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BOTULINUM TOXIN

Mechanisms of action

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ABSTRACT - This review describes therapeutically relevant mechanisms of action of botulinum toxin (BT). BT's molecular mode of action includes extracellular binding to glycoproteine structures on cholinergic nerve terminals and intracellular blockade of the acetylcholine secretion. BT affects the spinal stretch reflex by blockade of intrafusal muscle fibres with consecutive reduction of Ia/II afferent signals and muscle tone without affecting muscle strength (reflex inhibition). This mechanism allows for antidystonic effects not only caused by target muscle paresis. BT also blocks efferent autonomic fibres to smooth muscles and to exocrine glands. Direct central nervous system effects are not observed, since BT does not cross the blood-brain-barrier and since it is inactivated during its retrograde axonal transport. Indirect central nervous system effects include reflex inhibition, normalisation of reciprocal inhibition, intracortical inhibition and somatosensory evoked potentials. Reduction of formalin-induced pain suggests direct analgesic BT effects possibly mediated through blockade of substance P, glutamate and calcitonin gene related peptide.

KEY WORDS: botulinum toxin, mechanisms of action, acetylcholine, muscle spindles, stretch reflex, smooth muscles, exocrine glands, retrograde axonal transport, blood-brain-barrier, substance P.

Toxina botulínica: mecanismos de ação

RESUMO - O propósito deste artigo é uma revisão dos mecanismos de ação da toxina botulínica (TB) relevantes para a compreensão do seu uso terapêutico. A ação da TB a nível molecular consiste na sua ligação extracelular a estruturas glicoprotéicas em terminais nervosos colinérgicos e no bloqueio intracelular da secreção de acetilcolina. A TB interfere no reflexo espinal de estiramento através do bloqueio de fibras musculares intrafusais causando redução da sinalização aferente veiculada por fibras Ia e II e do tono muscular. Portanto, o efeito da TB pode estar relacionado não somente à paresia muscular mas também à inibição reflexa espinal. A TB promove ainda o bloqueio de fibras autonômicas para músculos lisos e glândulas exócrinas. Apesar de ocorrer alguma difusão sistêmica após a aplicação intramuscular a TB não atinge o sistema nervoso central (SNC) devido ao seu peso molecular (não atravessa a barreira hematoencefálica) e à lentidão do seu transporte axonal retrógrado que permite a sua inativação. Os efeitos indiretos sobre o SNC são: inibição reflexa, reversão das alterações da inibição recíproca, da inibição intracortical e de potenciais evocados somatosensoriais. A redução da dor induzida por formalina sugere que a TB tenha efeito analgésico direto possivelmente mediado por bloqueio da substância P, do glutamato e do peptídeo relacionado ao gene da calcitonina.

PALAVRAS-CHAVE: toxina botulínica, mecanismos de ação, acetilcolina, fusos musculares; reflexo de estiramento, músculos lisos, glândulas exócrinas, transporte axonal retrógrado, barreira hematoencefálica, substância P.

Botulinum toxin (BT) has been perceived as a lethal threat for many centuries. In medieval times guild regulations were used to control sausage production as a major source of botulism. In the 19th century the German district physician Justinus Kerner published two monographs describing the clinical features of botulism with a precision still unsurpassed today[1,2]. In the 1970's the perception of BT began to change when it was used as a research tool to study spinal cord physiology[3]. In the early 1980's BT's perception changed completely when its therapeutic potential suddenly became apparent. Over the past 20 years BT has been shown to be useful for the treatment of many conditions (Table)[4].

We wish to give an overview over BT's mechanisms of action relevant for understanding its therapeutic use.

Botulinum toxin structure

BT is produced by *Clostridium botulinum* and

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consists, of a complex mixture of proteins containing botulinum neurotoxin and various non-toxic proteins. Botulinum neurotoxin consists of a heavy chain and a light chain linked together by a single disulfide bond. It is synthesised as a relatively inactive single chain polypeptide with a molecular mass of approximately 150kD. It is activated when the polypeptide chain is proteolytically cleaved into the 100kD heavy chain and the 50kD light chain. Botulinum neurotoxin exists in seven different serotypes named A, B, C, D, E, F and G. Although all of these serotypes inhibit acetylcholine release from nerve terminals, their intracellular target proteins, their characteristics of action and their potencies vary substantially. BT type A (BT-A) has been the most widely studied serotype for therapeutic purposes. More recently BT type B (BT-B) became commercially available.

