


# Mechanisms of the analgesic effect of calcitonin on chronic pain by alteration of receptor or channel expression

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## Abstract

The polypeptide hormone calcitonin is well known clinically for its ability to relieve osteoporotic back pain and neuropathic pain such as spinal canal stenosis, diabetic neuropathy, chemotherapy-induced neuropathy, and complex regional pain syndrome. Because the analgesic effects of calcitonin have a broad range, the underlying mechanisms of pain relief by calcitonin are largely unknown. However, recent studies using several types of chronic pain models combined with various methods have been gradually clarifying the mechanism. Here, we review the mechanisms of the analgesic action of calcitonin on ovariectomy-induced osteoporotic and neuropathic pain. The analgesic action of calcitonin may be mediated by restoration of serotonin receptors that control selective glutamate release from C-afferent fibers in ovariectomized rats and by normalization of sodium channel expression in damaged peripheral nerves. Serotonin receptors are reduced or eliminated by the relatively rapid reduction in estrogen during the postmenopausal period, and damaged nerves exhibit hyperexcitability due to abnormal expression of Na<sup>+</sup> channel subtypes. In addition, in chemotherapy-induced peripheral neuropathy, inhibition of signals related to transient receptor potential ankyrin-1 and melastatin-8 is proposed to participate in the anti-allodynic action of calcitonin. Further, an unknown calcitonin-dependent signal appears to be present in peripheral nervous tissues and may be activated by nerve injury, resulting in regulation of the excitability of primary afferents by control of sodium channel transcription in dorsal root ganglion neurons. The calcitonin signal in normal conditions may be non-functional because no target is present, and ovariectomy or nerve injury may induce a target. Moreover, it has been reported that calcitonin reduces serotonin transporter but increases serotonin receptor expression in the thalamus in ovariectomized rats. These data suggest that calcitonin could alleviate lower back pain in patients with osteoporosis or neuropathic pain by the alteration in receptor or channel expression.

## Keywords

Peripheral nerve excitability, neuropathic pain, osteoporosis, ovariectomy, estrogen, serotonin, chronic constriction injury model, Na<sup>+</sup> channel expression, analgesia

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## Introduction

Calcitonin (CT), a 32-amino acid polypeptide released from the parafollicular C cells of the mammalian thyroid gland, is a major regulatory hormone of calcium homeostasis<sup>1</sup> and plays only during periods of calcium stress, such as growth, pregnancy, lactation, and menopause. The effect is exerted via the activation of the CT receptor, a G-protein-coupled receptor with a seven-transmembrane domain. CT receptors are broadly distributed not only in osteoclasts but also in brain, ovary, kidney, stomach, and skeletal muscles, implying that CT may exert a wide range of effects on biological

functions. One of the main functions of CT is to provide relief from severe pain associated with fractures,<sup>2,3</sup> post-osteoporotic fractures,<sup>3,4</sup> and Paget's disease.<sup>4</sup> Thus, an

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early assumption was that the analgesic effect of CT is due to an increase in bone mass. However, the analgesic effect of CT is clearly distinct from the increase in bone mass as observed by the time course of these events. The analgesic effect becomes significant after three to four weeks, before an increase in bone mass. A different mechanism is, therefore, likely regarding the analgesic effect of CT on patients suffering from osteoporosis. In addition, CT ameliorates various types of neuropathic pain associated with lumbar spinal canal stenosis,<sup>5</sup> diabetic neuropathy,<sup>6</sup> reflex sympathetic dystrophy,<sup>7</sup> and post-herpetic neuralgia.<sup>8</sup> CT is recently shown to inhibit the development of complex regional pain syndrome after stroke.<sup>9</sup> The primary cause of these chronic types of pain may be the hyperexcitability of the peripheral nervous system. The excitability of nerves is preferentially controlled by the density of sodium and potassium channels, and by expression of particular sodium channel subtypes. Nevertheless, the analgesic mechanisms of CT remain poorly understood. On the other hand, accumulating evidence supports the idea that CT exerts its actions through changes in gene expression.<sup>10–14</sup> For instance, CT inhibits bone resorption by activation of protein kinase A and protein kinase C.<sup>12</sup> Activated protein kinase A induces a reduction in CT receptor mRNA, which negatively controls the effect of CT.<sup>13</sup>

