

Review

Botulinum Toxins – Cause of Botulism and Systemic Diseases?

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ABSTRACT

Toxins of *Clostridium botulinum* (types A–G) are known as ‘neurotoxins’, causing the clinically well-known picture of flaccid muscular paralysis. The molecular biological background is the blocking of acetylcholine secretion in neuromuscular junctions by enzymatic cleavage of molecules forming the machinery of exocytosis. Two ‘non-neurotoxins’ (types C2, C3) are produced by some strains of *C. botulinum* types C and D. These affect the cytoskeleton by ribosylating actin filaments. All these toxins are used as cell biological tools for the study of specific actions and effects in different eukaryotic cells. Pharmaceutical and molecular biological research has shown their influence on several crucial organs (or cell cultures thereof) of humans and animals (brain and spinal cord, cerebellum, hippocampus, hypophysis, pancreas, adrenal glands, salivary glands and others). Under natural conditions, botulinum toxins may pass the intestinal barrier and circulate in the bloodstream for a certain time. Carriers occurring naturally in food, such as wheat germ agglutinin, digitonin or saponin, and bacterial toxins such as streptolysin O, perfringolysins, C2 toxin or botulinolysin may also form pores in cell walls. They facilitate the entry of botulinum toxins into cells that may not have natural binding receptors. It is concluded that *in vivo* actions of different botulinum toxins after their entry into the organism may contribute to the onset of different diseases of hitherto cryptogenic origin. Some examples are given and future problems are discussed.

Keywords: botulinum toxin, botulism, *Clostridium botulinum*, systemic disease

Abbreviations: BoNT, botulinum neurotoxins; BoT, botulinum toxins; DIG, digitonin; SAP, saponins; SLO, streptolysin O; WGA, wheat germ agglutinin

BASICS

The species *Clostridium botulinum* comprises a group of different spore-forming anaerobic bacteria which are characterized by their metabolic capacity to produce highly lethal exotoxins.

At least seven types of botulinum neurotoxins (BoNT) are known (A, B, C1, D, E, F, G). There are some reports of mixed types: two toxins in one bacterium, or moieties of different toxins in one molecule (for review see Cato *et al.*, 1986; Smith and Sugiyama,

1988). BoNT block the exocytosis of acetylcholine in neuromuscular junctions by proteolytic cleavage of so called SNARE molecules (for reviews see Logan *et al.*, 2003; Szule and Coorssen, 2003). Changes in the expression of neurotransmitter genes in motoneurons may occur as well (Jung *et al.*, 1997; Humm *et al.*, 2000).

Some strains of *C. botulinum* types C and D produce two 'non-neurotoxins', C2 and C3 (Gunnison and Meyer, 1929; Aktories *et al.*, 1986a,b, 1987). C2 is a binary toxin. C2 and C3 exist in two different molecular forms (Oguma *et al.*, 1981, 1984; Moriishi *et al.*, 1993). C2 is recognized as 'toxin' and it has been classified as cytotoxic enterotoxin (Ohishi *et al.*, 1984), whereas the role of C3 as a virulence factor is still unclear. It is usually described as an 'exoenzyme'. In this review, both neurotoxins and C2 and C3 are referred to as botulinum toxins (BoT).

BoNT lead to the well-known clinical picture of muscular paralysis in humans and animals ('botulism'), which is used as the standard diagnostic tool in the mouse bioassay (CDC, 1998). Other effects, already partly described in the first human artificial intoxication (Kerner, 1820) (e.g. 'dry mouth'), were considered as atypical or as almost negligible side-effects, although they were the main clinical symptoms in various outbreaks (Jenzer *et al.*, 1974). C2 is lethal for mice, rats, guinea-pigs and chickens and causes increased enterotoxin-like permeability of blood vessels (Ohishi *et al.*, 1980; Ohishi, 1983a,b; Ohishi and DasGupta, 1987) and haemorrhages and oedema in the lungs (Simpson, 1982; Ermert *et al.*, 1995, 1996, 1997). C3 even has a ribosylation effect on alfalfa plant cells (Minic *et al.*, 1999).

The standard aetiology of botulism is food poisoning in which BoT has been produced outside the body. Since 1967, toxicoinfections have been described in which BoT are produced inside the intestinal tract (foal botulism, infant botulism) (Rooney and Prickett, 1967; Midura and Arnon, 1976). It was shown that BoNT type A is an inhalation toxin and may penetrate human and murine pulmonary epithelial cells and canine kidney (MDCK) cells *in vivo* and *in vitro* (Park and Simpson, 2003). BoT are thought to be directed via the bloodstream to the neuromuscular junctions. Rare cases of wound botulism, an infection, have been reported. All these forms are considered to lead to muscular paralysis.

There are reports that BoT may cause types of clinical disease other than flaccid paralysis. For general aspects see Jenzer *et al.* (1974), Smith and Sugiyama (1988), Kriek and Odendaal (1994); for sudden infant death see Arnon and Chin (1979), Böhnel *et al.* (2001b); for equine grass sickness see Tocher *et al.* (1923), Hunter *et al.* (1999); and for visceral botulism in cattle see Böhnel *et al.* (2001a). As well as in neuromuscular junctions, BoT binding sites have been described in the central nervous system, especially in the hippocampus and cerebellum as well as in the cerebral cortex (Black and Dolly, 1987; Simpson, 1989; Li and Singh, 1998). It is possible to measure postsynaptic retrograde actions of BoT in the hippocampus (Lledo *et al.*, 1998). Berardelli and colleagues (2002) point out that BoT can transiently alter the excitability of the cortical motor areas, and alter sensory input, producing secondary changes at central level. Heart rate variations, sympathetic skin responses and low plasma noradrenaline (norepinephrine) levels as well as hypotension were manifestations of autonomic dysfunction in botulism patients (Simpson, 1982; Chen *et al.*, 1999). There is one report of pure autonomic dysfunction as the leading clinical symptom with type

B botulism in humans (Merz *et al.*, 2003). A general symptom of botulism in dairy cattle is a pronounced reduction in milk yield (Moeller *et al.*, 2003).

In 1981, BoNT were introduced as therapeutic agent to treat strabismus (Scott, 1981). Subsequent publications on BoNT treatment ('chemodenervation') of quite different diseases are numerous (see Münchau and Bhatia, 2000; Brin *et al.*, 2002). However, complications and adverse reactions should be taken into account (Klein, 2003).

In natural diseases, various toxins may remain detectable in the blood up to 3 weeks after uptake in humans (Finegold, 1987); in animals, BoNT may not be found in the circulation for several days (Smith and Sugiyama, 1988; Moeller *et al.*, 2003), although death may occur in cattle as long as 3 weeks after uptake of toxic feed (Kriek and Odendaal, 1994). The therapeutic neuromuscular duration of BoNT in small amounts is dose-dependent, being long for type A (half-life of inhibition $\gg 31$ days), followed by types C1, B, F and E with half-lives $\gg 25$, ~ 10 , ~ 2 , and ~ 0.8 days, respectively (Foran *et al.*, 2003b).

In neurons, the destruction of the motor endplate was considered as being irreversible (Smith and Sugiyama, 1988). Recent reports show that acetylcholine release is abolished by BoNT action in neuronal synapses; new sprouts of the nerve terminal will take over their functions. However after 90 days (BoNT type A) or 7 (type F) or 5 days (type E) the parent nerves take on their original functions and the sprouts are eliminated (Foran *et al.*, 2003a; Meunier *et al.*, 2003).

MOLECULAR BIOLOGICAL RESEARCH USING BOTULINUM TOXINS AS TOOLS

Intensive pharmacological research has revealed that exocytosis in practically all eukaryotic cell systems is based on various types of molecules – the SNARE proteins – although many questions remain unanswered (for reviews see Bock and Scheller, 1997; Jahn and Niemann, 1994; Graham *et al.*, 2002). Different isoforms of SNARE molecules, such as SNAP-23, SNAP-25 or SNAP-29, are found in many organs and cells, including brain, placenta and haematopoietic cells (Ravichandran *et al.*, 1996; Wong *et al.*, 1997; Logan *et al.*, 2003). These SNARE proteins are major research targets. BoNT are used as a biological tool in dissecting the exocytic machinery (Ludger and Galli, 1998; Linial, 2000). C2 and C3 toxins affect some but not all members of the Rho family by ADP-ribosylating the actin filaments and are used in research for intracellular processes (Nishi *et al.*, 2002; for review see Aktories and Wegner, 1989; Schmid *et al.*, 1994; Aktories *et al.*, 2000; Wilde and Aktories, 2001; Barth *et al.*, 2002 a,b).

In general, this basic research was obviously not connected with the clinical disease of 'botulism' and clinicians hardly considered the possibility that naturally occurring BoT, produced outside or inside the body (except to some extent C2 toxin), might also trigger cellular processes *in vivo* that may initiate or aggravate clinical diseases other than muscular paralysis. It seems that BoT could play a central physiological role in connection with exocytosis and cell structure.

The goal of this review was to compile results of publications available to us that have been presented under pharmacological or basic biological headings and that might explain non-muscular effects of BoT.

Penetration of the toxins into the cells was accomplished either by direct contact or, for those cells lacking effective BoT receptors, artificially by conjugation to pore-forming adjuvants. The pore-forming substances used (and selectively recorded here) do not destroy the cells. Most of them are naturally occurring products. Wheat germ agglutinin, most frequently used, may be found not only in wheat, but also in groundnuts, soybeans, tomatoes, potatoes and many other plants. This compound might help to increase the permeability of the intestinal wall and mediate the uptake of larger molecules, which apparently occurs in the upper gastrointestinal tract, and it maintains its biological activity in the blood (Wang *et al.*, 1998; Watzl *et al.*, 2001; Wirth *et al.*, 2002; Gabor *et al.*, 2002). Streptolysin O is produced by most strains of Lancefield group A β -haemolytic streptococci. It may cause toxaemias and generates large transmembrane channels exceeding 15 nm. It may attack a wide variety of cells (Bhakdi *et al.*, 1985). Digitonin is contained within the seeds of *Digitalis purpurea*. Saponins are a type of glycoside widely distributed in plants. Other substances such as lipopolysaccharides (for review see Caroff *et al.*, 2002), perfringolysin (a toxin of *C. perfringens*) and botulinolysin (a haemagglutinin of *C. botulinum*) (Sekiya *et al.*, 1998), apparently have not been used by molecular biologists but may be present under natural conditions in the intestines and may perforate the intestinal wall and blood vessels (Granum, 1990; Steinhorsdottir *et al.*, 2000). Staphylococcal α -toxin or *Escherichia coli* haemolysins may also be present (Bhakdi *et al.*, 1996). C2 is a binary toxin; one part (C2-II) forms channels in lipid bilayer membranes, enabling the other part (C2-I) to traverse the cell membrane directly (Schmid *et al.*, 1994; Stiles *et al.*, 2002) or to help other proteins such as C3 toxin to overcome the intestinal barrier (Barth *et al.*, 1998; Nilius *et al.*, 1999; Barth *et al.*, 2002a; for review see Barth *et al.*, 2002b; Blöcker *et al.*, 2003).

Sometimes the molecules of BoNT are split by dithiothreitol to procure the enzymatically active light chain of the neurotoxin molecules. Toxins may penetrate the cells even without specific binding receptors under conditions of long time and low concentration combined with low affinity (Shone and Melling, 1992).

Tests regarding BoT action with electroporation, microinjection or genetically manipulated cells were omitted from this review as well as C3 toxins produced by *C. limosum*.

OVERVIEW

The effects of BoNT on exocytosis of cells others than those of neuromuscular junctions of striated muscles are shown in Tables I to III (neuronal cells and organs, endocrine cells and organs, and non-neuronal and non-endocrine cells and organs). Almost all types of BoNT were used in a variety of primary and secondary cell cultures or preparations including those which are ordinarily resistant to the effect of neurotoxins (Chaddock *et al.*, 2000). Summarizing, it is possible that BoNT are

TABLE I
Effect of botulinum 'neurotoxin' action on exocytosis of neuronal cell types and organs

Organ	Cell type, cell line	Origin	Botulinum toxin		Effect*	Reference
			Type	Input		
Brain	Synaptosomes	Mouse	A	Direct	■ Acetylcholine	Gundersen and Howard (1978)
	Cerebrocortical synaptosomes	Guinea pig	A	Direct	■ Quetylcholine	Wonnacott and Marchbanks (1976)
	Cerebrocortical synaptosomes	Rat	A	Direct	■ Acetylcholine, dopamine, noradrenaline, γ -aminobutyric acid	Ashton and Dolly (1988)
	Cortex synaptosomes	Rat	D, A	Direct	■ Glutamate	Yamasaki <i>et al.</i> (1994)
	Cortex synaptosomes	Rat	A, B, E	Direct	□ Competitive binding on receptors	Li and Singh (1998)
	Synaptosomes	Rat	A, A ^b	Direct	■ Serotonin	Najib <i>et al.</i> (1999)
Pineal body	Synaptosomes	Rat	B	Direct	■ Noradrenaline	Kohda <i>et al.</i> (2000)
	Somatodendritic cells	Rat	A, B	Direct	■ Dopamine release	Bergquist <i>et al.</i> (2002)
	Pinealocytes	Rat	E	Direct	■ 5-Hydroxytryptamine (partly)	Yamada <i>et al.</i> (2002)
	Interneurons	Rat	D ^b	Direct	□ Effect on on Ca ²⁺ signalling	Zilberter <i>et al.</i> (1999)
Neocortex	Primary pyramidal cells	Rat	D ^b	Direct	■ Retrograde messenger (glutamate) secretion in neurons (partly)	Zilberter (2000)
	Cerebellar granule cells	Mouse	C	Direct	□ Effect on Ca2+ signaling	Leist <i>et al.</i> (1997)
Cerebellum	Cerebellar granule cells	Rat	A, B, Cl, E, F	Direct	□ Prevention of apoptosis by peroxynitrite and nitric oxide donors	Foran <i>et al.</i> (2003b)
	Cerebellar granule cells	Rat	A, B, Cl, E, F	Direct	□ Inhibition of evoked exocytosis	
Hippocampus	Nerve terminals	Rabbit	A	Direct	■ Acetylcholine	Nakov <i>et al.</i> (1989)
	Embryonic neuronal cells, Astrocytes	Rat	B, F	Direct	■ Synaptobrevin	Verderio <i>et al.</i> (1999)
	Embryonic neuronal cells, Astrocytes	Rat	A, C	Direct	□ Cleavage of synaptic vesicle	
	Embryonic neuronal cells, Astrocytes	Rat	A, C	Direct	■ Exo-endocytic recycling	Verderio <i>et al.</i> (1999)
Embryonal cells	Embryonal cells	Mouse	A	Direct	□ Reduced number of vesicles in neuronal terminals	Grosse <i>et al.</i> (1999)
	Embryonal cells	Mouse	A	Direct	□ Impairment of elongation of the dendrites	

