Presence of tropomyosin in adrenal chromaffin cells and its association with chromaffin granule membranes

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The presence of tropomyosins in adrenal chromaffin cells was demonstrated by immunoperoxidase staining of cultured chromaffin cells by an anti-tropomyosin antibody. In immunoblotting the antibody labelled polypeptides of M_r 39000, 36500 and 31000 in $100000 \times g$ supernatants and heat-stable fractions of adrenal medulla. The adrenal medullary tropomyosins did not comigrate with smooth muscle tropomyosins. The 39-kDa and 31-kDa polypeptides comigrated with polypeptides labelled by anti-tropomyosin in brain supernatants. Chromaffin granule membranes possessed a 39-kDa polypeptide that was labelled by anti-tropomyosin.

Chromaffin cell *Tropomyosin* *Adrenal medulla* *Secretory granule* *Cytoskeleton*

1. INTRODUCTION

Muscle tropomyosin functions with troponin in the calcium-dependent regulation of contractile activity [1]. Tropomyosins are located along the thin filaments of muscle [1] and on the stress fibres of cultured cells [2]. The size of the tropomyosin subunits is tissue-specific [3,4] though the various polypeptides are antigenically and biochemically related [2,3,5]. In the case of fibroblasts and other cultured cells multiple forms of tropomyosin are present [5–7].

Adrenal chromaffin cells secrete catecholamines by exocytosis in response to an increase in the concentration of intracellular free calcium [8]. Chromaffin cells contain both actin [9–11] and myosin [10,12,13] and it is conceivable that interactions of secretory granules with the cytoskeleton could be important for granule movement to the plasma membrane in stimulated cells. If the chromaffin cell cytoskeleton is regulated by calcium it might be expected that components similar to the regulatory elements in muscle would be present. We report here that one such regulatory component, tropomyosin, is present in adrenal chromaffin cells and a tropomyosin-like polypeptide is associated with the chromaffin granule membrane.

2. MATERIALS AND METHODS

2.1. *Immunoperoxidase staining of chromaffin cell cultures*

Bovine adrenal chromaffin cells were prepared by enzymatic digestion of medullary fragments [14]. Dissociated cells were suspended in Dulbecco's modified Eagles medium with 10% foetal calf serum, 50 μM fluorodeoxyuridine, 50 μg/ml gentamycin and cultured in 24-well culture plates. Cell cultures were fixed and permeabilised by treatment with methanol at −20°C for 10 min. For immunoperoxidase staining cultures were rehydrated in phosphate-buffered saline (PBS) and incubated with rabbit antibody [6,7] against chicken gizzard tropomyosin (a gift from Dr J.J.-C. Lin) diluted to 1/50 in PBS + 3% BSA for 1 h at room temperature. After washing in PBS the cultures were incubated with anti-rabbit peroxidase (Sigma) for 1 h, followed by incubation in 0.2 mg/ml diaminobenzidine, 0.006% H_2O_2 for 10 min. All incubations and photography were carried out on cells in the wells in which they were cultured.

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2.2. *Preparation of tissue extracts*

100000 × *g* supernatants of rat brain and bovine adrenal medulla were prepared by homogenisation in 10 mM Hepes, 150 mM NaCl, 5 mM EGTA, 1 μ M bacitracin, pH 8.0, and centrifugation at 100000 × *g* for 1 h at 4°C. Chicken gizzard extract was prepared by homogenisation in 1 M KCl, 10 mM Tris, pH 7.5, and centrifugation at 14000 × *g* for 3 min in an MSE microcentaur centrifuge. Heat-stable proteins were prepared by boiling 100000 × *g* supernatants and gizzard extract for 10 min, and removal of precipitates by centrifugation in an MSE microcentaur for 3 min. Chromaffin granule membrane fractions were prepared as in [15].