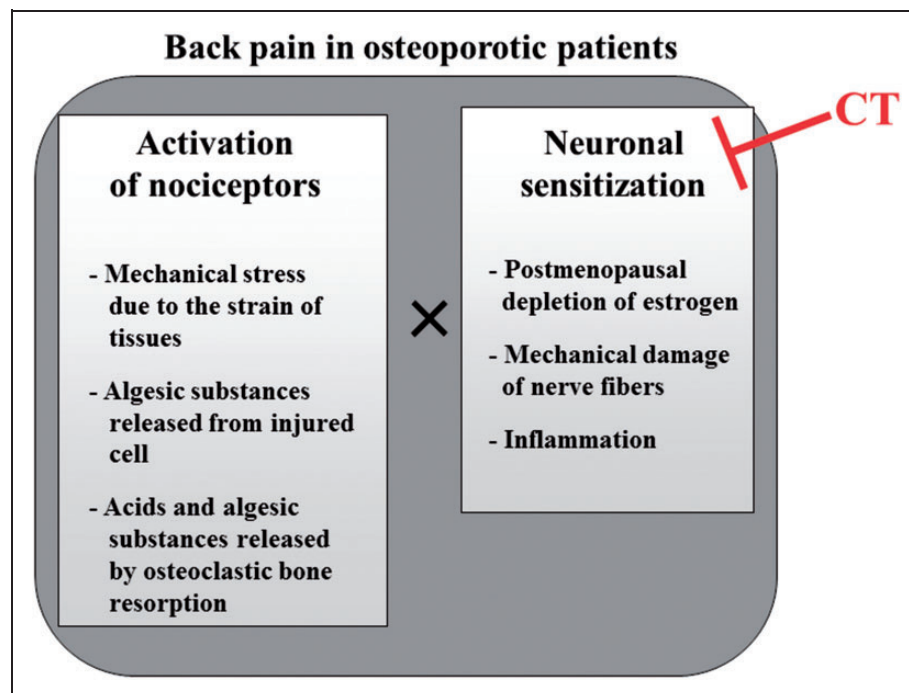
Furthermore, in the rat thalamus, CT reduces serotonin (5-HT) transporters and increases 5-HT<sub>1A</sub> receptors.<sup>14</sup> These data suggest that a CT-activated signaling pathway regulates nociceptive transmission by controlling expression of various genes.

Recent electrophysiological studies combined with various techniques, especially molecular biology, suggest possible mechanisms for the action of CT in the peripheral or central nervous system. In this paper, we review the underlying mechanisms and efficacies of CT as an analgesic substance, based on observations in various types of chronic pain models.

### Mechanisms of the analgesic effect of CT on ovariectomized (OVX) rats

#### Background for the analysis of OVX rats

As shown in Figure 1, the back pain in osteoporotic patients may occur as a result of mechanical stress that leads to direct stimulation of nociceptors that innervate bone marrow<sup>15</sup> and algescic substances released from injured cells following fractures. In addition, back pain in the absence of fractures may occur as a result of activation of nociceptors by acids and algescic substances released by osteoclastic bone resorption.



**Figure 1.** Analgesic effect of CT against the pain that accompanies postmenopausal osteoporosis.

Back pain in osteoporotic patients may occur as results of mechanical stress, algescic substances, and acids that directly stimulate nociceptors innervating bone marrow. Neuronal sensitization enhances back pain in osteoporotic patients. CT may alleviate the back pain via inhibition of neuronal sensitization induced by postmenopausal depletion of estrogen and nerve damage.

Menopause is a major cause of osteoporosis in humans.<sup>16</sup> One important change following menopause is depletion of estrogen, which regulates expression of various genes.<sup>17</sup> Thus, depletion of estrogen influences the expression of receptors and channels that are required for the modulation of nociceptive transmission.

Based on the results obtained from OVX rats,<sup>18–21</sup> OVX-induced hyperalgesia may be due to a dysfunction of the descending serotonergic inhibitory system that controls nociceptive transmission at the spinal dorsal horn level. This dysfunction likely enhances the back pain in osteoporotic patients (Figure 1).

When CT is administered to patients, a period of about a month is required before the back pain is relieved.<sup>22</sup> Repeated administration of CT inhibits OVX-induced hyperalgesia in rats with a similar time course as that seen in patients.

