1986). The antiopioid action exerted by cholecystokinin appears to be tonic as cholecystokinin receptor antagonists enhance opioid-induced antinociception (see Wiesenfeld-Hallin and Xu, 1996 for review). Results obtained with cholecystokinin receptor antagonists and receptor antisense oligonucleotides have indicated that the CCK_2 receptor mediates for the interaction between the cholecystokinin and opioid systems in rodents (Dourish et al., 1990; Wiesenfeld-Hallin et al., 1990a,b; Vanderah et al., 1994). The efficacy of endogenously released opioids following the administration of endopeptidases and electroacupuncture is also potentiated by cholecystokinin receptor antagonists (Han et al., 1986; Valverde et al., 1994). Even clinical placebo analgesia which is opioid in nature is potentiated by a cholecystokinin receptor antagonist (Benedetti, 1996).

The mechanism by which cholecystokinin antagonizes opioid analgesia is not fully understood. The blockade of morphine analgesia by cholecystokinin is not due to a direct hyperalgesic effect of cholecystokinin as cholecystokinin does not alter baseline pain threshold and most receptor binding studies failed to show an affinity of cholecystokinin for opioid receptors (Wang and Han, 1989). However, there is evidence that binding of CCK-8 to the cholecystokinin receptor reduces the binding affinity of μ-opioid receptor ligands (Wang and Han, 1989). Furthermore, cholecystokinin may counteract intracellular events after opioid receptor activation (Wang et al., 1992).

2.2. Cholecystokinin and opioid sensitivity in rats with nerve injury or inflammation

The clinical analgesic effect of morphine varies in different pain states. Patients with neuropathic pain following injury to the nervous system usually responds poorly to opioids (Arnér and Meyerson, 1993). This is supported by experimental evidence as in rats morphine causes less spinal antinociception after peripheral nerve injury (Xu and Wiesenfeld-Hallin, 1991a; Xu et al., 1994; Lee et al., 1995; Mao et al., 1995; Ossipov et al., 1995). Axotomy causes an upregulation of cholecystokinin and CCK_2 receptor mRNA in rat dorsal root ganglion cells (Table 1) (Verge et al., 1993; Xu et al., 1993; Zhang et al., 1993). The role of cholecystokinin in modifying morphine-induced antinociception has been studied in electrophysiological and behavioral experiments in nerve-injured rats. Systemic morphine induced reduced antinociception in axotomized rats compared to normals and addition of the CCK_2 receptor antagonist CI-988 strongly potentiated the effect of morphine (Xu et al., 1994). Furthermore, CCK_2 receptor antagonists reversed the lack of effect of i.t. morphine in alleviating neuropathic pain-like symptoms in rats after complete or partial peripheral nerve injury (Xu et al., 1993; Nichols et al., 1995). CCK_2 receptor antagonist has been also shown to produce analgesia after constriction injury of the rat sciatic nerve (Yamamoto and Nozaki-taguchi, 1995). Thus, in rats, opioid insensitivity after nerve injury may be related to enhanced activity of the endogenous cholecystokinin system.

Sensitivity to opioids may be also modified by cholecystokinin in animal models of inflammation, although it is the opposite of that observed following nerve injury (Table 1). The antinociceptive effect of opiates is enhanced in animals with acute inflammation (Stanfa and Dickenson, 1993). While exogenous cholecystokinin still attenuates the antinociceptive effect of morphine, cholecystokinin receptor antagonists no longer enhance morphine's effect during inflammation. Thus, although the mechanism by which cholecystokinin reduces the action of morphine is still intact, there may be a decrease in the availability of cholecystokinin within the spinal cord following inflammation, either due to decreased release of cholecystokinin or reduced level of the peptide within the dorsal horn, underlying the lack of effect of the cholecystokinin antagonists.

Table 1
Summary of the changes in levels of CCK, CCK_2 receptor, galanin and GAL-1 and GAL-2 receptors following axotomy and inflammation compared to control levels in rats

	Axotomy		Inflammation	
	DRG	Dorsal horn	DRG	Dorsal horn
CCK	(+)	−	±	±
CCK_2R	+++	±	±	±
Galanin	+++	±	−	++
GAL-1R	−	±	−	±
GAL-2R	−	±	++	±

The table is based on Hökfelt et al. (1997), Xu et al. (2000) and Zhang et al. (2000a,b,c).
+ = increase, − = decrease, ± = unchanged, (+) = possible increase.

