# Evidence for Antinociceptive Activity of Botulinum Toxin Type A in Pain Management

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**The neurotoxin, botulinum toxin type A, has been used successfully, in some patients, as an analgesic for myofascial pain syndromes, migraine, and other headache types. The toxin inhibits the release of the neurotransmitter, acetylcholine, at the neuromuscular junction thereby inhibiting striated muscle contractions. In the majority of pain syndromes where botulinum toxin type A is effective, inhibiting muscle spasms is an important component of its activity. Even so, the reduction of pain often occurs before the decrease in muscle contractions suggesting that botulinum toxin type A has a more complex mechanism of action than initially hypothesized. Current data points to an antinociceptive effect of botulinum toxin type A that is separate from its neuromuscular activity. The common biochemical mechanism, however, remains the same between botulinum toxin type A's effect on the motor nerve or the sensory nerve: enzymatic blockade of neurotransmitter release. The antinociceptive effect of the toxin was reported to block substance P release using in vitro culture systems.[1]**

**The current investigation evaluated the in vivo mechanism of action for the antinociceptive action of botulinum toxin type A. In these studies, botulinum toxin type A was found to block the release of glutamate. Furthermore, Fos, a product of the immediate early gene, c-*fos*, expressed with neuronal stimuli was prevented upon peripheral exposure to the toxin.**

**These findings suggest that botulinum toxin type A blocks peripheral sensitization and, indirectly, reduces central sensitization. The recent hypothesis that migraine involves both peripheral and central sensitization may help explain how botulinum toxin type A inhibits migraine pain by acting on these two pathways. Further research is needed to determine whether the antinociceptive mechanism mediated by botulinum toxin type A affects the neuronal signaling pathways that are activated during migraine.**

**Key words: antinociceptive, botulinum, pain, mechanism**

**Abbreviations: BoNT botulinum toxin, BoNTA botulinum toxin type A, ACh acetylcholine, CNS central nervous system, DRG dorsal root ganglia**

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Botulinum toxin (BoNT), the most potent biological toxin known to man,[1] was first isolated in 1897. It is responsible for the severe food-borne illness, botulism, with lethal doses occurring at $10^{-9}$ g/kg of body weight.[2] Administration of therapeutic doses (nanogram quantities) of this same toxin manufactured for medical use has long been used as treatment for various neuromuscular disorders including focal dystonia and spasticity. In treatment of these neuromuscular disorders, marked analgesic activity was also observed. Recent research demonstrates that BoNT (specifically BoNT type A [BoNT/A]) may be an effective therapy for myofascial pain syndromes, migraine, and other headache types. The successful use of BoNT as a novel analgesic is providing renewed hope to patients with therapy-resistant chronic pain, particularly in migraine.

Botulinum toxin type A was initially thought to provide pain relief by reducing muscular activity. The efficacy of BoNT/A in muscular relaxation is due to its ability to act directly on muscle activity by inhibiting the release of the neurotransmitter, acetylcholine

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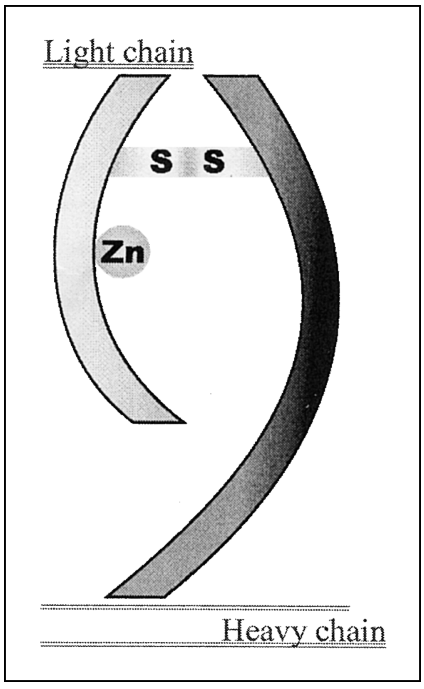
(ACh), at the neuromuscular junction.[3] As reviewed in Prof. Dolly's article in this supplement, BoNT/A-mediated inhibition of muscle contraction occurs via the classical mechanism of action (inhibition of ACh release) at the neuromuscular junction.[4]

Even so, clinicians have reported that the reduction of pain often occurs before muscular improvement, suggesting that BoNT/A may have a more complex mechanism of action on the pain system. Research investigating the BoNT/A mechanism of action at the cellular level suggests that this toxin has an antinociceptive effect that is independent of its neuromuscular activity.[5-7] This article will briefly summarize the mechanism by which BoNT/A affects muscular activity and focus primarily on BoNT/A-mediated antinociceptive effects.

