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Generation of a monoclonal anti-human β2-adrenergic receptor antibody using GST-β-adrenergic receptor C-terminal fusion proteins expressed in E.Coli.

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Among the various receptor molecules discovered so far, the β 2-adrenergic receptors have been regarded as excellent model systems for the so called 7 transmembrane helix receptor and have been the focus of extensive studies. For the analysis of receptor structure and function, a monoclonal antibody plays a crucial role, thus providing useful tools for the study of receptor. However, because of the minute quantity of receptor molecules which could be obtained from natural sources, the generation of specific monoclonal antibody against receptor molecules from the purified receptors has been regarded as virtually impractical in consideration of cost and experimental times. The purpose of the present study was to generate and characterize a monoclonal antibody against human β2-adrenergic receptor. For the production of antibody, C-terminal regions of the human β 2-adrenergic receptor was produced as a fusion protein with Glutathion S-transferase (GST) in E. Coli. The expression of the fusion protein was identified by SDS-PAGE and Western blot using monoclonal anti-GST antibody. The fusion protein was purified to an apparent homogeniety by affinity chromatography with Glutathion Sepharose CL-4B and used as an antigen for the immunization of BALB/C mice. The Production of monoclonal antibody was achieved by fusion of the immunized spleen cells and SP/2-0 myeloma cells. Positive hybridomas were screened by ELISA and were cloned by two consecutive rounds of limiting dilution. The monoclonal antibody produced in this study (mAbβC02) was IgM type and purified by immunoaffinity chromatography using anti-mouse IgM agarose as an affinity matrix. MAbβC02 showed strong and specific immunoreactivity against both the fusion protein and human β2-adrenergic receptor in ELISA and Western blot. The molecular weight of immunoreactive band was 64 kDa and exactly coincided with the previously reported molecular weight of β 2-adrenergic receptors. The results of the present study suggest that mAbβ C02 may be used for the study of receptor function and regulation in normal or nonphysiological status.