

DETAILED DESCRIPTION

[0021] The present invention provides antibodies which bind to epitopes of BoNT/A and BoNT/A Hc. These antibodies can be used to purify BoNT/A from an impure solution containing BoNT/A, and to detect BoNT/A in by a sample and as a treatment for BoNT/Z intoxication. In addition, the antibodies can be used in kits for using in the methods described.

[0022] The term “antibody” is art-recognized terminology and is intended to include molecules or active fragments of molecules that bind to known antigens. Examples of active fragments of molecules that bind to known antigens include Fab and F(ab')₂ fragments. These active fragments can be derived from an antibody of the present invention by a number of techniques. For example, purified monoclonal antibodies can be cleaved with an enzyme, such as pepsin, and subjected to HPLC gel filtration. The appropriate fraction containing Fab fragments can then be collected and concentrated by membrane filtration and the like. For further description of general techniques for the isolation of active fragments of antibodies, see for example, Khaw, B. A. et al. *J. Nucl. Med.* 23:1011-1019 (1982). The term “antibody” also includes bispecific and chimeric antibodies.

[0023] The language “monoclonal antibody” is art-recognized terminology. The monoclonal antibodies of the present invention can be prepared using classical cloning and cell fusion techniques. The immunogen (antigen) of interest, e.g. different portions of BoNT/A or BoNT/A Hc, is typically administered (e.g. intraperitoneal injection) to wild type mice or transgenic mice which produce desired antibodies, such as human antibodies, rats, rabbits or other animal species which can produce native or human antibodies. The immunogen can be administered alone or as a fusion protein to induce an immune response. Fusion proteins comprise the peptide against which an immune response is desired coupled to carrier proteins, such as β -galactosidase, glutathione S-transferase, keyhole limpet hemocyanin (KLH), and bovine serum albumin, to name a few. In these cases, the peptides serve as haptens with the carrier proteins. After the animal is boosted, for example, three or four times, the spleen is removed and splenocytes are extracted and fused with myeloma cells using the well-known processes of Kohler and Milstein (*Nature* 256: 495-497 (1975)) and Harlow and Lane (*Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York 1988)). The resulting hybrid cells are then cloned in the conventional manner, e.g. using limiting dilution, and the resulting clones, which produce the desired monoclonal antibodies, cultured.

[0024] Examples of monoclonal antibodies raised against BoNT/A or BoNT/A Hc using this method include MAb 4A2-2, MAb 6B2-2 and MAb 6C2-4. The monoclonal antibodies MAb 4A2-2 is produced by the hybridoma deposited under American Type Culture Collection (ATCC) Accession No. PTA-971 and recognizes BoNT/A and BoNT/A Hc. MAb 6B2-2 is produced by the hybridoma deposited under American Type Culture Collection (ATCC) Accession No. PTA-969 and recognizes BoNT/A and BoNT/A Hc, and MAb 6C2-4 is produced by the hybridoma deposited under American Type Culture Collection (ATCC) Accession No. PTA-970 recognizes BoNT/A and BoNT/A Hc. Because these monoclonals recognize the carboxy terminal of the BoNT/A Hc, a region of the Hc found to form

an a principal protective antigenic determinant of BoNT/A, these monoclonals may be useful for passive immunization or for reducing the symptoms of botulinum intoxication. The monoclonals and their derivatives can be administered to a subject in an amount effective to produce protection or reduce symptoms. The amount administered will depend upon the age, weight and condition of the subject as described below.

[0025] The language “polyclonal antibody” is art-recognized terminology. The immunogen used to produce the polyclonals of the present invention was the 25 kDa heavy chain of BoNT/A. The polyclonal recognizes BoNT/A. These antibodies are, therefore, useful for studying the topology of BoNT/A. In addition, these antibodies can be used to determine the orientation of BoNT/A reconstituted into artificial liposomes or virosomes. The separation of correctly-oriented from incorrectly-oriented liposomes or virosomes can be achieved using affinity chromatography. Anholt et al. *J. Biol. Chem.* 256:4377 (1981). Because the epitopes recognized by the polyclonal antibody are solvent exposed, it is likely a useful antibody for immunoprecipitation experiments.

[0026] A common method for preparing polyclonal antibodies to an immunogen of interest, such as BoNT/A or a fragment thereof, includes injecting (e.g. intradermally, intramuscularly) an animal, such as a rabbit, with an the immunogen emulsified in Freund’s complete adjuvant. This process is repeated after two weeks. Two weeks later, monthly subcutaneous booster injections can begin with the immunogen in Freund’s incomplete adjuvant. The animals are bled biweekly by a marginal ear vein technique beginning six weeks after the primary immunization. The collected blood is refrigerated, allowing clots to form, and the supernatant (antiserum) retained.

[0027] The term “epitope” is art-recognized. It is generally understood by those of skill in the art to refer to the region of an antigen, such as BoNT/A, that interacts with an antibody. An epitope of a peptide or protein antigen can be formed by contiguous or noncontiguous amino acid sequences of the antigen. BoNT/A, like many large proteins, contains many epitopes. Examples of BoNT/A epitopes recognized by antibodies of the present invention include the amino acid sequences 1150-1289 of BoNT/A Hc (SEQ ID NO:1), amino acids 1157-1181 (SEQ ID NO:2), and amino acids 1230-1253 (SEQ ID NO:3). These peptides offer a convenient method for eluting BoNT/A bound to either MAb 4A2-2, 4A2-4, 6E9-3, 6E9-4, 6E10-4, 6E10-5, 6E10-8, 6E10-10, 6B2-2, and 6C2-4 on immunoaffinity columns. For example, when an antibody which recognizes the epitope for either MAb 4A2-2, MAb 6C2-4, or MAb 6B2-2, is used in an immunoaffinity column to purify BoNT/A, the peptide recognized by the antibody can be added to the immunoaffinity column to elute the BoNT/A. See below for a more detailed description of the purification of BoNT/A.

[0028] Epitope mapping studies described in this application defined three groups of MABs, corresponding to two-distinct and one overlapping protective-epitope regions on BoNT/A Hc. One particular region of the antigen was defined by MAb 6B2-2, while 4A2-2, 4A2-4, 6E10-5, 6E10-8, 6E10-10, 6E9-3, 6E9-4, 6E9-12, and 6E10-4 MABs bound a distinct site. The MAb 6C2-4 defined a site that overlaps with 6E10-5, 6E10-8, 6E10-10, 6E9-3, 6E9-4,