TABLE I (continued)

Organ	Cell type, cell line	Origin	Botulinum toxin		Effect*	Reference
			Type	Input		
Spinal cord	Pyramidal cells	Rat	B ^b	Direct	<input type="checkbox"/> Reduced responsiveness of post-synaptic AMPA receptors to glutamate	Lüscher <i>et al.</i> (1999)
	Neuronal cells	Rat	C	Direct	<input type="checkbox"/> Downregulation of specific GABA transmitter	Horton and Quick (2001)
	Embryonic pyramidal neurons	Rat	A	Direct	<input type="checkbox"/> Fast synaptic transmission	Demarque <i>et al.</i> (2002)
	Synaptosomes	Rat	C1, E, F	Direct	<input type="checkbox"/> Catecholamine	Stigliani <i>et al.</i> (2003)
	Embryonic neuronal cells	Rat	E	Direct	<input checked="" type="checkbox"/> Block of glutamate signal transmission	Schenk <i>et al.</i> (2003)
	Spinal cord neuron	Mouse	A	Direct	<input type="checkbox"/> Block of transmitter systems	Bigalke <i>et al.</i> (1985)
	Embryonic spinal cord cells	Mouse	A, C	Direct	<input checked="" type="checkbox"/> Glycine and glutamate	Williamson <i>et al.</i> (1996)
	Spinal cord cells	Mouse	A, E	Direct	<input checked="" type="checkbox"/> Glycine	Keller and Neale (2001)
	Primary embryonic dorsal root ganglia neurons	Rat	A, B, C, F	Direct	<input checked="" type="checkbox"/> Substance P	Welch <i>et al.</i> (2000)
	Primary embryonic dorsal root ganglia neurons	Rat	A	Direct	<input checked="" type="checkbox"/> Glutamate	Purkiss <i>et al.</i> (2000)
Abducens motoneurons	Abducens motoneurons	Cat	A	Direct ^c	<input type="checkbox"/> Different firing patterns	Moreno Lopez <i>et al.</i> (1994)
	Motoneurons	Rat	A	Direct ^c	<input type="checkbox"/> Gene upregulation of enkephalin and beta-calcitonin-gene-related peptide, <input type="checkbox"/> Focal upregulation of choline acetyltransferase, <input type="checkbox"/> focal down-regulation of cholecystokinin	Jung <i>et al.</i> (1997)
Motoneurons	Motoneurons	Rat	A	Direct ^c	<input type="checkbox"/> Gene upregulation and down-regulation of processes of degeneration and regeneration	Humm <i>et al.</i> (2000)
Coeliac ganglion	Coeliac ganglion	Guinea-pig	A	Direct	<input type="checkbox"/> Inhibition of synaptic transmission	Gibbins <i>et al.</i> (2003)

TABLE I (continued)

Organ	Cell type, cell line	Origin	Botulinum toxin			Effect*	Reference
			Type	Input			
Adeno-hypophysis	Anterior pituitary cells + GH3	Rat	A	Direct ^d	■ Neurotransmitters	Aguado <i>et al.</i> (1996)	
	Anterior pituitary cell	Mouse Rat	A, A ^b F	Direct ^d SLO ^d	■ Adenocorticotrophic hormone ■ Growth hormone	Aguado <i>et al.</i> (1997) Jacobsson <i>et al.</i> (1997)	
Intermediate hypophysis	Neurointermediate cells	Rat	A	Direct ^d	■ Neurotransmitters	Aguado <i>et al.</i> (1996)	
Neuro-hypophysis	Neurosecretory nerve terminals	Rat	A ^b	DIG	■ Vasopressin	Dayanithi <i>et al.</i> (1990)	

*■ = inhibition of secretion/release of . . . ; □ = other effects

^aDirect contact with toxin or pretreated with WGA (wheat germ agglutinin); SLO (streptolysin O); DIG (digitonin); SAP (saponin)

^bLight chain

^cAfter intramuscular injection

^dActivated by dithiothreitol

TABLE II
Effect of botulinum 'neurotoxin' action on exocytosis of endocrine cell types and organs

Organ	Cell type, cell line	Origin	Botulinum toxin			Effect*	Reference
			Type	Input			
Pancreas	HIT	Rat and hamster	A, E	SLO ^b	■ Insulin	Sadoul <i>et al.</i> (1995)	
	Pancreatic β -cells, INS-1	Rat	B	SLO ^b	■ Insulin	Regazzi <i>et al.</i> (1995)	
	Pancreatic islet cells	Rat	C1, A ^c	SLO ^b	■ Insulin	Wheeler <i>et al.</i> (1996)	
	HIT-T15	Hamster	C1, E, C1 ^c	SLO	■ Insulin	Lang <i>et al.</i> (1997)	
	HIT-T15	Hamster	A ^d	WGA ^b	■ Insulin	Chaddock <i>et al.</i> (2000)	
	HIT	Hamster	E	SLO	■ Insulin	Gonelle-Gispert <i>et al.</i> (2000)	
	α TC6	Mouse	E	Direct	■ Glutamate	Yamada <i>et al.</i> (2001)	
	Adrenal glands	Chromaffin medullary cells	Bovine	D	Direct	■ Catecholamine	Knight <i>et al.</i> (1985)
		Medullary cells	Bovine	A, B, D	Direct	■ Catecholamine	Knight (1986)
		Chromaffin cells	Bovine	A, A ^c	SLO ^b	■ Catecholamine	Stecher <i>et al.</i> (1989)
Chromaffin cells		Bovine	A, E, A ^c	DIG ^b	■ Catecholamine	Bittner <i>et al.</i> (1989)	
PC12		Rat	A, A ^c	DIG	■ Noradrenaline	McInnes and Dolly (1990)	
PC12		Rat	A	Direct	■ Noradrenaline	Shone and Melling (1992)	
PC12		Rat	F, G	Direct ^b	■ Neurotransmitter	Papini <i>et al.</i> (1995)	

TABLE II (continued)

Organ	Cell type, cell line	Origin	Botulinum toxin			Effect*	Reference
			Type	Input			
Parotid	PC12	Rat	A	DID	■ Catecholamine	Shimazaki <i>et al.</i> (1996)	
	Chromaffin cells	Bovine	A, B	Direct,	■ Catecholamine		
			A	DIG	■ Catecholamine	Lawrence <i>et al.</i> (1996)	
	Chromaffin cells	Bovine	C1, D ^c , E ^c	DIG	■ Catecholamine	Glenn and Burgoyne (1996)	
	Chromaffin cells	Bovine	A	DIG ^b	■ Catecholamine	Gutierrez <i>et al.</i> (1997)	
	PC12	Rat	A, E	Direct ^{b,e}	■ Noradrenaline	Gerona <i>et al.</i> (2000)	
	(i) PC12	Rat	A ^d	WGA ^b	■ Noradrenaline (i, ii), glycine (iii)	Chaddock <i>et al.</i> (2000)	
	(ii) SH-SY5Y	Human	A				
	(iii) eSC neurons	Human	A				
	PC12	Rat	E	Direct	■ D-Aspartate	Nakatsuka <i>et al.</i> (2001)	
	Primary acinar cells	Rat	B	Direct ^b , SLO	■ Amylase	Fujita-Yoshigaki <i>et al.</i> (1996, 1998)	

*■ = inhibition of secretion/release of . . . ; □ = other effects

^aDirect contact with toxin or pretreated with WGA (wheat germ agglutinin); SLO (streptolysin O); DIG (digitonin); SAP (saponin)

^bActivated by dithiothreitol

^cLight chain

^dDerivative non-cell binding moiety

^eMechanically disrupted

TABLE III
Effect of botulinum 'neurotoxin' action on exocytosis of different non-neuronal and non-endocrine cell types and organs

Organ	Cell type, cell line	Origin	Botulinum toxin			Effect*	Reference
			Type	Input			
Parotid	Primary acinar cells	Rat	B	Direct ^b , SLO	■ Amylase	Fujita-Yoshigaki <i>et al.</i> (1996, 1998)	
Blood	Erythrocytes	Human	A ^c	Direct	■ Binding to erythrocytes	Fujinaga <i>et al.</i> (2000)	
Nasal mucosa	Mucosal glands	Guinea-pig	A	Direct	□ Apoptosis	Rohrbach <i>et al.</i> (2001)	
Lungs	Endothelial cells	Rat	B	Direct ^b	□ Reduction of endocytosis and transcytosis	Schnitzer <i>et al.</i> (1995)	
Kidney	MDCK	Canine	E	SLO ^b	■ Transcytosis and recycling	Apodaca <i>et al.</i> (1996)	
	MDCK	Canine	E	SLO ^b	■ Transferrin recycling	Leung <i>et al.</i> (1998)	
	Inner medullary collecting duct cells	Rat	A, E	Direct ^b	■ H ⁺ -ATPase	Banerjee <i>et al.</i> (2001)	
Urinary bladder	Parasympathetic neurons	Guinea-pig	A	Direct	■ Excitatory response	MacKenzie <i>et al.</i> (1982)	
Genital tract	Spermatozoa	Human	A, C, E, F	SLO ^b	■ Sperm acrosome reaction	Tomes <i>et al.</i> (2002)	
Vas deferens	Smooth muscles	Guinea-pig, mouse	A	Direct	■ Neuronal transmission	Holman and Spitzer (1973)	
Uterus	Myometrium	Rat	A	Direct	■ Myometrial activity	Garza <i>et al.</i> (2003)	
Uterine arteries	Autonomic vasodilator neurons, arterial segments	Guinea-pig	A	Direct	■ Neurotransmitters, acetylcholine, Isometric contraction	Morris <i>et al.</i> (2001)	
Uterine arteries, vena cava	Sympathetic vasoconstrictor neurons	Guinea-pig	A	Direct	■ Norepinephrine	Morris <i>et al.</i> (2002)	
Intestinal tract	Endothelial cells	Guinea-pig	A ^c	Direct	■ Binding to endothelial cells	Fujinaga <i>et al.</i> (2000)	
	Microvilli	Guinea-pig	A ^c	Direct	■ Binding to microvilli		

TABLE III (continued)

Organ	Cell type, cell line	Origin	Botulinum toxin		Effect*	Reference
			Type	Input		
Ileum long muscle	Smooth muscle cells	Guinea-pig	A	Direct ^d	■ Stimulation of cholinergic neurons	MacKenzie <i>et al.</i> (1982)
Intestinum	Smooth muscle cells	Guinea-pig	B	Direct ^d	■ Contractile responses, ³ H]choline	Olgart <i>et al.</i> (2000)
Anococcygeus muscle	smooth muscle cells	Rat	A	Direct ^d	■ Stimulation of noradrenergic neurons	MacKenzie <i>et al.</i> (1982)
Iris sphincter	Muscle preparation	Rabbit	A	Direct	■ Acetylcholine (parasympathetic nerve terminals)	Ishikawa <i>et al.</i> (2000)
Fat tissue	3T3-L1	Mouse	B	SLO ^b	■ Substance P (trigeminal nerve ending) □ Translocation of GLUT 4 (insulin-responsive glucose transporter), glucose transport activity	Tamori <i>et al.</i> (1996)

*■ = inhibition of secretion/release of . . . ; □ = other effects

^aDirect contact with toxin or pretreated with WGA (wheat germ agglutinin); SLO (streptolysin O); DIG (digitonin); SAP (saponin) light chain

^bActivated by dithiothreitol

^cProgenitor toxin with haemagglutinin

^dAfter intramuscular injection

transported additionally into cells other than neurons under natural conditions. These naturally occurring substances and their presence/absence may provide an explanation of the obviously uneven distribution of botulinum diseases. Streptolysin O and staphylococcal or *C. perfringens* toxins could help to overcome the blood–brain barrier. In the central nervous system of the rat there are selective locations of receptors for BoT, especially in the hippocampus and the cerebellum, although toxin uptake is minimal and its penetration into intact cells is restricted (Black and Dolly, 1987). In bovine brain it was shown that specific types of gangliosides enhance adherence of BoNT (Schengrund *et al.*, 1993). The results of tests with C2 toxin affecting actin filaments of the cell skeleton in different organs or cell cultures are given in Table IV. Specific actions of C3 toxin are compiled in Table V.

Although an understanding of the mechanisms underlying these observations is clearly incomplete, there are sufficient data to suggest influences on clinical diseases. Owing to restrictions of space in this overview, the compiled tables cannot be comprehensive; for details, it is necessary to consult physiological and clinical textbooks. Any presentation of regulatory circuits, important as they are for general understanding, are beyond the scope of this review.

There is a wide range between complete and partial blockage of secretions. The effects on intact neurons may be different from those in cell culture (Williamson *et al.*, 1996). As shown in Table I to III, BoNT may interfere with many physiological features. However, the effects of different toxin types on different organs of different animal species and humans cannot be compared directly. Not every type of BoNT will produce the same physiological result. *In vivo* there may be different effects of BoNT. Type B neurotoxin causes drooling of liquid saliva, whereas type C/D results in mucous salivation in cattle (Kriek and Odendaal, 1994). Type A will reduce the clinical symptoms of salivary overproduction in humans when injected directly into the salivary glands (Ellies *et al.*, 2003). Injection of type A into the rat urinary tract will show different effects according to the neurotransmitter (MacKenzie *et al.*, 1982) and innervation (Smith *et al.*, 2003), whereas small amounts of BoNT type C may cause only frequent urination in cattle (Kriek and Odendaal, 1984; Moeller *et al.*, 2003).

Flaccid paralysis and some ‘atypical’ features are well accepted by the medical and veterinary professions. The data presented in this review, however, are sufficient to illustrate the relevant concepts. In the following, underrated influences of BoT on different organ systems are given as examples.

