LOCALIZATION OF THE CHOLINERGIC RECEPTOR PROTEIN BY IMMUNOFLUOROSCENCE IN EEL ELECTROPLAX

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1. Introduction

The electroplax, the elementary unit of the electric organ of *Electrophorus electricus*, is a flat syncytium particularly well designed for cytochemical studies on excitable membranes [1]. The cell is asymmetrical and shows two distinct faces. The caudal, or innervated, face receives nerve terminals and responds to both chemical and electrical stimuli. The rostral, or noninnervated, face does not receive nerve endings, is not excitable, but is specialized in the active transport of sodium and potassium. As expected from their different functions, the cytoplasmic membranes which limit these two faces differ by their enzyme content. As shown both *in situ* using a variety of cytochemical [2–5] and immunochemical techniques [6] and *in vitro* after separation by hand dissection of the two faces of the electroplax, the enzyme acetylcholinesterase (AcChE) is located almost exclusively on the innervated face. The noninnervated membrane, on the other hand, is particularly rich in another enzyme: the ouabain sensitive, Na^+–K^+ ATPase [7].

Purified α-toxins from a variety of snakes constitute highly specific, and often, irreversible, reagents of the cholinergic receptor protein both *in situ* and in solution [8]. Lee and Tseng [9] and Sato, Abe and Tamya [10] took advantage of this exceptional property to localize the cholinergic receptors at the end plates of the mouse diaphragm using ^{131}I-labelled toxins from *Bungarus multicinctus* or *Laticauda semifasciata*. In an attempt to localize the cholinergic receptor protein in the eel electroplax, we have used a pure α-toxin prepared from the venom of *Naja nigricollis* [11–13]. In addition we revealed the bound toxin by an indirect immunofluorescence technique. It is shown that the toxin binds exclusively to the caudal surface of the electroplax and, thereby, that the cholinergic receptor sites are located exclusively on the innervated membrane.

2. Material and methods

Electric organ. Fragments of the Sachs' organ of about 5 mm^3 were cut with scissors from slices of alive *Electrophorus electricus*. The fragments were sectioned along a plane parallel to the surface of the organ in a cryostat at −20°. The frozen sections, 16 μm thick, were collected on glass slides and stored in the dry at −15° until use. Before proceeding to the immunochemical reaction, the activity of AcChE was always tested by a modified Koelle's method [4].

α-toxin. The pure toxin was prepared by Boquet and associates [12–13] from crude venom of *Naja nigricollis* by the method of Karlsson, Eaker and Porath [14] except that Biorex 70 was used instead of Amberlite IRC 50. Solutions of toxin were always made in 10^{-1} M Na-phosphate buffer pH 7.1.

Serum. The anti α-toxin serum was prepared by injecting the pure toxin into rabbits following the technique of Boquet, Dumarey and Izard (to be published).

Purified sheep immunoglobulins directed against rabbit immunoglobulins were conjugated with fluorescein in the conditions described by Pillot and Gisler [15]. The number of fluorescein molecules bound was 2.7/molecule of immunoglobulin.

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Incubation. The electroplax sections on glass slides were incubated at room temperature in the following solutions, all made in 10^{-1} M Na phosphate buffer pH 7.1: (1) α-toxin. The concentration of toxin was varied from 0.1 to 50 μg/ml. (2) 0.2 or 2% neutral formaldehyde. (3) Rabbit anti α-toxin serum diluted 100- or 200-fold. (4) Sheep anti-rabbit IgG antibodies conjugated with fluorescein diluted 100- or 200-fold. The final concentrations were 72 and 36 μg protein/ml.

Exposure to each solution lasted 45 min. Between each step the slides were carefully washed (for 20 min) with 10^{-1} M Na phosphate buffer. The best results were obtained with 5 μg/ml α-toxin, 0.2% formaldehyde, rabbit and sheep serum diluted 100-fold.

3. Results and discussion

Fig. 1A shows the distribution pattern of fluorescent material after successive exposure of sections of electric organ to α-toxin, rabbit serum anti-toxin and fluorescent sheep anti-rabbit IgG in the conditions described in sect. 2. Only one of the two faces of the electroplax appears brightly, but not uniformly (fig. 1C and 1D), fluorescent along the external infoldings

