Vol. 85, No. 1, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 14, 1978 Pages 377-382

SCORPION TOXIN : SPECIFIC BINDING TO RAT SYNAPTOSOMES

Emmanuel Jover*, Nicole Martin-Moutot*, François Couraud** and Hervé Rochat*
*Laboratoire de Biochimie et U 172 de l'INSERM, Faculté de Médecine Secteur Nord, Boulevard Pierre Dramard, F 13326 Marseille Cédex 3
**Laboratoire de Biochimie Médicale et U 38 de l'INSERM, Faculté de Médecine, Boulevard Jean Moulin, F 13385 Marseille Cédex 4

Received September 18,1978

SUMMARY : The protein neurotoxin II from the venom of the scorpion Androctonus australis Hector was labeled with ^{125}I by the lactoperoxidase method to a specific radioactivity of about 100 μCi/μg without loss of biological activity. The labeled neurotoxin binds specifically to a single class of non interacting binding sites of high affinity (K_D = 0.20 - 0.35 nM) and low capacity (30 - 60 fmoles/mg protein) to rat synaptosomes. This binding is reversible : the values of the rate association and dissociation constants k_1 and k_{-1} are respectively 1.5×10^7 M^{-1} s^{-1} and 1.6×10^{-3} s^{-1} and are in good agreement with the equilibrium constant. Moreover the bound labeled scorpion toxin II was displaced by the toxin II from the sea anemone Anemonia Sulcata.

Synaptosomes prepared from rat brain are still able to retain resting membrane potential and also the ability to increase sodium permeability (1). On this preparation, veratridine and the venom of the scorpion Leirus quinquestriatus were able to increase calcium uptake and noradrenaline release (2). In the same way, toxin I from the scorpion Androctonus australis Hector stimulated the release of γ-amino butyric acid (3). These effects were all blocked by tetrodotoxin (2,3). From these results, we considered the possibility to use rat brain synaptosomes for the study of the mode of action and the binding of scorpion toxins, which affect selectively the closing of the action potential Na^+ ionophore in axonal preparations (4) and in neuroblastoma cells (5). Recent studies have demonstrated a potential dependent binding of a toxin from the venom of L. quinquestriatus to electrically excitable neuroblastoma cells (6,7) and, independently, the binding of the toxin II of A. australis to the same cells (8).

0006-291X/78/0851-0377$01.00/0

In this communication, we report the specific binding of toxin II from A. australis to rat brain synaptosomes and the comparison between results obtained by equilibrium and kinetic studies.

MATERIALS AND METHODS

Scorpion toxin II (ScTX II) from A. australis was purified according to the procedure set up by Miranda et al. (9). ScTX II was iodinated using the lactoperoxidase method of ^{125}I-iodide oxidation and purified by immunoprecipitation with a monospecific antiserum prepared against the native toxin (10). Specific radioactivities of about 100 µCi/µg were routinely obtained from reaction of 5 µg of scorpion toxin II in the presence of 0.5 mCi of carrier-free Na^{125}I. Toxin II from sea anemone Anemonia sulcata was kindly given to us by M. Lazdunski (Nice) and G. Wunderer (Munich). Synaptosomes were prepared from rat striatum, by a simplification of the method of Gray and Whittaker (11). Striatum from 150-200 g rats were homogenized in a teflon pestle. Homogenate was centrifuged at 850 g for 10 min ; supernatant was centrifuged at 11,000 g for 30 min and the pellet resuspended in the binding medium made of choline choride 140 mM ; KCl 5.4 mM ; $CaCl_2$ 1.8 mM ; Mg SO_4 1 mM ; bovine serum albumin 0.25 % ; HEPES 25 mM ; TRIS base to obtain pH 7.2 in the volume necessary to reach a final protein concentration of 0.5 to 0.7 mg per ml. Aliquots of 225 µl of the synaptosome suspension were distributed in tubes containing, where indicated, unlabeled ScTX II or sea anemone toxin II. After addition of 25 µl ^{125}I-ScTX II, synaptosomes were incubated at 37°C for the indicated time. The reaction was stopped by addition of 750 µl of the binding medium at 4°C and centrifugation at 11,000 g for 1 minute. The pellet was washed two times with the same medium and counted in a γ scintillation spectrometer. Values are means of assays in duplicate and curves were traced using linear regression calculation.

RESULTS AND DISCUSSION

Binding of ^{125}I-ScTX II to rat synaptosomes is a saturable phenomenon (Fig. 1). Scatchard plot (Fig. 2) revealed a single class of non interacting binding sites, the dissociation constant K_D^* being 0.2 nM. The dissociation constant, K_D, for unlabeled ScTX II, calculated by displacement of ^{125}I-ScTX II by increased concentrations of unlabeled ScTX II, is very similar : 0.3 nM (Fig. 3). Calculated from four independent experiments, the values of the K_D and of the capacities varied respectively between 0.20 and 0.35 nM and 30 and 60 fmoles per mg of protein.