Central and peripheral nervous systems

In the central nervous system, release of acetylcholine or noradrenaline might be blocked. After resorption of the toxin from the intestinal tract, the cranial nerves are affected first, causing diplopia and dilated pupils. Peripheral nerves are affected later, finally causing respiratory or cardiac failure (Hirokawa and Kitamura, 1979; Haberman *et al.*, 1988; Luvisetto *et al.*, 2003). It is predicted that blockage of signal cascades in pituitary cells by C3 might contribute to several human neurological diseases (Storey *et al.*, 2002). Mediator elaboration and cell activation in immune and inflammatory

TABLE IV
Effects of botulinum C2 toxin action on actin filaments of the cell skeleton of different organs

Organ	Cell type, cell line	Origin	Input ^a	Effect*	Reference
Embryo Pancreas	Embryo cells	Chicken	Direct	<input type="checkbox"/> Rounding up of cells	Reuner <i>et al.</i> (1987)
	HIT-T15 and pancreatic islet	Hamster, rat	Direct	<input checked="" type="checkbox"/> Insulin	Li <i>et al.</i> (1994)
Adrenal glands	Y-1	Mouse	Direct	<input type="checkbox"/> Increase in steroid release	Considine <i>et al.</i> (1992)
	Chromaffin cells	Bovine	Direct	<input type="checkbox"/> Disassembly of actin filaments	Gasman <i>et al.</i> (1999)
	PC12	Rat	Direct	<input type="checkbox"/> Short-term increase, long-term decrease of noradrenaline release	Matter <i>et al.</i> (1989)
Blood vessel	Endothelial cells	Pig	Direct	<input type="checkbox"/> Changes in surface morphology, intercellular gap formation, cell detachment	Schnittler <i>et al.</i> (2001)
Adenoma Blood	HeLa	Human	Direct	<input type="checkbox"/> Delay of mitosis	Barth <i>et al.</i> (1999)
	Erythrocytes	Human, rabbit, bovine, horse, sheep	Direct	<input type="checkbox"/> Change in agglutination and binding properties	Sugii and Kozaki (1990)
Neutrophils	Neutrophils	Rat	Direct	<input type="checkbox"/> Inhibition of phagocytosis, enhancement of oxidase activation	Al-Mohanna <i>et al.</i> (1987)
	Neutrophils	Human	Direct	<input type="checkbox"/> Blockage of migration, stimulation of O ₂ production	Norgauer <i>et al.</i> (1988)
Neutrophils Neutrophils Neutrophils Neutrophils B cells B cells	Neutrophils	Human	Direct	<input type="checkbox"/> Degranulation, reduced endocytosis	Norgauer <i>et al.</i> (1989)
	Neutrophils	Human	Direct	<input type="checkbox"/> Increase in secretory responses	Grimminger <i>et al.</i> (1991)
	Neutrophils	Human	Direct	<input type="checkbox"/> Modification of actin skeleton	Just <i>et al.</i> (1993)
	Neutrophils	Human	Direct	<input type="checkbox"/> Potentiation of lysozyme release	Wenzel-Seifert <i>et al.</i> (1997)
	B cells	Human	Direct	<input type="checkbox"/> Blockage of cell proliferation	Melamed <i>et al.</i> (1991)
	B cells	Human	Direct	<input type="checkbox"/> Inhibition of Epstein-Barr virus induced proliferation	Melamed <i>et al.</i> (1994)
B cells	Human	Direct	<input type="checkbox"/> Prevention of increase of filamentous actin	Melamed <i>et al.</i> (1995)	

TABLE IV (continued)

Organ	Cell type, cell line	Origin	Input ^a	Effect*	Reference
	RBL-2H3-hm1	Rat	Direct	<input type="checkbox"/> Degranulation, dramatic changes of morphology, increase of stimulated serotonin secretion	Prepens <i>et al.</i> (1998)
	B cells	Human	Direct	<input type="checkbox"/> Blocking signals for apoptosis	Melamed and Gelfand (1999)
	Platelets	Human	Direct	<input type="checkbox"/> Abolishment of formation of filament network	Aktorjes <i>et al.</i> (1986a)
	8CYTOSOLS9				
Lymphatic tissue	S49	Mouse	Direct	<input type="checkbox"/> Induction of ADP-ribosylation	Aktorjes <i>et al.</i> (1986b)
	Lymphoma cells	Mouse	Direct	<input type="checkbox"/> Rounding of cells, inhibition of movement and invasiveness	Verschueren <i>et al.</i> (1995)
	BW-O-Li				
Phagocytes	Macrophages	Mouse	Direct	<input type="checkbox"/> Inhibition of pseudopodia formation and phagocytic activity	Jun <i>et al.</i> (1996)
	L929	Mouse	Direct	<input type="checkbox"/> Rounding of cells	Ohishi <i>et al.</i> (1984)
Mammary gland	Fibroblasts, Myofibroblasts	Human	Direct	<input type="checkbox"/> Change in morphology, stop of migration	Rønnov-Jessen and Petersen (1996)
Lungs	Endothelial cells	Rabbit	Direct	<input type="checkbox"/> Oedema formation	Ermert <i>et al.</i> (1995)
	Endothelial cells	Rabbit	Direct	<input type="checkbox"/> Deterioration of capillary endothelial barrier function	Ermert <i>et al.</i> (1996)
	Endothelial cells	Rabbit	Direct	<input type="checkbox"/> Interstitial fluid accumulation, enhanced secretion	Ermert <i>et al.</i> (1997)
	Alveolar epithelial type II cells	Rat	Direct	<input type="checkbox"/> Increase of phosphatidyl secretion	Rose <i>et al.</i> (1999)
	Artery endothelial cells	Pig	Direct	<input type="checkbox"/> Increase of permeability	Suttorp <i>et al.</i> (1991)
Liver	Liver cells	Rabbit	Direct	<input type="checkbox"/> Induction of ADP-ribosylation, reduction of actin viscosity <i>in vitro</i>	Aktorjes <i>et al.</i> (1986b)
	Hepatocytes	Rat	Direct	<input type="checkbox"/> Conformational changes in actin forms	Reuner <i>et al.</i> (1991)
	FAO U 333	Rat	Direct	<input type="checkbox"/> Disappearance of microfilaments	Wieggers <i>et al.</i> (1991)

TABLE IV (continued)

Organ	Cell type, cell line	Origin	Input ^a	Effect*	Reference
Kidney	Vero	Green monkey	Direct	<input type="checkbox"/> Rounding of cells	Ohishi <i>et al.</i> (1984)
	BHK-21/WI-2	Hamster	Direct	<input type="checkbox"/> Rounding of cells	Ohishi <i>et al.</i> (1984)
	RK13	Rabbit	Direct	<input type="checkbox"/> Rounding of cells	Ohishi <i>et al.</i> (1984)
	Vero	Green monkey	Direct	<input type="checkbox"/> Induced channel formation in lipid bilayer membranes	Bachmeyer <i>et al.</i> (2001)
Intestine	Intestinal cells (<i>in vivo</i>)	Mouse	Direct	<input type="checkbox"/> Accumulation of fluid in the intestine, acute inflammation, vacuolation of epithelial cells	Ohishi and Odagiri (1984)
	Intestine 407	Human	Direct	<input type="checkbox"/> Rounding of cells	Ohishi <i>et al.</i> (1984)
Peritoneum	Mast cells	Mouse	Direct	<input type="checkbox"/> Inhibition of induced degranulation and serotonin release in suspended cells, stimulation of induced degranulation in attached cells	Wex <i>et al.</i> (1997)
	3T3-L1	Mouse	Direct	<input type="checkbox"/> Depolymerization of actin filaments	Schmid <i>et al.</i> (1998)
Amnion Uterus Ovary	FL	Human	Direct	<input type="checkbox"/> Rounding of cells	Ohishi <i>et al.</i> (1984)
	HeLa	Human	Direct	<input type="checkbox"/> Rounding of cells	Ohishi <i>et al.</i> (1984)
	CHO-K1	Hamster	Direct	<input type="checkbox"/> Rounding of cells	Ohishi <i>et al.</i> (1984)

*■ = inhibition of secretion/release of . . . ; □ = other effects

^aDirect contact with toxin

TABLE V
Effects of botulinum C3 toxin action on actin filaments of the cell skeleton of different organs

Organ	Cell type, cell line	Origin	Input ^a	Effect*	Reference
Spinal cord	Dorsal root ganglion	Chick		<input type="checkbox"/> Promotion of neurite outgrowth	Fournier <i>et al.</i> (2003)
	Outer hair cells	Guinea-pig	Direct	<input checked="" type="checkbox"/> Cochlear amplification and electromotility	Kalinek <i>et al.</i> (2000) Zhang <i>et al.</i> (2003)
Pancreas	Acinar cells	Rat	DIG	<input checked="" type="checkbox"/> Amylase	Kiehne <i>et al.</i> (2002)
	Chromaffin cells	Bovine	Direct	<input type="checkbox"/> Inactivating of Rho signalling	Gasman <i>et al.</i> (1999)
Adrenal glands	PC12	Rat	Direct	<input type="checkbox"/> Disappearance of actin stress fibres, rounding up of cell body	Chardin <i>et al.</i> (1989)
	HUVEC	Human	Direct	<input type="checkbox"/> Induction of apoptosis	Hippenstiel <i>et al.</i> (2002)
Blood vessel	Endothelial cells	Human	Direct	<input type="checkbox"/> Prevention of downregulation of eNOS by thrombin	Eto <i>et al.</i> (2001)
	Smooth muscle cells	Rat	Direct	<input type="checkbox"/> Reduction of AngII induced expression of monocyte chemoattractant protein-1	Funakoshi <i>et al.</i> (2001)
Lungs	Aortic epithelial cells	Pig	Direct	<input type="checkbox"/> Decrease of wound closure	Lee and Gotlieb (2002)
	Aortic endothelial cells	Bovine	Direct	<input checked="" type="checkbox"/> ATP release	Koyama <i>et al.</i> (2001)
Airways	Aortic endothelial cells	Mouse	Direct	<input type="checkbox"/> Increase of endothelial NO synthase, increased cerebral blood flow	Laufs <i>et al.</i> (2000)
	HMVEC	Human	Direct	<input type="checkbox"/> Inhibition of IL-8 induced vascular permeability	Schraufstatter <i>et al.</i> (2001)
Lungs	Neutrophils, HUVEC	Human	Direct	<input checked="" type="checkbox"/> Transendothelial migration, actin polymerization, myosin II filament formation, myosin light chain phosphorylation	Saito <i>et al.</i> (2002)
	Hep-2	Human	Direct	<input type="checkbox"/> Prevention of induced stress fibre formation	Gower <i>et al.</i> (2001)
Airways	Airway smooth-muscle cells	Rat	Direct	<input type="checkbox"/> Reduced stiffness	An <i>et al.</i> (2002)

TABLE V (continued)

Organ	Cell type, cell line	Origin	Input ^a	Effect*	Reference
Blood	Platelets	Human	SLO	<input type="checkbox"/> Thrombin-induced aggregation	Nishioka <i>et al.</i> (2001)
	Platelets	Human	Direct	<input type="checkbox"/> Prevention of platelet activation	Retzer <i>et al.</i> (2000)
	Platelets	Human	Direct	<input type="checkbox"/> Blockage of shape change	Retzer and Essler (2000)
Lymphatic tissue	NIH3T3	Mouse	Direct	<input type="checkbox"/> Disappearance of actin stress fibres, rounding up of cell body	Chardin <i>et al.</i> (1989)
Peritoneum	Mast cells	Rat	SLO	<input type="checkbox"/> Blockage of secretion	Sullivan <i>et al.</i> (1999)
Liver	FAO	Rat	Direct	<input type="checkbox"/> Disappearance of microfilaments, rounding up of cells	Wieggers <i>et al.</i> (1991)
	U 333	Rat	Direct	<input type="checkbox"/> Inhibition of pseudopodia formation	Imamura <i>et al.</i> (1999)
	MM1	Rat	Direct	<input type="checkbox"/> Blockage of transcellular migration	Yoshioka <i>et al.</i> (1999)
	MM1	Rat	SAP	<input type="checkbox"/> Inhibition of response to liver injury	Di Sario <i>et al.</i> (2002)
	Stellate cells	Rat	Direct	<input type="checkbox"/> Block of negative regulator in liver regeneration	Ikeda <i>et al.</i> (2003)
	Hepatocytes	Rat	Direct	<input type="checkbox"/> Block of negative regulator in liver regeneration	Ikeda <i>et al.</i> (2003)
Kidney	Vero	Green monkey	Direct	<input type="checkbox"/> Disappearance of actin stress fibres, rounding up of cell body	Chardin <i>et al.</i> (1989)
	MDCK LLC-PK1	Canine	Direct Direct	stress fibres formation <input checked="" type="checkbox"/> ³ H-thymidine uptake, <input type="checkbox"/> Stimulation of apoptosis	Kodama <i>et al.</i> (2000) Anderson <i>et al.</i> (2000)

TABLE V (continued)

Organ	Cell type, cell line	Origin	Input ^a	Effect*	Reference
Intestinum	Intestinal smooth muscle cells IEC-6	Rabbit Rat	Direct Direct	<input type="checkbox"/> Reduced phospholipase D activity <input type="checkbox"/> Reduction of cell migration	Murthy <i>et al.</i> (2001) Rao <i>et al.</i> (2001)
Colon	HT 29 cells	Human	Direct	<input type="checkbox"/> Blockage of induced invasion factor	Attoub <i>et al.</i> (2002)
Ovary	Granulosa cells	Rat	Direct	<input type="checkbox"/> Reduced survival and differentiation	Shiota <i>et al.</i> (2003)
Endometrium	Decidual cells	Human	Direct	<input type="checkbox"/> Loss of actin stress fibre formation, rounding up of cells, decreased embryo outgrowth <input type="checkbox"/> Inhibition of migration	Shiokawa <i>et al.</i> (2000) Shiokawa <i>et al.</i> (2002)
Mammary gland	Trophoblasts MCF-7	Human Human	Direct Direct	<input type="checkbox"/> Enhanced estrogen receptor activation	Su <i>et al.</i> (2001)
Fat tissue	3T3-L1	Mouse	SAP	<input type="checkbox"/> Induction of GLUT 1 and GLUT4 translocation (insulin responsive glucose transporters)	van den Berghe <i>et al.</i> (1996)

* = inhibition of secretion/release of . . . ; = other effects^aDirect contact with toxin or pretreated with SLO (streptolysin O); DIG (digitonin); SAP (saponin)

cells may also be affected by BoT (see Logan *et al.*, 2003). There may even be further effects due to retrograde actions on the central nervous system once the toxin has passed the intestinal barrier (Wiegand *et al.*, 1976; Ashton and Dolly 1988; Moreno Lopez *et al.*, 1994; Behari and Raju, 1996). Recent studies showed that alterations in adrenal and catecholaminergic pathways may lead to profound changes in autonomic, cardiovascular, neuroendocrine, metabolic, nociceptive and immune functions (Parmer and Zinder, 2002). The precise outcome of a blockage of the sympathetic nervous system cannot be predicted, however (Moynihan *et al.*, 2004). In cases of visceral botulism, affected cows appear blind and deaf or with reduced cognition; the whole herd presents a strange attitude if one enters the stable (B. Schwagerick, personal communication). Some animals may remain without any movement for several minutes, others show a staggering gait that is different from muscular paralysis. There could be a connection between BoT effects on the pancreas and cognitive impairment due to a leaking brain–blood-barrier, as is reported for type II diabetes (Bowler, 2003; Starr *et al.*, 2003).