In a recent series of studies, we examined the interaction of the μ-opioidergic and cholecystokinin systems in the rat spinal cord during inflammation and nerve injury and compared them to the normal state. In vivo studies were carried out with the microdialysis technique in the rat spinal cord and morphological studies examining the interaction of the μ-opioid receptor and cholecystokinin were carried out with single- and double-color immunofluorescence technique.

2.3. Extracellular levels of cholecystokinin in normal rats

As summarized above, the extracellular level of cholecystokinin under different conditions may be a factor in its modulation of opioid action. The microdialysis technique was used to measure the level of CCK in the rat spinal cord in vivo combined with radioimmunoassay (Gustafsson et al., 1998). The basal cholecystokinin level in normal rats was usually below or close to the detection limit of the radioimmunoassay. However, perfusion of the probe with Krebs–Ringer solution with increased K^+ concentration for 30 min induced a more than sixfold increase in the extracellular level of cholecystokinin-like immunoreactivity (Gustafsson et al., 1998).

2.4. Effect of morphine administration on cholecystokinin release in normal rats and after axotomy

In rats with intact sciatic nerves, morphine was injected intravenously or applied topically on the spinal cord, which resulted in a significant increase of cholecystokinin in the dialysate (Lucas et al., 1998), in agreement with several previous in vivo and in vitro studies (Benoliel et al., 1991; Zhou et al., 1993; Bourgoin et al., 1994). Morphine also increased cholecystokinin levels similarly after topical or intravenous (i.v.) administration in rats with unilateral sciatic nerve section (Lucas et al., 1998). The release of cholecystokinin by morphine was blocked by naloxone, indicating the involvement of opioid receptors in the release of cholecystokinin. In contrast, 100 mM K^+ did not release cholecystokinin in axotomized rats (Gustafsson et al., 1998).

It has been suggested that there may be increased activity of cholecystokinin after nerve injury (Xu et al., 1993; Stanfa et al., 1994) based on the upregulation of cholecystokinin and CCK_2 receptor mRNA in DRG neurons following axotomy (Verge et al., 1993; Xu et al., 1993; Zhang et al., 1993) and the increase in cholecystokinin activity may underly the reduced effectiveness of morphine in treating neuropathic pain. The results from the microdialysis studies suggest that the release of cholecystokinin by morphine after axotomy is unchanged, indicating that factors other than availability of cholecystokinin play a role in the activity of the cholecystokinin system. This is supported by data indicating that the translation of cholecystokinin mRNA into peptide is very low in axotomized dorsal root ganglion neurons (Verge et al., 1993; Brás et al., 1999). Furthermore, the lack of K^+ stimulation-induced release of cholecystokinin in axotomized rats also indicates that axotomy does not lead to increased availability of peptide (Gustafsson et al., 1998). A CCK_2 receptor-mediated inhibition of the release of cholecystokinin is more likely as there is an upregulation of the receptor on afferent terminals following axotomy (Zhang et al., 1993) and the addition of a CCK_2 receptor antagonist restores K^+-induced release (Gustafsson et al., 1998).

It is surprising that morphine released cholecystokinin in axotomized rats. μ-Opioid receptor-like immunoreactivity and cholecystokinin-like immunoreactivity are colocalized in dorsal horn neurons (Zhang et al., 2000c), which may indicate a direct release mechanism. However, neurotransmitter release following opioid receptor activation does not agree with the generally accepted inhibitory action of opioids. The ability of opioids to induce neurotransmitter release has been therefore explained by a disinhibitory process (Zieglgänsberger et al., 1979). However, there is evidence that opioid receptors may be coupled to phospholipase C (Cheng and Huang, 1991), resulting in increased inositol 1,4,5-phosphate (IP_3) level that is pertussis toxin sensitive and Ca^{2+} dependent (Jin et al., 1992; Smart et al., 1994). Thus, both direct and indirect routes of cholecystokinin release by morphine are possible.