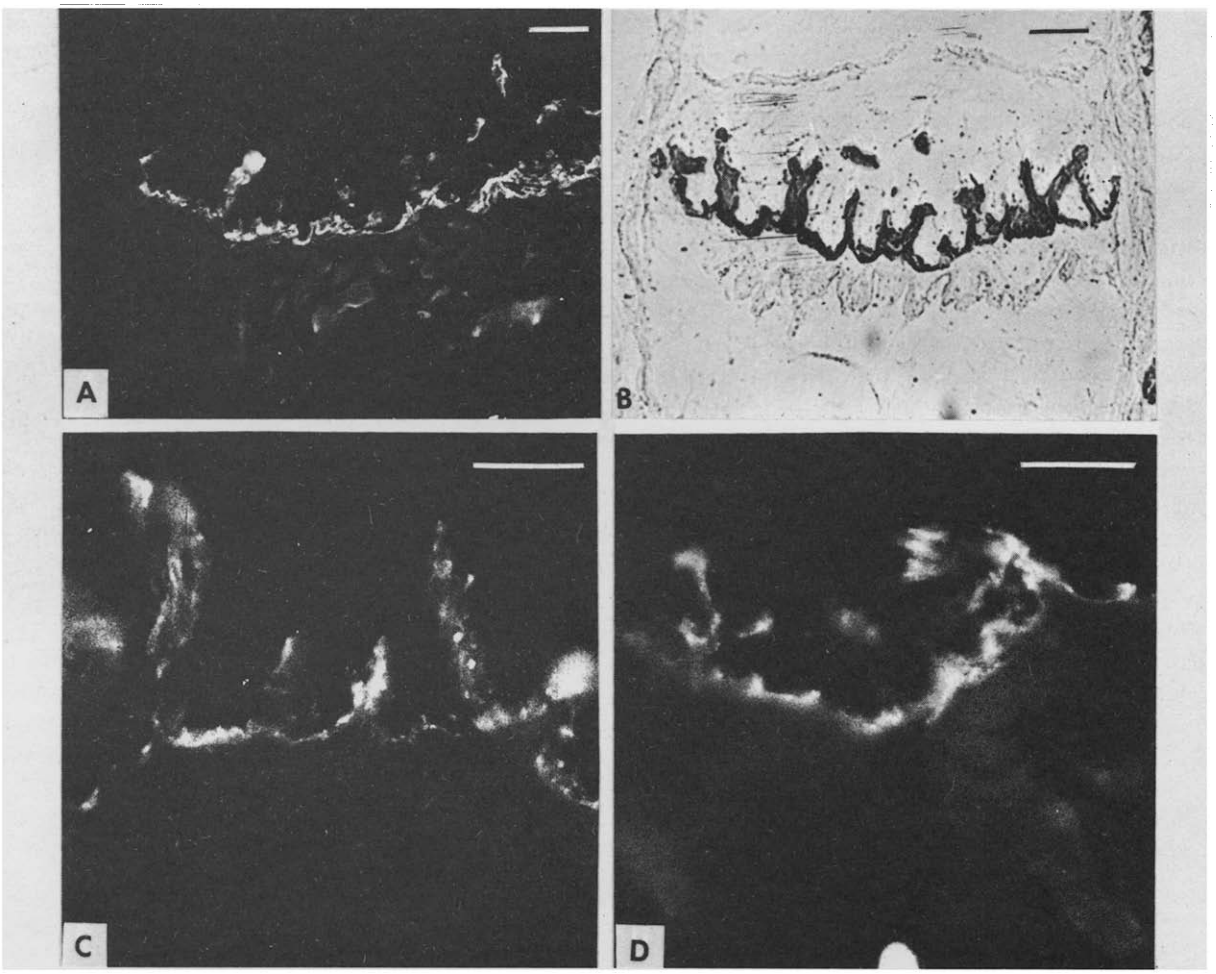

Fig. 1. (A), (C) and (D): Immunochemical localization of *Naja nigricollis* α-toxin bound to a section of electroplax. 5 μg/ml α-toxin, 0.2% formaldehyde, 100-fold dilution of rabbit and sheep serum. The length of the bar represents 100 μm. (B): Localization of acetylcholinesterase on a section of electroplax by Koelle's reaction. Orientation of section in fig. (A) and (B) are identical.

of the membrane. The fluorescence exhibited by the other side is in general very faint. It disappears completely when the concentration of α-toxin is reduced to 1 μg/ml. If exposure to the α-toxin (step 1) is omitted or if the anti α-toxin serum is exchanged with serum of nonimmune rabbits, then, the fluorescence almost completely disappears. The fluorescence thus reveals toxin molecules bound to some electroplax structure.

In order to identify which one of the two faces of the cell is labelled by the toxin, a Koelle reaction for AcChE was carried out on the same sections (fig. 1B). The faces which became fluorescent after the immunochemical reaction always stained for AcChE and were thus innervated faces. Since it has been previously shown that under our experimental conditions the α-toxin of *Naja nigricollis* binds, exclusively, to the cholinergic receptor protein, it is concluded that the receptor protein is only present on the innervated membrane of the elextroplax.

The resolution of our method is not good enough to give the precise distribution of the receptor protein within the innervated membrane. It would be of inportance to know if the receptor protein is present in the innervated membrane both under the synapses and between the synapses. Immunochemical techniques adapted to electron microscopy are being developed to answer this question.

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