Enteric nervous system

Damage to the enteric nervous system in the gut by BoT may influence motility, exocrine and endocrine secretions, microcirculation and immune and inflammatory processes. In particular, food intake, gallbladder and pancreas may be affected, and diarrhoea and constipation may be seen (for review see Hansen, 2003a,b,c). Although not specific to BoT, a broad pathology of clostridial constipation is hypothesized (Zoppi *et al.*, 1998; Johnson, 2001). BoT might even be suspected, like other clostridial toxins, to be connected with some forms of autism (Finegold *et al.*, 2002), as has been already hypothesized for the related tetanus toxin (Bolte, 1998).

Endocrine glands, such as pancreas

Diabetic autonomic neuropathy of uncertain origin is a well-known and well-characterized clinical manifestation (Vinik *et al.*, 2002). Almost all reported dysfunctions connected with this disease may be caused by or attributed to BoT, including the hypothalamus as a central site within the central nervous system and the clinical expression of diabetes (Elmqvist and Marcus, 2003).

In a small prospective study in obese adults, bacterial forms of *C. botulinum* were found in the stools of four out of five patients with diabetes. Out of 15 controls without diabetes, two were positive for BoNT and three for bacterial forms (Scholz, Krüger, and Böhnelt, unpublished data, 2001). Recent studies showed an increased blood–brain barrier permeability in type II diabetes eventually linked with cognitive impairment (Mayhan, 2001; Starr *et al.*, 2003; Bowler, 2003) and an effect of high glucose content on oxidative stress (Evans *et al.*, 2003).

Respiratory tract

In its first description, C2 was shown to cause lung oedema in cattle. This is true for other animals as well (Ohishi *et al.*, 1980; Simpson, 1982; Ohishi, 1983 a,b; Ohishi and DasGupta, 1987; Smith and Sugiyama, 1988; Ermert *et al.*, 1995, 1996, 1997). The pulmonary epithelium may act as an entry port for BoNT without destruction of the cells (Park and Simpson, 2003). C2 toxin may cause rounding of epithelial cells, hence facilitating the entry of other toxins. Aspiration pneumonia due to paralysis of the tongue and larynx, leading to a febrile reaction, may hide the real cause.

Genital tract, including fertility

Reduced calving rate, stillbirth of calves, placental retention and delayed involution of the uterus are frequently seen in cases of visceral botulism. This could be due to reduced muscular action and blood supply, and blockage of neuronal actions (Morris *et al.*, 2001, 2002). Reduced acrosome reaction by different types of BoNT (Tomes *et al.*, 2002), reduced survival of granulosa cells in the ovary (Shiota *et al.*, 2003) and decreased embryo outgrowth (Shiokawa *et al.*, 2000), both connected with C3 toxin, could be an explanation for reduced fertility. BoNT type B may have an influence on erectile dysfunction in humans (Jenzer *et al.*, 1974; Merz *et al.*, 2003) as it was reported as a side-effect in BoNT type A treatment (Papadonikolakis *et al.*, 2002). Similar clinical symptoms were observed in a farmer who showed antibodies against BoNT types A and B (unpublished data). The influence of C2 on fibroblasts (Rønnov-Jessen and Petersen, 1996), together with reduced tension of the teat sphincter muscle, could lead to an increased number of cases of mastitis. There is one report on an antibiotic-resistant mastitis with production of BoNT type B (Böhnel *et al.*, 2004), whereas there is no evidence for transfer of toxin from the blood into the milk (Moeller *et al.*, 2003).

Blood cells and blood vessels

Phagocytosis. There is ample evidence that blood cells are affected by BoT, with a mainly negative effect on phagocytosis (see Tables III to V). Changes in surface morphology of endothelial cells (Schnittler *et al.*, 2001), dissemination of C2 toxin throughout the body (Suttorp *et al.*, 1991), and reduced blood pressure may be the reason for formation of oedema, negative jugular vein pulsation, sterile laminitis (Schwagerick, 2004) and some stellate skin depigmentation (M. Aldinger, personal communication).

Allergic and inflammatory affections. Substance P and histamine increase cell cytotoxicity, whereas noradrenaline reduces natural killer cell activity. Cell migration is influenced in the opposite direction (Lang *et al.*, 2003). C2 toxin is reported to influence these regulatory circuits and hence may show an interconnection between immune and neuroendocrine systems (see Table IV).

Neuro-immuno-endocrine interactions. Neuro-immuno-endocrine interactions may be triggered by BoT (Besedovsky and del Rey, 1996; Majde and Krueger, 2002; for review see Ader *et al.*, 2001). A retrograde influence far from the site of the direct action of BoT injected into muscles changed the physiology of corresponding motoneurons (Moreno Lopez *et al.*, 1994; Jung *et al.*, 1997; Humm *et al.*, 2000).

There are reports of 'beneficial' effects of BoT: induced Epstein–Barr virus proliferation was inhibited by C2. C3 caused an overall decrease in both number and length of respiratory syncytial virus filaments in human cancer (Hep-2) cells (Melamed *et al.*, 1994; Gower *et al.*, 2001; McCurdy and Graham, 2003). C3 may have beneficial effects in ischaemic strokes (Laufs *et al.*, 2000) and may reduce the invasiveness of human and rat hepatoma cells (Genda *et al.*, 1999; Imamura *et al.*, 1999; Yoshioka *et al.*, 1999) and human ovarian cancer cells (Sawada *et al.*, 2002). Negative regulators of regeneration in liver cells and axonal cells in injured brain and spinal cord are blocked by C3 (Ikeda *et al.*, 2003; Fournier *et al.*, 2003). Dysfunctions of haematopoietic cells in allergies may be affected by BoT (see Logan *et al.*, 2003).

Although, under natural conditions, toxin concentrations in blood may not be as high as during some of the reported pharmacological tests, and low BoT concentrations may not diffuse through the blood–brain barrier (Boroff and Chen, 1975), a continuous uptake of toxin over weeks or even months could lead to completely different clinical pictures. It was for a long time accepted that in botulism no serum antibodies against BoT were to be found, on the presumption that the toxic level was lower than the antigenic level. The demonstration of antibodies under natural conditions (Ohishi *et al.*, 1979) and in visceral botulism (Böhnel *et al.*, 2001a) indicates that very low doses of BoT do have access to the body systems without obvious clinical disease.

There are no reports on the clinical effects of low doses of BoT and long-time absorption on eventual long-term mortality as has been shown for other foodborne gastrointestinal infections in humans (Helms *et al.*, 2003).

Outlook

From 1997 to 2003 our institute received almost 13 000 samples for botulism control. We tested for the presence of botulinum neurotoxins and bacterial forms in samples of stools/faeces and organs according to international standards (CDC, 1988). Almost 1000 outbreaks on farms were confirmed; 966 were positive, and an additional 57 could not be neutralized by available antitoxins. There was an increase from 60 cases in 1999 to 387 in 2003. According to veterinary practitioners, the disease has reached an unacceptable level (Böhnel and Gessler, 2003). From 208 human cases, 96 were positive and 14 could not be neutralized. Several cases of diagnosed botulism did not coincide with standard forms of botulism. In human cases with cryptogenic origin, such as cases of multiple sclerosis in infants, cryptogenic neurological failures, incontinence and sexual dysfunction in adults, periodic blurred vision in children and adults, and sudden death of a patient with necrotic wounds, we found the presence of BoT in the patients' stools. In 9 out of 49 apparently healthy infants, at least without

obvious clinical symptoms, BoT or bacterial forms of *C. botulinum* (five neutralized by antitoxins) were found in the faeces (unpublished results). Finally the effects of BoT have been implicated in cryptogenic pathological and clinical changes that led to visceral botulism in cattle (Böhnel *et al.*, 2001a) and equine grass sickness in horses (Tocher *et al.*, 1923; Hunter *et al.*, 1999; Böhnel *et al.*, 2003).

It is generally accepted that health risk will increase with increasing number of pathogens. Many substances that are claimed to be beneficial for soil structure and fertility may contain heavy loads of botulinum spores and even toxins (Popoff and Argente, 1995; Böhnel and Lube, 2000; Böhnel *et al.*, 2002; Böhnel, 2002). Consequently, any voluntary spreading of additional *C. botulinum* in the environment will add to possibly existing bacterial populations. Water and dust and direct contact with soil will increase the health risk. Modern waste management practices in which agricultural sites are used as dumping grounds for recycling products could therefore pose a threat to human and animal health, today and in the future.

Hence, the results of molecular-biological research indicate that BoT, both neurotoxic and non-neurotoxic, influence exocytosis and the intracellular skeleton in eukaryotic cells and may initiate or mediate unexplained systemic disorders. The long-used clinical term 'botulism' might not be replaced by flaccid paralysis or visceral botulism, but the general understanding has to be broadened to 'different clinical expressions of one systemic disease'. The increasing numbers of site-specific and/or hitherto locally unknown types of *C. botulinum* in the environment will increase both the number and types of diseases or syndromes of humans and animals. Antibiotic treatment to reduce the load of *C. botulinum* in the intestines is not entirely successful, and new health management systems need to be found to reduce the impact of *C. botulinum* on human and animal health.

REFERENCES

- Ader, R., Felten, D.L. and Cohen, N., 2001. *Psychoneuroimmunology*, 3rd edn, vol. 1, (Academic Press, San Diego)
- Aguado, F., Majó, G., Ruiz-Montasell, B., Canals, J.M., Casanova, A., Marsal, J. and Blasi, J., 1996. Expression of synaptosomal-associated protein SNAP-25 in endocrine anterior pituitary cells. *European Journal of Cell Biology*, **69**, 351–359
- Aguado, F., Gombau, L., Majó, G., Marsal, J., Blanco, J. and Blasi, J., 1997. Regulated secretion is impaired in AtT-20 endocrine cells stably transfected with botulinum neurotoxin type A light chain. *Journal of Biological Chemistry*, **272**, 26005–26008
- Aktories, K. and Wegner, A., 1989. ADP-ribosylation of actin by clostridial toxins. *Journal of Cell Biology*, **109**, 1385–1387
- Aktories, K., Ankenbauer, T., Schering, B. and Jakobs, K.H., 1986a. ADP-ribosylation of platelet actin by botulinum C2 toxin. *European Journal of Biochemistry*, **161**, 155–162
- Aktories, K., Bärmann, M., Ohishi, I., Tsuyama, S., Jakobs, K.H. and Habermann, E., 1986b. Botulinum C2 toxin ADP-ribosylates actin. *Nature*, **322**, 390–392
- Aktories, K., Weller, U. and Chhatwal, G.S., 1987. *Clostridium botulinum* type C produces a novel ADP-ribosyltransferase distinct from botulinum C2 toxin. *FEBS Letters*, **212**, 109–113
- Aktories, K., Schmidt, G. and Just, I., 2000. Rho GTPases as targets of bacterial protein toxins. *Biological Chemistry*, **381**, 421–426
- Al-Mohanna, F.A., Ohishi, I. and Hallett, M.B., 1987. Botulinum C2 toxin potentiates activation of the neutrophil oxidase. *FEBS Letters*, **219**, 40–44

- An, S.S., Laudadio, R.E., Lai, J., Rogers, R.A. and Fredberg, J.J., 2002. Stiffness changes in cultured airway smooth muscle cells. *American Journal of Physiology*, **283**, C792–C801
- Anderson, R.J., Ray, C.J. and Popoff, M.R., 2000. Evidence for Rho protein regulation of renal tubular epithelial cell function. *Kidney International*, **58**, 1996–2006
- Apodaca, G., Cardone, M.H., Whiteheart, S.W., DasGupta, B.R. and Mostov, K.E., 1996. Reconstitution of transcytosis in SLO-permeabilized MDCK cells, existence of an NSF-dependent fusion mechanism with the apical surface of MDCK cells. *EMBO Journal*, **15**, 1471–1481
- Arnon, S.S. and Chin, J., 1979. The clinical spectrum of infant botulism. *Review of Infectious Diseases*, **1**, 614–624
- Ashton, A.C. and Dolly, J.O., 1988. Characterization of the inhibitory action of botulinum neurotoxin type A in the release of several transmitters from rat cerebro-cortical synaptosomes. *Journal of Neurochemistry*, **50**, 1808–1816
- Attoub, S., Rivat, C., Rodrigues, S., van Bocxlaer, S., Bedin, M., Bruyneel, E., Louvet, C., Kornprobst, M., Andre, T., Mareel, M., Mester, J. and Gespach, C., 2002. The c-kit tyrosine kinase inhibitor STI571 for colorectal cancer therapy. *Cancer Research*, **62**, 4879–4883
- Bachmeyer, C., Benz, R., Barth, H., Aktories, K., Gilbert, M. and Popoff, M.R., 2001. Interaction of *Clostridium botulinum* C2 toxin with lipid bilayer membranes and Vero cells, inhibition of channel function by chloroquine and related compounds *in vitro* and intoxication *in vivo*. *FASEB Journal*, **15**, 1658–1660
- Banerjee, A., Li, G., Alexander, E.A. and Schwartz, J.H., 2001. Role of SNAP-23 in trafficking of H⁺-ATPase in cultured inner medullary collecting duct cells. *American Journal of Physiology and Cell Physiology*, **280**, C775–C781
- Barth, H., Hofmann, F., Olenik, C., Just, I. and Aktories, K., 1998. The N-terminal part of the enzyme component C2I of the binary *Clostridium botulinum* C2 toxin interacts with the binding component C2II and functions as a carrier system for rho ADP-ribosylating C3-like fusion toxin. *Infection and Immunity*, **66**, 1364–1369
- Barth, H., Klingler, M., Aktories, K. and Kinzel, V., 1999. *Clostridium botulinum* C2 toxin delays entry into mitosis and activation of p34cdc2 kinase and cdc25-C phosphatase in HeLa cells. *Infection and Immunity*, **67**, 5083–5090
- Barth, H., Blöcker, D. and Aktories, K., 2002a. The uptake machinery of clostridial actin ADP-ribosylating toxins – a cell delivery system for fusion proteins and polypeptide drugs. *Naunyn-Schmiedeberg's Archive of Pharmacology*, **366**, 501–512
- Barth, H., Roebeling, R., Fritz, M. and Aktories, K., 2002b. The binary *Clostridium botulinum* C2 toxin as a protein delivery system. *Journal of Biological Chemistry*, **277**, 5074–5081
- Behari, M. and Raju, G.B., 1996. Electrophysiological studies in patients with blepharospasms before and after botulinum A therapy. *Journal of Neurological Science*, **135**, 74–77
- Berardelli, A., Gilio, F. and Currà, A., 2002. Effects of botulinum toxin type A on central nervous system function. In: M.F. Brin, M. Hallett and J. Jankovic (eds), *Scientific and Therapeutic Aspects of Botulinum Toxin*, (Lippincott Williams and Wilkins, Philadelphia), 171–177
- Bergquist, F., Niazi, H.S. and Nissbrandt, H., 2002. Evidence for different exocytosis pathways in dendritic and terminal dopamine release *in vivo*. *Brain Research*, **950**, 245–253
- Besedovsky, H.O. and del Rey, A., 1996. Immune-neuro-endocrine interactions, facts and hypotheses. *Endocrine Reviews*, **17**, 64–102
- Bhakdi, S., Tranum-Jensen, J. and Sziegoleit, A., 1985. Mechanism of membrane damage by streptolysin-O. *Infection and Immunity*, **47**, 52–60
- Bhakdi, S., Bayley, H., Valeva, A., Walev, I., Walker, B., Weller, U., Kehoe, M. and Palmer, M., 1996. Staphylococcal alpha-toxin, streptolysin-O, and *Escherichia coli* hemolysin, prototypes of pore-forming bacterial cytolysins. *Archive of Microbiology*, **165**, 73–79
- Bigalke, H., Dreyer, F. and Bergey, G., 1985. Botulinum A neurotoxin inhibits non-cholinergic synaptic transmission in mouse spinal cord neurons in culture. *Brain Research*, **360**, 318–324
- Bittner, M.A., DasGupta, B.R. and Holz, R.W., 1989. Isolated light chains of botulinum neurotoxins inhibit exocytosis. *Journal of Biological Chemistry*, **264**, 10354–10360
- Black, J.D. and Dolly, J.O., 1987. Selective location of acceptors for botulinum neurotoxin A in the central and peripheral nervous systems. *Neuroscience*, **23**, 767–779
- Blöcker, D., Bachmeyer, C., Benz, R., Aktories, K. and Barth, H., 2003. Channel formation by the binding component of *Clostridium botulinum* C2 toxin: glutamate 307 of C2II affects channel properties *in vitro* and pH-dependent C2I translocation *in vivo*. *Biochemistry*, **42**, 5368–5377
- Bock, J.B. and Scheller, R.H., 1997. A fusion of new ideas. *Nature*, **387**, 133–135
- Böhnel, H., 2002. Household biowaste containers (bio-bins) – potential incubators for *Clostridium botulinum* and botulinum neurotoxins. *Water, Air, and Soil Pollution*, **140**, 335–341