2.3. *Polyacrylamide gel electrophoresis and immunoblotting*

Samples were solubilised and separated on 12.5% SDS-polyacrylamide slab gels as in [16]. For immunoblotting, proteins were transferred from gels onto nitrocellulose paper (Millipore) by transverse electrophoresis. Protein transfer was confirmed by staining with 0.2% Ponceaus S (Sigma) and immunoperoxidase staining carried out under essentially the same conditions as those used for cultures, except that all antibody solutions contained 0.2% Triton X-100.

3. RESULTS AND DISCUSSION

To test the possibility that chromaffin cells contained tropomyosin, chromaffin cells in primary culture were examined by immunoperoxidase staining with a rabbit antibody directed against tropomyosin. Immunoreactivity (fig.1) was present in all cells in culture with the typical chromaffin cell morphology, with a particulate distribution throughout the cell body and processes. The nuclei were unstained by the antibody.

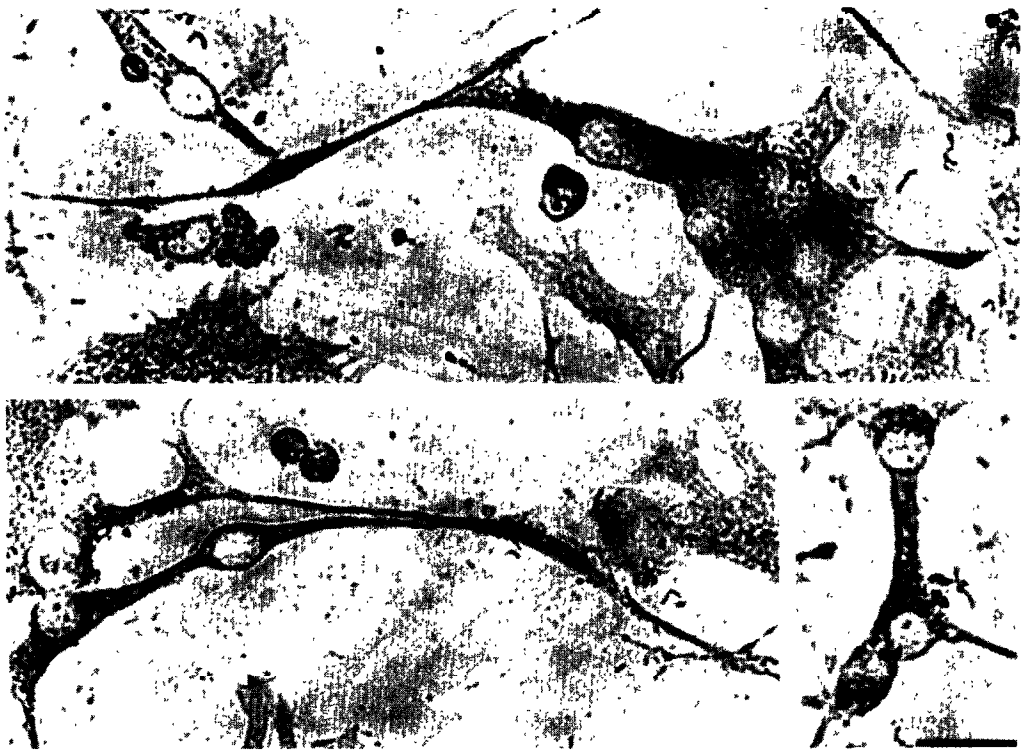

Fig.1. Bovine adrenal chromaffin cells in culture stained with rabbit antibody against tropomyosin using immunoperoxidase. Chromaffin cells with a range of morphologies are shown with particulate immunoreactivity in cell body and processes. Marker bar 20 μm.

The nature of tropomyosin in the adrenal medulla was examined by immunoblotting of the 100000 × *g* supernatants of bovine adrenal medulla. Such supernatants were found to contain polypeptides of M_r 39000, 36500 and a minor component of M_r 31000 which were consistently stained by the antibody against chicken gizzard tropomyosin (fig.2A). Both brain and adrenal medullary chromaffin cells are derived embryologically from the neural crest and therefore a 100000 × *g* supernatant of brain was also examined for the purposes of comparison. Brain supernatants differed from the medullary supernatants in containing 4 polypeptides of M_r 44000, 39000, 31000 and 26000 that were stained by the antibody (fig.2B). The results from brain supernatants are consistent with the demonstration that brain tropomyosin preparations contained a doublet of approx. 30 kDa as well as a doublet of higher molecular mass [17,18].

