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Updates on the antinociceptive mechanism hypothesis of botulinum toxin A

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A B S T R A C T

Botulinum toxin A has been traditionally viewed as a motor nerve specific treatment. However, clinical uses for botulinum toxin A have continued to expand, with increased use in conditions implicating sensory pain nerve dysfunction. Chronic pain is associated with excess pain fiber activity. When the site of this excess activity resides in the peripheral portion of the pain pathway, a condition of peripheral sensitization can establish. During this state, excess pain signaling reaches the central nervous system, which can then lead to a condition of central sensitization, manifesting as the symptoms associated with chronic pain (i.e. burning, electric pain, lowered pain threshold to normal stimuli, etc). *Experimentally*, botulinum toxin type A has been shown to reduce neuropeptides and neurotransmitter release from treated cells or nerve endings and to attenuate nociception in both neuropathic and non-neuropathic pain models. This review summarizes the literature to update the hypothesis for the mechanism by which botulinum toxin type A can modulate chronic pain.

1. Introduction

Botulinum toxin type A (BoNT-A) was first approved for use in certain movement disorders (i.e., benign essential blepharospasm and hemifacial spasm) in 1989. Since then, it has been used to control various unwanted movements and excess secretions of patients worldwide [1,2]. Early observations in cervical dystonia patients noted that pain relief preceded muscle relaxation [3,4]. This observation regarding pain relief remained unexplained, beyond the obvious hypothesis that reduction of muscle contractions reduced pain. A report of pain relief in a condition with no perceived muscle contraction (i.e., migraine pain) [5] increased interest in testing and in understanding the mechanism by which a motor-nerve-specific therapeutic agent could reduce chronic pain symptoms.

Onabotulinum toxinA (BOTOX®, a BoNT-A-based product) was approved in 2010 for the treatment of patients with chronic migraine (headaches on 15 or more days per month) in the UK, United States and other countries; outcomes from submissions to additional countries are pending. The details of the clinical trials supporting the approval of onabotulinumtoxinA have been published in separate articles [6–8] and will not be discussed in this review. Briefly, clinical trials evaluated the safety and effectiveness of onabotulinumtoxinA compared with placebo in 1384 adults from 122 study sites in North America and Europe. Patients with a history of migraine and suffering from headaches on 15 or more days per month with at least 50% of the headache days being migraine or probable migraine were studied in two double-blind, randomised placebo-controlled trials. Results from these studies have established that onabotulinumtoxinA is effective in controlling the pain associated with chronic migraine. In the same issue, Schoenen [9] commented that understanding the mechanism of action related to the use of onabotulinumtoxinA in the treatment of chronic migraine is desired.

Botulinum toxin's (BoNT) mechanisms of action on motor and autonomic nerves have been described in detail in other reports [10,11]. Thus, this review will expand on previously published reports on the hypothesised mechanism for the antinociceptive action of BoNT-A (specifically onabotlinumtoxinA) [12,13]. The basic biochemical mechanism of BoNT in cleaving its intracellular protein target in its antinociceptive mechanism of action hypothesis is identical to the motor-nerve cleavage of a specific SNARE (SNAP (Soluble NSF-Attachment Protein) Receptor) protein. For botulinum toxin serotype A (BoNT-A), the SNARE target is SNAP-25 (synaptosomal-associated protein 25). The only difference between the neuromuscular/autonomic system and the antinociceptive response is the physiological response to the temporary, albeit an extended duration, inhibition of neurotransmission.

2. Antinociceptive hypothesis

The BoNT-A (onabotulinumtoxinA) antinociceptive mechanism combines its known intracellular effects on neurotransmitter release and the mechanism of chronic pain induction and propagation.

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Patient responsiveness to therapeutic intervention will depend on the mechanism of chronic pain maintenance [14]. As onabotulinumtoxinA is administered locally to a peripheral location, its antinociceptive mechanism hypothesis requires a peripheral source of chronic pain nerve stimulation leading to a central change which manifests as chronic pain. The nerve targets for pain are the C-fibres (afferent unmyelinated nerves, 0.4–1.2 μm in diameter with nerve conduction velocities of approximately 0.7–2.3 m s^{-1}). Therefore, there are two components to the hypothesised antinociceptive mechanism of onabotulinumtoxinA (Fig. 1):

(1) direct temporary inhibition of pain neurotransmitter release at the peripheral dosing location reducing peripheral pain nerve sensitisation and
(2) indirect reduction of central sensitisation associated with chronic pain through the reduction of the peripheral nerve over-activity.