- Böhnel, H. and Gessler, F., 2003. Botulismusdiagnose seit 1995. Ein Erfahrungsbericht. *Berliner und Münchener Tierärztliche Wochenschrift*, **116**, 269–273
- Böhnel, H. and Lube, K., 2000. *Clostridium botulinum* and biocompost. A contribution to the analysis of potential health hazards caused by bio-waste recycling. *Journal of Veterinary Medicine B*, **47**, 785–795
- Böhnel, H., Schwagerick, B. and Gessler, F., 2001 a. Visceral botulism – a new form of bovine *Clostridium botulinum* toxication. *Journal of Veterinary Medicine A*, **48**, 375–383
- Böhnel, H., Behrens, S., Loch, P., Lube, K. and Gessler, F., 2001b. Is there a link between infant botulism and sudden infant death? Bacteriological results obtained in Central Germany. *European Journal of Pediatrics*, **160**, 623–628
- Böhnel, H., Briese, B.H. and Gessler, F., 2002. Methods for health risk assessment by *Clostridium botulinum* in bio-compost. In: H. Insam, N. Riddech, and S. Klammer (eds), *Microbiology of Composting*, (Springer, Heidelberg), 517–526
- Böhnel, H., Wernery, U. and Gessler, F., 2003. Two cases of equine grass sickness with evidence for soil borne origin involving botulinum neurotoxin. *Journal of Veterinary Medicine B*, **50**, 178–182
- Böhnel, H., Neufeld, B. and Gessler, F., 2004. Botulinum neurotoxin type B in milk from a cow affected by visceral botulism. *The Veterinary Journal*. doi.10.1016/j.tvjl.2004.01.006
- Bolte, E.R., 1998. Autism and *Clostridium tetani*. *Medical Hypotheses*, **51**, 133–144
- Boroff, D.A. and Chen, G.S., 1975. On the question of permeability of the blood–brain barrier to BoNT. *International Archive of Allergy and Applied Immunology*, **48**, 495–504
- Bowler, J.V. 2003. Blood–brain barrier permeability in type II diabetes. *Journal of Neurology, Neurosurgery and Psychiatry*, **74**, 6
- Brin, M.F., Hallett, M. and Jankovic, J. (eds), 2002. *Scientific and Therapeutic Aspects of Botulinum Toxin*, (Lippincott Williams and Wilkins, Philadelphia)
- Caroff, M., Karibian, D., Cavaillon, J.-M. and Haeffner-Cavaillon, N., 2002. Structural and functional analyses of bacterial lipopolysaccharides. *Microbes and Infection*, **4**, 915–926
- Cato, E.P., George, W.L. and Finegold, S.M., 1986. Genus *Clostridium* Prazmowski 1880. In: P.H.A. Sneath, N.S. Mair, M.E. Sharpe and J. Holt (eds), *Bergey's Manual of Systematic Bacteriology*, vol. 2, (Williams and Wilkins, Baltimore), 1141–1200
- CDC, 1998. Botulism in the United States, 1899–1996. *Handbook for Epidemiologists, Clinicians, and Laboratory Workers*, (Centers for Disease Control and Prevention, Atlanta, GA)
- Chaddock, J.A., Purkiss, J.R., Friis, L.M., Broadbridge, J.D., Duggan, M.J., Fooks, S.J., Shone, C.C., Quinn, C.P. and Foster, K.A., 2000. Inhibition of vesicular secretion in both neuronal and nonneuronal cells by a retargeted endopeptidase derivative of *Clostridium botulinum* neurotoxin type A. *Infection and Immunity*, **68**, 2587–2593
- Chardin, P., Boquet, P., Madaule, P., Popoff, M.R., Rubin, R.J. and Gill, D.M., 1989. The mammalian G protein rhoC is ADP-ribosylated by *Clostridium botulinum* exoenzyme C3 and affects actin microfilaments in Vero cells. *EMBO Journal*, **8**, 1087–1092
- Chen, J.T., Chen, C.C., Lin, K.P., Wang, S.J., Wu, Z.A. and Liao, K.K., 1999. Botulism, heart rate variation, sympathetic skin responses, and plasma norepinephrine. *Canadian Journal of Neurological Science*, **26**, 123–126
- Considine, R.V., Simpson, L.L. and Sherwin, J.R., 1992. Botulinum C2 toxin and steroid production in adrenal Y-1 cells, the role of microfilaments in the toxin-induced increase in steroid release. *Journal of Pharmacology and Experimental Therapy*, **260**, 859–864
- Dayanithi, G., Ahnert-Hilger, G., Weller, U., Nordmann, J.J. and Gratzl, M., 1990. Release of vasopressin from isolated permeabilized neurosecretory nerve terminals is blocked by the light chain of botulinum A toxin. *Neuroscience*, **39**, 711–715
- Demarque, M., Represa, A., Becq, H., Khalilov, I., Ben-Ari, Y. and Aniksztejn, L., 2002. Paracrine intercellular communication by a Ca²⁺- and SNARE-independent release of GABA and glutamate prior to synapse formation. *Neuron*, **36**, 1051–1061
- Di Sario, A., Bendia, E., Svegliati-Baroni, G., Marzioni, M., Ridolfi, F., Trozzi, L., Ugili, L., Saccomanno, S., Jezequel, A.M. and Benedetti, A., 2002. Rearrangement of the cytoskeletal network induced by platelet-derived growth factor in rat hepatic stellate cells, role of different intracellular signalling pathways. *Journal of Hepatology*, **36**, 179–190
- Ellies, M., Laskawi, R., Rohrbach-Volland, S. and Arglebe, C., 2003. Up-to-date report of botulinum toxin therapy in patients with drooling caused by different etiologies. *Journal of Oral and Maxillofacial Surgery*, **61**, 454–457
- Elmqvist, J.K. and Marcus, J.N., 2003. Rethinking the central causes of diabetes. *Nature Medicine*, **9**, 645–647

- Ermert, L., Brückner, H., Walmrath, D., Grimminger, F., Aktories, K., Suttorp, N., Duncker, H.R. and Seeger, W., 1995. Role of endothelial cytoskeleton in high-permeability edema due to botulinum C2 toxin in perfused rabbit lungs. *American Journal of Physiology*, **268**, L753–L761
- Ermert, L., Rössig, R., Hansen, T., Schütte, H., Aktories, K. and Seeger, W., 1996. Differential role of actin in lung endothelial and epithelial barrier properties in perfused rabbit lungs. *European Respiratory Journal*, **9**, 93–99
- Ermert, L., Duncker, H.R., Brückner, H., Grimminger, F., Hansen, T., Rössig, R., Aktories, K. and Seeger, W., 1997. Ultrastructural changes of lung capillary endothelium in response to botulinum C2 toxin. *Journal of Applied Physiology*, **82**, 382–388
- Eto, M., Barandiér, C., Rathgeb, L., Kozai, T., Joch, H., Yang, Z. and Lüscher, T., 2001. Thrombin suppresses endothelial nitric oxide synthase and upregulates endothelin-converting enzyme-1 expression by distinct pathways. Role of Rho/ROCK and mitogen-activated protein kinase. *Circulation Research*, **89**, 583–590
- Evans, J.L., Goldfine, I.D., Maddux, B.A. and Grodsky, G.M., 2003. Are oxidative stress-activated signalling pathways mediators of insulin resistance and beta-cell dysfunction? *Diabetes*, **52**, 1–8
- Finegold, S.M., 1987. *Anaerobic Bacteria in Human Disease*, (Academic Press, New York)
- Finegold, S.M., Molitoris, D., Song, Y., Liu, C., Vaisanen, M.-L., Bolte, E., McTeague, M., Sandler, R., Wexler, H., Marlowe, E.M., Collins, M.D., Lawson, P.A., Summanen, P., Baysallar, M., Tomzynski, T.J., Read, E., Johnson, E., Rolfe, R., Nasir, P., Shah, H., Haake, D.A., Manning, P. and Kaul, A., 2002. Gastrointestinal microflora studies in late-onset autism. *Clinical Infectious Diseases*, **35**(Supplement 1), S6–S16
- Foran, P.G., Davletov, B. and Meunier, F.A., 2003a. Getting muscles moving again after botulinum toxin: novel therapeutic challenges. *Trends in Molecular Medicine*, **9**, 291–299
- Foran, P.G., Mohammed, N., Lisk, G.O., Nagwaney, S., Lawrence, G.W., Johnson, E., Smith, L., Aoki, K.R. and Dolly, J.O., 2003b. Evaluation of the therapeutic usefulness of botulinum neurotoxin B, C1, E, and F compared with the long lasting type A. *Journal of Biological Chemistry*, **278**, 1363–1371
- Fournier, A.E., Takizawa, B.T. and Strittmatter, S.M., 2003. Rho kinase inhibition enhances axonal regeneration in the injured CNS. *Journal of Neuroscience*, **23**, 1416–1423
- Fujinaga, Y., Inoue, K., Nomura, T., Sasaki, J., Marvaud, J.C., Popoff, M.R., Kozaki, S. and Oguma, K., 2000. Identification and characterization of functional subunits of *Clostridium botulinum* type A progenitor toxin involved in binding to intestinal microvilli and erythrocytes. *FEBS Letters*, **467**, 179–183
- Fujita-Yoshigaki, J., Dohke, Y., Hara-Yokoyama, M., Kamata, Y., Kozaki, S., Furuyama, S. and Sugiya, H., 1996. Vesicle associated membrane protein 2 is essential for cAMP-regulated exocytosis in rat parotid acinar cells, the inhibition of cAMP-dependent amylase release by botulinum neurotoxin B. *Journal of Biological Chemistry*, **271**, 13130–13134
- Fujita-Yoshigaki, J., Dohke, Y., Hara-Yokoyama, M., Furuyama, S. and Sugiya, H., 1998. Snare proteins essential for cyclic AMP-regulated exocytosis in salivary glands. *European Journal of Morphology*, **36**(Supplement), 46–49
- Funakoshi, Y., Ichiki, T., Shimokawa, H., Egashira, K., Takeda, K., Kaibuchi, K., Takeya, M., Yoshimura, T. and Takeshita, A., 2001. Rho-kinase mediates angiotensin II-induced monocyte chemoattractant protein-1 expression in rat vascular smooth muscle cells. *Hypertension*, **38**, 100–104
- Gabor, F., Schwarzbauer, A. and Wirth, M., 2002. Lectin-mediated drug delivery, binding and uptake of BSA-WGA conjugates using the Caco-2 model. *International Journal of Pharmaceutics*, **237**, 227–239
- Garza, J.J., Downard, C.D., Clayton, N., Maher, T.J., and Fauza, D.O., 2003. *Clostridium botulinum* toxin inhibits myometrial activity *in vitro*: possible application on the prevention of preterm labor after fetal surgery. *Journal of Pediatric Surgery*, **38**, 511–513
- Gasman, S., Chasserot-Golaz, S., Popoff, M.R., Aunis, D. and Bader, M.F., 1999. Involvement of Rho GTPases in calcium-regulated exocytosis from adrenal chromaffin cells. *Journal of Cell Science*, **112**, 4763–4771
- Genda, T., Sakamoto, M., Ichida, T., Asakura, H., Kojiro, M., Narumya, S. and Hirohashi, S., 1999. Cell motility mediated by the rho and rho-associated protein kinase plays a critical role in intrahepatic metastasis of human hepatocellular carcinoma. *Hepatology*, **30**, 1027–1036
- Gerona, R.R.L., Larsen, E.C., Kowalchuk, J.A. and Martin, T.F.J., 2000. The C terminus of SNAP25 is essential for Ca²⁺-dependent binding of synaptotagmin to SNARE complexes. *Journal of Biological Chemistry*, **275**, 6328–6336
- Gibbins, I.L., Jobling, P., Teo, E.H., Matthew, S.E., Morris, J.L., 2003. Heterogeneous expression of SNAP-25 and synaptic vesicle proteins by central and peripheral inputs to sympathetic neurons. *Journal of Comparative Neurology*, **459**, 25–43