## BOTULINUM TOXIN

The anaerobic bacterium, *Clostridium botulinum*, produces 7 serologically distinct neurotoxins that are designated A, B, C1, D, E, F, and G. Botulinum toxin serotypes are synthesized as single-chain polypeptides with a molecular mass of approximately 150 kDa. When isolated from bacterial cultures, BoNTs are found as macromolecular complexes, ranging from approximately 300 kDa to 900 kDa, consisting of the 150-kDa exotoxin molecule and one or more nontoxin proteins.[2,8] The creation of active BoNT requires a 2-step process. The first step occurs after the toxin is released from the bacterium, whereby the 150-kDA single-chain polypeptide and accessory proteins are released during autolysis of the bacterium. The second step involves the cleavage



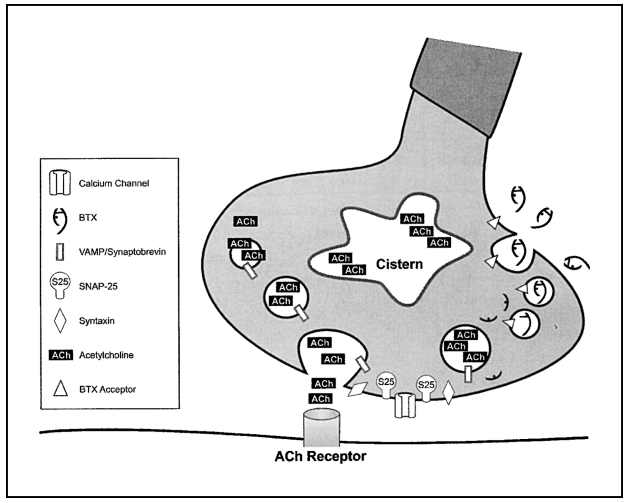




**Fig 1A.—Botulinum toxin type A is synthesized as a single polypeptide chain (150 kDa). The polypeptide is cleaved to form the light and heavy chains that remain connected by a disulfide bridge. In the dichain form, the toxin is active. Adapted from Brin, 1997.[4] 1B.—Pharmacology of botulinum toxin type A at the neuromuscular junction. Botulinum toxin type A is internalized by endocytosis into the axon terminal. After internalization, the light chain is translocated into the cytoplasm and acts as an endopeptidase. In the cytoplasm, the light chain cleaves a number of cellular proteins (specific for each serotype neurotoxin) that are involved in the fusion of synaptic vesicles to the plasma membrane. These cellular proteins include synaptosome-associated protein of 25 kDa (SNAP-25), syntaxin, and vesicle-associated membrane (VAMP) also known as synaptobrevin, each of which is important for acetylcholine secretion. Each serotype cleaves a specific peptide bond of a soluble *N*-ethylmaleimide sensitive factor attachment receptor (SNARE) protein. Adapted from Brin, 1997.[4]**

of this single polypeptide into a disulfide-linked dichain molecule consisting of a light chain (50 kDa) and a heavy chain (100 kDa) (Figure 1A). While the biological activity of each exotoxin is similar in that they produce temporary chemical denervation and relaxation of striated muscle, the intracellular molecular targets of each of these toxins differ, and they exhibit different durations of effect and different potencies.[2,8] It is the BoNT/A complex that is commercially available and for which most clinical experience exists.