### *The analgesic effect of CT on OVX rats*

OVX rats with osteoporosis exhibit hyperalgesia as seen with the tail withdrawal test. The analgesic effect is alleviated by repetitive, subcutaneous injections of CT in a dose-dependent manner<sup>18</sup>; this effect becomes significant after three to four weeks of treatment, before an increase in bone mass. Because these results are similar to the clinical effect of CT in patients, this rat model seems appropriate for studying the analgesic mechanisms of CT.

c-Fos expression in spinal cord neurons has been used as a functional marker of nociception in many studies.<sup>23–28</sup> Takayama et al.<sup>21</sup> demonstrate that the number of c-Fos-immunoreactive neurons in the spinal dorsal horn is significantly increased in response to acute noxious stimuli in OVX rats, particularly in the superficial laminae, compared to sham rats, indicating that OVX enhances nociception in rats. Repeated CT injections suppress the number of c-Fos-positive neurons in the superficial laminae as seen with the formalin test.<sup>21</sup> These results behaviorally confirm the OVX-induced hyperalgesia and CT-induced anti-hyperalgesia.<sup>18,20</sup>

### *Involvement of the serotonergic system in the CT-induced analgesic effect in OVX rats*

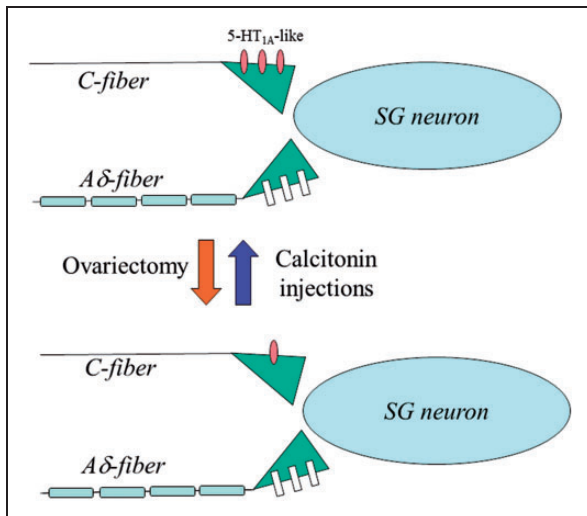
The central serotonergic and noradrenergic systems originating from the brain stem contribute to modulation of nociceptive transmission.<sup>29–33</sup> In particular, the serotonergic system likely contributes to the analgesic effect of CT.<sup>34,35</sup> Thus, involvement of the serotonergic system is examined using p-chlorophenylalanine (PCPA) and methiothepin, an inhibitor of 5-HT biosynthesis and a broad 5-HT receptor antagonist, respectively. In OVX rats, the analgesic effect of CT is completely inhibited by intraperitoneal injection of PCPA.<sup>18</sup> Moreover,

suppression of c-Fos expression by CT in OVX rats is abolished by PCPA.<sup>21</sup> Consistently, the amount of 5-HT is decreased, and the activity of the serotonergic system is decreased after PCPA injection.<sup>18</sup> Furthermore, the CT-induced analgesic effect is completely eliminated 60 min after methiothepin injection.<sup>36</sup> These data support the idea that the serotonergic system may be involved in CT-mediated analgesia in postmenopausal patients.

### *Serotonergic dysfunction in the spinal dorsal horn of OVX rats and its recovery following CT treatment*

The substantia gelatinosa (SG) (lamina II) of the spinal dorsal horn plays a critical role in the modulation of nociceptive transmission from the periphery to the central nervous system.<sup>37–41</sup> Fine myelinated A $\delta$ - and unmyelinated C-afferents, many of which carry nociceptive information, terminate preferentially in the SG,<sup>37,42–46</sup> and the majority of SG neurons are excited by noxious stimulation.<sup>47,48</sup> Further, SG is the main target of the descending serotonergic system.<sup>49</sup>

