CHARACTERISTIC LOCALIZATION OF CALMODULIN IN HUMAN TISSUES: IMMUNOHISTOCHEMICAL STUDY IN THE PARAFFIN SECTIONS

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A calcium-binding protein, calmodulin is thought to be an intracellular mediator for many calcium-dependent processes. In this report, to see the immunohistochemical localization of calmodulin in human tissues, routine paraffin sections were studied using either indirect immunoperoxidase or immunofluorescent method, and the characteristic staining was observed in some types of the epithelial cells, the neurons and others. After the preservation of the tissues in the formalin and in the paraffin sections, the immunoreactivity of calmodulin could still be demonstrated, which allows the accumulation of the knowledge concerning the pathological as well as normal distribution of calmodulin in human tissues for the elucidation of calmodulin functions in the human body.

Calmodulin, a calcium-binding protein ubiquitous in the plant and animal kingdoms, is thought to be an intracellular mediator for many calcium-dependent processes (for review, 7). In spite of its importance, its immunohistochemical localization in human tissues has been reported only in the brain (11). This report gives the results of the demonstration of calmodulin in routine paraffin sections of human tissues using a specific anti-calmodulin antibody visualized by either indirect immunoperoxidase or immunofluorescent technique.

MATERIALS AND METHODS

The specific antibody against calmodulin was elicited in rabbits with bovine brain calmodulin as an antigen and was purified using affinity-chromatography as described previously (5). Its immunological specificity has also been described previously (5). The human tissues were obtained from 3 autopsy cases. Thin tissue blocks less than 2 mm-thick were fixed for 3 days in 10% formalin buffered with phosphate buffer (pH 7.4) and embedded in paraffin (Histosec; Merck) after processing through graded alcohols and chloroform following the routine method. Some tissues were fixed for 2.5 months and then, embedded in paraffin. The sections (2-4 μm-thick) from these tissues were mounted on the slide glass and kept at room temperature for 2 months. Other sections on the slide glass were stocked at 4°C and used for the immunostaining within 2 weeks. Following conventional deparaffinization and rehydration, the sections were stained with a specific anti-
calmodulin antibody by indirect immunoperoxidase method or indirect immunofluorescent method. In the former case, the sections were treated as described previously in the studies on cultured cells (9). The sections with many red cells were pretreated with methanol for 30 min and 0.01% hydrogen peroxide for another 30 min to eliminate the pseudoreaction. This treatment did not affect the immunostaining pattern. In the latter case, the localization of calmodulin was visualized with FITC-labeled anti-IgG (Cappel, 1:75 dilution) instead of the peroxidase-labeled anti-IgG. The fluorescent sections mounted with glycerin buffered with phosphate buffer after washing were examined for their morphology and fluorescent pattern microscopically (Nikon, Microflex, UFX-II). Kodak Tri X-Pan film was used for their photomicrographs. The concentration of specific anti-calmodulin antibody used in both method was 4 to 8 μg/ml.

For the control, non-immune rabbit IgG or specific anti-calmodulin antibody preadsorbed with calmodulin were used as the first antibody instead of anti-calmodulin at the same concentration. The completely positive staining was counted as the meaningful staining.

RESULTS

Calmodulin showed some characteristic immunohistochemical localization in various human tissues.

In the skin of the breast, the cornified layer was unstained, but the cells of the epidermis under the cornified layer showed intense immunostaining. The epithelial cells of the sweat gland were also heavily stained. The cells of the sebaceous glands containing relatively less lipid showed moderate staining. The intensity of the staining seemed to become weaker as the lipid was accumulated in the cells (Fig. 1). The lipid itself was unstained.

In the kidney, the cells in the glomeruli, probably the podocytes and/or mesangium cells, were stained intensely (Fig. 2). The type of the stained cells in the glomeruli, however, should be determined immunoelectron-microscopically. The cells of the walls of the collecting duct were also stained, especially intensely at the part of the papillary duct.

In the small intestine, the epithelial cells were heavily stained particularly at the surface microvilli. The mucus of goblet cells was unstained (Fig. 3). The visceral muscle and the ganglion cells were also stained moderately.

In the pancreas, the epithelial cells of the pancreatic ducts showed intense staining. The centroacinous cells were also moderately stained. The cells of the islet of Langerhans were stained weakly (Fig. 4).

In the lung, the bronchial epithelial cells gave clear staining with the unstained mucus of the goblet cells (Fig. 5). The cartilage cells showed some dot-like staining. The alveolar cells showed weak but heterogenous staining.

The cardiac muscle had the characteristic staining at the I-band and intercalated discs (Fig. 6). I-band of the skeletal muscle was also stained (Fig. 7).

In the spinal cord, the neurons including the large motor neurons were stained clearly with their processes. The neuronal fibers in the dorsal and ventral roots showed weak staining (Fig. 8).
In other tissues, the positive staining was shown in the Purkinje cells and the granular cells in the cerebellum and the neurons in the cerebral cortex. In the retina, the fibers of the outer and inner plexiform layers and many but not all the

**Figs. 1–8.** The immunohistochemical localization of calmodulin in the paraffin sections of various human tissues was demonstrated. The sections shown in Figs. 1a, 1b, 2b, and 7 were stained with immunofluorescent method and others, immunoperoxidase method.