Botulinum toxin molecular mode of action

When the motoneuron action potential depolarises the axon terminal, acetylcholine is released from the cytosol into the synaptic cleft. This acetylcholine release is performed by a transport protein chain, the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex. When BT is injected into a target tissue, the heavy chain of the botulinum neurotoxin binds to glycoprotein structures specifically found on cholinergic nerve terminals. This specific docking is the reason for BT's high selectivity for cholinergic synapses. After internalisation, the light chain of the botulinum neurotoxin binds with high specificity to the SNARE protein complex[5]. The target proteins vary amongst the BT serotypes. BT-A cleaves synaptosomal-associated proteins of 25kDa (SNAP-25)[6]. BT-B cleaves vesicle-associated membrane protein (VAMP), also known as synaptobrevin II. The light chain's proteolytic cleavage of the SNARE protein complex prevents the docking of the acetylcholine vesicle on the inner surface of the cellular membrane and results in blockade of vesicle fusion. When the target tissue is a muscle, paresis by chemical denervation occurs. When the target tissue is an exocrine gland, the glandular secretion is blocked. The inhibition of acetylcholine exocytosis by BT is terminated by restoration of the SNARE protein complex turnover. Axonal sprouting and endplate elongation occurs, but is believed to be a transient phenomenon not responsible for the termination of the BT effect[7].

Botulinum toxin action on the striate muscle

Duration of action - When BT is injected into a striate muscle, paresis occurs after two to five days and lasts from two to three months before it gradually starts to wear off. Figure 1 gives an example of BT's duration of action as reconstructed from a patient's treatment calendar. When antibodies against BT are formed, as in this example, the duration of action and the extent of the maximal therapeutic effect are usually reduced after few BT applications

Table. Conditions for which treatment with botulinum toxin has been tried (Dressler, 2000).

Movement Disorders
Dystonia
Hemifacial Spasm
Tremor
Tics
Bruxism
Re-innervation Synkinesias
Myokymia
Neuromyotonia
Stiff Person Syndrom
Spasticity
Hypersecretory Disorders
Hyperhidrosis
Sialorrhea
Hyperlacrimation
Rhinorrhea
Ophtalmic Disorders
Strabismus, Nystagmus
Exotropia, Esotropia, Entropium
Protective Ptosis
Pain
Tension Headache
Migraine
Myofacial Pain
Pelvic Floor and Gastrointestinal Disorders
Achalasia
Anal Fissures
Detrusor-Sphincter Dyssynergia
Vesical Sphincter Spasms
Sphyncter Odii Spasms
Anismus
Vaginismus
Cosmetic Applications
Muscular Facial Lines
Facial Assymetries
Others
Eye-Lid Opening Apraxia
Tetanus
Stuttering
Perioperative Fixations in Orthopedic Surgery

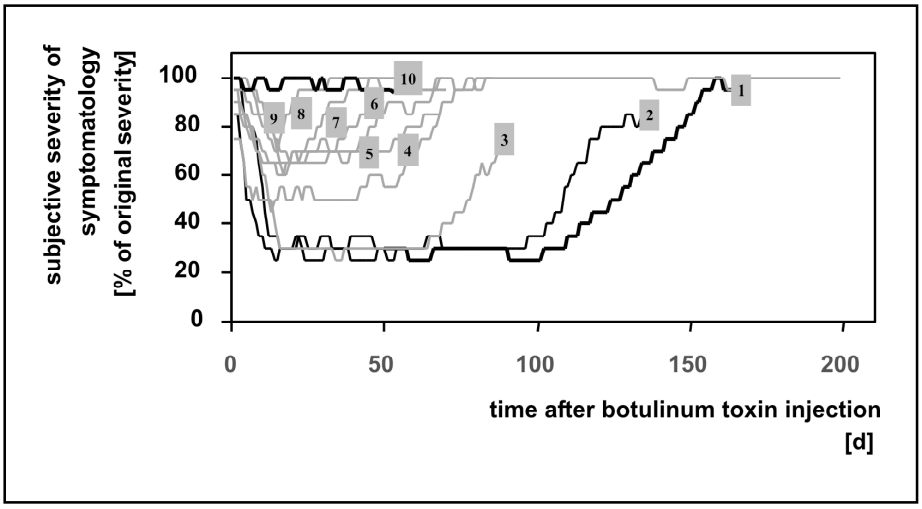

Fig 1. Treatment profile of a patient with cervical dystonia and antibody-induced botulinum tox - in therapy failure. The profile was reconstructed from a treatment calendar in which the patient was asked to document the overall severity of all cervical dystonia related complaints on a day to day basis. 100% reflects the untreated condition, 0% lack of any complaints. Injection series 1 and 2 produce adequate therapeutic effects, whereas injection series 10 does not produce any therapeutic effects (complete therapy failure). All other injection series produce reduced thera - peutic effects (partial therapy failure). From Dressler 2000.