A blind patch-clamp study in OVX rat spinal cord slices demonstrated that presynaptic 5-HT-induced inhibition of glutamatergic transmission evoked by stimulating C-afferent fibers is reduced. This effect is restored after repeated injections of CT. Intriguingly, no such effect was observed after A $\delta$ -afferent stimulation<sup>19</sup> (Figure 2). A similar loss of presynaptic inhibition is observed for spontaneous transmitter release.<sup>19</sup> This inhibition is mimicked by a 5-HT<sub>1A</sub> receptor agonist, 8-hydroxy-2-(di-n-propylamino) tetralin hydrobromide (8-OH-DPAT). Nevertheless, this effect is not antagonized by the 5-HT<sub>1A</sub> receptor antagonist, WAY100635.<sup>19</sup> This action of 8-OH-DPAT is consistent with the observation using autoradiography that the number of <sup>3</sup>H-8-OH-DPAT binding sites is decreased by 20% to 30% after either neonatal capsaicin treatment or dorsal rhizotomy, which eliminates C-fibers.<sup>50</sup> Furthermore, the binding of <sup>3</sup>H-8-OH-DPAT to the spinal cord is reduced in OVX rats. This reduction in <sup>3</sup>H-8-OH-DPAT binding is restored when OVX rats are chronically treated with CT.<sup>19,20</sup> Consistent with these observations, behavioral examination demonstrates that OVX rats exhibit hyperalgesia, which is alleviated by CT administration.<sup>18,20</sup> Altogether, these results indicate that hyperalgesia and CT-induced anti-hyperalgesia may be attributed to a change in the number of 5-HT receptors expressed at C-afferent terminals. As observed by the resistance to WAY100635, the participating receptor appears to be a 5-HT<sub>1A</sub>-like receptor but not 5-HT<sub>1A</sub>.<sup>19</sup> This idea was further confirmed by polymerase chain reaction studies, which showed no expression of 5-HT<sub>1A</sub> receptor mRNA in rat dorsal root ganglion (DRG) neurons.<sup>51,52</sup> This change in the number of 5-HT receptors could underlie



**Figure 2.** Possible mechanisms of OVX-induced hyperalgesia and its relief by CT treatment in spinal SG neurons in rats. 5-HT<sub>1A</sub>-like receptors present on C-fiber terminals, the activation of which decreases the release of glutamate, are reduced in OVX rats, leading to hyperalgesia. CT treatment of OVX rats restores the number of receptors to normal, which results in anti-hyperalgesia. On the other hand, the decrease in glutamate released from A $\delta$ -fiber terminals via the activation of other 5-HT receptors (white bar) does not change. SG: substantia gelatinosa.

the analgesic effects of CT on osteoporotic pain in humans.

CT may be an exceptional analgesic substance that acts by restoring the density of 5-HT<sub>1A</sub>-like receptors in C-fiber terminals (Figure 2). Considering that C-fibers convey predominantly diffuse and long-lasting pain information, 5-HT<sub>1A</sub>-like receptors may play an important role in chronic pain transmission.

### Ineffectiveness of estrogen treatment on hyperalgesia long term after OVX

In clinical studies, estrogen treatment is common in postmenopausal women with recurrent back pain, but this hormone replacement has no appreciable effect on continuous pain.<sup>53</sup> Most reviews have determined that CT treatment is preferable to hormone replacement for the alleviation of pain that accompanies osteoporosis,<sup>54–56</sup> despite the fact that osteoporosis and the accompanying pain are accelerated by the reduction in estrogen levels. Distinct from the clinical view, animal studies have shown that estrogen treatment reduces OVX-induced hyperalgesia.<sup>20,57</sup> The discrepancy in clinical and animal study outcomes may be due to the timing of administration of estrogen after depletion of the hormone. Estrogen injected three weeks after OVX mimics the effects of CT and prevents the OVX-induced decrease

in 5-HT<sub>1A</sub>-like receptor expression. Nevertheless, injection of estrogen 15 weeks after OVX has no significant effect, although the effects of CT are observed regardless of the timing of CT injection.<sup>20</sup> These results suggest that the estrogen receptor (ER) is down-regulated gradually after OVX.

ERs are expressed in rat Schwann cells<sup>58</sup> and DRG neurons.<sup>59</sup> Autoregulation of ER by its ligand has been the subject of many previous studies.<sup>60–66</sup> In rats 11 days after OVX, ER mRNA in rat DRG neurons is upregulated.<sup>61</sup> Following long-term OVX (three months post-operation), the levels of ER expression are significantly reduced in rat brain.<sup>66</sup> Another study showed that at 49 weeks after OVX, pituitary ER mRNA levels are decreased by 55%.<sup>65</sup> The long-term change in the degree of ER expression after OVX should be studied in the peripheral nervous system. The ineffectiveness of estradiol on OVX-induced hyperalgesia may be attributed to the down-regulation of ERs during the 15 weeks after the operation. A decrease in ERs may be one of the reasons that estrogen is not recommended for the treatment of back pain associated with osteoporosis.

