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CENTRAL ACTION OF BOTULINUM TOXIN TYPE A – IS IT POSSIBLE?

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Botulinum toxin (BoTx) is a product of gram-positive anaerobic bacteria of the *Clostridium* genus. At present, seven serotypes (A to G) of BoTx have been identified. Each of them functions as a zinc-dependent endopeptidase that hydrolyzes peptide bonds within soluble N-ethylmaleimide-sensitive factor attaching protein receptors. BoTx affects proteins required for neurotransmitter release through presynaptic membranes. As a result, muscle weakness develops, or complete paralysis of the muscles occurs. These effects are not only limited to striated muscles but also may have impact on smooth muscles and secretory glands. The observation that BoTx can diffuse from the site of administration may indicate the possibility of direct or indirect influence of the toxin on the CNS. Consequently, the question arises: What is the mechanism of the central action of BoTx? Several mechanisms of such action have been proposed. However, recent findings showed that the most probable mechanism responsible for the central effects of BoTx action is its anterograde transport. In this review, we describe and discuss the most important aspects related to BoTx action on the CNS.

Keywords: botulinum toxin (BoTx), proteins of the SNARE complex, neurotransmitters, effeects on the CNS, anterograde transport, antinociceptive effect.

INTRODUCTION

Botulinum toxins (BoTxs) are produced by gram-positive anaerobic bacteria of the *Clostridium* genus (*Clostridium botulinum*, *Clostridium butyricum*, and *Clostridium baratii*). These bacteria have been with mankind since the beginning of time. The interest of scientists in BoTx began at the end of the 18th century, when sausage poisoning in the German state of Wurttemberg was first described. A few years later, a German poet and district medical officer, Justin Kerner, published a complete description of food-borne botulism symptoms. However, the work done by Kerner was not enough to identify the deadly pathogen, although it allowed researchers to conclude that the disease was closely related to consumption of processed meat. The first to identify and describe the pathogen was the German Professor of bacteriology at the University of Ghent, Emile Van Ermengem [1–3]. Later on, research on BoTxs led to the identification of their seven serotypes (A to G). The structure of these toxins and the mechanisms of their effects were described, which allowed researchers to begin the therapeutic use of BoTx. In 1989, the US Food and Drug Administration approved the use of BoTx type A in the treatment of strabismus, blepharospasm, hemifacial spasm, and cervical dystonia [4]. Nowadays BoTxs are widely used both by the pharmaceutical industry and in cosmetic medicine/surgery. Also, many off-label uses of BoTx A are being developed. The list of medical conditions treated by BoTx administration is shown in Table 1.

Structure of BoTx; the Mechanism and Effects. Each serotype of BoTx is formed by a 150 kDa single polypeptide chain that is posttranslationally proteolysed by endogenous proteases to a dichain structure. As a result, an active form of BoTx is composed of two polypeptide chains, a heavy chain (HC, 100 kDa) and a light chain (LC, 50 kDa), which are connected by covalent disulfide and noncovalent bonds. The heavy chain of BoTx consists of two domains [5]. A C-terminal domain contributes to the binding of the BoTx molecule to membrane gangliosides and specific receptors, such as synaptotagmins I and II [6, 7]. An N-terminal domain,

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Medical conditions treated with botulinum toxin [34, 81-91]

Медичні показання для лікування з використанням ботулінового токсину [34, 81–90]

Conditions for which treatment with BoTx has been used	
Movement disorders:	dystonia; hemifacial spasm; tremor; tics; bruxism; re-innervation synkinesias; myokymia; neuromyotonia; stiff person syndrome.
Hypersecretory disorders:	hyperhidrosis; sialorrhea; hyperlacrimation; rhinorrhea.
Ophthalmic disorders:	strabismus, nystagmus; exotropia, esotropia, entropium; protective ptosis.
Pain:	neuropathic pain; tension headache; migraine; myofacial pain; musculoskeletal pain; arthritis.
Pelvic floor and gastrointestinal disorders:	achalasia; anal fissures; detrusor-sphincter dyssynergia; vesical sphincter spasms; sphincter oddi spasms; animus; vaginismus.
Cosmetic applications:	muscular facial lines; facial asymmetries.
Others:	eye-lid opening apraxia; tetanus; stuttering; multiple sclerosis; idiopathic bladder syndrome; perioperative fixations in orthopedic surgery.