## BOTULINUM TOXIN MUSCULAR ACTIVITY: EFFECT AT THE NEUROMUSCULAR JUNCTION AND MUSCLE SPINDLE

Botulinum toxin-mediated muscular paralysis, via inhibition of ACh release at the neuromuscular junction, has been well documented. As described in Prof. Dolly's chapter, BoNT/A is internalized by the motor neuron through binding to a high-affinity ectoacceptor. Once BoNT is internalized, cleavage of the disulfide bond between the heavy and light chain results in the translocation of the light chain into the cytoplasm. Here the light chain acts as a zinc endopeptidase and actively inhibits ACh release by cleaving intracellular proteins involved in ACh exocytosis.[4] These toxins cleave a number of intracellular proteins including synaptosome-associated protein of 25 kDa (SNAP-25). This cleavage prevents ACh vesicle fusion with the plasma membrane and, therefore, inhibits ACh neurotransmitter release at the neuromuscular junction (Figure 1B).[8]

Several reports have found that BoNT/A is effective at reducing muscle spasticity over a larger area than what is covered by the injection site. This observation may be explained by the fact that ACh is released by both alpha and gamma motor neurons[8]; these motor neurons are involved in voluntary (alpha) and involuntary (gamma) muscle contractions. Gamma motor neurons innervate the intrafusal fibers of the muscle spindles.[9] Rosales and colleagues found that BoNT/A caused atrophy of the intrafusal fibers, as well as the extrafusal fibers where alpha motor neurons are located.[10] Moreover, BoNT/A attenuates the output from the muscle spindle to the central nervous system (CNS) (Ia afferent). Filippi and colleagues demonstrated that in a model of rat's jaw muscle spindle, BoNT/A reduced the spindle afferent discharge.[11] This attenuated Ia afferent signal may then reduce the feedback to the alpha motor neurons to reduce muscle activity of the surrounding noninjected muscles.[5] The reduction of overall muscle contraction presumably, in turn, reduces excess muscle contraction associated with pain, thereby further contributing to the pain relief mechanism.

## BEYOND MUSCULAR EFFECTS: ANTINOCICEPTIVE ACTIVITY

Because local muscle paralysis and reduction in overall muscle contraction do not fully explain the pain relief mechanism of BoNT/A, it is postulated that BoNT/A inhibits peripheral sensitization of nociceptive fibers, thereby indirectly reducing central sensitization (Figures 2A and 2B). Botulinum toxin type A-mediated antinociceptive effects have been investigated using animal models and in vitro culture systems. In these studies, BoNTA has been shown to reduce peripheral sensitization by inhibiting the release of several neuronal signaling markers including glutamate and substance P, and reducing c-*fos* gene expression, as determined by immunohistochemical localization of the Fos protein.

We demonstrated the antinociceptive effect of subcutaneous (SC) BoNT/A (BOTOX was used in these studies) in a rat model with formalin-induced inflammatory pain.[7] There are two phases of formalin-induced pain. Phase I is the result of the response to acute pain due to chemical stimulation of nociceptor neurons by formalin. Directly following phase I, the pain response enters a quiet phase, which is thought to be the active inhibition of pain pathways. Phase II is initiated when the peripheral neurons become sensitized by the inflammatory response. Botulinum toxin type A (low dose, 3.5 U/kg; medium dose, 7 U/kg) or vehicle was administered subcutaneously to the plantar surface in each hind paw footpad. Rats were challenged with formalin injection 5 days post-BoNT/A injection in one hind paw, and 12 days postinjection in the other hind paw. Paw-licking and paw-lifting behaviors were monitored as indicators of pain.