### Other mechanisms of the anti-hyperalgesic effects of CT in OVX rats

A recent review shows that the voltage-gated sodium channels, Nav1.3, Nav1.7, Nav1.8, and Nav1.9, play pivotal roles in nociceptive transmission.<sup>67</sup> Nav1.3, a low-threshold, tetrodotoxin (TTX)-sensitive sodium channel, is expressed in embryonic but not adult DRG neurons.<sup>68</sup> Nav1.3 is, however, re-expressed in adult rat DRG neurons following peripheral nerve injury.<sup>68,69</sup> In contrast, the expression of Nav1.8 and Nav1.9, which are high-threshold, TTX-resistant sodium channels, is significantly attenuated in injured nerves.<sup>69–71</sup> As the threshold of Nav1.3 is much lower than that of Nav1.8 and Nav1.9, these observations suggest that the excitability of injured nerves becomes sensitive to small membrane potential changes, resulting in easy initiation of spontaneous action potentials (sensitization).

In addition to nerve injury, OVX also increases the transcription of Nav1.3 and reduces mRNA expression of TTX-resistant Na<sup>+</sup> channels in DRGs.<sup>72</sup> Therefore, OVX may lower the threshold for generation of action potentials in C-fibers. Furthermore, repeated injections of CT normalize the threshold<sup>73</sup> by restoring OVX-induced changes in transcription.<sup>72</sup> Thus, the changes in sodium channel transcription may be an additional mechanism of hyperalgesia in OVX rats.

Expression of tumor necrosis factor- $\alpha$ ,<sup>74</sup> P<sub>2</sub>X<sub>3</sub> receptors,<sup>75</sup> CT gene-related peptide receptors,<sup>15</sup> and transient receptor potential vanilloid 1<sup>15</sup> in DRGs is elevated in OVX rats. However, no studies have examined

whether CT ameliorates OVX-induced hyperalgesia by normalizing expression of those genes.

## **Analgesic mechanisms of neuropathic pain in model rats**

### *Analgesic effect of CT on pain behaviors in chronic constriction injury (CCI) model rats*

The analgesic effects of CT on pain behaviors in CCI-induced hyperalgesia in rats have been analyzed.<sup>76</sup> Mechanical and thermal hyperalgesia develop over time on the ipsilateral hind paw in CCI model rats. Subcutaneously applied CT after surgery gradually relieves hyperalgesia, and these effects persist for several days after cessation of the drug.<sup>76</sup> The effects of CT are dose-dependent on both mechanical and thermal hyperalgesia.<sup>76</sup>

### *Changes in Na<sup>+</sup> channel transcription in DRGs after CCI surgery*

Similar to OVX rats, CCI causes induction of abnormal gene expression of Na<sup>+</sup> channels in DRG neurons.<sup>76</sup> Transcription of Nav1.3 but not Nav1.7 in the ipsilateral DRG in the CCI model<sup>76</sup> is significantly increased. In contrast, CCI causes a significant reduction in mRNA expression of the TTX-resistant Na<sup>+</sup> channels, Nav1.8 and Nav1.9.<sup>76</sup> Subcutaneous administration of CT restores the expression of Nav1.3, Nav1.8, and Nav1.9 mRNA without affecting Nav1.7 as measured with quantitative reverse transcriptase-polymerase chain reaction.<sup>76</sup> The abnormal expression of Nav channels following CCI is consistent with previous reports.<sup>68–71</sup>