2.5. Effect of morphine and K^+ on cholecystokinin release during peripheral inflammation

During unilateral carrageenan-induced inflammation, the unstimulated level of cholecystokinin overflow was similar in normal and axotomized animals, although the variability in cholecystokinin levels was larger than in rats with intact or axotomized nerves (Lucas et al., 1998). Surprisingly, morphine did not increase the level of extracellular cholecystokinin above basal levels following either i.t. or i.v. administration (Lucas et al., 1998). In contrast, 100 mM K^+ induced a significant increase in the release of cholecystokinin with a more than threefold increase over basal values in the inflamed rats (unpublished observations), similar to K^+-induced release in normal rats (Gustafsson et al., 1998). These data suggest that reduced level of cholecystokinin in the extracellular space plays a key role in the increased analgesic efficacy of morphine during inflammation (Stanfa and Dickenson, 1993). However, this is unlikely to be due to decreased synthesis of cholecystokinin during inflammation as the immunohistochemical study failed to show an inflammation-induced effect on cholecystokinin-like immunoreactivity (Zhang et al., 2000c, see below). Moreover, the results with K^+ stimulation also indicate that a releasable pool of cholecystokinin-containing vesicles is still present in the spinal dorsal horn. Thus, it is most likely that inflammation influences release mechanisms or exocytosis by morphine.

2.6. Localization and colocalization of μ-opioid receptor- and cholecystokinin-like immunoreactivities in the spinal dorsal horn

Single- and double-colour immunofluorescence was applied to study the relationship of μ-opioid receptor- and cholecystokinin-like immunoreactivities in superficial dorsal horn neurons to establish a morphological basis for the interaction between opioids and cholecystokinin (Zhang et al., 2000c). Fibers containing μ-opioid receptor- and cholecystokinin-like immunoreactivities were observed in laminae I and II. Many neurons in lamina II were μ-opioid receptor immunoreactive, whereas only a few cholecystokinin-positive neurons could be observed in lamina II without colchine treatment. In colchine-treated rats, μ-opioid receptor- and cholecystokinin-like immunoreactivities were colocalized in some neurons in the medial part of lamina II. μ-Opioid receptor-like immunoreactivity was present in 65% of counted cholecystokinin-positive neuron profiles, whereas 40% of counted MOR-positive neurons contained cholecystokinin-like immunoreactivity. Of 87 μ-opioid receptor-immunoreactive neuron profiles, 9 profiles also contained cholecystokinin-like immunoreactivity. However, most cholecystokinin-positive neuron profiles contained μ-opioid receptor (9 out of 12).

These results indicated considerable colocalization between cholecystokinin-like immunoreactivity and μ-opioid receptor-like immunoreactivity in lamina II dorsal horn neurons. Thus, some cholecystokinin-containing neurons may be directly activated by morphine and other μ-opioid receptor agonists as these neurons also possess μ-opioid receptor. Morphine-induced in vivo release of cholecystokinin in dorsal spinal cord, suggesting that the opioid may directly act on these cholecystokinin-containing neurons, leading to cholecystokinin release. These results also suggested that there is an additional cholecystokinin-positive neuron population in lamina II of the dorsal horn which does not contain μ-opioid receptor-like immunoreactivity. This would provide a morphological basis for the differential release of cholecystokinin by morphine and K^+. These morphological data, however, do not exclude the possibility that opioid-induced cholecystokinin release may be mediated through indirect mechanisms, such as disinhibition.

2.7. Influence of axotomy, inflammation and systemic morphine on μ-opioid receptor- and cholecystokinin-like immunoreactivities

A moderate reduction in μ-opioid receptor- and cholecystokinin-like immunoreactivities was observed in laminae I and II of the ipsilateral dorsal horn 7 days after axotomy (Table 1) (Zhang et al., 2000c). Systemic morphine induced a dose-related temporary (less than 30 min) further decrease in μ-opioid receptor- and cholecystokinin-like immunoreactivities ipsilateral to the axotomy. Morphine also dose-relatedly further reduced cholecystokinin-like immunoreactivity in the superficial dorsal horn more than 30 min after drug administration. Pretreatment with naloxone blocked this effect of morphine on μ-opioid receptor- and cholecystokinin-like immunoreactivities in some rats. In contrast, carrageenan-induced inflammation did not influence the distribution or intensity of μ-opioid receptor- and cholecystokinin-like immunoreactivities in the dorsal spinal cord. Moreover, morphine had no effect on μ-opioid receptor- and cholecystokinin-like immunoreactivities in inflamed rats (Zhang et al., 2000c).

