

Botulinum Toxin: Mechanisms of Action

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Key Words

Botulinum toxin · Mechanisms of action · Acetylcholine · Muscle spindles · Stretch reflex · Smooth muscles · Exocrine glands · Retrograde axonal transport · Blood-brain barrier · Substance P

Abstract

Botulinum toxin (BT) has been perceived as a lethal threat for many centuries. In the early 1980s, this perception completely changed when BT's therapeutic potential suddenly became apparent. We wish to give an overview over BT's mechanisms of action relevant for understanding its therapeutic use. BT's molecular mode of action includes extracellular binding to glycoprotein 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 by blockade of substance P, glutamate and calcitonin gene-related peptide.

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Introduction

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 [Kerner, 1820, 1822]. In the 1970s, the perception of BT began to change when it was used as a research tool to study spinal cord physiology [Hagenah et al., 1977]. In the early 1980s, BT's perception changed completely when its therapeutic potential suddenly became apparent. We wish to give an overview over BT's mechanisms of action relevant for understanding its therapeutic use.

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0014-3022/05/0531-0003\$22.00/0

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Table 1. Botulinum toxin components

Neurotoxin	light chain	50 kDa
	heavy chain	100 kDa
Non-toxic proteins	haemagglutinin complex	60 kDa
	non-haemagglutinating proteins	130 kDa

BT Structure

BT is produced by *Clostridium botulinum* and consists, as shown in table 1, 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 disulphide bond. It is synthesised as a relatively inactive single-chain polypeptide with a molecular mass of approximately 150 kDa. It is activated when the polypeptide chain is proteolytically cleaved into the 100-kDa heavy chain and the 50-kDa light chain. Botulinum neurotoxin exists in 7 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) has become commercially available.

BT 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 [Rizo and Sudhof, 1998]. The target proteins vary amongst the BT serotypes. BT-A cleaves synaptosome-associated proteins of 25 kDa (SNAP25) [Blasi et al., 1993]. 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 [de Paiva et al., 1999].

BT Action on the Striate Muscle

Duration of Action

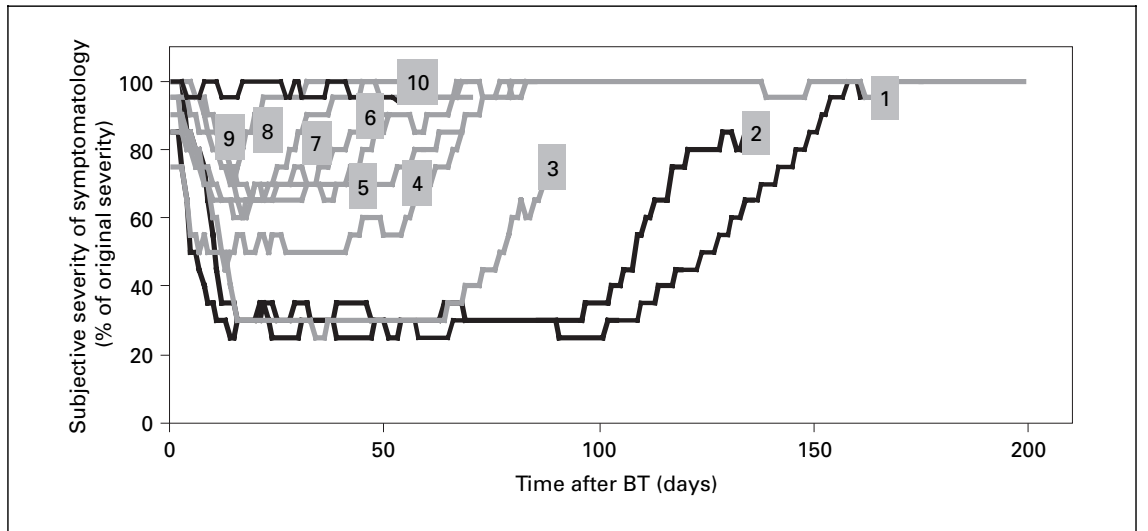
When BT is injected into a striate muscle, paresis occurs after 2–5 days and lasts for 2–3 months before it gradually starts to wear off. Figure 1 gives an example of BT's duration of action as reconstructed from a patient's treatment calendar. When antibodies against BT are formed, as in this example, the duration of action and the extent of the maximal therapeutic effect are usually reduced after few BT applications (partial therapy failure) [Dressler, 1997] before complete therapy failure occurs. The subjective duration of action varies between patients suffering from the same condition and between patients suffering from different 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 [Dressler and Rothwell, 2000]. 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