### *Analgesic mechanisms of chemotherapy-induced peripheral neuropathy in rats*

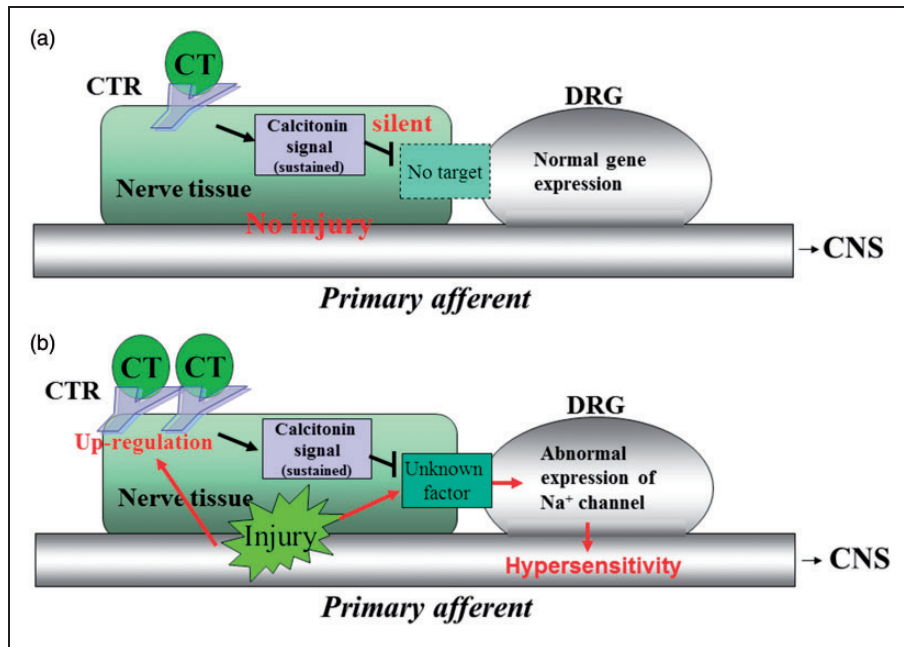
Oxaliplatin is commonly used to treat advanced metastatic colorectal cancer<sup>77</sup> and induces both acute and chronic neuropathy. In particular, cold allodynia appears soon after administration. Paclitaxel, which is commonly used to treat ovarian, breast, and non-small cell lung cancer,<sup>78,79</sup> induces sensory neuropathy.<sup>80,81</sup> Indeed, the neurotoxicity due to oxaliplatin or paclitaxel is often the reason for discontinuation of treatment, dose reduction, or hospitalization rather than tumor progression.<sup>82</sup> Although many studies have investigated methods to relieve neurotoxicity induced by anti-cancer drugs, no successful treatments for neuropathy have been developed to date. Aoki et al.<sup>83</sup> reported that CT almost completely reverses the effects of both cold and mechanical allodynia induced by oxaliplatin and paclitaxel.

Transient receptor potential ankyrin-1 (TRPA1) and the melastatin-8 (TRPM8) receptor, which are activated by temperatures less than 17°C and 25°C, respectively, are expressed in nociceptors and may be involved in pain perception.<sup>84,85</sup> Oxaliplatin and paclitaxel do not induce cold allodynia, and oxaliplatin and cisplatin do not induce mechanical allodynia to as great an extent in TRPA1-deficient mice.<sup>86,87</sup> Cold allodynia induced by oxaliplatin is inhibited by capsazepine, a blocker of TRPM8,<sup>88</sup> and oxaliplatin increases the expression of TRPA1<sup>86</sup> and TRPM8<sup>88,89</sup> mRNA in DRG neurons. These findings suggest that thermo-sensitive TRPA1 and TRPM8 are involved in the mechanism of neuropathy induced by anti-cancer drugs. CT prevents the cold and mechanical allodynia induced by intra-plantar allyl isothiocyanate and menthol, which are TRPA1 and TRPM8 agonists, respectively.<sup>83</sup> Thus, inhibition of cellular signaling related to TRPA1 and TRPM8 may be involved in the analgesic action of CT.<sup>83</sup> However, no convincing data support this possibility.

## **Site of action of CT in CCI rats**

CT receptors are expressed in peripheral nervous tissues, including Schwann cells, blood vessels, connective tissues, and others, but not in DRG neurons.<sup>76</sup> Although, CT receptors are expressed at higher levels in the spinal cord and hypothalamus than in peripheral nervous tissues, the analgesic effects and normalization of Na<sup>+</sup> channel mRNA by CT occur in parallel to the increase in CT receptor mRNA expression in peripheral nervous tissues but not in the spinal cord and hypothalamus.<sup>76</sup> On the contrary, down-regulation of CT receptor expression is well known. CT causes not only inhibition of bone resorption via activation of protein kinase A,<sup>12</sup> but also a decrease in <sup>125</sup>I-CT binding, which is related to the amount of CT receptor mRNA.<sup>90</sup> Therefore, down-regulation of CT receptor mRNA may be mediated by a signal following the activation of CT receptors.

Injection of CT induces down-regulation of CT receptor mRNA expression in peripheral nervous tissues. This result suggests the existence of a peripheral CT receptor-mediated system that serves as a feedback mechanism to regulate the levels of the CT signal.<sup>76</sup> On the other hand, CT injections do not influence CT receptor mRNA expression in the spinal cord and hypothalamus<sup>76</sup> because CT cannot pass through the blood–brain barrier. Accordingly, these studies suggest that CT-induced normalization of the expression of Na<sup>+</sup> channel mRNA in DRG neurons may be mediated by a “CT signal” released as a result of the activation of CT receptors in injured peripheral nervous tissues but not DRG neurons. This may contribute to the analgesic effect of CT on neuropathic pain.