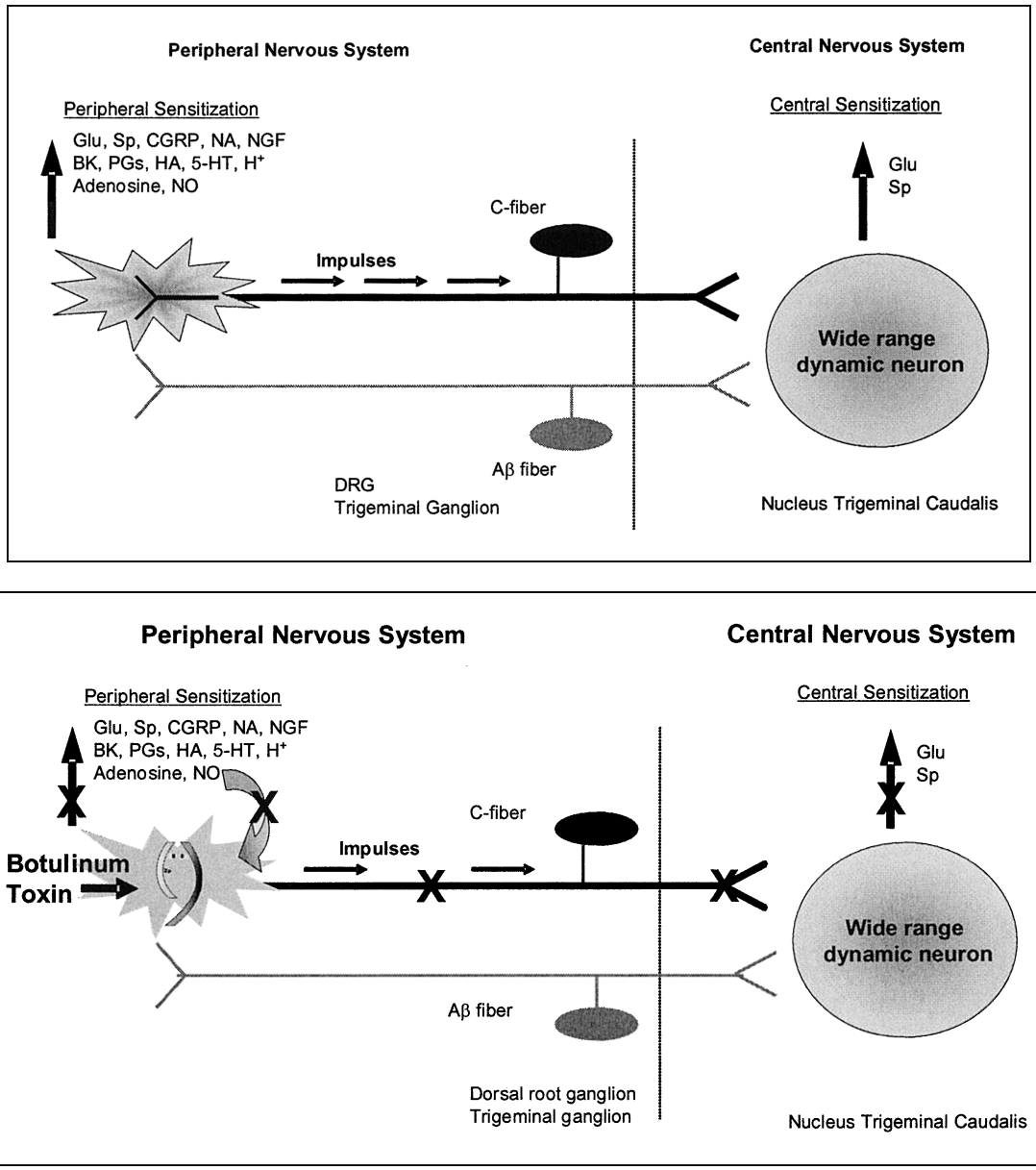



**Fig 2A.—Peripheral and central nervous system (CNS) sensitization. Peripheral sensitization occurs when neuropeptides and inflammatory mediators are released in response to injury or other stimuli. This peripheral sensitization increases the amount of signal transmitted to the spinal cord or into the trigeminal nucleus resulting in sensitization of the CNS. Glu indicates glutamate; Sp, substance P; CGRP, calcitonin gene-related peptide; NA, noradrenaline; NGF, nerve growth factor; BK, bradykinin; PGs, prostaglandins; HA, histamine; 5-HT, serotonin; $H^+$, hydrogen; NO, nitric oxide; DRG, dorsal root ganglia. 2B.—Botulinum toxin type A effects on sensitization. Botulinum toxin inhibits the release of a variety of neurotransmitters that would be secreted upon nociceptive stimulation and peripheral nerve injury. The toxin blocks peripheral sensitization directly and central sensitization indirectly via the inhibition of release of neurotransmitters, including glutamate and substance P. Within the CNS, glutamate and substance P secretion are blocked, thereby down-regulating immediate early gene expression at the level of the dorsal horn.**