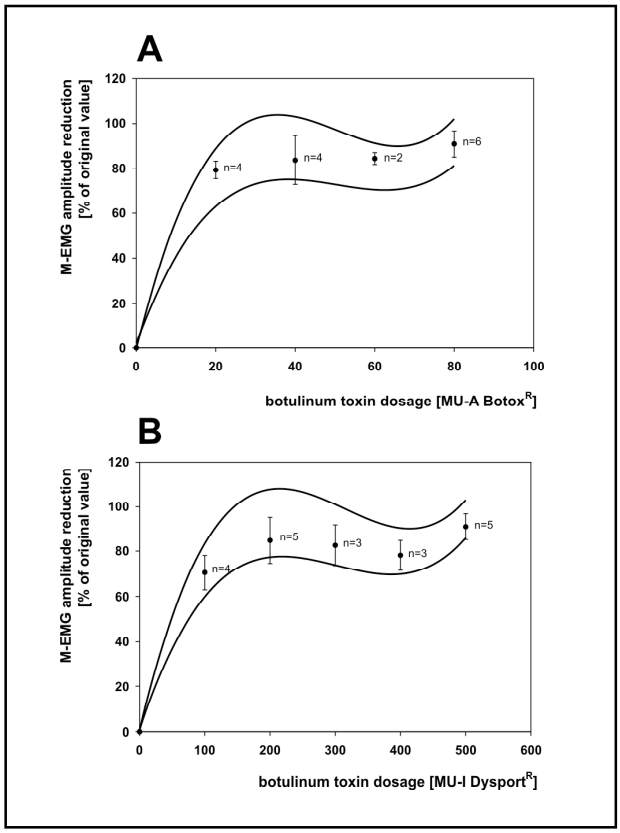

Fig 2. Correlation between botulinum toxin type A dose and induced reduction of the maximal electromyographic (M-EMG) amplitude in the sternocleidomastoid muscle. Mean values with two standard deviations. Curves are polynomal trendcurves (n=3, Microsoft Excel) of the two standard deviation values. A Botox®. B Dysport®. From Dressler & Rothwell, 2000.

(partial therapy failure) before complete therapy failure occurs[8]. The subjective duration of action varies between patients suffering from the same condition and between patients suffering from d i f-ferent conditions. When the same patient is treated with identical treatment parameters the duration of action is usually stable.

Dose-effect correlation - As shown in figure 2, there is a correlation between the amount of BT applied and the extent of paresis provoked[9]. However, relatively low BT doses already produce substantial paresis. Dose-effect correlation curves can be used to optimise BT doses in muscle tissue, dose-duration correlations, however, have to be kept in mind.

Dose-duration correlation - There is also a correlation between the amount of BT applied and the duration of its action[9]. However, this correlation seems to exist only when relative low BT doses are used. With higher BT doses the duration of action seems to saturate at about three months.

Muscle atrophy - When BT is injected into a hyperactive muscle the induced paresis produces a reduction of the diameter of the target muscle. When the target muscle is hypertrophic due to long lasting hyperactivity BT-induced paresis can normalise its size. When BT is given over a prolonged period of time real muscle atrophy can occur. However, mus-