on the other hand, allows BoTx to enter nerve cells by translocation [8, 9]. Once the light chain of BoTx is translocated into the neuron, it impairs the release of neurotransmitters affecting a multiple-step cascade of protein-protein interactions, which is as follows. The arrival of an action potential (AP) at the axon terminal activates voltage-gated calcium channels, causing an influx of localized calcium ions into the cell. This, in turn, according to the SNARE hypothesis, triggers interaction of synaptotagmins with SNARE proteins (soluble N-ethylmaleimide attachment to the protein receptor). These events lead to fusion of the synaptic vesicles with the presynaptic membrane within an active zone. Briefly, once calcium ions attach to synaptotagmins, binding of synaptobrevin (VAMP, vesicle-associated membrane protein) to SNAP-25 and syntaxin is facilitated. Then, Rab-mediated n-Sec1 dissociation from synataxin is observed. As a consequence, a core complex is formed. Calcium-dependent oligomerization of the core complex leads

to the formation of a fusion pore; the latter is stabilized by oligomerization of synaptotagmin I. As the result, the membrane collapses, and the vesicle content is released into the synaptic gap [10–12].

After the released neurotransmitters reach the postsynaptic membrane, they bind to postsynaptic receptors, ionotropic (ligand-gated ion channels) or metabotropic (receptors coupled to G proteins). This causes changes in the postsynaptic membrane permeability followed by a shift of the potential on the postsynaptic membrane. As a result, excitatory or inhibitory postsynaptic potentials (EPSPs and IPSPs, respectively) develop. If the postsynaptic membrane undergoes depolarization, an EPSP occurs, and this may lead to generation of an action potential (AP).

A milestone in understanding the process described above was the following interpretation. The BoTx light chain functions as a zinc-dependent endopeptidase and hydrolyzes the SNARE protein complex [5]. Toxins of the B, D, F, and G types act on synaptobrevin [13–16]. The serotypes A and E hydrolyze SNAP25, while the serotype C is able to degrade both SNAP25 and syntaxin [17, 18] (for review, see [5, 19–21]). As a result, neurotransmitter exocytosis is blocked at different levels. Due to SNAP25 hydrolysis, BoTx type A blocks the post-docking priming step of exocytosis. Serotypes D, E, and F, on the other hand, prevent the formation of the fusion complex, while BoTxs B, C, and G uncouple fusion particles from the vesicle or plasma membrane [22].

Regardless of the hydrolyzed SNARE-type protein, the effect of BoTx on the nerve cell is the dose-dependent inhibition of neurotransmitter release through the presynaptic membrane (mainly that of acetylcholine). This causes inhibition of striated-muscle neuromuscular junctions with consequent muscle weakness and possible complete paralysis. Further experiments have shown that the action of BoTx is not limited to acetylcholine release and striated muscle weakness, but also influences the secretion of neurotransmitters, including glutamate, GABA, aspartate, met-enkephalin, noradrenaline [23, 24], serotonin [25], and glycine [26]. These effects cause the respective electrophysiological alterations [27] and can influence smooth muscles and secretory glands [28].

Initially, it was thought that the BoTx-specific effects are local. However, it was shown that BoTxs are able to diffuse and act on other remote tissues, perhaps even on those of the CNS, causing long-lasting structural and functional changes in the vicinity of the site of BoTx administration [29–32].

As was mentioned, therapeutics containing BoTx A (e.g., Botox ® and Dysport ®) or BoTx B (Myobloc®) are widely used in the treatment of medical conditions associated with excessive activity of the striated muscles, smooth muscles, or secretory glands, and also in cosmetic medicine for wrinkle reduction. Additionally, there are attempts to use BoTxs of types A or B in the treatment of off-label medical conditions. With increase in the therapeutic use of BoTx A and BoTx B, an increased number of reports concerning side effects of BoTx administration is observed. Among the most frequently mentioned, there are markers of neurological impairment (impaired vision, conjunctival irritation, reduced sweating, swallowing difficulties, mouth dryness, and others) [33, 34]. This may suggest that, in addition to the peripheral action correlated with side effects of toxin administration, BoTx is likely to directly affect the nervous system by influencing peripheral or central pathways.