- Glenn, D.E. and Burgoyne, R.D., 1996. Botulinum neurotoxin light chains inhibit both Ca^{2+} -induced and GTP analogue-induced catecholamine release from permeabilised adrenal chromaffin cells. *FEBS Letters*, **386**, 137–140
- Gonelle-Gispert, C., Molinete, M., Halban, P.A. and Sadoul, K., 2000. Membrane localization and biological activity of SNAP-25 cysteine mutants in insulin-secreting cells. *Journal of Cell Science*, **113**, 3197–3205
- Gower, T.L., Peeples, M.E., Collins, P.L. and Graham, B.S., 2001. RhoA is activated during respiratory syncytial virus infection. *Virology*, **283**, 188–196
- Graham, M.E., Washbourne, P., Wilson, M.C. and Burgoyne, R.D., 2002. Molecular analysis of SNAP-25 function in exocytosis. *Annals of the New York Academy of Sciences*, **971**, 210–221
- Granum, P.E., 1990. *Clostridium perfringens* toxins involved in food poisoning. *International Journal of Food Microbiology*, **10**, 101–112
- Grimminger, F., Sibelius, U., Aktories, K., Just, I. and Seeger, W., 1991. Suppression of cytoskeletal rearrangement in activated human neutrophils by botulinum C2 toxin. Impact on cellular signal transduction. *Journal of Biological Chemistry*, **266**, 19276–19282
- Grosse, G., Grosse, J., Tapp, R., Kuchinke, J., Gorsleben, M., Fetter, I., Höhne-Zell, B., Gratzl, M. and Bergmann, M., 1999. SNAP-25 requirement for dendritic growth of hippocampal neurons. *Journal of Neuroscience Research*, **56**, 539–546
- Gundersen, C.B. and Howard, B.D., 1978. The effect of botulinum toxin on acetylcholine metabolism in mouse brain slices and synaptosomes. *Journal of Neurochemistry*, **31**, 1005–1013
- Gunnison, J.B. and Meyer, K.F., 1929. The occurrence of non-toxic strains of *Clostridium parobotulinum*. *Journal of Infectious Diseases*, **34**, 79–80
- Gutierrez, L.M., Rueda, J., Ferrer-Montiel, A.V., Canaves, J.M. and Montal, M., 1997. A peptide that mimics the C-terminal sequence of SNAP-25 inhibits secretory vesicle docking in chromaffin cells. *Journal of Biological Chemistry*, **272**, 2634–2639
- Haberman, E., Müller, H. and Tudel, M., 1988. Tetanus toxin and botulinum A and C neurotoxins inhibit noradrenergic release from cultured mouse brain. *Journal of Neurochemistry*, **51**, 522–527
- Hansen, H.B., 2003a. The enteric nervous system I: organisation and classification. *Pharmacology and Toxicology*, **92**, 105–113
- Hansen, H.B., 2003b. The enteric nervous system II: gastrointestinal functions. *Pharmacology and Toxicology*, **92**, 249–257
- Hansen, H.B., 2003c. The enteric nervous system III: a target for pharmacological treatment. *Pharmacology and Toxicology*, **93**, 1–13
- Helms, M., Vastrup, P., Gerner-Smidt, P. and Molbak, K., 2003. Short and long term mortality associated with foodborne bacterial gastrointestinal infections, registry based study. *British Medical Journal*, **326**, 357–360
- Hippenstiel, S., Schmeck, B., N'Guessan, P.D., Seybold, J., Krüll, M., Preissner, K., Eichel-Streiber, C.V. and Suttrop, N., 2002. Rho protein inactivation induced apoptosis of cultured human endothelial cells. *American Journal of Physiology*, **283**, L830–L838
- Hirokawa, N. and Kitamura, M., 1979. Binding of *Clostridium botulinum* neurotoxin to the presynaptic membrane in the central nervous system. *Journal of Cell Biology*, **81**, 43–49
- Holman, M.E. and Spitzer, N.C., 1973. Action of botulinum toxin on transmission from sympathetic nerves to the vas deferens. *British Journal of Pharmacology*, **47**, 431–433
- Horton, N. and Quick, M.W., 2001. Syntaxin 1A up-regulates GABA transporter expression by subcellular redistribution. *Molecular Membrane Biology*, **18**, 39–44
- Humm, A.M., Pabst, C., Lauterburg, T. and Burgunder, J.-M., 2000. Enkephalin and aFGF are differentially regulated in rat spinal motoneurons after chemodeneration with botulinum toxin. *Experimental Neurology*, **161**, 361–372
- Hunter, L.C., Miller, J.K. and Poxton, I.R., 1999. The association of *Clostridium botulinum* type C with equine grass sickness, a toxicoinfection? *Equine Veterinary Journal*, **31**, 492–499
- Ikedo, H., Satoh, H., Yanase, M., Inoue, Y., Tomiya, T., Arai, M., Tejima, K., Nagashima, K., Maekawa, H., Yahagi, N., Yatomi, Y., Sakurada, S., Takuwa, Y., Ogata, I., Kimura, S. and Fujiwara, K., 2003. Antiproliferative property of sphingosine 1-phosphate in rat hepatocytes involves activation of Rho via Edg-5. *Gastroenterology*, **124**, 459–469
- Imamura, F., Mukai, M., Ayaki, M., Takemura, K., Horai, T., Shinkai, K., Nakamura, H. and Akedo, H., 1999. Involvement of small GTPases Rho and Rac in the invasion of rat ascites hepatoma cells. *Clinical and Experimental Metastasis*, **17**, 141–148
- Ishikawa, H., Mitsui, Y., Yoshitomi, T., Mashimo, K., Aoki, S., Mukuno, K. and Shimizu, K., 2000. Presynaptic effects of botulinum toxin type A on the neuronally evoked response of albino and pigmented rabbit iris sphincter and dilator muscles. *Japanese Journal of Ophthalmology*, **44**, 106–109

- Jacobsson, G., Håkansson, M.-L., Hulting, A.-L. and Meister, B., 1997. Botulinum neurotoxin F, a VAMP-specific endopeptidase, inhibits Ca^{2+} -stimulated GH secretion from rat pituitary cells. *Regulatory Peptides*, **71**, 37–44
- Jahn, R. and Niemann, H., 1994. Molecular mechanisms of clostridial neurotoxins. *Annals of the New York Academy of Science*, **733**, 245–255
- Jenzer, G., Mumenthaler, M., Ludin, H.P. and Robert, F., 1974. Botulismus Typ B. Bericht über milde Verlaufsformen mit vorwiegend autonomen Innervationsstörungen. *Schweizer Medizinische Wochenschrift*, **104**, 685–693
- Johnson, E.A. and Bradshaw, M., 2001. *Clostridium botulinum* and its neurotoxins: a metabolic and cellular perspective. *Toxicon*, **39**, 1703–1722
- Johnson, S., 2001. Clostridial constipation's broad pathology. *Medical Hypotheses*, **56**, 532–536
- Jun, C.D., Han, M.K., Kim, U.H. and Chung, H.T., 1996. Nitric oxide induces ADP-ribosylation of actin in murine macrophages, association with the inhibition of pseudopodia formation, phagocytic activity, and adherence on a laminin substratum. *Cellular Immunology*, **174**, 25–34
- Jung, H.H., Lauterburg, T. and Burgunder, J.M., 1997. Expression of neurotransmitter genes in rat spinal motorneurons after chemodenervation with botulinum toxin. *Neuroscience*, **78**, 469–479
- Just, I., Wille, M., Chaponnier, C. and Aktories, K., 1993. Gelsolin-actin complex is target for ADP-ribosylation by *Clostridium botulinum* C2 toxin in intact human neutrophils. *European Journal of Pharmacology – Molecular Pharmacology Section*, **246**, 293–297
- Kalinec, F., Zhang, M., Urrutia, R. and Kalinec, G., 2000. Rho GTPases mediate the regulation of cochlear outer hair cell motility by acetylcholine. *Journal of Biological Chemistry*, **275**, 28000–28005
- Keller, J.E. and Neale, E.A., 2001. The role of the synaptic protein SNAP-25 in the potency of botulinum neurotoxin type A. *Journal of Biological Chemistry*, **276**, 13476–13482
- Kiehne, K., Herzig, K.H. and Fölsch, U.R., 2002. CCK-stimulated changes in pancreatic acinar morphology are mediated by rho. *Digestion*, **65**, 47–55
- Kerner, J., 1820. *New Observations on a Lethal Intoxication by the Consumption of Smoked Sausages, Often Appearing in Würtemberg in Germany*, (EF Osiander, Tübingen)
- Klein, A.W., 2003. Complications, adverse reactions, and insights with the use of botulinum toxin. *Dermatologic Surgery*, **29**, 549–556
- Knight, D.E., 1986. Botulinum toxin types A, B and D inhibit catecholamine secretion from bovine adrenal medullary cells. *FEBS Letters*, **207**, 222–226
- Knight, D.E., Tonge, D.A. and Baker, P.F., 1985. Inhibition of exocytosis in bovine adrenal medullary cells by botulinum toxin type D. *Nature*, **317**, 719–721
- Kodama, A., Matozaki, T., Fukuhara, A., Kikyo, M., Ichihashi, M. and Takai, Y., 2000. Involvement of an SHP-2-Rho small G protein pathway in hepatocyte growth factor/scatter factor-induced cell scattering. *Molecular Biology of the Cell*, **11**, 2565–2575
- Kohda, T., Kamata, Y. and Kozaki, S., 2000. Endocytosis of *Clostridium botulinum* type B neurotoxin into rat brain synaptosomes. *Journal of Veterinary Medical Science*, **62**, 1133–1138
- Koyama, T., Oike, M. and Ito, Y., 2001. Involvement of Rho-kinase and tyrosine kinase in hypotonic stress-induced ATP release in bovine aortic endothelial cells. *Journal of Physiology*, **532**, 759–769
- Kriek, N.P.J. and Odendaal, M.W., 1994. Botulism. In: J.A.W. Coetzer, G.R. Thomson and R.C. Tustin (eds), *Infectious Diseases of Livestock with Special Reference to Southern Africa*, (Oxford University Press, Capetown), 1354–1371
- Lang, J., Zhang, H., Vaidyanathan, V.-V., Sadoul, K., Niemann, H. and Wollheim, C.B., 1997. Transient expression of botulinum neurotoxin C1 light chain differentially inhibits calcium and glucose induced insulin secretion in clonal β -cells. *FEBS Letters*, **419**, 13–17
- Lang, K., Drell, T.L., Niggemann, B., Zänker, K.S. and Entschladen, F., 2003. Neurotransmitters regulate the migration and cytotoxicity in natural killer cells. *Immunology Letters*, **90**, 165–172
- Laufs, U., Endres, M., Stagliano, N., Amin-Hanjani, S., Chui, D.S., Yang, S.X., Simoncini, T., Yamada, M., Rabkin, E., Allen, P.G., Huang, P.L., Böhm, M., Schoen, F.J., Moskowitz, M.A. and Liao, J.K., 2000. Neuroprotection mediated by changes in endothelial actin cytoskeleton. *Journal of Clinical Investigation*, **106**, 15–24
- Lawrence, G.W., Foran, P. and Dolly, J.O., 1996. Distinct exocytotic responses of intact and permeabilised chromaffin cells after cleavage of the 25-kDa synaptosomal-associated protein SNAP-25 or synaptobrevin by botulinum toxin A or B. *European Journal of Biochemistry*, **236**, 877–886
- Lee, T.Y.J. and Gotlieb, A.I., 2002. Rho and basic fibroblast growth factor involvement in centrosome redistribution and actin microfilament remodelling during early endothelial wound repair. *Journal of Vascular Surgery*, **35**, 1242–1252
- Leist, M., Fava, E., Montecucco, C. and Nicotera, P., 1997. Peroxynitrite and nitric oxide donors induce neuronal apoptosis by eliciting autocrine excitotoxicity. *European Journal of Neuroscience*, **9**, 1488–1498

- Leung, S.M., Chen, D., DasGupta, B.R., Whiteheart, S.W. and Apodaca, G., 1998. SNAP-23 requirement for transferrin recycling in streptolysin-O-permeabilized Madin-Darby canine kidney cells. *Journal of Biological Chemistry*, **273**, 17732–17741
- Li, G., Rungger-Brändle, E., Just, I., Jonas, J.-C., Aktories, K. and Wollheim, C.B., 1994. Effect of disruption of actin filaments by *Clostridium botulinum* C2 toxin on insulin secretion in HIT-T15 cells and pancreatic islets. *Molecular Biology of the Cell*, **5**, 1199–1213
- Li, L. and Singh, B.R., 1998. Isolation of synaptotagmin as a receptor for types A and E botulinum neurotoxin and analysis of their comparative binding using a new microtiter plate assay. *Journal of Natural Toxins*, **7**, 215–226
- Linal, M., 2000. Neurotoxins as tools in dissecting the exocytic machinery. In: H. Hilderson and S. Fuller (eds), *Biological Membranes and Related Problems*, vol. 34, (Kluwer Academic/Plenum Publishers, New York), 39–72
- Lledo, P.M., Zhang, X., Südhof, T.C., Malenka, R.C. and Nicoll, R.A., 1998. Postsynaptic membrane fusion and long-term potentiation. *Science*, **279**, 399–403
- Logan, M.R., Odemuyiwa, S.O. and Moqbel, R., 2003. Understanding exocytosis in immune and inflammatory cells: the molecular basis of mediator secretion. *Journal of Allergy and Clinical Immunology*, **111**, 923–932
- Lüscher, C., Xia, H., Beattie, E.C., Carroll, R.C., Zastrow, M.V., Malenka, R.C. and Nicoll, R.A., 1999. Role of AMPA receptor cycling in synaptic transmission and plasticity. *Neuron*, **24**, 649–658
- Ludger, J. and Galli, T., 1998. Mini review. Exocytosis, SNAREs drum up! *European Journal of Neuroscience*, **10**, 415–422.
- Luisetto, S., Rossetto, O., Montecucco, C. and Pavone, F., 2003. Toxicity of botulinum neurotoxins in central nervous system of mice. *Toxicon*, **41**, 475–481
- MacKenzie, I., Burnstoc, G. and Dolly, J.O., 1982. The effects of purified botulinum neurotoxin type A on cholinergic, adrenergic and non-adrenergic, atropine-resistant autonomic neuromuscular transmission. *Neuroscience*, **7**, 997–1006
- Majde, J.A. and Krueger, J.M., 2002. Neuroimmunology of sleep. In: H. D'haenen, J.A. den Boer, H. Westenberg, and P. Willner (eds), *Textbook of Biological Psychiatry*, (Wiley, London), 1247–1257
- Matter, K., Dreyer, F. and Aktories, K., 1989. Actin involvement in exocytosis from PC12 cells, studies on the influence of botulinum C2 toxin on stimulated noradrenaline release. *Journal of Neurochemistry*, **52**, 370–376
- Mayhan, W.G., 2001. Regulation of blood–brain barrier permeability. *Microcirculation*, **8**, 89–104
- McCurdy, L.H. and Graham, B.S., 2003. Role of plasma membrane lipid microdomains in respiratory syncytial virus filament formation. *Journal of Virology*, **77**, 1747–1756
- McInnes, C. and Dolly, J.O., 1990. Ca²⁺-dependent noradrenaline release from permeabilised PC12 cells is blocked by botulinum neurotoxin A or its light chain. *FEBS Letters*, **261**, 323–326
- Melamed, I. and Gelfand, E.W., 1999. Microfilament assembly is involved in B-cell apoptosis. *Cellular Immunology*, **194**, 136–142
- Melamed, I., Downey, G.P., Aktories, K. and Roifman, C.M., 1991. Microfilament assembly is required for antigen-receptor-mediated activation of human B lymphocytes. *Journal of Immunology*, **147**, 1139–1146
- Melamed, I., Stein, L. and Roifman, C.M., 1994. Epstein–Barr virus induces actin polymerization in human B cells. *Journal of Immunology*, **153**, 1998–2003
- Melamed, I., Franklin, R.A. and Gelfand, E.W., 1995. Microfilament assembly is required for anti-IgM dependent MAPK and p90rsk activation in human B lymphocytes. *Biochemical and Biophysical Research Communications*, **209**, 1102–1110
- Merz, B., Bigalke, H., Stoll, G. and Naumann, M., 2003. Botulism type B presenting as pure autonomic dysfunction. *Clinical Autonomic Research*, **13**, 337–338
- Meunier, F.A., Lisk, G., Sesardic, D. and Dolly, J.O., 2003. Dynamics of motor nerve terminal remodelling unveiled using SNARE-cleaving botulinum toxins: the extent and duration are dictated by the sites of SNAP-25 truncation. *Molecular and Cellular Neuroscience*, **22**, 454–466
- Midura, T.F. and Arnon, S.S., 1976. Infant botulism. Identification of *Clostridium botulinum* and its toxins in faeces. *Lancet*, **ii**, 934–936
- Minic, Z., Laporte, J., Couchy, I., Popoff, M.R., Satiat-Jeunemaitre, B. and Brown, S., 1999. Probing the action of *Clostridium* toxins B and exoenzyme C3 for detection of Rho-like motifs of alfalfa proteins. *Plant Physiology and Biochemistry*, **37**, 775–787
- Moeller, R.B., Puschner, B., Walker, R.L., Rocke, T., Galey, F.D., Cullor, J.S. and Ardans, A.A., 2003. Determination of the median toxic dose of type C botulinum toxin in lactating dairy cows. *Journal of Veterinary Diagnosis and Investigation*, **15**, 523–526

