DISTRIBUTION OF MACROMOLECULES FROM THE INTERCELLULAR MATRIX IN THE ELECTROPLAQUE OF *ELECTROPHORUS ELECTRICUS*

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1. Introduction

The elementary unit of intercellular communication in the nervous system, the synapse, is characterized by a well-defined and fixed spatial distribution of a few macromolecular species. In a peripheral cholinergic synapse like the motor endplate [1,2] or the electroplaque synapse [3,4], the enzyme of acetylcholine biosynthesis, choline acetyltransferase, is located exclusively in the nerve ending; the acetylcholine receptor is concentrated with a very high density in the cytoplasmic membrane which faces the nerve terminal [2–5]; and acetylcholinesterase is bound to the basal lamina which covers the postsynaptic membrane [6]. Denervation and muscle regeneration experiments carried out with adult skeletal muscle have led to the proposal that the basal lamina, or one of its components, might interact with the receptor protein and, therefore, play a role as an 'organizing factor' for the receptor's dense and stable accumulation in the subsynaptic membrane [6–12].

Chemical studies on basal lamina and interstitial connective tissue have shown that these structures are composed of several genetically distinct types of collagens and structural glycoproteins. Collagens of type I and III are present in interstitial connective tissues [13] and collagens of type IV and V in basement membranes [14,15]. Fibronectin [16,17] and laminin [18,19] are the two major structural glycoproteins present in basal lamina.

The electroplaque from the electric organ of *Electrophorus electricus* is a highly specialised cell derived from skeletal muscle fibers but deprived of contractile myofibrils. In addition, it exhibits an asymmetrical organization: the nerve terminals contact only one of the two faces of the cell, which is referred to as the 'innervated' face. Light microscopy discloses that acetylcholinesterase [3,4] and the acetylcholine receptor [3,4] are located exclusively on the innervated face of the electroplaque. Because of this particularly simple organization, we undertook the study of the distribution of basal lamina and matrix macromolecules in this system using specific antisera to purified collagens and structural glycoproteins present in these structures. We report results obtained by the indirect immunofluorescence method for the identification and localization of these molecules.

2. Materials and methods

A slice of electric organ of *E. electricus* was dissected from a fresh animal and fixed in 2% paraformaldehyde in 0.2 M cacodylate buffer (pH 7.4) for 30 min. Frozen sections were prepared with a cryostat for histochemistry and immunofluorescence. Acetylcholinesterase was histochemically localized according to [20]. Indirect immunofluorescence tests were carried out using the appropriate dilutions of the antisera (1:50 or 1:100) for 30 min followed by rinsing with phosphate-buffered saline (PBS). The appropriate anti-IgG antiserum (Pasteur Institute, Paris) was then added for 30 min followed by rinsing in PBS and mounting in 50% glycerol. Photographs were taken with a Zeiss III RS microscope using Kodachrome ASA 400 film all at identical magnifica-

tion (× 120). The following antisera were used: goat and guinea pig antisera to human and pig collagens type I and III; rabbit and goat anti-bovine collagens type IV (lens capsule) and type V (placenta). Anti-fibronectin serum was prepared in rabbits against purified human plasma cold insoluble globulin (CIG) essentially as in [21]. The serum was purified by double-immunoabsorption first on a gelatin column to eliminate the insoluble globulin of the rabbit immune serum and then on plasma proteins depleted of fibronectin linked to CNBr–Sepharose column.

This serum had a high titer (1.066 mg/ml) and exhibited a wide interspecies cross reactivity [22].

Antilaminin (mouse, tumor, [18,19]) antiserum from rabbit was kindly provided by Dr R. Timpl (Max-Planck-Institut, Martinsried). Rabbit anti-actin anti-sera were kindly provided by Professor Gabbiani (Dept. Pathology, Univ. of Geneva). Anti-collagen anti-sera were kindly provided by Dr J. Rauterberg (Institut für Arteriosklerosеforschung an der Universität Münster) [31].

Control experiments for immunofluorescence were carried out by adding the corresponding antigen to the section before the specific anti-sera. The extinction of the fluorescence was taken as an indication of the specificity of the reaction.

3. Results

Fig.1 shows the fluorescent staining patterns obtained with the anti-collagen antisera in comparison to the histochemical staining for acetylcholinesterase. For type I collagen (fig.1a), no preferential localization in the innervated membrane (BM) was observed; the staining was diffuse, both within the cell and on the intercellular matrix side of the innervated membrane. For type III collagen (fig.1b), the fluorescent staining was also diffuse but with a predilection for the cytoplasmic side of the innervated membrane. For type IV collagen (fig.1c) the fluorescent staining coincided with that of acetylcholinesterase and, therefore, with the innervated membrane although some staining was observed on the cytoplasmic side. For type V collagen (fig.1d), the fluorescence underlined exactly the acetylcholinesterase-labelled membrane without any detectable staining on its extracellular or cytoplasmic sides.

Fig.2 shows the laminin (a), fibronectin (b), and actin (c) fluorescent staining patterns. The fibronectin labelling precisely superimposed with that of acetylcholinesterase. Laminin and actin staining were somewhat more diffuse but also covered the innervated membrane. All the control experiments were negative, confirming the specificity of the immunohistochemical localizations.