It was found that acute pain (phase I) is not relieved by BoNT/A, but inflammatory pain (phase II) was inhibited by BoNT/A (Figure 3). Low-dose BoNT/A decreased the phase-II pain response by 29% and medium-dose BoNT/A by 46% in comparison to the vehicle. Antinociceptive efficacy was maintained at day 12 with the higher BoNT/A dose (30% pain inhibition versus vehicle). Muscle weakness at

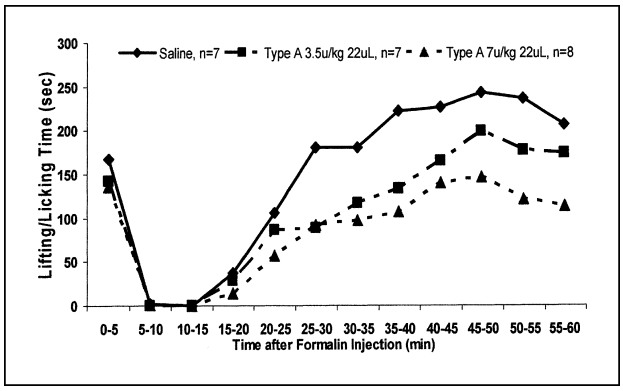



**Fig 3.—Antinociceptive activity of botulinum toxin type A in formalin-challenged rats.**

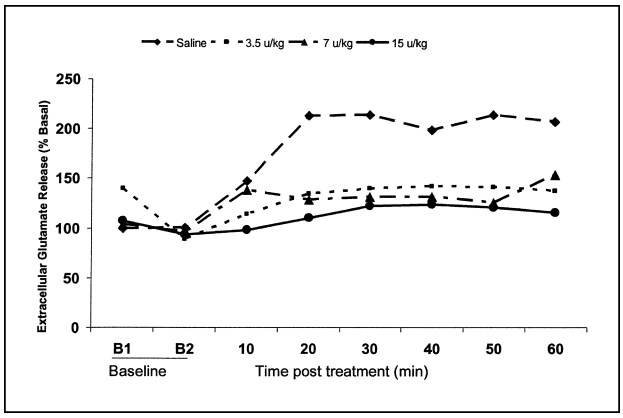



**Fig 4.—Reduction of formalin-mediated glutamate release with botulinum toxin type A (BoNTA). Subcutaneous BoNTA injection reduced formalin-induced glutamate release in the rat paw in a formalin-challenged inflammatory pain animal model. Five days post-BoNTA injection, formalin was injected into the footpad of the hind paw. Three dosages of BoNTA were used: 3.5 U/kg, 7 U/kg, and 15 U/kg. Subcutaneous BoNTA injection prevented formalin-induced increase of peripheral glutamate release compared to saline.**

the injection site was not apparent in any of the rats treated unilaterally with BoNT/A. In addition, BoNT/A injection followed by formalin challenge in the footpad produced a significant ($P<.05$), dose-related inhibition of the paw-licking and paw-lifting pain behaviors. These results suggest that BoNT/A inhibits pain by preventing peripheral sensitization in a dose-dependent manner.

Research investigating how BoNT/A inhibits peripheral sensitization of nociceptive fibers is ongoing. Data from primary culture and animal models indicate that BoNT/A inhibits the release of glutamate and neuropeptides, such as substance P, from the nociceptive neurons. Using the rat model of formalin-induced inflammatory pain, we have found that formalin-induced peripheral glutamate release in the rat footpad was significantly reduced by BoNT/A compared to vehicle.[7] Upon formalin challenge 5 days post-BoNT/A injection, a dose-dependent decrease in glutamate release was observed with an approximately 2-fold decrease at the medium dose (7 U/kg) (Figure 4).

