A spider toxin (JSTX)-binding protein in rat hippocampus

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ABSTRACT

In order to isolate the glutamate receptor of mammalian brain, a spider toxin (JSTX-3) which blocks glutaminergic transmission in various nervous systems was employed. A protein which bound specifically to JSTX-3 was solubilized with Triton X-100 and isolated from rat hippocampal tissue. Isolation of JSTX-3 binding protein was performed using an avidin D agarose column with iminobiotinyl JSTX-3. On sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, the purified protein was found in a single band and estimated to be a 60-70 K dalton molecule.

Glutamate-mediated neurotransmission has been the focus of many current studies in neuroscience, since it appears to play a key role in the brain function, particularly in learning, memory and epileptic diseases. However, detailed neurochemical studies of glutamate receptors have been hampered by a lack of specific ligands. A spider toxin (JSTX-3), derived from the Joro spider (*Nephila clavata*) has been found to block glutamate receptors in invertebrate (1) and vertebrate (7) nervous system with a high degree of specificity. Since the blocking action of JSTX-3 on the glutamate receptors is practically irreversible, labeling of the toxin is a useful approach in the study of the glutamate receptors. We recently reported an autoradiographic study of JSTX binding to lobster muscle using ¹²⁵I-labeled JSTX (8). In the present experiments, we prepared iminobiotinyl JSTX-3 for the purpose of studying the toxin-binding protein in mammalian brain tissue. Using avidin-iminobiotin interaction, we were able to isolate the protein which bound to iminobiotinyl JSTX-3 in a synaptic membrane fraction derived from the rat hippocampus.

The strategy for isolation of JSTX-binding

protein is illustrated in Fig. 1. The solubilized synaptic membrane fraction is incubated with iminobiotinyl JSTX-3. At pH 8.5 JSTX-3 bound protein is trapped on an avidin-D agarose column by means of the molecular interaction between iminobiotin and avidin (6). The trapped protein is eluted with iminobiotinyl JSTX-3 at pH 4.0 since iminobiotin interacts poorly with avidin at acidic pH. For affinity purification of the toxin-binding protein, synthetic JSTX-3 was coupled with an equimolar amount of *N*-hydroxysuccinimide iminobiotin HBr (Pierce Chemical) by the

Fig. 2 Separation of iminobiotinyl JSTX-3 binding proteins in rat hippocampus on an avidin D agarose column. The column was equilibrated with buffer A (0.1 M NaCl, 0.1% Triton X-100 in 0.1 M borate buffer, pH 8.5). After the column was washed with 60 ml of buffer A, the eluent was switched to buffer B (1.0 M NaCl in buffer A). This was followed by buffer C (1.0 M NaCl and 0.1% Triton X-100 in 0.1 M acetate buffer, pH 4.0).

Fig. 3 SDS-polyacrylamide gel electrophoresis of fraction C eluted by buffer C in Fig. 2. Samples were applied to 8–25% linear gradient gels using the buffer system of Laemmli (5). Lane 1: fraction C without iminobiotinyl JSTX-3 treatment. Lane 2: fraction C incubated with iminobiotinyl JSTX-3. Lane 3: Triton X-100 soluble P2 fraction before affinity purification. Lane 4: The molecular size markers were phosphorylase *b* (94 K), albumin (67 K), ovalbumin (43 K), carbonic anhydrase (30 K), trypsin inhibitor (20.1 K), lactalbumin (14.4 K).

usual condensation method (3). The biological activity of iminobiotinyl JSTX-3 was tested on the lobster neuromuscular synapse. Although the potency for blocking glutamate receptors was reduced to approximately 1/10 of that of native JSTX-3, iminobiotinyl JSTX-3 preserved an irreversible blocking action.

A crude synaptic membrane fraction (P2) of

rat hippocampal tissues was prepared according to the method described by Kessler *et al.* (4). In the rat hippocampal P2 fraction, 60% of membrane protein was solubilized by 0.5% Triton X-100 in borate buffer (pH 8.5). Iminobiotinyl JSTX-3 was added to Triton X-100 solubilized P2 fraction (final concentration; 0.5 mM iminobiotinyl JSTX-3) and incubated at 25°C for 30 min. After free iminobiotinyl JSTX-3 was removed by gel filtration through Sephadex G-50, the protein fraction (20 mg/10 ml) was applied to agarose avidin D (Vectastain) column (3 × 7.5 cm). The chromatographic elution profile with two steps buffer changes is shown in Fig. 2. The fraction C eluted by buffer C was concentrated with YM10 membrane (Amicon) to a final volume of 50 μ l before SDS-gel electrophoretic analysis (Fig. 3).

The presence of a single polypeptide which bound to JSTX-3 was demonstrated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. The protein bands were visualized by silver stain method (2). While fraction C without iminobiotinyl JSTX-3 showed no visible trace (Fig. 3, lane 1), a discrete band appeared in fraction C incubated with iminobiotinyl JSTX-3 (Fig. 3, lane 2). Therefore the 60–70 K polypeptide was derived from that binds specifically to JSTX-3. Since JSTX preferentially blocks non-NMDA receptors (1, 7), the protein obtained may correspond to the quisqualate/kainate type receptor-ion channel complex or a part of it. Sequencing of the protein is in progress for

the purpose of determining the complete amino acid sequence.

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