Myosin-like protein in the human parathyroid glands: An immunohistochemical study

F. Amenta and C. Cavallotti

Istituto di Anatomia Umana Normale, Università di Roma, I-00161 Roma (Italy), 27 July 1978

Summary. The immunofluorescent staining of the human parathyroid glands shows that the principal cells bind antimyosin-like antibodies. The presence of a myosin-like protein in the principal cells of the human parathyroid glands is presumably in rapport with parathormone secretion.

In the recent years, several studies have been carried out on the presence of molecules with notables similarity to muscle actin and myosin in numerous nonmuscle cell types[1-3].

The acto-myosin complex, or actin-like or myosin-like proteins, have been demonstrated and/or isolated by several mammalian tissues, such as cat, cow and rat brain[4-6], liver cells[7], nonmuscular ocular cells[8], ovaries, kidney and testis[9-13].

The present observations show that the principal cells of the human parathyroid glands contain a myosin-like protein. Observations were made in human parathyroid glands. We have examined 10 specimens of the parathyroid glands obtained at autopsy within 24–30 h of death in 4 male and 6 female subjects, and 4 biopsy samples taken from 3 male and 1 female. All specimens were free of parathyroid disease.

Some specimens were frozen in freon 12 cooled in liquid nitrogen, and then cut in serial sections 6–8 µm in thickness with a cryostat at a temperature of −20 °C. Unfrozen samples were fixed in 95% ethanol and embedded in paraffin according to the procedure of Sainte Marie[14]. The sections were cut serially in 5–8 µm in thickness and deparaffinized with petroleumether (b.p. 40–60 °C).

Fluorescein-conjugated rabbit's anti-myosin-like antibodies (F-AMA) were isolated and purified in our laboratory by methods reported previously[9,10,13]. The specificity of these F-AMA for myosin-like protein was confirmed by blocking studies by absorption and immunodiffusion. 4 consecutive sections from the cryostat and/or from paraffin were mounted on each slide. The first section was treated for 30 min with F-AMA diluted in phosphate buffered saline (PBS) 1:50. The second section was treated with nonfluorescent AMA, in PBS. The third section was treated with F-AMA previously absorbed by myosin-like protein. The fourth section was treated with normal rabbit serum in PBS.

A Zeiss photomicroscope II with HBO high pressure mercury lamp was used for the observations. A FITC filter was used as an exciter filter, while a combination of barrier filters (44, 50 and 65) were employed. A dark field condensor was used to improve the contrast. The sections were then stained with hematoxylin-eosin. Sections treated with F-AMA demonstrated obvious specific fluorescence in the human parathyroid glands, in correspondence to principal cells (figure 1).

At higher magnifications, the immunofluorescent staining is well-localized inside the cellular membrane, arranged in irregular, filamentary pictures (figure 2). Owing to the low magnification, it is not possible to demonstrate whether the staining corresponds exactly to the microfilamentous structures. The pattern of fluorescence is similar in the samples obtained at autopsy and in fresh samples, indicating that the myosin-like proteins maintain their characteristics for a period at least as long as this after death.

The binding between the myosin-like protein and F-AMA is not altered in ethanol-fixed and paraffin-embedded

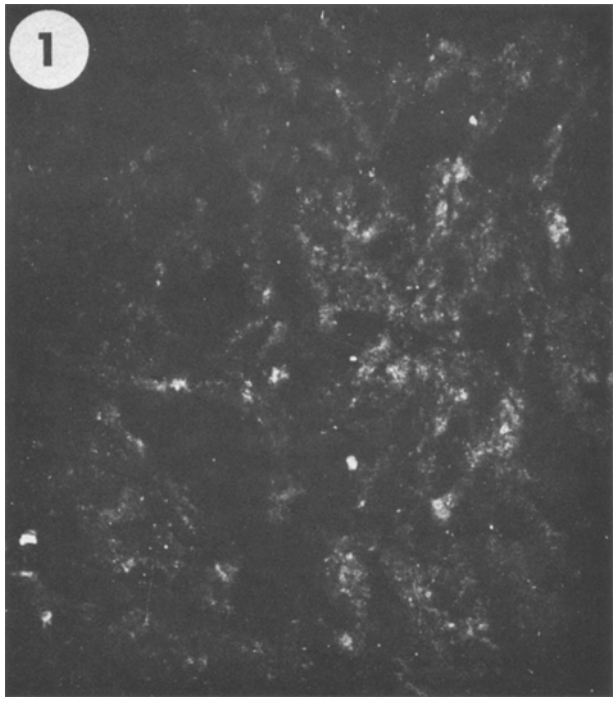

Fig. 1. Immunofluorescence of human parathyroid gland treated with F-AMA. The fluorescence is localized in correspondence of principal cells. × 40.