- Moreno Lopez, B., de la Cruz, R., Pastor, A.M. and Delgado, G.J., 1994. Botulinum neurotoxin alters the discharge characteristics of abducens motoneurons in the alert cat. *Journal of Neurophysiology*, **72**, 2041–2044
- Moriishi, K., Syuto, B., Saito, M., Oguma, K., Fujii, N., Abe, N. and Naiki, M., 1993. Two different types of ADP-ribosyltransferase C3 from *Clostridium botulinum* type D lysogenized organisms. *Infection and Immunity*, **61**, 5309–5314
- Morris, J.L., Jobling, P. and Gibbins, I.L., 2001. Differential inhibition by botulinum neurotoxin A of cotransmitters released from autonomic vasodilator neurons. *American Journal of Physiology and Heart Circulation Physiology*, **281**, H2124–H2132
- Morris, J.L., Jobling, P. and Gibbins, I.L., 2002. Botulinum neurotoxin A attenuates release of norepinephrine but not NPY from vasoconstrictor neurons. *American Journal of Physiology and Heart Circulation Physiology*, **283**, H2627–H2635
- Moynihan, J., Kruszezwska, B., Madden, K. and Callahan, T., 2004. Sympathetic nervous system regulation of immunity. *Journal of Neuroimmunology*, **147**, 87–90
- Münchau, A. and Bhatia, K.P., 2000. Uses of botulinum toxin injection in medicine today. *British Medical Journal*, **320**, 161–165
- Murthy, K.S., Zhou, H., Grider, J.R. and Makhlof, G.M., 2001. Sequential activation of heterotrimeric and monomeric G proteins mediates PLD activity in smooth muscle. *American Journal of Physiology*, **280**, G381–G388
- Najib, A., Pelliccioni, P., Gil, C. and Aguilera, J., 1999. Clostridium neurotoxins influence serotonin uptake and release differently in rat brain synaptosomes. *Journal of Neurochemistry*, **72**, 1991–1998
- Nakatsuka, S., Hayashi, M., Muroyama, A., Otsuka, M., Kozaki, S., Yamada, H. and Moriyama, Y., 2001. D-Aspartate is stored in secretory granules and released through a Ca²⁺-dependent pathway in a subset of rat pheochromocytoma PC12 cells. *Journal of Biological Chemistry*, **276**, 26589–26596
- Nakov, R., Habermann, E., Hertting, G., Wurster, S. and Allgaier, C., 1989. Effects of botulinum A toxin on presynaptic modulation of evoked transmitter release. *European Journal of Pharmacology*, **164**, 45–53
- Nilius, B., Voets, T., Prenen, J., Barth, H., Aktories, K., Kaibuchi, K., Broogmans, G. and Eggermont, J., 1999. Role of Rho and Rho kinase in the activation of volume-regulated anion channels in bovine endothelial cells. *Journal of Physiology*, **516**(Supplement 1), 67–74
- Nishi, M., Takeshima, H., Houtani, T., Nakagawara, K.I., Noda, T. and Sugimoto, T., 2002. RhoN, a novel small GTP-binding protein expressed predominantly in neurons and hepatic stellate cells. *Molecular Brain Research*, **67**, 74–81
- Nishioka, H., Horiuchi, H., Tabuchi, A., Yoshioka, A., Shirakawa, R. and Kita, T., 2001. Small GTPase Rho regulates thrombin-induced platelet aggregation. *Biochemical and Biophysical Research Communications*, **280**, 970–975
- Norgauer, J., Kownatzki, E., Seifert, R. and Aktories, K., 1988. Botulinum C2 toxin ADP-ribosylates actin and enhances O₂ production and secretion but inhibits migration of activated human neutrophils. *Journal of Clinical Investigation*, **82**, 1376–1382
- Norgauer, J., Just, I., Aktories, K. and Sklar, L.A., 1989. Influence of botulinum C2 toxin on F-actin and N-formyl peptide receptor dynamics in human neutrophils. *Journal of Cell Biology*, **109**, 1133–1140
- Oguma, K., Syoto, B., Iida, H. and Kubo, S., 1981. Homogeneity and heterogeneity of toxins produced by *Clostridium botulinum* type C and D strains. *Infection and Immunity*, **34**, 382–388
- Oguma, K., Murayama, S., Syuto, B., Iida, H. and Kubo, S., 1984. Analysis of antigenicity of *Clostridium botulinum* type C1 and D toxins by polyclonal and monoclonal antibodies. *Infection and Immunity*, **43**, 584–588
- Ohishi, I., Sakaguchi, G., Riemann, H., Behymer, D. and Hurvell, B., 1979. Antibodies to *Clostridium botulinum* toxins in free-living birds and mammals. *Journal of Wildlife Diseases*, **15**, 3–9
- Ohishi, I., 1983b. Response of mouse intestinal loop to botulinum C2 toxin, enterotoxigenic activity induced by cooperation of nonlinked protein components. *Infection and Immunity*, **40**, 691–695
- Ohishi, I. and DasGupta, G.R., 1987. Molecular structure and biological activities of *Clostridium botulinum* C2 toxin. In: M.W. Eklund and V.R. Dowell (eds), *Avian Botulism. An International Perspective*, (Charles C. Thomas, Springfield, IL), 223–247
- Ohishi, I. and Odagiri, Y., 1984. Histopathological effect of botulinum C2 toxin on mouse intestines. *Infection and Immunity*, **43**, 54–58
- Ohishi, I., Iwasaki, M. and Sakaguchi, G., 1980. Vascular permeability activity of botulinum C2 toxin elicited by cooperation of two dissimilar protein components. *Infection and Immunity*, **31**, 890–895
- Ohishi, I., Miyake, M., Ogura, H. and Nakamura, S., 1984. Cytopathic effect of botulinum C2 toxin on tissue-culture cells. *FEMS Microbiological Letters*, **23**, 281–284

- Olgart, C., Gustafsson, L.E. and Wiklund, N.P., 2000. Evidence for nonvesicular nitric oxide release evoked by nerve activation. *European Journal of Neuroscience*, **12**, 1303–1309
- Papadonikolakis, A.S., Vekris, M.D., Kostas, J.P., Korompilias, A.V. and Soucanos, P.N., 2002. Transient erectile dysfunction associated with intramuscular injection of botulinum toxin type A. *Journal of the Southern Orthopaedic Association*, **11**, 116–118
- Papini, E., Rossetto, O. and Cutler, D.F., 1995. Vesicle-associated membrane protein VAMP/synaptobrevin-2 is associated with dense core secretory granules in PC12 neuroendocrine cells. *Journal of Biological Chemistry*, **270**, 1332–1336
- Park, J.B. and Simpson, L.L., 2003. Inhalation poisoning of botulinum toxin and inhalation vaccination with its heavy-chain component. *Infection and Immunity*, **71**, 1147–1154
- Parmer, R.J. and Zinder, O., 2002. Catecholaminic pathways, chromaffin cells, and human disease. *Annals of the New York Academy of Sciences*, **971**, 497–505
- Popoff, M.R. and Argente, G., 1995. Le botulisme animal – est-il une menace pour l'homme? *Bulletin de la Academie Vétérinaire Française*, **69**, 373–382
- Prepens, U., Barth, H. and Wilting, J., 1998. Influence of *Clostridium botulinum* C2 toxin on FcεRI-mediated secretion and tyrosine phosphorylation in RBL cells. *Naunyn-Schmiedeberg's Archive of Pharmacology*, **357**, 323–330
- Purkiss, J.R., Welch, M.J., Doward, S., Foster, K.A. and Quinn, C.P., 2000. A method for the measurement of [³H]-glutamate release from cultured dorsal root ganglion neurons. *Biochemical Society Transactions*, **26**, S108
- Rao, J.N., Li, L., Golovina, V.A., Platoshyn, O., Strauch, E.D., Yuan, J.X.J. and Wang, J.Y., 2001. Ca²⁺-Rho signalling pathway required for polyamine-dependent intestinal epithelial cell migration. *American Journal of Physiology*, **280**, C993–C1007
- Ravichandran, V., Chawla, A. and Roche, P.A., 1996. Identification of a novel syntaxin- and synaptobrevin/VAMP-binding protein, SNAP-23, expressed in non-neuronal tissues. *Journal of Biological Chemistry*, **271**, 13300–13303
- Regazzi, R., Wollheim, C.B., Lang, J., Theler, J.-M., Rossetto, O., Montecucco, C., Sadoul, K., Weller, U., Palmer, M. and Thorens, B., 1995. VAMP-2 and cellubrevin are expressed in pancreatic β-cells and are essential for Ca²⁺ but not for GTPγS-induced insulin secretion. *EMBO Journal*, **14**, 2723–2730
- Retzer, M. and Essler, M., 2000. Lysophosphatidic acid-induced platelet shape change proceeds via Rho/Rho kinase-mediated myosin light-chain and moesin phosphorylation. *Cell Signal*, **12**, 645–648
- Retzer, M., Siess, W. and Essler, M., 2000. Mildly oxidised low density lipoprotein induces platelet shape change via Rho-kinase-dependent phosphorylation of myosin light chain and moesin. *FEBS Letters*, **466**, 70–74
- Reuner, K.H., Presek, P., Boschek, C.B. and Aktories, K., 1987. Botulinum C2 toxin ADP-ribosylates actin and disorganizes the microfilament network in intact cells. *European Journal of Cell Biology*, **43**, 134–140
- Reuner, K.H., Schlegel, K., Just, I., Aktories, K. and Katz, N., 1991. Autoregulatory control of actin synthesis in cultured rat hepatocytes. *FEBS Letters*, **286**, 100–104
- Rohrbach, S., Olthoff, A., Laskawi, R., Giefer, B. and Götz, W., 2001. Botulinum toxin type A induces apoptosis in nasal glands of guinea pigs. *Annals of Otology, Rhinology, and Laryngology*, **110**, 1045–1050
- Ronnov-Jessen, L. and Petersen, O.W., 1996. ADP-ribosylation of actins in fibroblasts and myofibroblasts by botulinum C2 toxin. Influence on microfilament morphology and migratory behaviour. *Electrophoresis*, **17**, 1776–1780
- Rose, F., Kürth-Landwehr, C., Sibelius, U., Reuner, K.H., Aktories, K., Seeger, W. and Grimminger, F., 1999. Role of actin depolymerization in the surfactant secretory response of alveolar epithelial type II cells. *American Journal of Respiration and Critical Care Medicine*, **159**, 206–212
- Rooney, J.R. and Prickett, M.E., 1967. Shaker foal syndrome. *Modern Veterinary Practice*, **48**, 44–45
- Sadoul, K., Lang, J., Montecucco, C., Weller, U., Regazzi, R., Catsicas, S., Wollheim, C.B. and Halban, P.A., 1995. SNAP-25 is expressed in islets of Langerhans and is involved in insulin release. *Journal of Cell Biology*, **128**, 1019–1028
- Saito, H., Minamiya, Y., Saito, S. and Ogawa, J., 2002. Endothelial Rho and rho kinases regulate neutrophil migration via endothelial myosin light chain phosphorylation. *Journal of Leukocyte Biology*, **72**, 829–836
- Sawada, K., Morishige, K.-I., Tahara, M., Ikebuchi, Y., Kawagishi, R., Tasaka, K. and Murata, Y., 2002. Lysophosphatidic acid induces focal adhesion assembly through Rho/Rho-associated kinase pathway in human ovarian cancer cells. *Gynecology – Oncology*, **87**, 252–259
- Schengrund, C.-L., DasGupta B.R. and Ringler, N.J., 1993. Ganglioside GD3 enhances adherence of botulinum and tetanus neurotoxins to bovine brain synapsin I. *Neuroscience Letters*, **158**, 159–162