**Figure 3.** Possible mechanisms of inhibition of abnormal expression of sodium channels by CT. (a) The action of CT under normal conditions. The CT-induced signal via CT receptors is silent under normal conditions because no target is present. (b) Nerve injury activates an unknown factor that induces the abnormal expression of Na<sup>+</sup> channels. CT may exert anti-hyperalgesic effects following normalization of Na<sup>+</sup> channel expression via inactivation of the unknown factor. CTR: calcitonin receptor; CT: calcitonin; DRG: dorsal root ganglion; CNS: central nervous system.

CT signals may also be induced under normal conditions by CT because CT suppresses CT receptor mRNA in intact nervous tissue.<sup>76</sup> However, this signal is thought to be non-functional because CT has no influence on the expression of Na<sup>+</sup> channels<sup>76</sup> or on behavioral responses<sup>76,83</sup> because no target is present (Figure 3(a)). On the other hand, in injured nervous tissue, CT signals may inhibit an unknown, injury-activated factor that induces abnormal expression of Na<sup>+</sup> channels (Figure 3(b)). Therefore, application of CT activates the CT signal, which prevents activation of the unknown factor, resulting in normalization of Na<sup>+</sup> channel expression (Figure 3(b)).

### CT signal

The cellular localization of CT receptors in peripheral nervous tissues and the CT signal has not been identified. After nerve injury, demyelination and proliferation of Schwann cells are induced.<sup>91,92</sup> Thus, CT receptors are likely expressed in Schwann cells, and a decrease or increase in expression of CT receptors may contribute to demyelination or proliferation of Schwann cells. Glial cell line-derived neurotrophic factor or nerve growth factor regulates the expression of Na<sup>+</sup> channels or the Na<sup>+</sup> current density in DRG neurons.<sup>93,94</sup> Schwann cells produce these neurotrophic factors.<sup>95,96</sup> The CT-induced signal that occurs via activation of CT

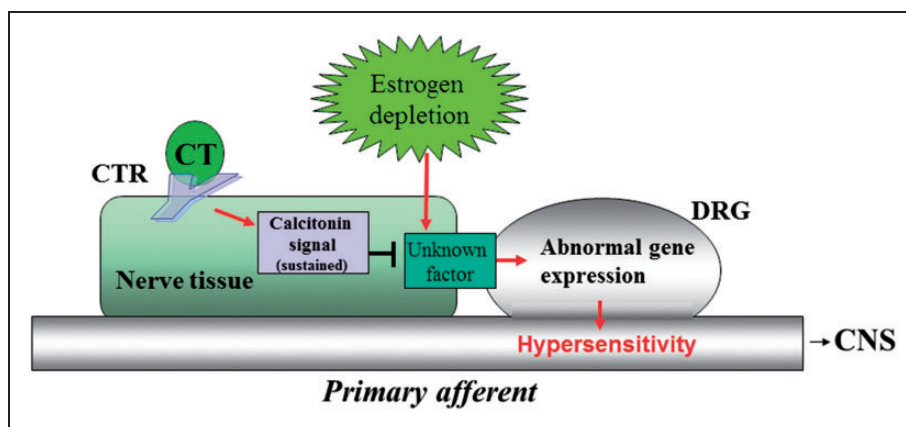
receptors in peripheral nervous tissue may be glial cell line-derived neurotrophic factor or nerve growth factor released from Schwann cells.

### Site of action of CT in other models

The site of action in OVX- and chemotherapy-induced peripheral neuropathy has not been reported. Considering the site of action in CCI model rats, CT produces anti-hyperalgesic and anti-allodynic effects via CT receptors in peripheral nervous tissue in rats (Figures 4 and 5). Meanwhile, CT has both acute and chronic anti-allodynic effects in oxaliplatin- and paclitaxel-injected rats.<sup>83</sup> This is in contrast to studies in OVX and CCI rats, which showed that repeated systemic administration of CT is needed to produce analgesia.<sup>3,4,18,76,97–99</sup> Therefore, other unknown factors may be involved in the underlying mechanism of CT in relieving oxaliplatin- and paclitaxel-induced neuropathy (Figure 5).