Botulinum toxin type A has also been reported to inhibit both substance P- and ACh-mediated responses in cholinergic neurons that innervate the iris sphincter and dilator muscles of rabbits[12], demonstrating the ability of BoNT/A to block release of substance P, a molecule that is involved in pain perception, promotes vasodilation, neurogenic inflammation, and migraine.[13] Welch and colleagues developed an in vitro primary culture system of embryonic rat dorsal root ganglia (DRG) neurons to examine the sensitivity of DRG neurons to BoNT/A.[14] In this model system, isolated primary DRG neurons exhibit calcium-dependent substance P secretion upon depolarization and are sensitive to BoNT/A. The authors found that incubation of primary DRG cells with BoNT/A resulted in synaptosome-associated protein of 25 kDa (SNAP-25) cleavage within 2 hours and the inhibition of substance P secretion within 4 hours. In this system, the $IC_{50}$ was 0.05 nM for BoNT/A. The overall effects of BoNT/A on primary DRG cells lasted for 15 days in culture. The finding that BoNT/A inhibited substance P release suggests that noncholinergic neurons may respond to BoNT/A. These studies support the hypothesis that BoNT/A mediates an antinociceptive activity via the peripheral inhibition of transmitters such as glutamate and substance P.

As illustrated in Figure 3, inhibition of peripheral sensitization would result in decreased sensitization of the CNS. We evaluated the amount of afferent input to the spinal cord following BoNT/A in rats injected with formalin into each hind paw.[7] Botulinum toxin type A (15 U/kg) or vehicle was administered SC to the plantar surface in each hind paw footpad. Rats were challenged with formalin injection 1 day

post-BoNTA injection. Using a probe into the dorsal horn, recordings of wide dynamic range (WDR) neurons up to 100 minutes postformalin injection were evaluated. We found that BoNT/A reduced the peripheral formalin-mediated activity in the WDR neurons of the dorsal horn compared to vehicle.

We have further demonstrated the indirect CNS effect of BoNT/A by investigating the stimulation of the immediate early gene, c-*fos*, using the formalin-challenged rat model.[7] Activation of the c-*fos* gene and expression of its protein product, Fos, indicate rapid neuronal firing in response to stimuli.[15] Botulinum toxin type A (7 U/kg, 15 U/kg, and 30 U/kg) or vehicle was administered SC to the plantar surface in each hind paw footpad prior to formalin injection. Immunostaining for Fos expression demonstrated a reduction of Fos-positive cells within lamina I and II of the dorsal horn in BoNT/A-treated animals compared to vehicle. Moreover, BoNT/A-mediated reduction of Fos expression after formalin challenge occurred in a dose-dependent manner. Results of this study would appear to confirm the inhibition of BoNT/A on nociceptive input into the CNS.

Results from these studies using various models demonstrated decreased CNS sensitization with BoNT/A in response to pain stimuli, and is likely due to BoNT/A inhibition of peripheral sensitization.

## ANTINOCICEPTIVE CONNECTION IN MIGRAINE PAIN

Migraine is a disorder of the brain that results from dysfunction of the brain stem and nociceptive neuronal changes.[16] Malick and Burstein have proposed that migraine pain is mediated through both peripheral and CNS sensitization.[17] They have demonstrated in an animal model of migraine that intracranial pain is accompanied by increased skin sensitivity (cutaneous allodynia—pain resulting from a non-noxious stimulus to normal skin). Their data suggests that the throbbing pain of migraine is mediated through both peripheral and central sensitization. Confirming the animal data, Burstein and colleagues[18] found that a majority (79%) of migraineurs had associated cutaneous allodynia. This data would suggest that the pathophysiology of migraine involves both peripheral and central sensitization.

The hypothesis that migraine involves both peripheral and central sensitization may explain how BoNT/A inhibits migraine pain. Research investigating the antinociceptive effects of BoNT/A indicates that BoNT/A inhibits peripheral sensitization thereby resulting in a reduction of central sensitization. Further research is required to determine if the antinociceptive mechanism of BoNT/A affects the same neuronal pathways that are activated during migraine.

## CONCLUSION

Studies of BoNT/A for the treatment of painful disorders have led to the understanding of additional mechanisms of action beyond the classical mechanism of inhibiting neurotransmitter release at the neuromuscular junction. The antinociceptive activity of BoNT/A—inhibition of peripheral sensitization resulting in an indirect reduction of central sensitization—may be a possible mechanism for its clinical efficacy in treating inflammatory and chronic pain. Clinical studies have strongly suggested BoNT/A efficacy in the treatment of these chronic pain syndromes such as migraine and other headache types. Future investigations will further define the role of BoNT/A in chronic pain management.

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