Actions of botulinum toxin on the human brain and human neuromuscular junction. N. Bakry, R. Zhang, J. Coffield, A. Maksymowych and L. L. Simpson (Jefferson Medical College, Philadelphia, Pennsylvania, U.S.A.).

The actions of several serotypes of botulinum neurotoxin were studied on the human nervous system. Experiments were done on a model neuromuscular preparation (pyramidalis muscle) and on several regions of brain (frontal cortex, cerebellar cortex, globus pallidus). The results with serotypes A and B, which are frequently implicated in human illness, and serotype C, which is rarely if ever associated with human illness, can be taken as representative. The human brain was shown to possess receptors for botulinum neurotoxin, and both K_d and B_{max} values varied with serotype. In addition, regional binding varied with serotype. The human nervous system possesses substrates for serotypes A (SNAP-25), B (synaptobrevin) and C (syntaxin). Cloning and sequencing of human syntaxin revealed substantial homology with rat syntaxin A ($\sim$98%) and syntaxin B ($\sim$89%). Standard electrophysiological techniques were used to examine the sensitivity of the pyramidalis muscle to toxin-induced blockade. At a toxin concentration of 10^{-8} M, spontaneous and evoked endplate activity was decreased ($\geq$90%) within 90 min by serotype A and 60 min by serotype B. In addition to experiments on tissues from patients without apparent neurologic disorders, studies were performed on tissues from patients with well-characterized disorders. The most striking finding was that Alzheimer's disease produced a marked reduction in toxin binding in the globus pallidus but not in other regions of the brain.

The mechanism of action of botulinolysin, a thiol-activated cytolysin of Clostridium botulinum. M. Matsuda, A. Haque, T. Senda, Y. Horiguchi and N. Sugimoto (Department of Bacterial Toxinology, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565, Japan).

Botulinolysin was highly purified, to a homogeneity form (2100 $HD_{50}/\mu g$ protein), from the culture supernatant of *Clostridium botulinum* type C strain C-203 by a combination of ammonium sulfate fractionation, ion exchange chromatographies on DEAE-Sepharose CL-6B and SP-Toyopearl 650 M columns, and Sephadex G-75 gel permeation chromatography. Cytotoxicity of the purified botulinolysin on erythrocytes and cultured cells, HeLa and Vero cells, was studied by hemoglobin release and trypan blue staining assays, respectively. Botulinolysin caused hemolysis in erythrocytes and induced characteristic morphological alterations (bleb and balloon formation) in HeLa and Vero cells. These cultured cells were stained with trypan blue after treatment with the toxin (CD_{50} was 120 ng/ml). Erythrocytes of rabbit, human, and guinea-pig were much more sensitive to the toxin than those of mouse, rat, and chicken. At 0°C, botulinolysin bound to the erythrocytes and cultured cells but did not show any cytotoxic effects, hemoglobin release and trypan blue staining. Dextran with mol. wts of larger than 19,500 inhibited both hemolysis and morphological alterations. Electron microscopical study with negative staining showed formation of ring structures of definite size (10 nm inner diameter) and large pore-like structures with various diameters on botulinolysin-bound erythrocytes. The lethal action of the toxin on rats (Sprague–Dawley) and mice (ddY) was studied electrophysiologically. Mouse i.v. LD_{50} of botulinolysin was 370 ng. Rats injected with 10,000 HD_{50} (5 μg) and 1000 HD_{50} (0.5 μg) of botulinolysin died in 2 and 15 min, respectively, while those injected with 100 HD_{50} (50 ng) survived. Rats injected i.v. with botulinolysin showed a rapid fall in arterial blood pressure. Toxicity *in vitro* on isolated heart, atrium, kidney, lung and liver was studied. An increase in resistance of systemic microcirculation was observed in rat isolated heart, lung, liver, and kidney. Isolated rat heart, but not atrium and isolated aortic ring, was extremely sensitive to botulinolysin. The above results show that botulinolysin is a large-pore forming toxin and coronary vasospasm is a cause of acute cardiac dysfunction leading to lethal hypotension in animals intoxicated with botulinolysin.

Efficacy of a zinc chelator, N,N,N′,N′-*tetrakis* (2-*pyridyl-methyl*) *ethylene-diamine* (*TPEN*), *against botulinum type A or B neurotoxin* (*BoNT/A or BoNT/B*) *in mice.* R. W. Wannemacher,[1] R. E. Dinterman,[1] S. S. Deshpande[2] and M. Adler[2] ([1]Toxinology Division, USAMRIID, Fort Detrick, Frederick, MD 21702-50, U.S.A.; and [2]Neurotoxicology Branch, Pathophysiology Division, USAMRICD, APG, MD 21010, U.S.A.).

Botulinum neurotoxins have protease activity, which can be blocked by zinc chelators or known inhibitors of zinc endopeptidases. An *in vitro* isolated neuromuscular preparation was used to demonstrate that TPEN will block the effects of BoNT/A or BoNT/B on neuromuscular transmission. The major objective of our research was to evaluate the *in vivo* efficacy of TPEN against BoNT. We determined that the median lethal dose (MLD) was 34.4 mg/kg and the median illness dose of TPEN was 18.6 mg/kg in mice injected i.p. Mice were challenged i.v. with BoNT/A or BoNT/B and lethality and mean time to death (MTD) were observed. When 10 mg/kg of TPEN was injected i.p. 1 hr before an i.v. challenge with either 20 or 2000 MLD of BoNT/A or BoNT/B, all the mice died; but the MTD was increased compared to controls injected with saline and challenged with the neurotoxins. If the mice were injected i.p. with 10 mg/kg of TPEN 30 min before and 15 min after exposure to either neurotoxin, all died. The MTD was increased from 1.03 ± 0.03 and 0.97 ± 0.03 to 2.21 ± 0.17 and 2.31 ± 0.11 hr, respectively, for mice that were challenged with 2000 MLD of BoNT/A or BoNT/B. With 20 MLDs, MTD increased from 8.2 ± 0.6 and 7.9 ± 0.1 to 10.6 ± 0.3 and 10.5 ± 0.3 hr, respectively. Thus, we