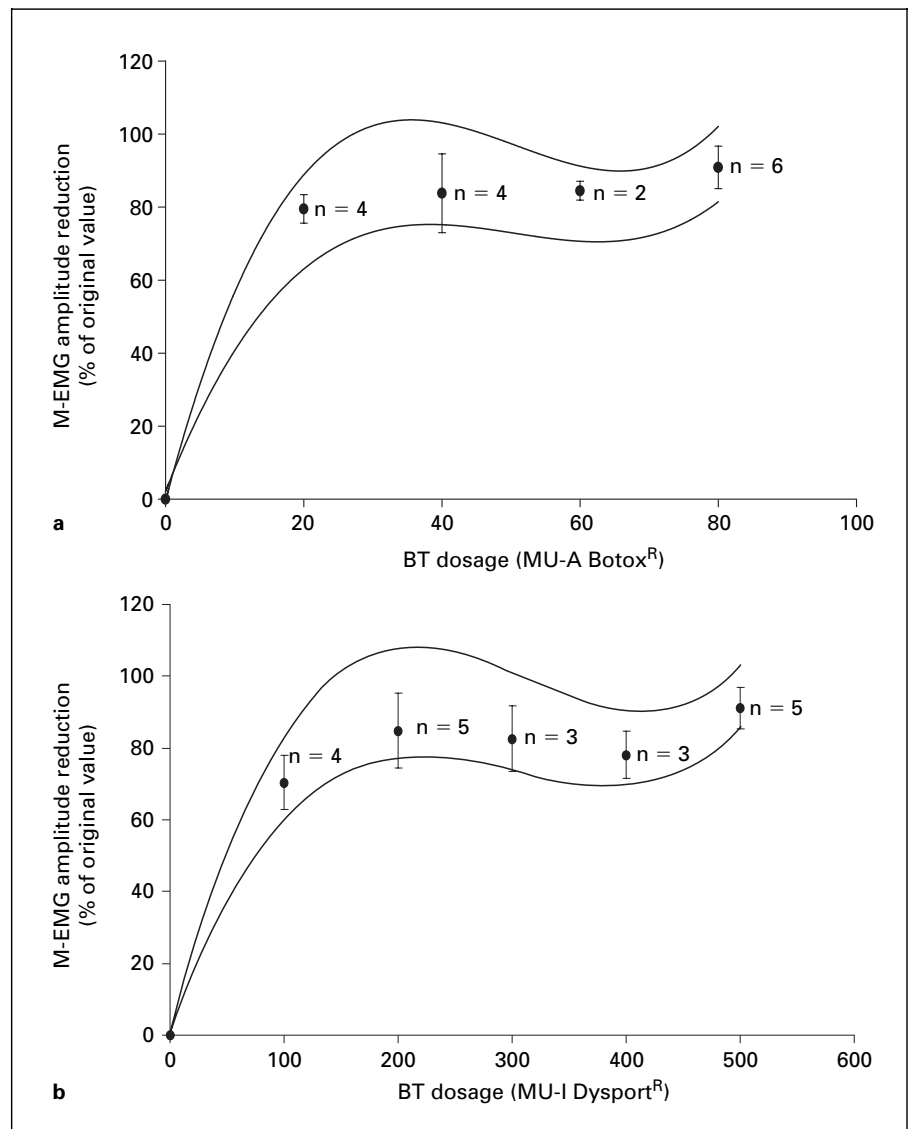
As shown in figure 3, there is also a correlation between the amount of BT applied and the duration of its action. However, this correlation seems to exist only when relatively low BT doses are used. With higher BT doses, the duration of action seems to saturate at about 3 months.



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Fig. 1. Treatment profile of a patient with cervical dystonia and antibody-induced BT 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 therapeutic effects (partial therapy failure). From Dressler [2000].

Fig. 2. Correlation between BT-A dose and induced reduction of the maximal electromyographic (M-EMG) amplitude in the sternocleidomastoid muscle. Mean values with 2 standard deviations. Curves are polynomial trend curves ($n = 3$, Microsoft Excel) of the 2 standard deviation values. **a** Botox[®]. MU-A = Allergan mouse units. **b** Dysport[®]. MU-I = Ipsen mouse units. From Dressler and Rothwell [2000].



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Fig. 3. Correlation between BT-A dose and duration of action. The maximal electromyographic (M-EMG) amplitude reduction describes the reduction of the surface electromyographic amplitude of the sternocleidomastoid muscle under maximal voluntary activation after BT application. MU = Mouse units. From Dressler and Rothwell [2000].