Central Effects of BoTx Administration. The hypothesis of the central BoTx action is consistent with the results of research conducted in the 1960–1970s. In 1963, Tyler [35] observed changes in the Hoffman reflex (H reflex) in a patient with botulism; a few years later, Polley et al. [36] found BoTx-related changes in cortical electrical activity in monkeys. Although the above authors [35, 36] showed that peripherally administered BoTx can cause symptoms associated with the CNS, there was no direct evidence for the possibility of the presence of BoTx in the CNS structures. The lack of this direct evidence made it impossible to link neurological effects of peripheral administration of BoTx with the appearance of the toxin in the CNS. One of the first research groups to prove that the peripherally administered BoTx can be transported to the brain or spinal structures was that of Wiegand et al. [31]. In their experiments, radioactively labeled BoTx was injected into the cat *m. gastrocnemius*. As a result, a distal-proximal gradient of radioactivity in the sciatic nerve developed. In addition, ipsilateral ventral roots innervating the injected muscle exhibited significantly higher levels of radioactivity than ventral roots of the contralateral (control) side. Direct stimulation of the muscle injected with BoTx type A caused an increase in the radioactivity level in the spinal half-segments ipsilateral to the injected muscle [31]. Unfortunately, the experiments using radioactively labeled BoTx type A demonstrated only that the peripherally administered BoTx A is, in principle, able to reach the

CNS. In addition, as in the case of all animal studies, it was impossible to rule out the influence of anatomical (e.g., muscle innervation) and physiological (e.g., metabolic rate) differences on the results obtained. Similarly, the administered BoTx dose was significantly higher than the doses used in the treatment of humans. Taking this into consideration, we doubt that BoTx type A found by Wiegand et al. in the CNS [31] possessed significant enzymatic activity, and that the observed results reflect changes occurring after peripheral administration of the toxin in humans. To resolve this, a few experimental and clinical studies were conducted. As a result, the main CNS structures, whose functions can be modulated by peripherally administered BoTx, have been identified. Among them, are the spinal cord, brainstem structures, and motor cortex [37-40].

The influence of peripherally injected BoTx on the spinal cord was confirmed by a series of electrophysiological experiments carried out by Hamjian and Walker [41]. Recording from the *extensor digitorum brevis* (EDB) muscle before and after administration of 10 U BoTx A in ten human subjects demonstrated that i.m. injection of the toxin caused a transient increase in the F-wave amplitude and a long-term increase in the F/M amplitude ratio. The authors suggested that the observed changes may result from a tetanus-like inhibition of acetylcholine release from Renshaw cells and reduced inhibition of ventral horn cells. Moreover, since the peripheral silent period remained unchanged, this could indicate that BoTx type A did not influence afferent inputs from muscle spindle receptors [41].

Moreno-Lopez et al. [42] tried to estimate the impact of BoTx type A on the brainstem functions. The authors recorded dose-dependent changes in impulsation in cranial nerves after administration of BoTx A to the lateral *rectus* muscle of the cat's eye. A single administration of the type A toxin in a dose of 3 ng/kg changed the discharge pattern of *abducens* motoneurons for three months. Based on these results, it was hypothesized that the effect of a high dose of BoTx A may be associated with the toxin transport from early endosomes via the trans-Golgi network and intra-Golgi transport to the endoplasmic reticulum by retrograde transport [43] or due to transneuronal changes [42]. Slawek and Reclawowicz [44] attempted to assess the possible central action of BoTx type A by recording auditory and somatosensory EPs in patients with cervical dystonia. The patients taking part in the experiment received BoTx A injections into the *mm. sternocleidomastoideus* (*splenius capitis*), *trapezius*, and *lavator scapulae*. In 4 to 6 weeks post-injection, examination of the brainstem auditory evoked responses (BAERs) and somatosensory evoked potentials (SSEPs) induced by stimulation of upper limbs was conducted. The results were compared with those obtained before BoTx A injections, and no statistically significant differences were observed. This may suggest that a direct central action of BoTx type A does not exist, and the neurological effect observed after BoTx administration may result from the impact of toxin on somatic elements of the peripheral nervous system [44].

In order to determine the impact of the toxin on the motor cortex, long-latency reflexes (LLRs) induced by electrical nerve stimulation were examined. After electrical stimulation of the median nerve, two types of EMG responses could be recorded, LLR1 and LRR2. The LLR1 is analogous to the stretch reflex, while the LLR2 is considered to reflect the activity of a cortical generator including the supplementary motor area. Naumann and Reiners [45] noted that patients with idiopathic focal dystonia manifested after BoTx A injections a significant reduction of the LLR2 amplitude on the clinically affected side, suggesting that BoTx A is capabile of modifying afferent outputs coming from the injected muscle and of modulating the central motor pattern in focal dystonia [45].