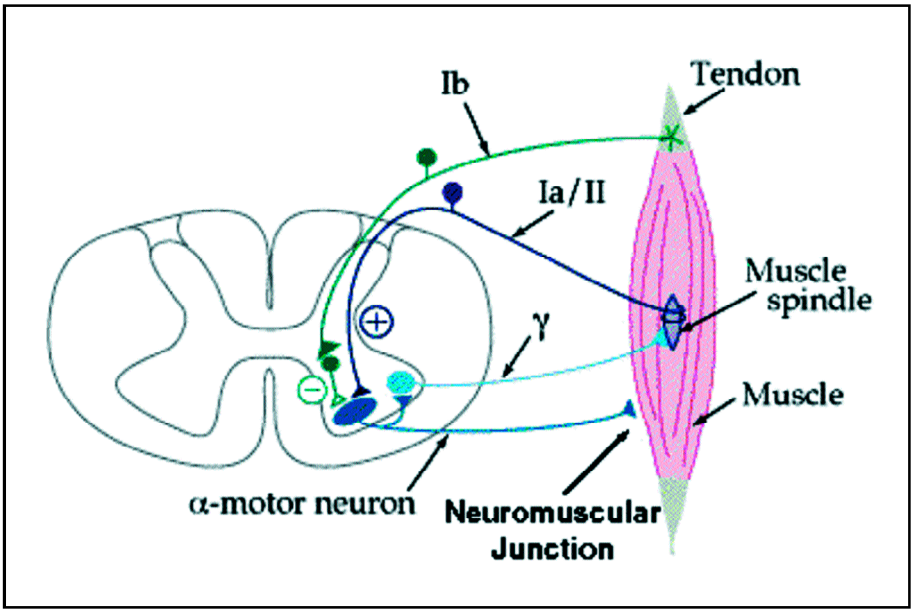

Fig 3. Spinal stretch reflex. Afferences from the muscle spindle organs and the Golgi tendon organs control the alpha motoneuron activity innervating the skeletal muscles. When the skeletal muscle is stretched muscle spindles convey a signal to the alpha motoneuron which then stimulates the contraction of both intrafusal and extrafusal muscle fibres.

cle atrophy is not an obligate BT effect and can therefore not be used to test BT efficacy.

Dilution effect - It has been assumed that with higher BT dilutions the tissue diffusion of BT can be increased, thus influencing the therapeutic effect and the side effects of a BT therapy. So far, no valid studies are available to estimate optimal dilution for different therapeutic situations. Defining a conversion ratio between Botox and Dysport mouse units to compare their therapeutic potencies has puzzled neurologists for years. Initial studies used inadequate clinical models, such as blepharospasm hemifacial spasm or spasmodic dysphonia, which are extremely dose insensitive with respect to their therapeutic outcome and side effects. Recently, Ranoux et al[10] used cervical dystonia as a more sensitive model and applied a crossover design to compare the potencies of the two products. This study has methodological advantages over previous ones, but referred to independent patients groups, thus provoking criticism because of vast interindividual cervical dystonia differences[11]. The more interesting result observed by Ranoux et al[10] cervical dystonia was that Dysport produce more swallowing difficulties than Botox. In the light of the conversion ratio discussion the logical argument was usually that Dysport was relatively overdosed compared to Botox. However the authors suggest that the two products have different diffusion pattern, but admitted that determining most appropriate conversion factors may be a never ending history[12].

Botulinum toxin action on the spinal stretch reflex

Human striate muscles contain cholinergic neuromuscular junctions not only between the alpha motoneurons and extrafusal muscle fibres, but also between the gamma motoneurons and intrafusal muscle fibres forming the muscle spindle organ. When a muscle stretch occurs afferent signals from the muscle spindle organs travelling in Ia and II fibres excite the alpha motoneurons of the stretched muscle as well as interneurons inhibiting the alpha motor neurons of its antagonistic muscles. Gamma motoneurons of the stretched muscle are activated by alpha motoneuron collaterals (alpha-gamma co-activation). This circuitry is shown in Figure 3. Signals from muscle spindle afferences are also relayed to supraspinal structures involved in long latency responses to the stretch reflex and in generation of a body image in space.

Recently, the role of afferent signals in the pathophysiology of dystonia has been stressed. After feedback mechanisms were suggested to play a role in laryngeal dystonia[13], it was demonstrated that Ia afference facilitation by tendon vibration can increase the severity of writer's cramp and that this increase can be blocked by lidocaine injections preferentially

affecting the muscle spindle function[14]. With this 'muscle afferent block' not only writer's cramp but also mandibular dystonia could be treated[15] .

BT produces different effects on the muscle spindle organs. Rosales et al.[16] demonstrated atrophy in both extrafusal and intrafusal muscle fibres in the biceps femoris of Wistar rats after injection of botulinum toxin type A. After BT injection muscle action potentials elicited by stimulation were abolished in both, extrafusal and intrafusal fibres, and spindle afferent discharges were progressively reduced[16]. Filippi et al.[17] demonstrated that gamma motoneuron terminals of isolated rat masseter muscles could be blocked by BT, thereby reducing the Ia and II afferent signal from the muscle spindle organs and the muscle tone by reflex inhibition without affecting muscle strength. The antidystonic effect of BT may, therefore, be caused not only by target muscle paresis but also by spinal reflex inhibition.