These results confirm that peripheral axotomy induces a moderate decrease in μ-opioid receptor- and cholecystokinin-like immunoreactivities in the ipsilateral dorsal horn of the spinal cord (Porreca et al., 1998; Zhang et al., 1998a). It was surprising that systemic morphine further acutely reduced the levels of μ-opioid receptor- and cholecystokinin-like immunoreactivities in the dorsal horn ipsilateral to axotomy. This effect was mediated by opioid receptors as it was dose dependent and was prevented by naloxone. As morphine had no effect on μ-opioid receptor-like immunoreactivity in normal or inflamed rats or in the dorsal horn contralateral to the axotomy, it is unlikely that this is due to an unspecific action on dorsal horn neurons. Internalization of μ-opioid receptors following morphine treatment did not occur, in agreement with earlier studies showing that morphine does not cause internalization of μ-opioid receptors (Afifiy et al., 1998; Arden et al., 1995), suggests that internalization of μ-opioid receptors cannot explain the reduction in μ-opioid receptor-like immunoreactivity following morphine administration.

The antibody used in this study was raised against the carboxy-terminus of the μ-opioid receptor (Arvidsson et al., 1995). This part of the μ-opioid receptor is involved in agonist-induced internalization and de- and resensitization (Segredo et al., 1997; Afifiy et al., 1998). It is therefore possible that the rapid and brief decrease in μ-opioid receptor-like immunoreactivity is due to an interaction between the carboxy-terminus of the μ-opioid receptor and proteins which are 'activated' by μ-opioid receptor stimulation and subsequently block the binding sites of the antibody. The fact that the decrease in μ-opioid receptor-like immunoreactivity only occurs after axotomy suggests that this effect of morphine is related to changes in the local dorsal horn microenvironment as a result of axotomy. Peripheral nerve injury is known to induce marked changes in dorsal root ganglion neurons and in the dorsal horn in terms of expression of neuropeptides, nitric oxide synthase, neurotrophins and many other substances, including gangliosides, which in turn may affect the local environment and activities of dorsal horn neurons (Hökfelt et al., 1994, 1997), which may 'prime' the cholecystokinin/μ-opioid receptor neurons in the superficial dorsal horn to respond to morphine differently from normal states. However, the ability of morphine to induce cholecystokinin release after axotomy remains intact, indicating that this

phenomenon does not play a significant role in the process of peptide release.

Similar to μ-opioid receptor-like immunoreactivity, the administration of morphine also reduced cholecystokinin-like immunoreactivity in a dose- and time-related manner ipsilateral to the nerve injury, but not contralaterally in intact or inflamed rats. Moreover, this effect was observed over 30 min without recovery, supporting that a release of the peptide may have taken place and that this release may be related to activation of μ-opioid receptors and the decrease in μ-opioid receptor-like immunoreactivity. This interpretation is supported by data obtained with the microdialysis technique.

3. Galanin

Galanin is a neuropeptide of 29 or 30 (in humans) amino acids originally isolated from porcine intestine (Tatemoto et al., 1983). Galanin is widely distributed in the nervous system and, like cholecystokinin, has been implicated in a number of functions, including feeding, cognition, endocrine modulation and nociception (see Bartfai et al., 1993; Wiesenfeld-Hallin et al., 1992a; Merchenthaler et al., 1993; Crawley, 1996; Ögren et al., 1998 for review). Galanin acts on specific G-protein coupled membrane receptors. At least three human and rodent galanin receptor subtypes ($galanin_1$, $galanin_2$ and $galanin_3$ receptor) have been cloned (Habert-Ortoli et al., 1994; see Branchek et al., 2000 for review) which are coupled to G_i/G_o-proteins (see Bartfai et al., 1993; Merchenthaler et al., 1993; Branchek et al., 2000 for review).

3.1. Galanin and galanin receptors in DRG neurons and spinal cord

Galanin is normally expressed in few small-sized sensory neurons in rat dorsal root ganglia, which also contain substance P and calcitonin gene-related peptide (Ch'ng et al., 1985; Skofitsch and Jacobowitz, 1985; Ju et al., 1987a). Galanin-like immunoreactivity has also been localized in dorsal horn neurons, mainly in lamina II where it coexists with GABA, enkephalin and neuropeptide Y (Simmons et al., 1995; Zhang et al., 1995a). Galanin-immunoreactive neurons have been also identified around the central canal (Ju et al., 1987b) and these neurons contain cholecystokinin and project to contralateral medial posterior thalamic structures (Ju et al., 1987b).