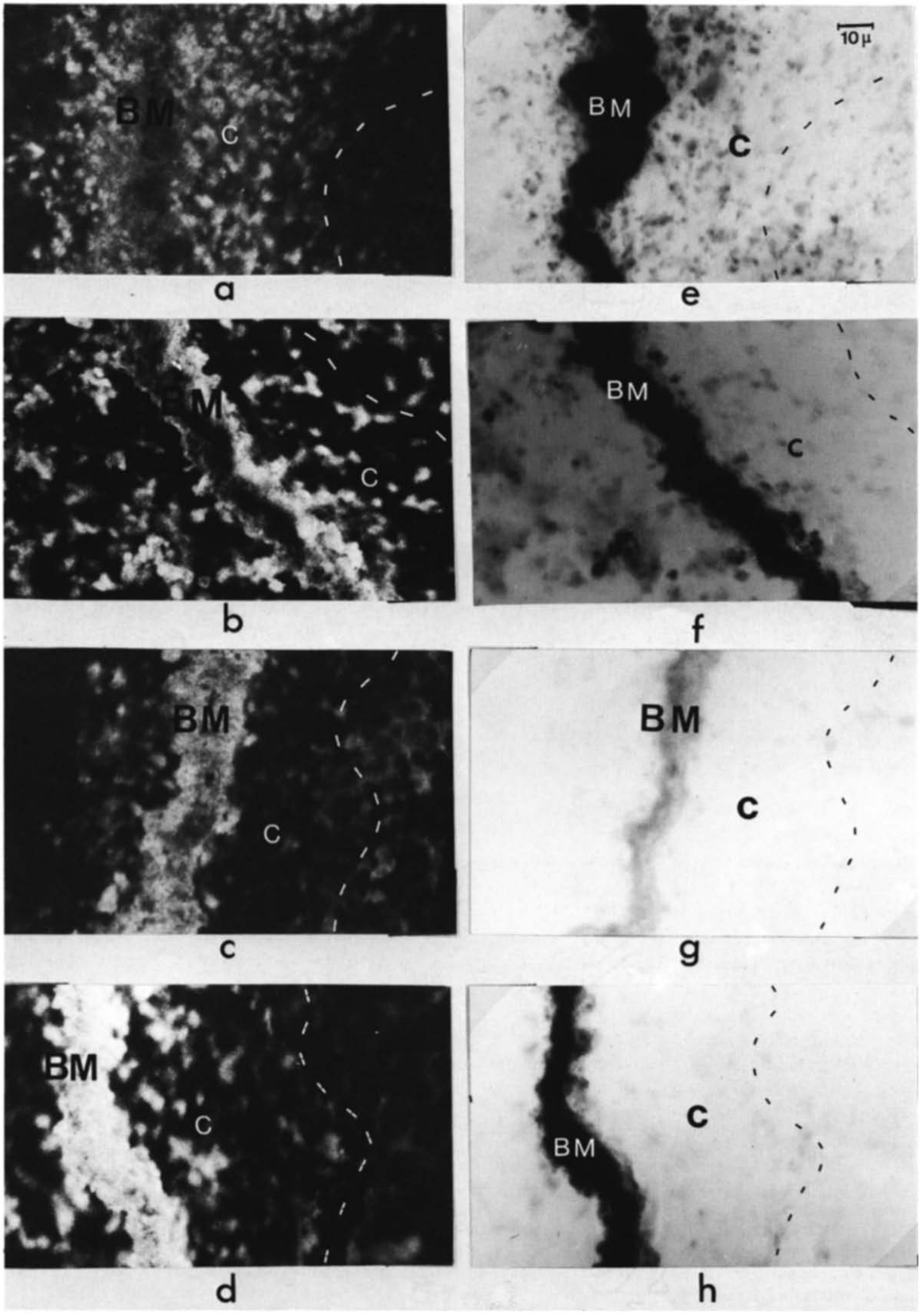
4. Discussion

The above results show that intercellular matrix macromolecules exhibit an uneven distribution on *E. electricus* electroplaque. Although the antisera used were not prepared against *E. electricus* matrix proteins, sufficient cross-reactivity was present to achieve a reliable histo-immunochemical localization. High levels of interspecies cross reactivity have indeed been noted with most of the anti-matrix macromolecule antisera [8,19,23,24]; anti-human fibronectin antisera even react with the lowest metazoans (sponges) [22].

The precise coincidence of fibronectin and acetylcholinesterase distribution on the innervated side of the electroplaque may suggest that fibronectin plays a role in the localization of acetylcholinesterase which possesses a collagen-like tail (references cited in [25]) and, therefore, might interact directly with fibronectin; an unambiguous demonstration of this point, however, is still lacking.