An another way to determine the cortical aspects of BoTx peripheral administration was to use transcranial magnetic stimulation (TMS) and analysis of motor evoked potentials (MEPs). Byrnes et al. [46] used this approach to plot an electrical activity map of primary motor cortex projections to the hand and forearm muscles in patients with writer's cramp during isometric contractions. It was found that i.m. BoTx type A injections did not cause any long-term improvement of the electrical activity map. However, BoTx A treatment was accompanied by transient reversal changes of abnormalities in the cortical electrical activity map, and the duration of such shifts depended on the clinical improvement associated with administration of the toxin. After the BoTx A effect declined, the cortical electrical activity map returned to the state observed before the treatment. These results suggest that changes within the primary motor cortex occurring in patients with dystonia may be due to abnormalities in afferent inputs, which can be temporarily modulated by i.m. administration of BoTx type A [46]. Experiments carried out on animals also supported a possibility of motor reorganization

following BoTx A injections. Using intracortical microstimulations of the vibrissa motor cortex area, Franchi [47] showed that BoTx A administration into a rat vibrissal pad caused a decrease in the dimensions of ipsilateral vibrissa representations in both hemispheres with simultaneous expansion of the forelimb and eye representations. This resulted in modulation of the forelimb, eye, and neck movements induced by electrical stimulation inside the former vibrissa region [47].

Other CNS elements that may be affected by the toxin are the structures involved in higher functions of the CNS, in particular the unimodal and heteromodal cortices, as well as structures of the paralimbic and limbic systems [48]. Although Haaland and Davis [50] did not succeed in proving the influence of BoTx on memory in patients with botulism [50], there are a few reports confirming such action. Experiments with positron emission tomography (PET) conducted on a group of patients diagnosed with adductor spasmodic dysphonia (ADSD) showed that speech-related responses of the CNS regions, involved in pathophysiology of the disorder, were significantly affected after administration of BoTx A [50]. In these experiments, Ali et al. [50] compared the results of PET examination in the course of the narrative speech test, narrative whispering test, and during a rest period. This was performed in a group of patients before and after treatment with unilateral injection of BoTx type A into the left thyroarytenoid muscle (16 ± 2.2 U, five patients) or injection of the toxin into both left and right thyroarytenoid muscles (2.9 ± 0.3 U, four patients). Additionally, to determine CNS markers of spasmodic dysphonia, the PET scan results of ADSD patients were compared with the results of healthy volunteers. Analysis of results of the narrative and whispering speech tasks revealed that BoTx treatment decreased the activity in the regions where responses demonstrated hyperactivity in the ADSD patients, including the right dorsal precentral gyrus, cerebellar hemispheres, vermis, primary auditory cortex, anterior cingulate cortex, and right anterior insula. The toxin administered also augmented the activity in the regions hypoactive in dysphonic patients. Among these regions were the dorsal postcentral gyrus, right anterior audidtory association cortex, posterior supramarginal gyrus, and posterior middle temporal gyrus, i.e., the parts of the unimodal and heteromodal sensory association areas. Moreover, the authors noticed a correlation between most regions where the BoTx treatment exerted its effects (increased or decreased activity) and clinical improvement. Although the toxin influenced a wide range of the CNS regions, it did not affect the activity in other regions, which were hypo- or hyperactive in ADSD. Among these regions, the supplementary motor area, anterior middle temporal gyrus, periaqueductal gray matter, posterior auditory association cortex, and right ventral precentral gyrus should be mentioned. According to the authors, these findings suggest that peripherally adminstered BoTx may, in fact, influence CNS activity. Unfortunately, the validity of these results can be brought into question. The obtained differences between healthy volunteers and patients may not be determined by the illness itself but reflect compensatory events or secondary responses to a primary pathophysiological process. This would explain the decrease of hyperactivity in the motor areas of people with ADSD, which are not typically associated with direct control of the oral and laryngeal muscles. Another troubleshooting issue, besides differentiation of the real impact of the toxin on spasmodic dysphonia pathophysiology related to the influence on compensatory mechanisms, is the dose and the side of toxin administration [52]. As was mentioned, five of nine patients received single unilateral injections of BoTx into the left thyroarytenoid muscle, while the remaining four patients were bilaterally injected with the toxin into both left and right thyroarytenoid muscles. Since it has been proven that the toxin's action is dose-dependent [51], it seems to be unlikely that the dose and side of administration did not affect the observed clinical improvement and PET scans in the above-mentioned experiments.