The following sections summarise the evidence supporting this hypothesis.

3. Chronic pain

Pain can serve as a valuable warning to an individual to avoid a potentially damaging situation. Adaptive (nociceptive), or acute, pain is therefore an important function for self-preservation. Chronic pain, or maladaptive pain, uncoupled from the noxious stimulus, is an altered state of central nervous system (CNS) responsiveness due to repeated painful stimuli. In contrast to adaptive pain, maladaptive pain is a disease state that can severely impact a patient's quality of life. Changes in the pain response state are caused by alternations in neuronal plasticity of the pain sensory system, which may be driven by a peripheral source [15–17]. The peripheral source of the stimulation can be inflammation, tissue damage or peripheral nerve damage.

The mechanism for this peripheral sensitisation includes: (1) increased sensitivity of existing receptors via activation of kinases and/or (2) increased surface expression of additional receptors (e.g., the receptors TRPV1 and P2X3) [15,18]. Current understanding of the mechanism for the up-regulation of specific receptors, such as TRPV1, on sensitised nociceptive nerves implicates a SNARE-mediated vesicle-fusion pathway in protein kinase C potentiation of vanilloid receptor activity [18–20]. Botulinum toxin type A blocks the surface expression of receptors through its ability to disrupt the SNARE-mediated vesicle-fusion process.

Increased stimulation of the second-order neurons in the dorsal horn can lead to the sensitisation of these cells, resulting in a windup of action-potential discharge and, thus, an increased perception of pain. The net effect is an altered sensory system (e.g., lower threshold for activation) that signals a painful sensation in