**Fig. 1.** The skin. (a) The cells of the epidermis under the cornified layer were stained intensely but the cornified layer was unstained. The epithelial cells of the sweat gland were also heavily stained. The cells of the sebaceous glands showed moderate staining with unstained lipid. ×80

(b) Negative control. The section next to that shown in (a) was treated with non-immune rabbit IgG which showed no characteristic staining. ×80

**Fig. 2.** The kidney. (a) The DAB reaction product was observed in the cells of the glomeruli. ×470

(b) The cells of the glomeruli showed immunofluorescent staining. ×330

**Fig. 3.** The small intestine. The epithelial cells were heavily stained particularly at their surface microvilli. ×235

**Fig. 4.** The pancreas. The epithelial cells of the pancreatic ducts, especially their apical part were stained intensely and the centroacinous cells, moderately. ×235

**Fig. 5.** The lung. The bronchial epithelial cells showed the heavy staining while the mucus of the goblet cells were negative in the immunoreaction. ×120

**Fig. 6.** The cardiac muscle. The characteristic staining was observed at the I-band. ×470

**Fig. 7.** The skeletal muscle. The focus was put on the I-band stained. ×330

**Fig. 8.** The spinal cord. The large motor neurons and other neurons had the clear staining. ×470
cells in the inner nuclear layer were stained. Some of the ganglion cells were also stained.

In the oral cavity, the mucosal epithelial cells of the stratified epithelium were intensely stained but at the palatine tonsil, only the basal cells layer showed moderate staining. The epithelial cells of the salivary gland were also stained well.

In the liver, the epithelial cells of the bile duct and some cell located at the wall of the sinusoids, probably the Kupffer, were stained. The staining of the hepatocytes was not clear. In the thyroid gland, the cuboidal and flattened follicular epithelial cells were stained clearly, but the colloid in the follicles had no immunoreaction. In the adrenal gland, the cells of the medulla had positive staining. In the cortex, the nucleus of some cells were slightly stained. In the spleen, the cells scattered in the red pulp were stained intensely which seemed to be the splenocytes. In the thymus, the epithelial reticular cells and outer cells of the Hassal bodies in the medulla were stained.

At the wall of the blood vessels, the smooth muscle was stained with the intense staining of the internal endothelium of arteries. The wall of the artery was sometimes heavily stained which might be the nerve fibers around it. The connective tissue was generally unstained.

The specific staining patterns were virtually identical in the sections stained with the immunoperoxidase method or with the immunofluorescent method with a somewhat intensified staining on the rim of the cells by the latter. Calmodulin could still be demonstrated in the tissues investigated—the kidney, oral cavity, retina, pancreas and thymus—which were fixed in formalin for 2.5 months and kept as the paraffin sections for 2 months at room temperature.

DISCUSSION

A calcium-binding protein, calmodulin is thought to mediate the control in a large number of calcium-dependent cellular processes by calcium, through the interaction with enzymes and non-enzymatic proteins. Its amino acid sequence has been conserved, almost perfectly, throughout evolution, which indicates its biological importance (see review, 7). However, its morphological distribution in the tissues of living things is not understood well, especially in human tissues. In the case of human tissues, it is often necessary to investigate the samples after the long-term preservation, and the paraffin embedding method is used in many laboratories for the conventional histopathological and also histochemical observations. With this method, the samples can be preserved in paraffin blocks in which they are easily set in the direction desired for the best observation, even if the tissues have nodule structures or are very tiny. They are difficult to deal with by other floating methods. Although some antigens more or less lose their immunoreactivity during the processes of the paraffin method, the usefulness of this method should be certainly estimated. With the immunohistochemistry of calmodulin, only the brain and kidney has been studied from the paraffin sections of the tissues of experimental animals reported previously. These included studies of the brain, liver, adrenal gland, skeletal muscle, thyroid gland, trachea and kidney (1–4, 6, 10–13). With human tissues, the report on the brain as paraffin sections (11) was the sole case that the authors found. We have also tried the paraffin method on
the retina, testis, lymph node and ganglion of rodents and have obtained the characteristic findings (8). Now, this report showed the localization of calmodulin in human tissues with the paraffin sections.

In this report, the localization of the immunoreactivity of calmodulin was found in various cells. Some of them were comparable to the previous findings obtained mainly on experimental animals by several methods. The localization in the glomeruli of the kidney was common in the rat and human (2). The staining of the neurons in the brain and the retina of the rodents (1, 6, 8, 10, 13) and the follicular cells of the dog thyroid gland (12) were similar to our results on the corresponding tissues, though the former were observed in the frozen- or vibratome-sections. The staining of the neurons of the human brain in the paraffin sections was also reported by another group (11). The staining in the epithelial cells of the trachea reported on hamsters (3) seemed likely to continue to the bronchus found in our results on humans, though the methods used were different. The staining at the I-band of the cardiac and skeletal muscle was probably similar to that of the frozen sections of the skeletal muscle of the rat (4). The cells of the medulla of the adrenal gland were stained in the human as in the rat (4) but the intensity might not be so strong as described on the frozen section of rat tissue (4). In our report, the hepatocytes were not stained clearly. This differs from the results of the frozen sections of the rat liver (4). This might be due to the pathological and/or physiological state of our case or to the effect of the procedures in preparing the paraffin sections, especially with the glycogen granules, since it is possible that the specific immunoreactivity might be reduced to some extents by the paraffin method.

In addition to these locations, this report showed the characteristic immunoreactivity in some but not all types of epithelial cells; the stratified epithelium of the skin and oral cavity, the epithelial cells of the ductus structures such as those in the pancreas, liver, kidney, of the sweat gland, and salivary gland and so on. For the ciliated cells of hamster tracheal epithelium, Gordon et al. suggested the function of calmodulin in ciliary beating in their immunoelectron microscopic study of the respiratory tract (3). The functions of calmodulin in the cells which showed a strong immunoreactivity were future problems to be studied. The results show that calmodulin can still be demonstrated in the tissues preserved in formalin and since the paraffin sections, as well as the normal ones, allow the accumulation of the pathological data, this will provide valuable information on the functions of calmodulin in the human body.

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REFERENCES