Besides human studies, the influence of BoTx on higher functions of the CNS seems to be confirmed by animal studies. Ando et al. [52] used administration of BoTx B to the entorhinal cortex to develop the rat model of dementia. Tests in the Hebb–Williams maze, AKON-1 maze, and a continuous alternation task in the T maze revealed the cognitive impairment in old rats and changes in learning and memory in adult rats. In addition, the analysis of induction of long-term potentiation (LTP) indicated significant suppression of this process in old rats [52]. Similar changes of the cognitive functions in CD1 mice after intracerebroventricular (i.c.v.) injection of the toxin types A and B were observed. During conditioning of active avoidance and of object recognition in the respective tests, BoTx-treated mice showed a reduced capacity to discriminate a novel object within a familiar environment [53]. In other experimental model with i.c.v. injections of the toxin type A

in rats, the Rotarod and Morris water maze tests were used to evaluate motor activity and spatial memory, respectively. The temporal characteristics of spatial memory impairment obtained during the experiment suggested a slower onset (during up to 3 months after i.c.v. BoTx A injections) and a long-term spatial memory disorder [54]. These results contradict the existing knowledge on the temporal characteristics of BoTx action. In most clinical cases, a measurable effect of BoTx action appears 3 to 30 days after administration [55]. In an animal model, products of BoTx A proteolysis were confirmed in the hippocampus 3 days after intrahippocampal injections [56].

Taken together, the results of experimental and clinical observations suggest that, in principle, the peripherally administered toxin can affect the CNS functions. Due to these results, a question arises: What is the mechanism of BoTx's central action?

Possible Mechanisms of the Central Action of BoTx. Currently, several possible major mechanisms of BoTx central action have been hypothesized. One of the most probable mechanisms of central action is related to blocking of acetylcholine release from γ-motoneurons connected with intrafusal muscle fibre endings. This leads to reduction of the input from Ia afferents with possible changes in presynaptic inhibitory effects of Ia afferents of antagonistic muscles or to reorganization of the cortical motor maps. The second mechanism involves the blockade of neuromuscular connections between α-motoneurons and extrafusal muscle fibers inducing plastic changes in motoneurons. Another possibility is transportation of the toxin from the cell body to axon terminals by anterograde transport followed by BoTx conveyance from one cell to another by membrane-bound carriers (a process called transcitosis) [38, 57]. Antonucci et al. [56] conducted an experiment in which BoTx type A was introduced in the hippocampus of C57BL/6N mice and Sprague–Dawley rats. To establish the central effect of such intrahippocampal injections, the emergence of SNAP25 breakdown products was recorded by immunohistochemical staining. The staining procedure was preceded by Western blot experiments; the latter demonstrated that polyclonal antibodies used in the experiment were recognized to be specifically cleaved by BoTx A or BoTx E SNAP- 25 and not by the whole protein. Interestingly, the SNAP25 hydrolysis product was found in a contralateral hemisphere. The staining spots were observed in the neuropil of superficial layers II-III. Since the II/III-layer neurons of the entorhinal cortex project to the hippocampus, retrograde transport can be assumed to be realized. In the same study, injections of BoTx toxin type A into a rat whisker muscle were performed. As a result, the SNAP25 proteolysis products appeared in the facial nucleus [56]. These results are strongly supported by the work by Restani et al. [58], who performed a multistep experiment on the rat visual pathways. Using an immunostaining technique and Western blotting, the authors managed to confirm the presence of BoTx A-truncated SNAP25 in the retinorecipient layer of the *colliculus superior*, as well as to rule out the possibility of the systemic spread of the toxin. Furthermore, the intraocular injection of colchicine revealed lack of BoTx A-truncated SNAP25 in the tectum after blockade of the anterograde transport. This fact, together with the lack of immunorectivity in retinal terminals after double immunostaining by markers of excitatory and inhibitory synapses, strongly suggests transcitosis and anterograde transport of the toxin. Since it could be argued that the conducted experiments showed the anterograde transport of cleaved SNAP25 rather than that of BoTx A itself, Restani et al. [58] combined intravitreal administration of toxin type A followed by section of the optic nerve with BoTx E intratectal injection. Similarly to BoTx A, the type-E toxin cleaves SNAP25. As a result, a 26-residue fragment is removed, while the BoTx type A removes only nine residues from the same region. Additionally, the action of BoTx E is short-lasting [59]. As was expected, the amount of BoTx A-truncated SNAP25 in the superior colliculus initially decreased. After the completion of BoTx type E effects, the catalytic activity of BoTx A, however, reappeared by means of increased BoTx A-truncated SNAP25, undeniably showing that toxin type A is transported anterogradely to the CNS structures [58]. Results presented by Bogucki [60], on the other hand, undermine the possibility of BoTx A retrograde transport to the CNS elements. To eliminate the possibility of BoTx A retrograde transport, peripheral nerve dissection was performed in an animal model. Single muscle fiber-EMG confirmed the presence of neuromuscular transmission disturbance in distant muscles, suggesting that hematogenic spreading of toxin type A occurs [60].