High-density galanin binding sites are in laminae I, II and X of the normal rat and monkey spinal cord (Fisone et al., 1989; Kar and Quirion, 1994; Zhang et al., 1995a,b) which are unaffected or modestly increased by dorsal rhizotomy and neonatal capsaicin, suggesting that these receptors are mainly localized on postsynaptic neurons (Kar and Quirion, 1994; Zhang et al., 1995a,b). Many intrinsic neurons in the dorsal horn express $galanin_1$ receptor mRNA (Parker et al., 1995; Gustafson et al., 1996; O'Donnell et al., 1999), only few express detectable $galanin_2$ receptor mRNA (O'Donnell et al., 1999; Waters and Krause, 2000) and $galanin_3$ receptor mRNA has so far only been described in spinal cord using blot techniques (Smith et al., 1998; Waters and Krause, 2000).

In dorsal root ganglia, $galanin_1$ receptor mRNA is mainly found in medium-sized and large, often CGRP-positive neurons (O'Donnell et al., 1999; Xu et al., 1996a,b) and $galanin_2$ receptor mRNA mostly in small CGRP-positive neurons (O'Donnell et al., 1999; Shi et al., 1997). Both $galanin_1$ and $galanin_2$ receptor mRNAs are downregulated after axotomy (Xu et al., 1996a,b; Shi et al., 1997) and $galanin_2$ receptor mRNA is transiently upregulated after inflammation (Shi et al., 1997). Only lower levels of $galanin_3$ receptor mRNAs have been found in DRGs (Waters and Krause, 2000; see Branchek et al., 2000; Zhang et al., 1998b for review).

3.2. Effect of galanin on spinal excitability

Behavioral and electrophysiological studies have shown that galanin produces complex effects with a predominantly inhibitory action on spinal nociception. The antinociceptive effect of i.t. galanin is usually observed only at high doses and is moderate. I.t. galanin potentiates the antinociceptive effect of morphine (Wiesenfeld-Hallin et al., 1990b), particularly when coadministered with a CCK_2 receptor antagonist (Wiesenfeld-Hallin et al., 1990a). Galanin receptor antagonists reduce the spinal effect of morphine and several other antinociceptive agents (Reimann et al., 1994; Selve et al., 1996; Zhang et al., 2000c). Galanin reduces spinal hyperexcitability (Wiesenfeld-Hallin et al., 1989a; Xu et al., 1990, 1991) and reduces the pronociceptive behavioral effect of substance P (Kuraishi et al., 1991a). However, low-dose i.t. galanin causes spinal reflex facilitation (Wiesenfeld-Hallin et al., 1988, 1989a; Xu et al., 1990, 1991) and nociceptive behaviors have been also reported (Cridland and Henry, 1988; Kuraishi et al., 1991a,b; Kerr et al., 2000).

The spinal effect of galanin is antagonized by chimeric peptide antagonists (Bartfai et al., 1991; Wiesenfeld-Hallin et al., 1992a,b; Xu et al., 1995), but no receptor antagonists against specific receptor subtypes are available. Peptide nucleic acid antisense reagents coupled to a cellular transporter protein against $galanin_1$ receptor induced a 40% reduction in galanin binding in the dorsal horn and reduced the depressive effect of i.t. galanin on spinal hyperexcitability by two orders of magnitude (Pooga et al., 1998). No effect was observed with the control scrambled peptide nucleic acid probe (Pooga et al., 1998). In contrast, the initial excitatory effect of galanin was not reduced, but rather enhanced, in the $galanin_1$ receptor peptide nucleic acid antisense-treated rats. Thus, the depressive effect of galanin in the spinal cord may be mediated by $galanin_1$ receptors presumably localized on dorsal horn interneurons

(O'Donnell et al., 1999). This result was confirmed in a more recent study using unmodified peptide nucleic acid against galanin$_1$ receptor (Rezaei et al., 2001). Thus, other subtypes of galanin receptors may mediate its excitatory effect.

The mechanisms underlying the spinal effect of galanin are still unclear. Galanin hyperpolarizes most dorsal horn neurons in vitro (Randic et al., 1987), which may be related to its postsynaptic inhibitory effect (Xu et al., 1990). Galanin may also have presynaptic effects (Yanagisawa et al., 1986; Nussbaumer et al., 1989). Galanin, like GABA, induces an inward current in cultured dorsal root ganglion neurons (Puttick et al., 1994), which may reflect presynaptic afferent depolarization underlying presynaptic inhibition (Curtis et al., 1977). Thus, galanin, by depolarizing the membrane terminals of sensory afferents, may reduce the amount of transmitter released from nociceptive afferents.