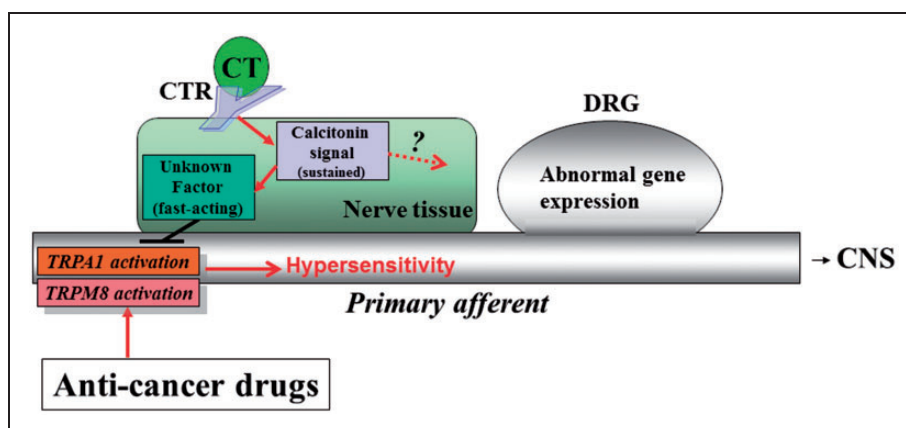
### Prolonged CT-induced signal and anti-hyperalgesic effect

Although CT disappears within 2h of injection from human<sup>100</sup> and rat plasma (in house data), subsequent injection of CT gradually enhances the anti-hyperalgesic effect.<sup>76</sup> In addition, the anti-



**Figure 4.** Proposed mechanism of inhibition of abnormal expression of 5-HT<sub>1A</sub>-like receptors and sodium channels by CT in OVX rats. Estrogen depletion may induce abnormal gene expression of Na<sup>+</sup> channels and the depletion of 5-HT<sub>1A</sub>-like receptors in DRG neurons via unknown factor(s) in nervous tissues. CT may exert anti-hyperalgesic effects by recovery of the abnormal gene expression via the CT-induced signal.

CTR: calcitonin receptor; CT: calcitonin; DRG: dorsal root ganglion; CNS: central nervous system.



**Figure 5.** Proposed mechanism of inhibition of TRPA1 and TRPM8 by CT in chemotherapy-induced peripheral neuropathy in rats. CT may exert anti-allodynic effects by inhibiting the activation of TRPA1 and TRPM8 channels via the CT-induced signal.

CTR: calcitonin receptor; CT: calcitonin; DRG: dorsal root ganglion; CNS: central nervous system.

hyperalgesic and anti-allodynic effects are maintained for several days after cessation of CT administration.<sup>76,83</sup> Therefore, the CT signal may be sustained for several days, and the accumulation of signals probably increases the strength of the anti-hyperalgesic effect.

### Central action of CT

Although this review focuses preferentially on the peripheral action of CT, there is some evidence suggesting of central action of CT. Systemic injections of CT reduces neural serotonin transporter but increase 5-HT<sub>1A</sub> receptors expression in the thalamus in OVX rats.<sup>14</sup> In formalin-induced hyperalgesia in rats, the ascending serotonergic system, rather than the

noradrenergic or opioidergic, mediate the anti-hyperalgesia of CT.<sup>34</sup> CT injected systemically binds to the CT receptors at blood–brain barrier free regions, median eminence, area postrema, and lamina terminals.<sup>101</sup> Therefore, it cannot be excluded a possibility that CT exert its analgesic actions through interacting with the central nerve system.

### Conclusions

Mechanisms of the analgesic effect of CT have been gradually elucidated by recent studies using several types of pain model animals and diverse methods. The anti-hyperalgesic action of CT appears to be mediated by restoration of 5-HT receptors that control excitatory

transmitter release from C-afferent terminals, and by normalization of expression of voltage-dependent sodium channels in damaged DRG neurons in OVX or CCI rats. Chemotherapy-induced peripheral neuropathy models have also revealed that inhibition of signals related to TRPA1 and TRPM8 participate in the anti-allodynic actions of CT. Further, a CT-dependent system appears to be present in peripheral nervous tissues and may be activated by nerve injury, resulting in regulation of excitability of primary afferents controlled by sodium channel transcription in DRG neurons. The CT-dependent systems may be silent in normal conditions, and OVX or nerve injury triggers their activation. In addition, CT reduces serotonin transporter but increases 5-HT receptors expression in the thalamus in OVX rats. However, these central actions of CT have not been rigorously addressed. These data suggest that CT may alleviate lower back pain in osteoporotic or neuropathic pain patients via normalization of neuronal hyperexcitability induced by depletion of estrogen or nerve injury. Although CT is an old medicine, its mechanisms of analgesia are only now being elucidated. Investigation of the CT-induced signal and further analysis of the CT receptor-mediated system in peripheral nervous tissue may lead to plausible strategies for alleviating peripheral neuropathic pain.

#### Declaration of Conflicting Interests

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