The third proposed mechanism seems to be the least likely since it implies toxin transport through the blood-brain barrier (BBB). Although Boroff and Chen [61] showed the presence of BoTx A in parenchyma and brain blood vessels after peripheral administration

in mice [62], the molecular size of botulinum toxin (150 kDa) makes the diffusion of BoTx through the BBB poorly probable.

Despite obtained evidence confirming the central action of BoTx and a few proposed mechanisms of such action, it seems that new experimental approaches in this respect are needed.

Antinociceptive Action of BoTx – New Insight into its Central Action. Unexpectedly, a new insight into the central effect of BoTx type A was provided due to explanation of an antinociceptive effect of the toxin. It is well known that pain transmission depends on the release of certain neurotransmitters and neuropeptides, including glutamate, substance P (SP), and calcitonin gene-related peptide (CGRP) [62]. Since it was shown that BoTx A inhibits the release all these agents (glutamate [63], substance P [64] and calcitonin gene-related peptide [65]), it seems reasonable to conclude that BoTx type A is capable of inducing the antinonciceptive effect by blocking the release of the above neurotransmitters and neuropeptides. This "pain relief" action does not always correlate with muscle weakness (if such occurs) and strongly suggests that the antinonciceptive BoTx A action depends on a mechanism differing from that responsible for muscle weakness. In subjects diagnosed with a muscle-centered temporomandibular disorder, i.m. injection of BoTx type A caused weakness in chewing muscles and reduction in subjective pain sensation. Eight weeks after toxin administration, the muscle power returned to baseline, while pain relief was maintained [67]. Similarly, Tarsy and First [68] found that the treatment of cervical dystonia with BoTx type A induced pain relief without improvement in the head positioning [67]. Further evidence for the antinonciceptive action of BoTx A has been provided using experimental inflammatory pain models. Pretreatment with BoTx type A reduced the formalin-induced grooming response [69] and licking/lifting behavior within the second phase of inflammatory pain [69] independently of the dose and route of administration [70, 71]. Also, pretreatment with BoTx A in carrageenan- and capsaicin-induced inflammatory pain models induced suppression of thermal and mechanical hyperalgesia [72, 73]. Neuropathic pain animal models confirmed the analgesic effect of BoTx type A. In the neuropathy model induced by sciatic nerve transection, single subcutaneous (s.c.) administration of BoTx A reduced or even completely abolished thermal and mechanical hyperalgesia [74]. In the chronic constriction-injury model of neuropathic pain, single intraplantar injection of the toxin reduced mechanical allodynia in mice [75] and rats [76], as well as thermal hyperalgesia [76] and allodynia [77] in rats. The peripheral polyneuropathic rat model also provided information on the analgesic effect of BoTx type A administration. In this model, paclitaxel-induced mechanical hyperalgesia was inhibited in both paws after BoTx A administration ipsilaterally to the paclitaxel injection side. The threshold for paw withdrawal pressure 3 days after the toxin injection was comparable with that observed in rats injected with saline [72].