3.3. Morphological changes in the galanin system after peripheral nerve injury

Sciatic nerve transection induces marked increase in galanin-like immunoreactivity in rat dorsal root ganglion neurons, with detection of galanin-like immunoreactivity in about 50% of dorsal root ganglion neurons of all sizes (Hökfelt et al., 1987; Villar et al., 1989). Increase of both galanin-like immunoreactivity and galanin mRNA is seen within 24 h after nerve injury, reaches maximum within a week and is maintained in the absence of nerve regeneration (Villar et al., 1989). After axotomy, galanin coexists with vasoactive intestinal peptide (Xu et al., 1990; Kashiba et al., 1992) and neuropeptide Y (Landry et al., 2000), both of which are also upregulated following axotomy (Shahab and Atkinson, 1986; Wakisaka et al., 1991). There is a moderate increase in the number of galanin-positive primary afferent terminals in laminae I and II, with a limited expansion of galanin-like immunoreactivity into lamina III of the spinal cord (Zhang et al., 1998b). Disruption of axonal transport by local application of vinblastine can also induce galanin upregulation, indicating that some factors synthesized in peripheral target tissues may tonically inhibit galanin production (Kashiba et al., 1992). Nerve growth factor has been shown to partly counteract axotomy-induced galanin upregulation both in vivo (Verge et al., 1995) and in vitro (Kerekes et al., 1997). However, leukemia inhibitory factor has turned out to be a key molecule in controlling galanin expression after axotomy (Rao et al., 1993; Corness et al., 1996; Sun and Zigmond, 1996). In fact, a close interaction between these two molecules, that is an increase in leukemia inhibitory factor combined with decrease in nerve growth factor, seems to be essential for nerve injury-induced galanin upregulation (Corness et al., 1998; Hökfelt et al., 1997).

In contrast to the marked upregulation of galanin in primary afferents after axotomy, no change in galanin expression in dorsal horn interneurons can be detected (Zhang et al., 1998b; Hökfelt et al., 1997). Moreover, there is no change in the expression of galanin$_1$ and galanin$_2$ receptors in dorsal horn neurons (Zhang et al., 1998b; Hökfelt et al., 1997) (Table 1). The number of dorsal root ganglion neurons expressing galanin$_1$ and galanin$_2$ receptors are moderately reduced in axotomized rats (Xu et al., 1996b; Shi et al., 1997).

3.3.1. Galanin upregulation after nerve injury: functional implications

Nerve injury leads to increased synthesis, central transport and terminal storage of galanin, suggesting that increased levels of galanin may be released from damaged sensory neurons. With the antibody microprobe technique, increased unstimulated galanin release in rats following peripheral nerve injury has been shown, which was further increased by electrical stimulation of C-fibers in injured peripheral nerves (Colvin and Duggan, 1998; Colvin et al., 1997).

As galanin is upregulated and released from sensory neurons after nerve injury and considering the mainly inhibitory function of this peptide, it is possible that galanin is tonically active in suppressing painful input from injured sensory fibers. Thus, low level of galanergic control may contribute to the development of neuropathic pain. Several lines of evidence support this hypothesis. Chronic i.t. infusion of the galanin receptor antagonist M-35 increased autotomy, an animal model of neuropathic pain, in axotomized rats (Verge et al., 1993). Furthermore, application of galanin antisense oligonucleotide to the distal stump of the transected sciatic nerve effectively reduced the upregulation of galanin in sensory neurons and exaggerated autotomy behavior (Ji et al., 1994). Thus, an inverse correlation exists between galanin level in DRG and the severity of autotomy in axotomized rats treated with galanin antisense. Galanin level also increases in dorsal root ganglion neurons after partial peripheral nerve injury (Nahin et al., 1994; Ma and Bisby, 1997; Hao et al., 1999). Rats with chronic nerve constriction injury were treated with antibody to nerve growth factor, which induced further increase in galanin upregulation in sensory neurons and alleviated pain-like behavior associated with this model (Ramer et al., 1998), supporting an antinociceptive role for galanin. In another study using nerve constriction, the extent of galanin upregulation was inversely correlated to the severity of pain-like behavior among individual rats subjected to the same type of injury (Shi et al., 1999).