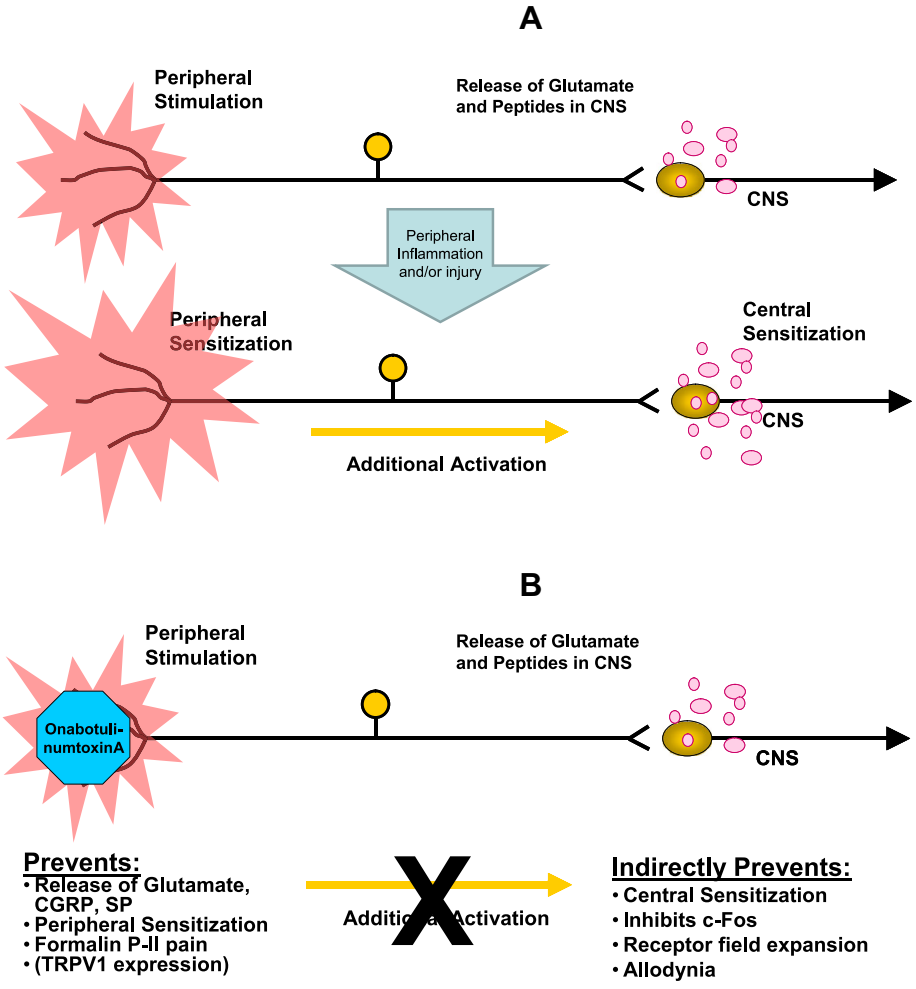

Fig. 1. (A) A pain perception (top) transmits information from periphery to CNS. Repeated stimulation, inflammation or nerve injury may sensitize peripheral nerve endings resulting in excess stimulation of CNS leading to central sensitization. (B) Hypothesized antinociceptive mechanism of botulinum neurotoxin . Botulinum toxin type A may directly inhibit primary sensory fibers, leading to a reduction of peripheral sensitization, and an indirect reduction in central sensitization, receptor field expansion, and allodynia.

response to an innocuous stimulus (allodynia) or an exaggerated response to pain (hyperalgesia). Botulinum toxin type A (onabotulinumtoxinA) reduces peripheral sensitisation, and this reduces the afferent signals to the dorsal horn and thus indirectly reduces central sensitisation.

4. Pain and sensitisation in headache patients

Migraine patients often demonstrate symptoms associated with chronic pain which appears to be the result of a complex pathophysiology involving nociceptive activation or perception of activation of trigeminal cranial afferents [21]. Many patients report migraine headache pain and associated cutaneous allodynia [22,23], which is effectively relieved by pharmacologic intervention only when administered before the full development of allodynia [22,24].

Currently, only two pharmacological treatments have been shown to be effective in placebo-controlled randomised trials of chronic migraine patients: topiramate (an antiepileptic) [25] and local injection of onabotulinumtoxinA [6–8]. Both treatments were effective in patients with and without medication overuse.

5. Preclinical antinociceptive activity of BoNT

The majority of *in vivo* evidence supporting the hypothesis was obtained from preclinical studies. A recent review has summarised the publications describing the efficacy of BoNT for pain management in animal models [26]. The authors concluded that BoNT-A is able to induce long-term analgesic effects in many different pain models (from inflammatory to neuropathic). Their proposed mechanism of action for botulinum toxin analgesia is consistent with the hypothesis provided in this current article and other publications [12,13]. As Pavone and Luvisetto provide a recent summary of the preclinical literature, a selected subset of our preclinical results will be summarised below to support the proposed onabotulinumtoxinA antinociceptive mechanism of action hypothesis.

In vivo, pre-treatment with onabotulinumtoxinA (3.5–30 U kg^{-1}, s.c.) prevented formalin-induced pain (phase 2 only) and the release of glutamate from the rat hindpaw [12,27]. Doses $<$15 U kg^{-1} administered to the plantar surface did not affect muscle activity. In the dorsal horn, onabotulinumtoxinA prevented the expression of Fos-like immunoreactivity (Fos-LI) and the activation of the wide dynamic range (WDR) neurons [12]. Consistent with previous studies [28–30], our results demonstrated that hindpaw intraplantar formalin injection induced the expression of Fos protein in laminae I, II, V and VI and a biphasic increase in the firing rate of spinal nociceptive dorsal-horn neurons. Pre-treatment of rats with subcutaneous onabotulinumtoxinA dose-dependently (3.5–30 U kg^{-1}) inhibited formalin-evoked Fos expression and evoked excitation of WDR cells during phase 2. As the second phase of the formalin test is maintained by peripheral input and/or central sensitisation [31–34], it is highly likely that the inhibition of inflammatory pain by subcutaneous injection of onabotulinumtoxinA was mediated by a reduction of peripheral input and, therefore, of central sensitisation. These data, together with a previous study [35], support the hypothesis that the preclinical antinociceptive effect of onabotulinumtoxinA can be attributed to the direct inhibition of peripheral sensitisation, and indirect inhibition of central sensitisation, through prevention of the release of neuromodulators at peripheral nociceptive terminals.

Additional studies from our laboratory (other authors have also published on this model, as summarised by Pavone and Luvisetto) have focussed on capsaicin-induced preclinical pain models. Consistent with the above hypothesis, onabotulinumtoxinA pre-treatment prevented the development of thermal hyperalgesia, mechanical allodynia and increased blood flow, WDR activation and Fos-LI [36,37]. In addition, our results also demonstrated that administration of onabotulinumtoxinA (15 U kg^{-1}, s.c.) reversed established local allodynia in a diabetic rat model [36]. At this dose, there was no measurable muscle weakness and, therefore, no interference with the measurement of allodynia (e.g., the animals were able to withdraw their hindpaw when challenged with a cutaneous stimulus).