The localization of type IV and V collagens on the innervated face of the electroplaque is consistent with the observation that they are associated with particular types of basal lamina [26] for instance the endomysium of striated muscle [27] and the part of the endoneurium derived from the Schwann cell basement membrane [28]. The presence of actin on the cytoplasmic side of the innervated membrane is in agreement with [24] and with the postulated associa-

Fig.1. Comparison of the immunofluorescent localization of type I (a), III (b), IV (c) and V (d) collagens to the histochemical detection of acetylcholinesterase by the Koelle method (e,f,g,h) obtained on successive serial sections of the same fragment of *Electrophorus electricus* electric organ. For details see section 2 (× 120): BM, innervated membrane; C, cytoplasm; – – –, non-innervated membrane.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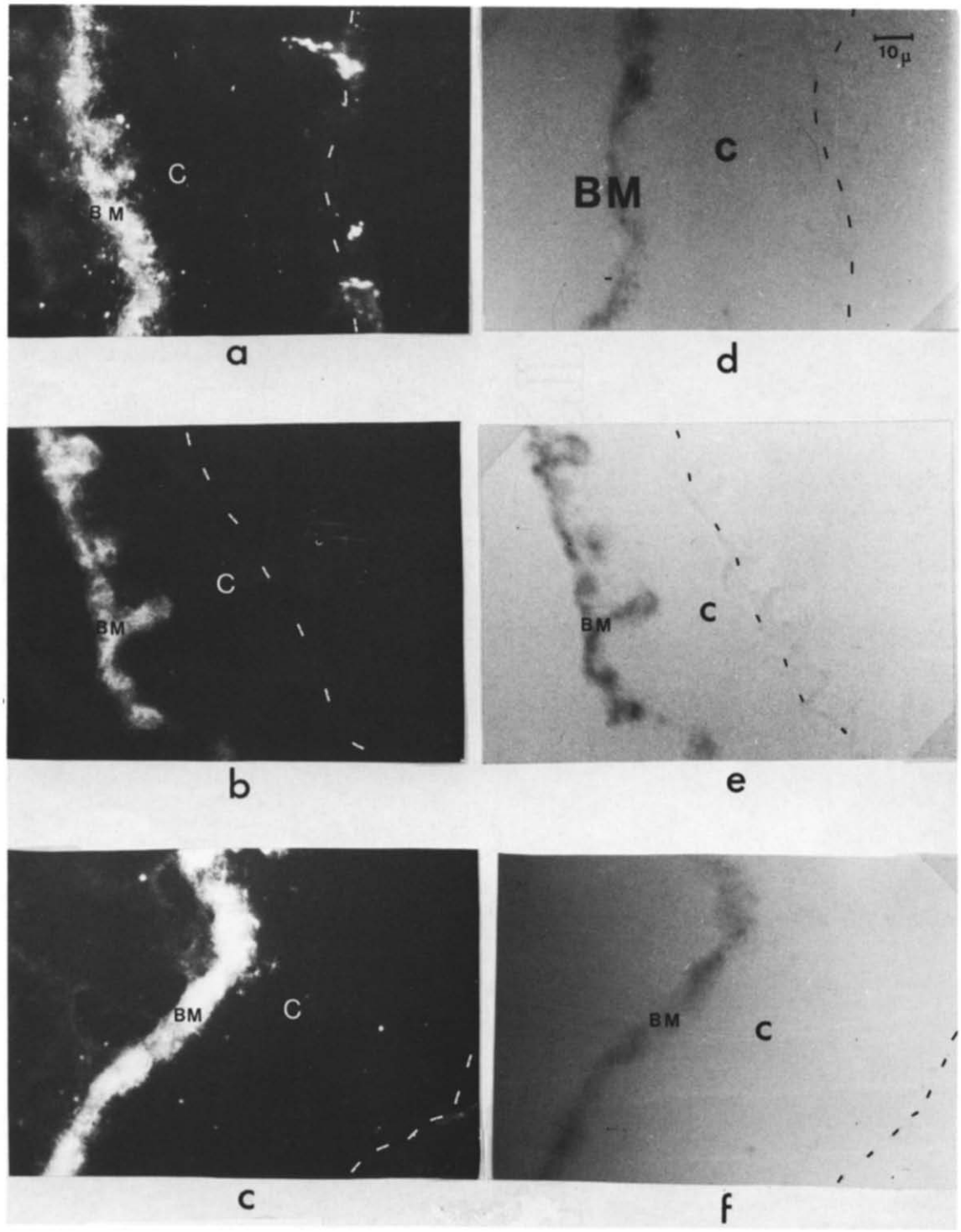

Fig.2. Immunofluorescent localization of laminin (a), fibronectin (b), and actin (c) compared to the histochemical staining of acetylcholinesterase (d,e,f) on successive serial sections of the same fragment of *Electrophorus electricus* electric organ (× 120).

tion of intracellular actin with extra-cellular fibronectin through the formation of a 'fibronexus' [29].

Our results confirm the presence of basement membrane-associated macromolecules such as collagen type IV and V, fibronectin, and laminin on the innervated side of the electroplaque although no data yet are available on their relative distribution in the synaptic and non-synaptic areas of this membrane. None of the above proteins, however, are specific for the synaptic region. The presence of some protein components characteristic of the synaptic sites of the basement membranes was, however, recently postulated on the basis of indirect immunochemical evidence [9,11].

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