- Schenk, U., Verderio, C., Benfenati, F. and Matteoli, M., 2003. Regulated delivery of AMPA receptor subunits to the presynaptic membrane. *EMBO Journal*, **22**, 558–568
- Schmid, A., Benz, R., Just, I. and Aktories, K., 1994. Interaction of *Clostridium botulinum* C2 toxin with lipid bilayer membranes. *Journal of Biological Chemistry*, **269**, 16706–16711
- Schmid, G., Schürmann, A., Huppertz, C., Hofmann, F., Aktories, K. and Joost, H.G., 1998. Inhibition of insulin-stimulated glucose transport in 3T3-L1 cells by *Clostridium difficile* toxin B, *Clostridium sordelli* lethal toxin, and *Clostridium botulinum* C2 toxin. *Naunyn-Schmiedeberg's Archive of Pharmacology*, **357**, 385–392
- Schnittler, H.J., Schneider, S.W., Raifer, H., Luo, F., Dieterich, P., Just, I. and Aktories, K., 2001. Role of actin filaments in endothelial cell-cell adhesion and membrane stability under fluid shear stress. *Pflügers Archiv – European Journal of Physiology*, **442**, 675–687
- Schnitzer, J.E., Liu, J. and Oh, P., 1995. Endothelial caveolae have the molecular transport machinery for vesicle budding, docking, and fusion including VAMP, NSF, SNAP, annexins, and GTPases. *Journal of Biological Chemistry*, **270**, 14399–14404
- Schraufstatter, I.U., Chung, J. and Burger, M., 2001. IL-8 activates endothelial cell CXCR1 and CXCR2 through Rho and Rac signaling pathways. *American Journal of Physiology*, **280**, L1094–L1103
- Schwagerick, B., 2004. Klinische Fälle von Viszeralem Botulismus bei Milchrindern in Mecklenburg-Vorpommern. *Tierärztliche Umschau*, **59**, 25–29
- Scott, A.B., 1981. Botulinum toxin injection of eye muscles to correct strabismus. *Transactions of the American Ophthalmological Society*, **79**, 734–770
- Sekiya, K., Danbara, H., Futaesaku, Y., Haque, A., Sugimoto, N. and Matsuda, M., 1998. Formation of ring-shaped structures on erythrocyte membranes after treatment with botulinolysin, a thiol-activated hemolysin from *Clostridium botulinum*. *Infection and Immunity*, **66**, 2987–2990
- Shimazaki, Y., Nishiki, T.-i. Omori, A., Sekiguchi, M., Kamata, Y., Kozaki, S. and Takahashi, M., 1996. Phosphorylation of 25-kDa synaptosome-associated protein. *Journal of Biological Chemistry*, **271**, 14548–14553
- Shiokawa, S., Sakai, K., Akimoto, Y., Suzuki, N., Hanashi, H., Nagamatsu, S., Iwashita, M., Nakamura, Y., Hirano, H. and Yoshimura, Y., 2000. Function of the small guanosine triphosphate-binding protein RhoA in the process of implantation. *Journal of Clinical Endocrinology and Metabolism*, **85**, 4742–4749
- Shiokawa, S., Iwashita, M., Akimoto, Y., Nagamatsu, S., Sakai, K., Hanashi, H., Kabir-Salmani, M., Nakamura, Y., Uehata, M. and Yoshimura, Y., 2002. Small guanosine triphosphatase RhoA and Rho-associated kinase as regulators of trophoblast migration. *Journal of Clinical Endocrinology and Metabolism*, **87**, 5808–5816
- Shiota, M., Tanihiro, T., Nakagawa, Y., Aoki, N., Ishida, N., Miyazaki, K., Ullrich, A. and Miyazaki, H., 2003. Protein tyrosine phosphatase PTP20 induces actin cytoskeleton reorganization by dephosphorylating p190 RhoGAP in rat ovarian granulosa cells stimulated with follicle-stimulating hormone. *Molecular Endocrinology*, **17**, 534–549
- Shone, C.C. and Melling, J., 1992. Inhibition of calcium-dependent release of noradrenaline from PC12 cells by botulinum type-A neurotoxin. Long-term effects of the neurotoxin on intact cells. *European Journal of Biochemistry*, **207**, 1009–1016
- Simpson, L.L., 1982. A comparison of the pharmacological properties of *Clostridium botulinum* type C1 and C2 toxins. *Journal of Pharmacology and Experimental Therapy*, **223**, 695–701
- Simpson, L.L., 1989. Peripheral actions of the BoNT. In: Simpson, L.L. (ed). *Botulinum Neurotoxin and Tetanus Toxin*, (Academic Press, San Diego), 153–178
- Smith, C.P., Franks, M.E., McNeil, B.K., Ghosh, R., de Groat, W.C., Chancellor, M.B. and Somogyi, G.T., 2003. Effect of botulinum toxin A on the autonomic nervous system of the rat lower urinary tract. *Journal of Urology*, **169**, 1896–1900
- Smith, L. and Sugiyama, H. 1988. *Botulism. The Organism, Its Toxins, The Disease*, 2nd edn., (Charles C. Thomas, Springfield, IL)
- Starr, J.M., Wardlaw, J., Ferguson, K., MacLulich, A., Deary, I.J. and Marshall, I., 2003. Increased blood–brain barrier permeability in type II diabetes demonstrated by gadolinium magnetic resonance imaging. *Journal of Neurology, Neurosurgery and Psychiatry*, **74**, 70–76
- Stecher, B., Weller, U., Habermann, E., Gratzl, M. and Ahnert-Hilger, G., 1989. The light chain but not the heavy chain of botulinum A toxin inhibits exocytosis from permeabilised adrenal chromaffin cells. *FEBS Letters*, **255**, 391–394
- Steinthorsdottir, V., Halldórsson, H. and Andrésson, Ó.S., 2000. *Clostridium perfringens* beta-toxin forms multimeric transmembrane pores in human endothelial cells. *Microbial Pathogenesis*, **28**, 45–50
- Stigliani, S., Raiteri, L., Fassio, A. and Bonanno, G., 2003. The sensitivity of catecholamine release to botulinum toxin C1 and E suggests selective targeting of vesicles set into the readily releasable pool. *Journal of Neurochemistry*, **85**, 409–421

- Stiles, B.G., Blöcker, D., Hale, M.L., Guethhoff, M.A. and Barth, H., 2002. *Clostridium botulinum* C2 toxin, binding studies with fluorescence-activated cytometry. *Toxicon*, **40**, 1135–1140
- Storey, N.M., O'Bryan, J.P. and Armstrong, D.L., 2002. Rac and Rho mediate opposing hormonal regulation of the ether-a-go-go-related potassium channel. *Current Biology*, **12**, 27–33
- Su, L.F., Knoblauch, R. and Garabedian, M.J., 2001. Rho GTPases as modulators of the estrogen receptor transcriptional response. *Journal of Biological Chemistry*, **276**, 3231–3237
- Sugii, S. and Kozaki, S., 1990. Hemagglutination and binding properties of botulinum C2 toxin. *Biochimica et Biophysica Acta*, **1034**, 176–179
- Sullivan, R., Price, L.S. and Koffer, A., 1999. Rho controls cortical F-actin disassembly in addition to, but independently of, secretion in mast cells. *Journal of Biological Chemistry*, **274**, 38140–38146
- Suttorp, N., Polley, M., Seybold, J., Schnittler, H., Seeger, W., Grimminger, F. and Aktories, K., 1991. Adenosine diphosphate-ribosylation of G-actin by botulinum C2 toxin increases endothelial permeability *in vitro*. *Journal of Clinical Investigations*, **87**, 1575–1584
- Szule, J.A. and Coorsen, J.R., 2003. Revisiting the role of SNAREs in exocytosis and membrane fusion. *Biochimica et Biophysica Acta*, **1641**, 121–135
- Tamori, Y., Hashiramoto, M., Araki, S., Kamata, Y., Takahashi, M., Kozaki, S. and Kasuga, M., 1996. Cleavage of vesicle-associated membrane protein VAMP-2 and cellubrevin on GLUT4-containing vesicles inhibits the translocation of GLUT4 in 3T3-L1 adipocytes. *Biochemical and Biophysical Research Communications*, **220**, 740–745
- Tocher, J.F., Tocher, W., Brown, W. and Buxton, J.B., 1923. 'Grass sickness' investigation report. *Veterinary Record*, **3**, 37–45, 75–89
- Tomes, C.N., Michaut, M., de Blas, G., Visconti, P., Matti, U. and Mayorga, S., 2002. SNARE complex assembly is required for human sperm acrosome reaction. *Developmental Biology*, **243**, 326–338
- Van den Berghe, N., Barros, L.F., van Mackelenbergh, M.G.H. and Krans, H.M.J., 1996. *Clostridium botulinum* C3 exoenzyme stimulates GLUT4-mediated glucose transport, but not glycogen synthesis, in 3T3-L1 adipocytes – a potential role of rho? *Biochemical and Biophysical Research Communications*, **229**, 430–439
- Verderio, C., Coco, S., Rosetto, O., Montecucco, C. and Matteoli, M., 1999. Internalization and proteolytic action of botulinum toxins in CNS neurons and astrocytes. *Journal of Neurochemistry*, **73**, 372–379
- Verschueren, H., Taelen, I.V.D., Dewit, J., Braekeleer, J.D., Baetselier, P.D., Aktories, K. and Just, I., 1995. Effects of *Clostridium botulinum* C2 toxin and cytochalasin D on *in vitro* invasiveness, motility and F-actin content of a murine T-lymphoma cell line. *European Journal of Cell Biology*, **66**, 335–341
- Vinik, A.I., Freeman, R. and Erbas, T., 2003. Diabetic autonomic neuropathy. *Seminars in Neurology*, **23**, 365–372
- Wang, Q., Yu, L.G., Campbell, B.J., Milton, J.D. and Rhodes, J.M., 1998. Identification of intact peanut lectin in peripheral venous blood. *Lancet*, **352**, 1831–1832
- Watzl, B., Neudecker, C., Hänsch, G.M., Reckemmer, G. and Pool-Zobel, B.L., 2001. Dietary wheat agglutinin modulates ovalbumin-induced immune response in Brown Norway rats. *British Journal of Nutrition*, **85**, 483–490
- Welch, M.J., Purkiss, J.R. and Foster, K.A., 2000. Sensitivity of embryonic rat dorsal root ganglia neurons to *Clostridium botulinum* neurotoxins. *Toxicon*, **38**, 245–258
- Wenzel-Seifert, K., Lentzen, H., Aktories, K. and Seifert, R., 1997. Complex regulation of human neutrophil activation by actin filaments, dihydrocytochalasin B and botulinum C2 toxin uncover the existence of multiple cation entry pathways. *Journal of Leukocyte Biology*, **61**, 703–711
- Wex, C.B.A., Koch, G. and Aktories, K., 1997. Effects of *Clostridium botulinum* C2 toxin-induced depolymerisation of actin on degranulation of suspended and attached mast cells. *Naunyn-Schmiedeberg's Archive of Pharmacology*, **355**, 319–327
- Wheeler, M.B., Sheu, L., Ghai, M., Bouquillon, A., Grondin, G., Weller, U., Beaudoin, A.R., Bennett, M.K., Trimble, W.S. and Gaisano, H.Y., 1996. Characterization of SNARE protein expression in β cell lines and pancreatic islets. *Endocrinology*, **137**, 1340–1348
- Wiegand, H., Erdmann, G. and Wellhöner, H.H., 1976. ¹²⁵I-labelled botulinum toxin A neurotoxin pharmacokinetics in cats after neuromuscular injection. *Naunyn Schmiedeberg's Archive of Pharmacology*, **292**, 161–166
- Wieggers, W., Just, I., Müller, H., Hellwig, A., Traub, P. and Aktories, K., 1991. Alterations of the cytoskeleton of mammalian cells cultured *in vitro* by *Clostridium botulinum* C2 toxin and C3 ADP-ribosyltransferase. *European Journal of Cell Biology*, **54**, 237–245
- Wilde, C. and Aktories, K., 2001. The Rho-ADP-ribosylating C3 exoenzyme from *Clostridium botulinum* and related C3-like transferases. *Toxicon*, **39**, 1647–1660
- Williamson, L.C., Halpern, J.L., Montecucco, C. and Brown, J.E., 1996. Clostridial neurotoxins and substrate proteolysis in intact neurons. *Journal of Biological Chemistry*, **271**, 7695–7699

- Wirth, M., Gerhardt, K., Wurm, C. and Gabor, F., 2002. Lectin-mediated drug delivery, influence of mucin on cytoadhesion of plant lectins *in vitro*. *Journal of Controlled Release*, **79**, 183–191
- Wong, P.P.C., Daneman, N., Volchuk, A., Lassam, N., Wilson, M.C., Klip, A. and Trimble, W.S., 1997. Tissue distribution of SNAP-23 and its subcellular localization in 3T3-L1 cells. *Biochemical and Biophysical Research Communications*, **23/0**, 64–68
- Wonnacott, S. and Marchbanks, R.M., 1976. Inhibition by botulinum toxin of depolarization-evoked release of [¹⁴C]acetylcholine from synaptosomes *in vitro*. *Biochemical Journal*, **156**, 701–712
- Yamada, H., Otsuka, M., Hayashi, M., Nakatsuka, S., Hamaguchi, K., Yamamoto, A. and Moriyama, Y., 2001. Ca²⁺-dependent exocytosis of L-glutamate by α TC6, clonal mouse pancreatic α -cells. *Diabetes*, **50**, 1012–1020
- Yamada, H., Hayashi, M., Uehara, S., Kinoshita, M., Muroyama, A., Watanabe, M., Takei, K. and Motiyama, Y., 2002. Norepinephrine triggers Ca²⁺-dependent exocytosis of 5-hydroxytryptamine from rat pinealocytes in culture. *Journal of Neurochemistry*, **81**, 533–540
- Yamasaki, S., Baumeister, A., Binz, T., Blasi, J., Link, E., Cornille, F., Roques, B., Fykse, E.M., Südhof, T.C., Jahn, R. and Niemann, H., 1994. Cleavage of members of the synaptobrevin/VAMP family by types D and F botulinum neurotoxins and tetanus toxin. *Journal of Biological Chemistry*, **269**, 12764–12772
- Yoshioka, K., Nakamori, S. and Itoh, K., 1999. Overexpression of small GTP-binding protein RhoA promotes invasion of tumor cells. *Cancer Research*, **59**, 2004–2010
- Zhang, M., Kalinec, G.M., Urrutia, R., Billadeau, D.D. and Kalinec, F., 2003. ROCK-dependent and ROCK-independent control of cochlear outer hair cell electromotility. *Journal of Biological Chemistry*, **278**, 35644–35650
- Zilberter, Y., Kaiser, K.M.M. and Sakmann, B., 1999. Dendritic GABA release depresses excitatory transmission between L 2/3 pyramidal and bitufted neurons in rat neocortex. *Neuron*, **24**, 979–988
- Zilberter, Y., 2000. Dendritic release of glutamate suppresses synaptic inhibition of pyramidal neurons in rat neocortex. *Journal of Physiology*, **528**, 489–496
- Zoppi, G., Cinquetti, M., Luciano, A., Benini, A., Muner, A. and Bertazzoni-Minelli, E., 1998. The intestinal ecosystem in chronic functional constipation. *Acta Paediatrica*, **87**, 836–841

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