The experiments were done in a Na^+-free medium so that the binding of ScTX II did not modify the membrane potential of synaptosomes. Na^+ was not found necessary for the binding of scorpion toxin.

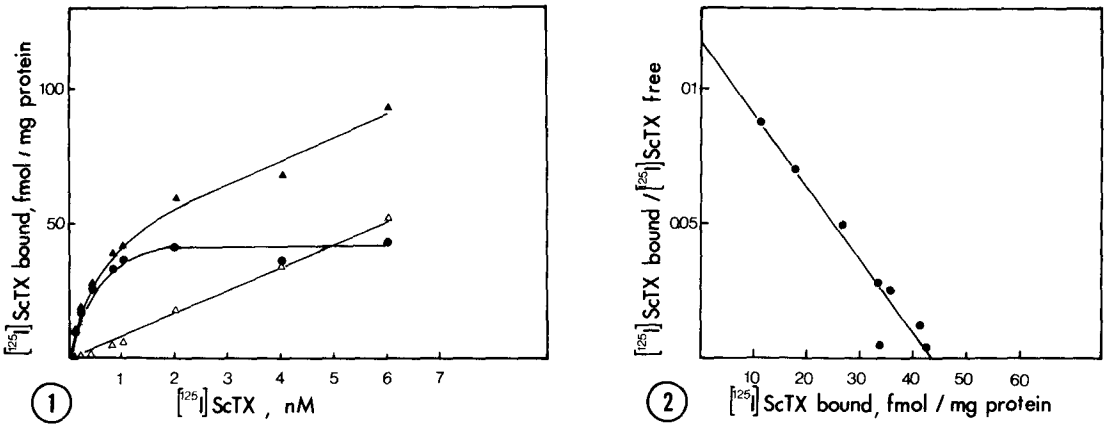

Fig.1 - Binding of ^{125}I-ScTX II to rat synaptosomes. The binding was measured after 30 min at 37°C incubation of synaptosomes (0.6 mg protein per ml) with increasing concentrations of labeled toxin, in a final volume of 250 µl, in the absence (▲) or the presence (Δ) of 200 nM unlabeled ScTX II. Specific binding (●) is the difference between the two curves.

Fig. 2 - Scatchard plot of specific binding. Data of Fig. 1.

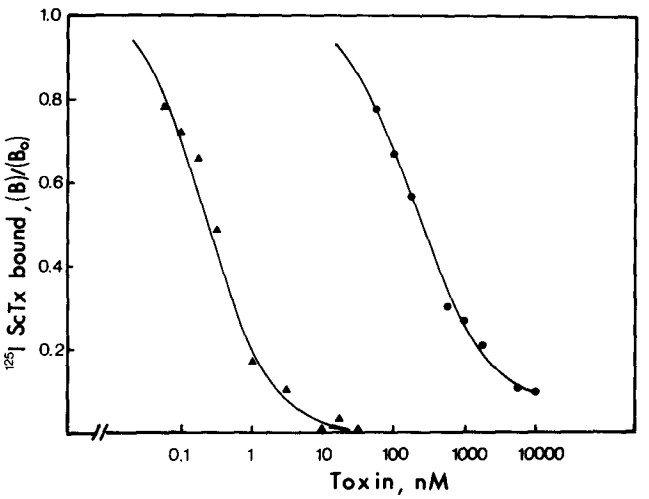

Fig. 3 - Displacement of ^{125}I-ScTX II binding by unlabeled ScTX II (▲) and sea anemone toxin II (●). Synaptosomes (0.5 mg protein per ml) were incubated with 0.2 nM ^{125}I-ScTX II at 37°C for 30 min in a final volume of 250 µl, in the presence of increasing concentrations of unlabeled ScTX II (▲) or sea anemone toxin II (●). [B_o] is the binding of ^{125}I-ScTX II in the absence of unlabeled toxin, and [B] the binding of ^{125}I- ScTX in the presence of the indicated unlabeled toxin. The nonspecific binding has been substracted.

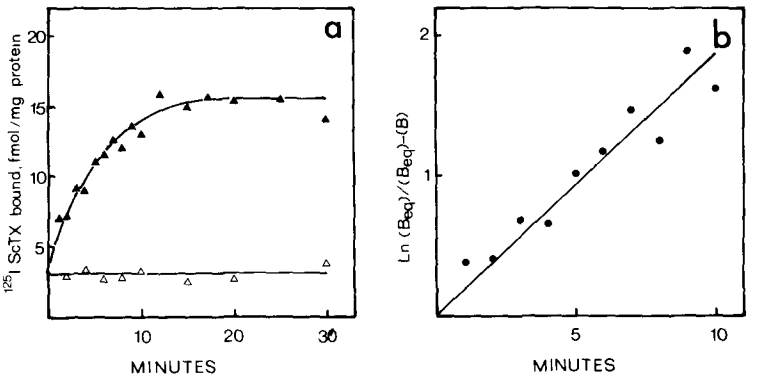

Fig. 4 - Time course of binding of ^{125}I-ScTX to synaptosomes.
a : 0.1 nM ^{125}I-ScTX II was incubated at 37° with synaptosomes (0.4 mg protein per ml) in the absence (▲) or the presence (△) of 200 nM unlabeled ScTX II. Binding was measured at the times indicated. Before adding ^{125}I-ScTX II, synaptosomes were preincubated at 37°C for 30 min.
b : the kinetic of ^{125}I-ScTX II binding was linearized according to the equation of a pseudo-first-order reaction as explained in the text.