The working hypothesis is further supported by results reported in a preclinical model of bladder pain in which onabotulinumtoxinA significantly reduced pain responses and inhibited local CGRP release from afferent nerve terminals [38]. In addition, human bladder samples from patients successfully treated with onabotulinumtoxinA demonstrated a reduction of P2X3 and TRPV1 immunoreactivity in the suburothelial plexus [39]. Patients treated for bladder over-activity with intradetrusor onabotulinumtoxinA have experienced efficacy exceeding that which would have been expected from a simple muscle relaxation effect. The dramatic reduction of urgency was an unexpected observation and suggests an alteration of the bladder sensory feedback system. Urinary bladder epithelial cells express the vanilloid receptor, TRPV1, and suggest their involvement in the sensory function of this tissue [40,41]. This has led to a proposed mechanism for onabotulinumtoxinA in the bladder involving inhibition of acetylcholine, adenosine triphosphate (ATP) and substance P release and reduction of axonal expression of capsaicin (TRPV1) and purinergic (P2X3) receptors [42]. This hypothesis is consistent with our proposal that onabotulinumtoxinA prevents peripheral sensitisation though the inhibition of local neuromodulator (neuropeptide) release.

6. Studies of BoNT-A on nociception in human volunteers

Reports on the antinociceptive effects of BoNT-A in human experimental models of pain have been mixed with negative and positive results (Table 1). These studies were double-blind, controlled designs and administered BoNT-A into the forearm [43–46], thigh [47] or forehead [48,49] of healthy volunteers. The experimental pain elicited in the volunteers varied (e.g., capsaicin [44], electrical stimulation [43,45] or ultraviolet B (UVB) irradiation [47]). These studies were focussed on BoNT-A-based products (abobotulinumtoxinA (Dysport®) [45,47] or onabotulinumtoxinA (BOTOX®) [43,44]). Important mechanism of action principles can be gleaned from the positive and negative reports.

Based the antinociceptive hypothesis [12,27,36], onabotulinumtoxinA or BoNT-A in general, is expected to prevent peripheral nerve sensitisation induced by local neurotransmitter (neuromodulator) release and indirectly attenuate central sensitisation (e.g., allodynia and/or hyperalgesia). Therefore, success or failure of a human experimental pain model to demonstrate an antinociceptive effect of BoNT-A must be reviewed in terms of the mechanism of pain initiation and/or the BoNT-A dose or location of the treatments, relative to the pain stimulus.

The electrical stimulation [43,45] used in two reports is not expected to be sensitive to inhibition by BoNT-A, as this afferent stimulus bypasses the nociceptive nerve terminal, where the toxin is active, and BoNT-A does not block action-potential conduction [50]. However, if the electrical stimulation led to local release of neuropeptides (e.g., CGRP), a change in peripheral blood flow (neurogenic flare) should be expected. This neurogenic flare, due to efferent exocytosis of CGRP from the peripheral nociceptive nerve terminals, is sensitive to inhibition by BoNT-A. Indeed, Kramer et al. observed that only neurogenic flare was moderately prevented by BoNT-A during the first 2 days of the observation period [43]. These results may be explained by the preclinical observation that neurogenic blood flow changes (i.e., CGRP mediated) are more sensitive to BoNT-A than is

Table 1
Human pain model summary.