We have analyzed the effects of galanin and galanin receptor antagonists in rats with nerve injury using the flexor reflex. Galanin maintained its inhibitory effect on the flexor reflex and its facilitation in axotomized rats, and there seemed to be an increased potency of galanin to depress the baseline flexor reflex (Wiesenfeld-Hallin et al., 1989b; Xu et al., 1990). The galanin antagonist M-35 in normal rats moderately potentiated reflex facilitation in-

duced by repetitive C-fiber stimulation, whereas such potentiating effect of M-35 was dramatically increased in axotomized rats (Wiesenfeld-Hallin et al., 1992a,b). These data suggest that the endogenous inhibitory role of galanin is enhanced in axotomized rats, probably as a result of increased galanin release. Galanin knock-out mice were shown to be slightly hyperalgesic to mechanical and thermal stimulation (Kerr et al., 2000), supporting an inhibitory function of galanin under normal conditions. In a very recent study, transgenic mice that overexpress galanin had decreased thermal nociception compared to wild-type controls (Blakeman et al., 2001), further supporting the antinociceptive role of galanin.

3.3.2. Plasticity of the galanin system after peripheral inflammation

Plasticities in the expression of neuropeptides in sensory neurons and spinal cord interneurons are often opposite in nerve injury and inflammation (Hökfelt et al., 1997). Thus, carrageenan-induced inflammation is associated with an upregulation of the expression of galanin and several other neuropeptides in the superficial dorsal horn, while the expression of galanin in sensory neurons, which is already low in normal rats, is further reduced (Ji et al., 1995) (Table 1). Galanin$_1$ receptor mRNA is downregulated in rat DRGs, whereas galanin$_2$ receptor mRNA is strongly, albeit transiently, upregulated and then downregulated (Xu et al., 1996a,b; Shi et al., 1997) (Table 1). Persistent inflammation in a rat model of polyarthritis results in upregulation of galanin, which may be secondary to structural damage to nerve fibers in the joints (Calza et al., 1998).

We have analyzed the effects of endogenous galanin after the induction of inflammation following subcutaneous injection of carrageenan into the rat hind paw (Xu et al., 1998). The ability of repetitive stimulation of C-fibers to induce "wind-up" and central sensitization was significantly reduced at the peak of inflammation. During inflammation, i.t. administration of the galanin receptor antagonist M-35 strongly enhanced "wind-up" and spinal sensitization. Both the reduction in C-fiber-induced spinal hyperexcitability and the effects of M-35 were reversed when the inflammation was resolved. These results indicate that endogenous galanin exerts a strong inhibitory control on C-fiber input at the peak of inflammation, which could be mediated by the increased expression of galanin in dorsal horn interneurons, leading to increased spontaneous release of the peptide during inflammation (Hope et al., 1994).

3.3.3. Is there a future for cholecystokininergic and galaninergic drugs as analgesics?

Although the management of nociceptive pain has been improved significantly in recent years, chronic neuropathic pain continues to be a major clinical problem. Nerve injury induces complex plasticity in sensory neurons, spinal cord and supraspinal structures (Hökfelt et al., 1997), indicating that our understanding of normal pain mechanisms and treatments based on such mechanisms may be inadequate for the treatment of neuropathic pain. For example, strong narcotic analgesics such as morphine appear to have limited effect on neuropathic pain. Therefore, alternative treatments based on potential mechanisms of neuropathic pain are needed. Thus, agonists of galanin receptors, possibly of the galanin$_1$ receptor subtype, deserve to be further evaluated for their analgesic potential for treating neuropathic pain. Similarly, cholecystokinin receptor antagonists, alone or in combination with opioids, may be useful for treating neuropathic pain. Interestingly, the analgesic effect of i.t. galanin and morphine in rats with neuropathy can be blocked by galanin and opioid receptor antagonists, respectively (Zhang et al., 2000a,b), supporting an interaction between the two systems that was documented in normal animals (Wiesenfeld-Hallin et al., 1990a; Reimann et al., 1994; Selve et al., 1996). Thus, the interactions between the galaninergic, cholecystokininergic and opioidergic systems may lead to the potentiation of analgesic effects in treating neuropathic pain. It remains to be seen whether the supraspinal effects of galaninergic and cholecystokininergic drugs, alone or in combination with opioids, leads to undesirable side-effects.

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