It is well known that i.p. injection of streptozotocin induces diabetic neuropathy, which results in increased sensitivity to mechanical and thermal stimuli three weeks after injection. Administration of BoTx type A not only reduced mechanical hypersensitivity ipsilaterally to the injection side, but contralaterally as well. The antinonciceptive effect of the toxin towards thermal stimuli was observed only on the ipsilateral side [71]. It is worth mentioning that the antinonciceptive effect of BoTx type A administration in all described pain models was observed without any changes in the muscle power, once again suggesting the existence of a different, than muscle weakness-inducing, mechanism of BoTx A action. If so, the question of the nervous system structures involved in BoTx type A action rearises. As was proposed by Pavone and Luvisetto [78], BoTx A can exert its action by inhibiting the neurotransmitter and/or neuropeptide release from nonciceptive endings. This, in turn, reduces peripheral and/or central sensization, as well as blocks neurotransmission from central terminals of nonciceptive afferents. The possibility of retrograde transport also should be taken into consideration [79]. To establish the BoTx A side of action, a series of experiments on the toxin-related antinonciceptive effect was conducted. Bach-Rojecky and Lackovic [79] employed acidic saline-induced pain, colchicine administration, and sciatic nerve transection to demonstrate the central analgesic action of BoTx A. In these experiments, unilateral intramuscular injection of an acidic saline into the rat's hindpaw induced bilateral mechanical hyperalgesia, which was latter reduced in both hindpaws by s.c. toxin administration. When BoTx type A was injected contralaterally to the pain induction side, the antinonciceptive effect was observed only on the above side. To eliminate the possibility of peripheral desensitization, the toxin was ipsilaterally injected after acidic saline administration into the sciatic nerve. Then, this nerve was cut distally to the side of injection, preventing BoTx A appearance in peripheral nerve endings. Surprisingly, a significant antinonciceptive effect on the contralateral side was induced after such BoTx type A injection. The authors hypothesized that

the decrease in mechanical hyperalgesia might reflect the central action of the toxin after its retrograde transport. To confirm this possibility, BoTx A injection was followed by colchicine administration. Both substances were injected ipsilaterally to the acidic saline administration side. Due to colchicine-induced blockade of the axonal transport, the toxin effect was abolished both ipsilaterally and contralaterally. The final evidence for the central BoTx type A action was the effect of intrathecal toxin injection, which resulted in the abolishment of mechanical hypersensitivity on both sides [80]. In another study, colchicine injection into the trigeminal ganglion decreased the analgesic effect of BoTx A in the formalin-induced pain model. Additionally, immunostaining revealed the presence of cleaved SNAP25 in the dorsal horn of the ipsilateral trigeminal *nucleus caudalis* after BoTx type A application into the rat whisker pad [68].

Taken together, these data strongly suggest a possibility ot the central action of BoTx A based on retrograde transport of the latter. However, additional work should be done to clarify possible BoTx-induced modifications of the CNS functions.

This publication is a review paper; it was not associated with any experiments on animals or tests involving human subjects; therefore, it does not require confirmation of compliance with existing ethical standards from this aspect.

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ЦЕНТРАЛЬНА ДІЯ БОТУЛІНОВОГО ТОКСИНУ ТИПУ А – ЧИ ВОНА МОЖЛИВА?

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Р е з ю м е

Ботуліновий токсин (BoTx) є продуктом життєдіяльності грампозитивних бактерій роду *Clostridium*. На теперішній час ідентифіковано сім серотипів BoTx (A–G). Всі вони функціонують як цинкзалежні ендопеотидази, що гідролізують пептидні зв'язки з розчинним N-етилмалеімідчутливим фактором, контактуючим з протеїновими рецепторами. BoTx впливає на протеїни, необхідні для вивільнення нейротрансмітерів через пресинаптичні мембрани. Як результат, розвиваються м'язова слабкість або повний параліч м'язів. Такі ефекти не обмежуються поперечносмугастими м'язами, вони виявляються також у гладеньких м'язах та секреторних залозах. Як спостерігалося, BoTx може дифундувати від місця свого введення; це може вказувати на принципову можливість прямих або непрямих впливів токсину на ЦНС. Відповідно, виникає питання: яким є механізм центральної дії BoTx. Було запропоновано декілька гіпотез про механізми такої дії. Результати нещодавніх досліджень, однак, свідчать про те, що найбільш вірогідним механізмом, відповідальним за центральні ефекти BoTx, є його дія на антероградний транспорт. У нашому огляді ми описуємо та обговорюємо найбільш важливі аспекти дії BoTx на ЦНС.

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