Botulinum toxin action on the autonomic nervous system

BT can be used to treat hyperactive smooth muscles, such as the distal oesophageal sphincter in achalasia, the sphincter Oddii in sphincter Oddii dysfunction, the internal anal sphincter in anal fissures and anismus, the vesical detrusor in detrusor-sphincter dyssynergia and the pylorus in gastroparesis. Systemic adverse effects of BT-B also demonstrate smooth muscle affection of BT when heart burn, accommodation difficulties and obstipation occurs[18]. When BT is used to treat hyperhidrosis, hypersalivation, hyperlacrimation, or when BT-B adverse effects, such as dryness of eye or mucosal dryness occurs[18], exocrine glandular tissue is affected by BT. BT, therefore can affect the efferent fibres of the autonomic nervous system as already meticulously described by Justinus Kerner in the early 19th century[1,2]. So far, it seems that BT action on the autonomic nervous system does not differ from its action on the striate neuromuscular synapse. Action on the autonomic nervous system offers an additional chance to study dose-effect and dose-duration relationships. Whether BT also affects the afferent transmission of the autonomic nervous system it needs to be studied.

Botulinum toxin action on the central nervous system (CNS)

Direct effects - When BT is injected into a target tissue it is almost completely bound to the axon terminal[19]. However, when BT-A is applied to treat cervical dystonia, small fractions of the applied BT are distributed systemically and can be detected by increase of neuromuscular jitter in non-injected muscles[20]. When BT-B is applied to treat cervical dystonia substantial systemic anticholinergic side effects can be clinically detected[18]. Despite its systemic distribution direct BT effects on the CNS have not been reported, since botulinum neurotoxin with its size of 150 kD cannot penetrate the blood brain barrier. Apart from systemic penetration BT could also reach the CNS by retrograde axonal transport. Indeed, such retrograde axonal transport has been detected for BT with radioactively-labelled botulinum neurotoxin[21]. However, the retrograde axonal transport was so slow that the applied BT was likely to be inactivated before it reached the CNS. Transsynaptic transport was not observed. BT action upon Renshaw cells was only demonstrated after intraspinal injection[3].

Indirect effects - Effects of BT on the neuromuscular synapse and on the muscle spindle organs can produce various indirect effects on the CNS. On the spinal level BT produces reflex inhibition of alpha motoneurons by gamma motoneuron blockade and subsequent Ia/II afferent input suppression1[16,17]. In patients with upper limb dystonia BT can normalise altered reciprocal inhibition between flexor and extensor muscles[22]. A similar effect was also demonstrated in patients with essential tremor[23]. EMG changes of the contralateral ocular muscles after injection of BT into the lateral rectus muscle also suggest central effects[24]. On the supraspinal level BT can normalise altered intracortical inhibition[25] and altered somatosensory evoked potentials[26]. Although BT can enhance some aspects of cortical activation it fails to improve the impaired activation of the primary motor cortex seen in writer's cramp[27].

Botulinum toxin action on pain

When BT is used to treat painful muscle hyperactivity disorders frequently substantial pain relief is reported. So far, this pain relief was attributed to the reduction of the muscle hyperactivity. However, formalin-induced pain in animals can be reduced by BT direct analgesic effect[28]. Probably such effect of BT is based on an action on neurotransmitters other than acetylcholine.

Substance P (SP), a neuropeptide involved in pain perception, vasodilation and neurogenic inflammation, can be blocked by BT together with

acetylcholine in the iris muscles of rabbits[29] as well as in cultured dorsal root ganglia neurons[30]. Association of this inhibition with a decrease of SNAP 25 suggests a direct BT effect. BT-induced suppression of SP can also be demonstrated in embryonic rat dorsal root ganglia neurons[31]. When different BT serotypes were tested, BT-A produced the strongest SP suppression[31].

BT has also been shown to suppress the release of glutamate, another neurotransmitter involved in nociception, in the periphery and in the dorsal horn[32] confirming earlier findings of BT-induced inhibition of glutamate release from cerebrocortical synaptosomes[33]. The release of noradrenaline in PC12 cells[34] and calcitonin gene related peptide (CGRP) in autonomic vascular nerve terminals[35] could also be reduced by BT suggesting additional possible mechanisms for BT effects on pain transmission[32,36]. Whether BT's action on Ia and II afferences can also modulate pain transmission it needs to be studied.

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