Additional evidence for the identification of the 3 adrenal medullary polypeptides as tropomyosins came from the finding that these polypeptides were enriched in the heat-stable fraction of a 100000 × *g* supernatant (fig.3A).

The tropomyosin-like polypeptides seen in 100000 × *g* supernatants of adrenal medulla could be derived from chromaffin cells, nerve fibres or smooth muscle cells of blood vessels. A comparison of the adrenal medullary polypeptides with smooth muscle tropomyosins indicated that polypeptides from the two tissues, that were stained by the antibody, did not comigrate (fig.3). Therefore smooth muscle cells can be eliminated as a source of the tropomyosins seen in adrenal medullary supernatants.

The 39-kDa polypeptide is almost certainly derived, at least in part, from the chromaffin cells themselves since a 39-kDa tropomyosin-like polypeptide was detected, by immunoblotting, associated with chromaffin granule membranes (fig.2C). A series of cytosolic proteins have been

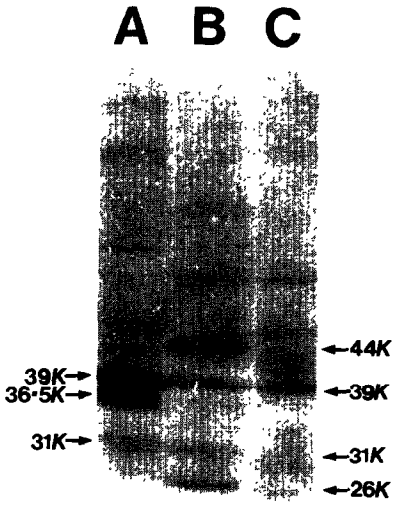

Fig.2. Immunoblotting with anti-tropomyosin of 100000 × *g* supernatant of adrenal medulla (A), 100000 × *g* supernatant of rat brain (B) and a chromaffin granule membrane fraction (C). Samples were separated on 12.5% gels, transferred to nitrocellulose and stained using the immunoperoxidase procedure. K, kDa.

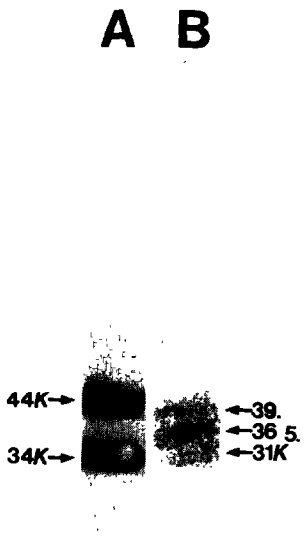

Fig.3. Immunoblotting with anti-tropomyosin of heat stable fractions of a chicken gizzard extract (A) and a 100000 × *g* supernatant of adrenal medulla (B).

shown to bind, in a calcium-dependent fashion to the granule membrane [19,20]. Several of these proteins are of a similar M_r to the adrenal medullary tropomyosins, however, they were not stained by the anti-tropomyosin antibody.

In agreement with the findings from immunoblotting, three polypeptides from adrenal medulla, of similar M_r to those described above, have been designated tropomyosins on the basis of heat-stability and chromatographic properties [21]. The largest of the polypeptides (M_r 39000) was detected in heat-stable fractions of isolated chromaffin cells by two-dimensional gel electrophoresis.

The demonstration that adrenal chromaffin cells and in particular chromaffin granule membranes contain a tropomyosin-like polypeptide suggests that tropomyosin may play a regulatory role in granule-cytoskeletal interactions. Of particular interest is the possibility that tropomyosin is involved in the control by calcium of the binding of actin filaments to the chromaffin granule membrane [22,23].

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