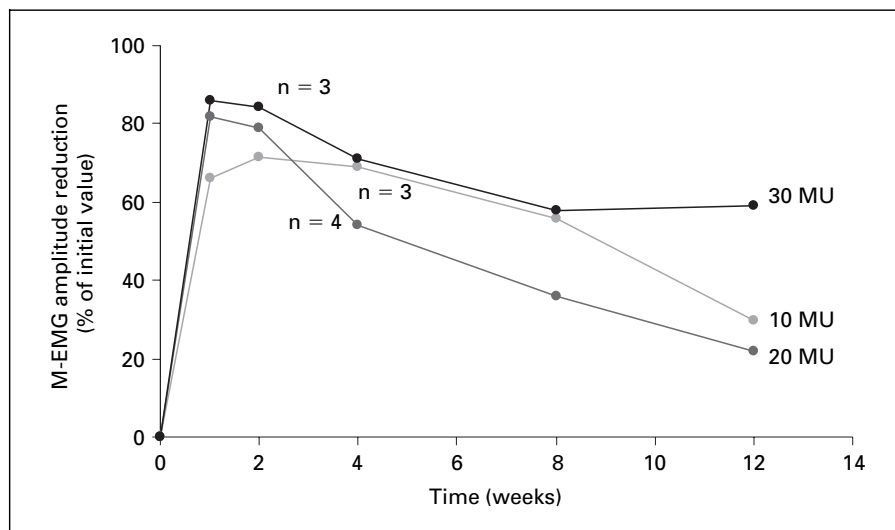
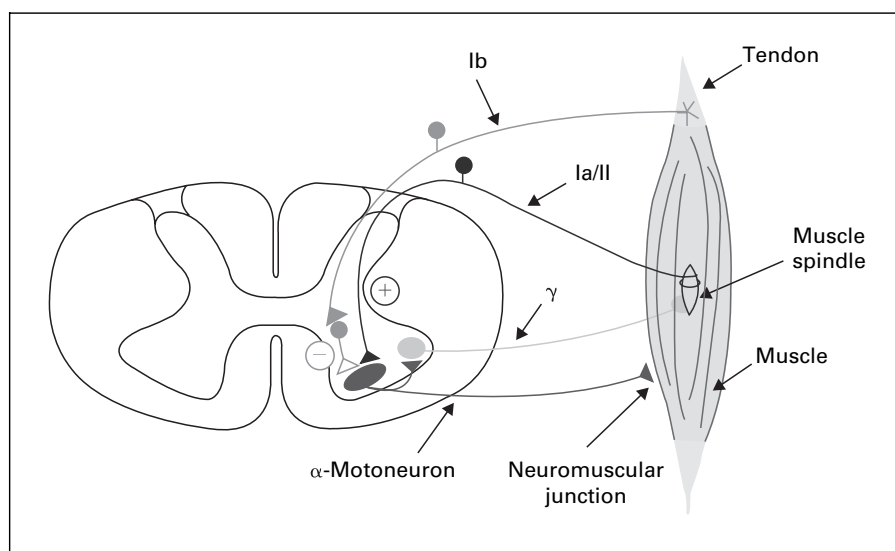


Fig. 4. Spinal stretch reflex. Afferences from the muscle spindle organs and the Golgi tendon organs control the α -motoneuron activity innervating the skeletal muscles. When the skeletal muscle is stretched, muscle spindles convey a signal to the α -motoneuron which then stimulates the contraction of both intrafusal and extrafusal muscle fibres.



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, muscle 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. Recently, the influence of the particular therapeutic BT preparation on the tissue diffusion has been pointed out [Dressler, 2002].

BT Action on the Spinal Stretch Reflex

Human striate muscles contain cholinergic neuromuscular junctions between the α -motoneurons and extrafusal muscle fibres, but also between the γ -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 α -motoneurons of the stretched muscle as well as interneurons inhibiting the α -motoneurons of its antagonistic muscles. γ -Motoneurons of the stretched muscle are activated by α -motoneuron collaterals (α - γ co-activation). This circuitry is shown in figure 4. Signals from muscle spindle afferents 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 had been suggested to play a role in laryngeal dystonia [Ludlow et al., 1990], 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 [Kaji et al., 1995]. With this 'muscle afferent block' not only writers's cramp, but also mandibular dystonia could be treated [Yoshida et al., 1998].