At 37°C and with 0.1 nM ^{125}I-ScTX II, the specific binding increased with time and reached a plateau at about 12 min (Fig. 4a), the non specific binding remaining constant throughout. The interaction of ^{125}I-ScTX II with synaptosomes can be analyzed as a pseudo first order reaction by the equation :

$$\ln \frac{[B_{eq}]}{[B_{eq}] - [B]} = ([L]k_1 + k_{-1})\ t$$

in which $[B_{eq}]$ is the concentration of bound ligand at equilibrium, $[B]$ is the concentration of the bound ligand at a given time t, $[L]$ is the concentration of ligand, k_1 is the rate constant of association and k_{-1} the rate constant of dissociation. When $\ln ([B_{eq}] / [B_{eq}] - [B])$ was plotted as a function of time (Fig. 4b) a straight line was obtained with a slope having a value of :

$$1 \times 10^{-10}\ k_1 + k_{-1} = 3.1 \times 10^{-3}\ s^{-1}$$

The value of k_{-1} was determined by measuring the dissociation of ^{125}I-ScTX II (Fig. 5a). Dissociation was linear when plotted according to the equation $\ln ([B]/[B_o]) = -k_{-1}\ t$ in which $[B_o]$ is the concentration

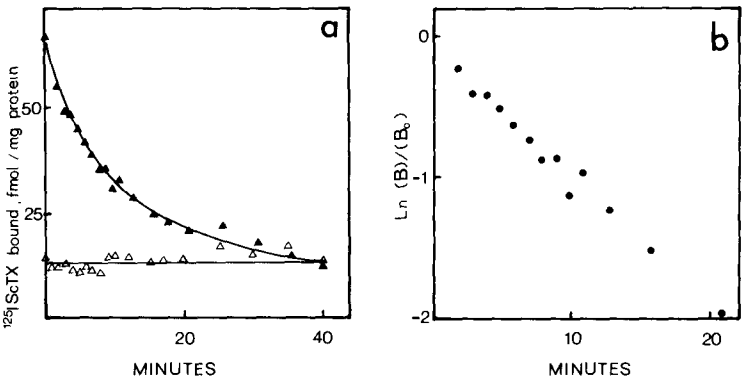

Fig. 5 - time course of dissociation of ^{125}I-ScTX II.
a : 0.8 nM ^{125}I-ScTX II was first incubated at 37° with synaptosomes (0.5 mg protein per ml). After 30 min, 1 µM unlabeled ScTX II was added in a negligible volume (2% of incubation total volume). Binding (▲) was measured at the times indicated, as described in Materials and methods. The non specific binding (Δ) was determined in a simultaneous experiment where unlabeled toxin at 1 µM was added to the incubation medium at the beginning of the first incubation period.
b: Dissociation was linearized according to the equation of a first-order kinetic reaction.

of bound toxin at time zero (Fig. 5b). The slope calculated from the dissociation curve gave $k_{-1} = 1.6 \times 10^{-3}\ s^{-1}$. From the association kinetic data, the rate constant of association was then calculated : $k_1 = 1.5 \times 10^{7}\ M^{-1}\ s$ Three other experiments at different concentrations of ligand, gave values of k_1 between 1 and $2 \times 10^{7}\ M^{-1}\ s^{-1}$. From the values of the rate constants, the dissociation constant was calculated : $K_D^* = k_{-1}/k_1 = 0.1$ nM. This value is in good agreement with the dissociation constant obtained by equilibrium experiments.

As the scorpion neurotoxins, the polypeptidic neurotoxins from the sea anemone A. sulcata affect specifically the closing of the Na^+ channel in myelinated and non myelinated axons (12,13). Fig. 3 shows that toxin II from sea anemone displaced the binding of ^{125}I-ScTX II to rat synaptosomes, the 50 % inhibition ($K_{0.5}$) being obtained for a concentration of 200 nM. This result confirms that, in rat synaptosomes as in neuroblastoma cells (8),

in a Na^+ free medium, scorpion toxin and sea anemone toxin bind to the same site, but with a great difference of affinity.

Preliminary experiments made with a better purified preparation of synaptosomes gave similar results. Thus synaptosomes are a good biological preparation to study the properties of the scorpion toxin binding and mainly its modification by membrane potential, ions and other toxins.

ACKNOWLEDGEMENTS : This work was supported in part by the DGRST (contract 77 7 0494), the DRET (contract 77 247), the CNRS (ERA 07 06 17) and the FRMF. The authors are deeply indebted to Professor S. Lissitzky and Professor F. Miranda for their constant interest and support during the course of the work.

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