Antinociceptive observation	Model	BoNT (product, dose)	Comments	Reference
Positive: Prevention of pain, increased blood flow and temperature at 7 and 14 days	Capsaicin (100 μg/0.1 ml, ID), forehead	BOTOX® 150 U (20 sites, head and neck region) 7, 14, 28 days or 22.5 U forehead	OnabotulinumtoxinA- and capsaicin-treated areas overlapped to allow pharmacologic effect.	Gazerani et al., 2006, 2009) [48,49]
Positive: Prevention of capsaicin-induced pain and neurogenic vasodilation	Capsaicin cream (0.5 ml of 0.075%), forearm (approx 24 cm^2)	BOTOX® 48 U (24 sites on forearm, approx 24 cm^2) vs. saline	OnabotulinumtoxinA effective only when administered in same area as capsaicin cream.	Tugnoli et al. (2007) [46]
Negative: No difference from placebo for pain perception or flare areas.	Capsaicin (0.1%, 0.02 ml, ID), forearm	BOTOX® 30 U (4 × 0.12 ml; 1.5 cm square) pre-treatment: 28 day	Interval between onabotulinumtoxinA and capsaicin may have been too long. In addition, the affected areas of BoNT-A vs. capsaicin may not have had sufficient overlap.	Voller et al., 2003 [44]
Negative: No direct antinociceptive effects in humans	Electrical (custom, concentric, 0.6–1.6 mA [55]), forearm	Dysport® 100 U (1 × 0.2 ml + 8 × 0.1 ml; 4 cm^2) pre-treatment: 28 & 56 day	Inappropriate pain stimulus for abobotulinumtoxinA mechanism.	Blersch et al., 2002 [45]
Negative: Lack of antinociceptive and anti-inflammatory effect	UVB (5 cm, 3 × minimal erythema dose), thigh	Dysport® 100U (5 × 0.1 ml; 3 cm^2) pre-treatment: 2 days	Pain stimulus may be inappropriate for abobotulinumtoxinA related mechanism or too strong for the dose of drug administered.	Sycha et al., 2006 [47]

pain (e.g., allodynia) [36]. In addition, the short duration of effect suggests that the BoNT-A dose for this particular stimulus was insufficient to produce a prolonged effect. The investigators may have observed additional inhibition of neurogenic flare if higher doses were administered. Blersch et al. used a custom concentric electrode designed to produce a "pinprick-like" pain, by selectively depolarising A-delta fibres in the superficial layer of the skin [45]. No neurogenic flare was reported with this stimulus. As expected, the results from this group demonstrated that a BoNT-A-based product, abobotulinumtoxinA (Dysport®), had no direct effect on non-inflammatory nociception. This is consistent with the preclinical observation that onabotulinumtoxinA did not inhibit pain transmission and was not effective in preventing the acute pain associated with the first phase (phase 1) of the rat formalin model [27].

Although BoNT-A was effective in blocking the second phase of an inflammatory stimulus in rats (e.g., formalin) [27] and as UVB is an inflammatory stimulus, the negative observation by Sycha et al. may be due to either: (1) the incorrect balance between the stimulus and the drug dose or (2) insufficient overlap between the drug and stimulus (unlikely, based on the methods description). The UVB model may be too severe and involve other mechanisms [51,52] that are not sensitive to inhibition by BoNT-A but sensitive to non-steroidal anti-inflammatory drug (NSAID) therapy [51–54].

Finally, the efficacy of BoNT-A in preventing capsaicin-induced hyperalgesia or allodynia was mixed. The study by Voller et al. [44] was negative while the studies by Gazerani et al. [48,49] and Tugnoli et al. [46] were positive. The differences in capsaicin dose, location and timing, relative to BoNT-A treatment, are summarised in Table 1. The interval between BoNT-A treatment and capsaicin challenge is 28 days for the negative study. In this time interval (28 days), Gazerani et al. found BoNT-A to have lost its antinociceptive effect. In addition, insufficient overlap between the BoNT-A-treated areas and the capsaicin treatment may have also resulted in the negative response in the Voller study, as Gazerani et al. used a larger injection volume (0.1 vs. 0.02 ml). Tugnoli et al. demonstrated conclusively that the onabotulinumtoxinA- and capsaicin-treated areas must overlap to demonstrate an inhibition of capsaicin-induced pain sensation, flare area and changes in cutaneous blood flow [46].

In summary, the antinociceptive effect of BoNT-A treatment has been observed in patients, preclinical models and in two clinical pain models. The observations of the negative clinical pain model may be due to inappropriate pain stimulus, insufficient area overlap between BoNT-A and the pain stimulus or an incorrect dose of BoNT-A. Further clinical trials in patients or pain models are needed to determine the appropriate conditions where BoNT-A can reduce chronic pain.