BT produces different effects on the muscle spindle organs. Atrophy in both extrafusal and intrafusal muscle fibres in the biceps femoris of Wistar rats has been demonstrated after BT-A injection [Rosales et al., 1996]. After BT injection, muscle action potentials elicited by stimulation were abolished in both, extrafusal and intrafusal fibres, and spindle afferent discharges were progressively reduced [Rosales et al., 1996]. Filippi et al [1993] demonstrated that γ -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.

BT 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 occur [Dressler and Benecke, 2003]. When BT is used to treat hyperhidrosis, hypersalivation, hyperlacrimation or when BT-B adverse effects, such as dryness of eye or mucosal

dryness occurs [Dressler and Benecke, 2003], exocrine glandular tissue is affected by BT. Therefore, BT can affect the efferent fibres of the autonomic nervous system as already meticulously described by Justinus Kerner in the early 19th century [Kerner, 1820, 1822]. 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 needs to be studied.

BT Action on the Central Nervous System

Direct Effects

When BT is injected into a target tissue, it is almost completely bound to the axon terminal [Takamizawa et al., 1987]. However, when BT-A is applied to treat cervical dystonia, small fractions of the applied BT are distributed systemically and can be detected by an increase in neuromuscular jitter in non-injected muscles [Sanders et al., 1986; Lange et al., 1987; Olney et al., 1988; Girlanda et al., 1992]. When BT-B is applied to treat cervical dystonia, substantial systemic anticholinergic side-effects can be detected clinically [Dressler and Benecke, 2003]. Despite its systemic distribution, direct BT effects on the central nervous system have not been reported, since botulinum neurotoxin with its size of 150 kDa cannot penetrate the blood-brain barrier. BT, however, could reach the central nervous system by retrograde axonal transport. Indeed, such retrograde axonal transport has been detected for BT with radioactively labelled botulinum neurotoxin [Wiegand et al., 1976]. However, the retrograde axonal transport was so slow that the applied BT was likely to be inactivated before it reached the central nervous system. Transsynaptic transport was not observed. BT action upon Renshaw cells was only demonstrated after intraspinal injection [Hagenah et al., 1977].

Indirect Effects

Effects of BT on the neuromuscular synapse and on the muscle spindle organs can produce various indirect effects on the central nervous system. On the spinal level, BT produces reflex inhibition of α -motoneurons by γ -motoneuron blockade and subsequent Ia/II afferent input suppression [Filippi et al., 1993; Rosales et al., 1996]. In patients with upper limb dystonia, BT can normalise the

altered reciprocal inhibition between flexor and extensor muscles [Priori et al., 1995]. A similar effect was also demonstrated in patients with essential tremor [Modugno et al., 1998]. EMG changes of the contralateral ocular muscles after injection of BT into the lateral rectus muscle also suggest central effects [Moreno-Lopez et al., 1994]. On the supraspinal level, BT can normalise altered intracortical inhibition [Gilio et al., 2000] and altered somatosensory evoked potentials [Dressler et al., 1995]. 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 [Ceballos-Baumann et al., 1997].

BT 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, when recently formalin-induced pain in animals could be reduced by BT [Cui and Aoki, 2000], direct analgesic effects of BT based on an action on neurotransmitters other than acetylcholine became likely.

Substance P, a neuropeptide involved in pain perception, vasodilation and neurogenic inflammation, can be blocked by BT together with acetylcholine in the iris muscles of rabbits [Ishikawa et al., 2000] as well as in cultured dorsal root ganglia neurons [Purkiss et al., 2000]. Association of this inhibition with a SNAP25 decrease suggests a direct BT effect. BT-induced suppression of substance P can also be demonstrated in embryonic rat dorsal root ganglia neurons [Welch et al., 2000]. When different BT serotypes were tested, BT-A produced the strongest substance P suppression [Welch et al., 2000].

BT has also been shown to suppress the release of glutamate, another neurotransmitter involved in nociception, in the periphery and in the dorsal horn [Cui et al., 2002] confirming earlier findings of BT-induced inhibition of glutamate release from cerebrocortical synaptosomes [McMahon et al., 1992]. The release of noradrenaline in PC12 cells [Shone and Melling, 1992] and calcitonin gene-related peptide in autonomic vascular nerve terminals [Morris et al., 2001] could also be reduced by BT suggesting additional possible mechanisms for BT effects on pain transmission [Cuesta et al., 1999; Cui et al., 2002]. Whether BT's action on Ia and II afferences can also modulate pain transmission needs to be studied.

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