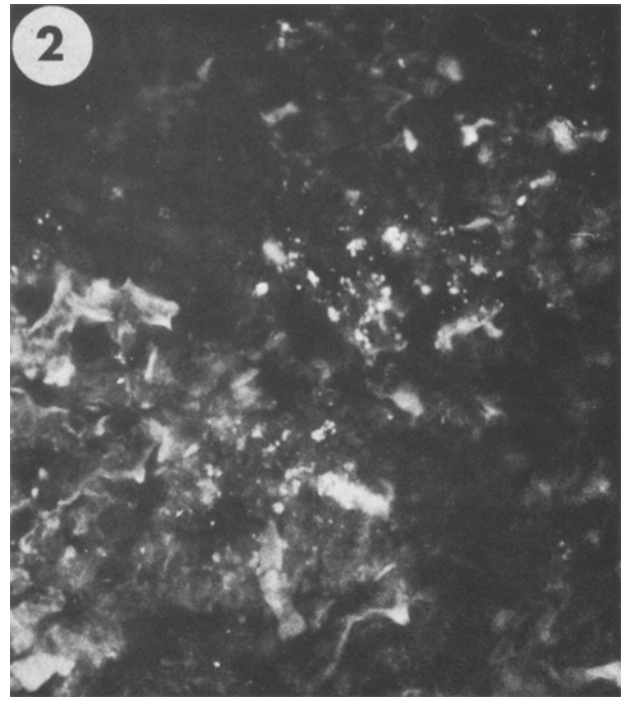

Fig. 2. Higher magnification of the fig. 1. F-AMA are localized inner the parathyroid principal cells. × 200.

sections, indicating that the fixation reduced only slightly the antigenicity of the myosin-like protein. The present results indicate that the parathyroid principal cells seem to be not kinetically passive but active, because they contain contractile elements; they might be able to produce discrete movements in the living organism.

The effective role of acto-myosin-like proteins in nonmuscular cells is not yet fully clear. In some types of cells, an actin-like protein is thought to take part in the microfilamentous structures seen close to cell membrane. Very little is know about the site and the functional role of the myosin-like protein.

Previous papers by our laboratory[9,10,13] have demonstrated the presence of a myosin-like protein in cells very rich in microfilamentous structures. Studies by the electron microscope have demonstrated that the cytoplasm of parathyroid principal cells contains microtubules and microfilaments[15-17]. The microtubule-microfilament system in some endocrine glands play a role in the secretory activity of several kinds of cells[18-20]. The correspondence between the immunofluorescence and the E.M. observations make it reasonable to suppose that the myosin-like protein could take part in the structure of the microtubular-microfilamentous cellular system.

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Evidence for changes in cell shape from a 2-dimensional to a 3-dimensional substrate

Marjorie A. England and Jennifer Wakely*

Department of Anatomy, University of Leicester, Leicester LE1 7RH (England), 21 December 1978

Summary. Chick embryo mesoderm cells were explanted to culture systems in vivo and in vitro and their subsequent movements were correlated with the external morphology as studied by SEM. In vitro cell movements are exaggerations of normal in vivo movements where a 2-dimensional substrate is encountered rather than a 3-dimensional environment.

Cell movement in vitro has been extensively studied in attempts to understand movement in vivo[1-3]. Various substrates, media and environmental conditions have been devised which allow cells to survive for long periods in culture. Numerous studies[4,5] have reported that various cell types in different culture systems have similar patterns of movement; lamellipodia, leading lamellae and ruffling activity. When new methods allowed further examination of cells in vivo it was reported by many experimenters that the characteristic morphological features of cell movement in vitro were largely absent from tissues in vivo[6].

Further studies using collagen in culture systems have shown that fibroblasts assume a bipolar spindle form on this substrate but do not invade the interior lattice[7].

We report here a series of experiments to demonstrate whether the differences between the 2 systems relate to the 2-dimensional substrate provided in an in vitro system and the 3-dimensional substrate in vivo. All specimens were examined on the advancing cell edge and on the internal aspect of the mass of cells. White Leghorn chick embryo mesoderm cells stage 4[8] were studied in ovo; in wounded New Culture systems[9]; in vivo on a cellular substrate; in vitro on glass coverslips and on Sterispon Absorbable Gelatin Sponge. In ovo specimens were incubated at 37.5 °C until stage 4. Other stage 4 embryos were mounted as for New Culture and an incision 0.2–0.3 mm long was made in the area pellucida endoderm adjacent to the area opaca border on the level of Hensen's node. 3 embryos were re-incubated at 37.5 °C and the healing wound examined at 30 min; 1 h and 2 h. In another group mesoderm cells to be grown on a cellular substrate were introduced onto the area opaca ventral ectoderm layer of a host chick embryo stage 4 mounted as for New Culture.

The overlying endoderm layer had previously been surgically removed in an area 0.5 mm × 0.5 mm. Host embryos were re-incubated at 37.5 °C for 1 h. Mesoderm cells were also cultured in vitro on glass coverslips and Sterispon Absorbable Gelatin Sponge BP (Allen and Hanburys Ltd., London) at 37.5 °C in TC 199 containing 10% serum, penicillin, streptomycin. All specimens were fixed in Karnovsky's fluid[10], buffered in cacodylate[11], osmicated in a 2% solution and dehydrated in a graded series of ethanol until 100%. They were then transferred to 100% acetone; critical point dried by acetone replacement with liquid CO_2, mounted on Cambridge stubs with colloidal silver paint and coated with 20 nm of gold. The specimens were examined in an ISI 60 scanning electron microscope.

Mesoderm cells in ovo on the advancing edge follow the ectoderm substrate first. They have numerous filopodia on the leading cell edge. Mesoderm cells in ovo internal to the layer are stellate and numerous filopodia on each cell contact approximately 8–12 adjacent cells in the mesoderm,