7. BoNT retrograde transport and transcytosis controversy

Unlike tetanus toxin, clinically relevant doses of the active portion of the botulinum toxin protein has been understood, by the scientific and medical field, that intact, active BoNT does not appear to undergo significant retrograde transport and transcytosis across neurons to exert effects in the central nervous system [56]. However, the laboratory of Caleo recently published non-clinical results and follow-on commentaries that appear to contradict this well-known distinction between botulinum and tetanus toxins [57–59]. The authors reported retrograde transport and transcytosis (i.e., adjacent spread) of botulinum toxin into the rat brain facial motor neuron nucleus following administration into the whisker pads. The authors concluded that "… Our findings provide the first evidence for a mechanism by which BoNT-A can gain access to the CNS after peripheral administration." [57]. The implications for this claim are important as direct administration of BoNT to the CNS can inhibit the activity many types of nerves. When administered to specific CNS sites, BoNT treatment prevented seizures in animals but impaired memory [60]. Thus, the issue of peripherally administered BoNT potentially reaching CNS neurons is important to understand.

The results of this study [57] are complicated by a number of important issues [61]. First, the authors used a high dose of a laboratory preparation of BoNT-A that was injected into a single site of the rat whisker pad. The dose used was 135 pg or approximately 450 pg kg^{-1}. For a clinical comparison, patients treated with onabotulinumtoxinA for cosmetic glabellar treatments typically receive approximately 20 units or approximately 3 pg kg^{-1} administered into the glabellar muscles, which is ~150-fold lower than the dose used by Antonucci and colleagues. Administration of this high dose in animals may have triggered a nonspecific uptake and could have overloaded the protein transport system of the neuron and transported material from the periphery to the central location of the treated neuron. The use of such high doses of a different type (i.e., laboratory preparation of BoNT-A) into a single

site negates the relevance of these results in a clinical setting with humans. In over 20 years of treatment with BoNT-A (onabotulinumtoxinA) of the facial area, no deleterious central effects have been observed. Alternatively, the dose/volume used by Caleo et al. may have exceeded the capacity of the injection location to retain BoNT-A locally and could have been absorbed systemically. Therefore, it is important for future studies to establish a dose–response relationship for each animal model to establish a locally effective dose with no significant systemic effect.

A second issue with this study [57] is that the authors used an incompletely characterised antibody to differentiate between cleaved and uncleaved SNAP-25, the key substrate for BoNT-A. The appearance of cleaved SNAP-25 in central neurons was taken to indicate retrograde transport and transcytosis of the active BoNT-A enzyme. That is, the authors did not attempt to directly determine the presence of BoNT-A in central neurons but rather assessed a fragment of the protein known to be cleaved by BoNT-A as a measure of toxin activity in tissues. They detected this protein fragment using Western blot or immunohistochemistry. However, the inference that a positive signal in these assays indicates the presence of cleaved SNAP-25 depends on the specificity of the antibody used. Because the antibody was not well characterised, it cannot be concluded with certainty that the protein binding to it was cleaved SNAP-25. Thus, the conclusion of the study that botulinum toxin type A can be undergo retrograde transport and transcytosis to other nerves in the CNS do not appear to be entirely justified. The relevance of the non-clinical report to the clinical use of botulinum neurotoxins remains controversial. Additional preclinical studies have been published, which also claim to have demonstrated botulinum toxin transport and transcytosis from the periphery to the CNS (reviewed by Pavone and Luvisetto [26]). These reports have not demonstrated that the doses used for the studies remained localised. Thus, resolution of this controversy requires further experimentation and the development of new methods to monitor the location of individual botulinum toxin molecules at clinically relevant doses.

Professor Berardelli has reviewed the potential clinical implications of BoNT-As long-distance effects [62] and concluded that there were no deleterious central effects in patients from therapeutic uses of BoNT-A. Berardelli and his co-author Dr. Curra opined that "… despite years of BT (BoNT-A) use in humans, no published reports has described a clinical event suggesting an uncontrollable, dangerous, or undesirable central action." These authors also supported the need for additional research to determine if there is a central action of BoNT-A from a peripheral administration.

8. Summary

The recent approval of onabotulinumtoxinA for the treatment of chronic migraine pain has increased the interest in understanding the mechanism by which a peripherally administered protein therapeutic can reduce chronic pain. This review has summarised non-clinical and clinical results to support the hypothesis that onabotulinumtoxinA reduces the symptoms associated with chronic pain through a two-step process:

(1) reduction of local pain nerve sensitisation through the local inhibition of neuropeptide release resulting in and
(2) indirect reduction of central sensitisation.

Conflict of interest statement

The authors are employees of Allergan, Inc, the manufacturer of BOTOX® (onabotulinumtoxinA).

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