Histochemistry (1987) 87:487–490

Histochemistry
© Springer-Verlag 1987

Immunohistochemical study of angiotensin-converting enzyme in human tissues using monoclonal antibodies

S.M. Danilov*, A.I. Faerman, O.Yu. Printseva, A.V. Martynov, I.Yu. Sakharov, and I.N. Trakht
Institute of Experimental Cardiology, USSR Cardiology Research Center, Moscow, 121552, USSR

Received November 12, 1986 / Accepted June 17, 1987

Summary. The localization of angiotensin-converting enzyme (ACE) in human tissues has been studied by the PAP-method with the use of monoclonal antibody 9B9 against human lung ACE. The enzyme was detected on the surface of endothelial cells in lung, myocardium, liver, intestine and testis as well as in the epithelial cells of the kidney proximal tubules and intestine. The monoclonal antibody 9B9 did not react with ACE in the epithelial cells of the testis seminiferous tubules. These data suggest that the antibody 9B9 recognizes epitope which is shared by the ACE molecule of endothelial cells and renal and intestinal epithelial cells but is not present in testicular ACE, or is not accessible there to the antibody.

Introduction

Angiotensin-converting enzyme (ACE) is a dipeptidyl carboxypeptidase (EC 3.4.15.1) which catalyzes the release of histidylleucine from angiotensin I to yield angiotensin II, a potent vasopressor agent. ACE also inactivates bradykinin, a vasodepressor nonapeptide (Cushman and Ondetti 1980). By converting hypertensive peptides and by inactivating hypotensive ones, ACE alters their concentration in blood and tissues. Therefore, ACE may be considered to be the key enzyme controlling the vascular tone (Bunning and Riordan 1981). The same enzyme hydrolyzes other biologically active substances such as natriuretic factor (Harris and Wilson 1985), substance P, and enkephalins (Erdos and Skidgel 1985).

In immunohistochemical studies on the distribution of ACE in tissues of the rabbit, rat, mouse and man, it was shown with polyclonal antibodies that the enzyme is localized mainly on the surface of vascular endothelial cells of the organs studied including lung, liver, thyroid gland and spleen. In addition, ACE was found on the surface of epithelial cells in kidney proximal tubules, testis seminiferous tubules, brain ventricles and intestine (Caldwell et al. 1976; Auerbach et al. 1982; Defendini et al. 1983).

Recent publications show structural and functional heterogeneity of ACE preparations obtained from different organs (Soffer and El-Dorry 1983; Stritmatter and Snyder 1984; Velletri 1985). However, the attempts to detect immunological differences of ACE preparations with the help of polyclonal antibodies were not successful (Polsky-Cynkin and Fanburg 1979). Monoclonal antibodies due to their high specificity and uniformity, are very useful for investigating the possible existance of ACE isoenzymes as well as for immunohistochemical revealing of ACE in human tissues.

Until now, few studies were made on the production of monoclonal antibodies to ACE. The monoclonal antibody 4F9 obtained by Moore et al. (1984) did not interact with human ACE, and the monoclonal antibody a-ACE 3.1.1 obtained by Auerbach et al. (1982), although showing cross-reactivity with human ACE, was not used for detailed visualization of ACE in human tissues.

Using human lung ACE as an antigen, we obtained a great number of monoclonal antibodies against ACE and showed that, in vitro, these highly specific antibodies interacted both with soluble and with immobilized ACE preparations without altering the catalytic activity of the enzyme (Danilov et al. 1987). In the present study, one of the monoclonal antibodies, 9B9, was used, immunocytochemically, in order to reveal the distribution of ACE in human tissues by the PAP-method with the help of monoclonal antibodies to peroxidase.

Materials and methods

Preparation of tissue samples. Tissue samples were dissected at autopsy 3–6 h post mortem (sudden death, men 28 to 65 years old), snap-frosen and stored in liquid nitrogen. In some cases, they were fixed by immersion in 10% formalin, washed, and then stored in liquid nitrogen. Cryostat sections (5 μm) of fresh-frozen or formalin-fixed tissues were mounted on slides, fixed in acetone for 5 min at room temperature, air-dried, wrapped in aluminium foil and stored at −20° C.

Monoclonal antibodies. The production of monoclonal antibodies against ACE has been described in detail elsewhere (Danilov et al. 1987). In brief, monoclonal antibody (MAb) 9B9 belonging to IgG_1 subclass was obtained by immunization of BALB/c mice with human lung ACE purified to homogeneity by affinity chromatography (Sakharov et al. 1987). The purified preparation had a specific activity of 13 μ/mg of protein. The activity of ACE was measured in 100 m*M* *Tris*-HCl buffer (pH 7.8) containing 600 m*M* NaCl and 2.5 m*M* Hippuryl-His-Leu at 37° C. One unit of enzyme activity is defined as the amount of ACE thet hydrolyzes 1 μmol Hippuryl-His-Leu per min under standard conditions. Mouse splenocytes were hybridized with murine myeloma (P_3O_1) cells according to Kohler and Milstein (1975). Hybrid cells were screened for antibody production by ELISA (Lanzillo and Fanburg 1982).

Primary populations of cells which reacted positively were

* To whom offprint requests should be sent

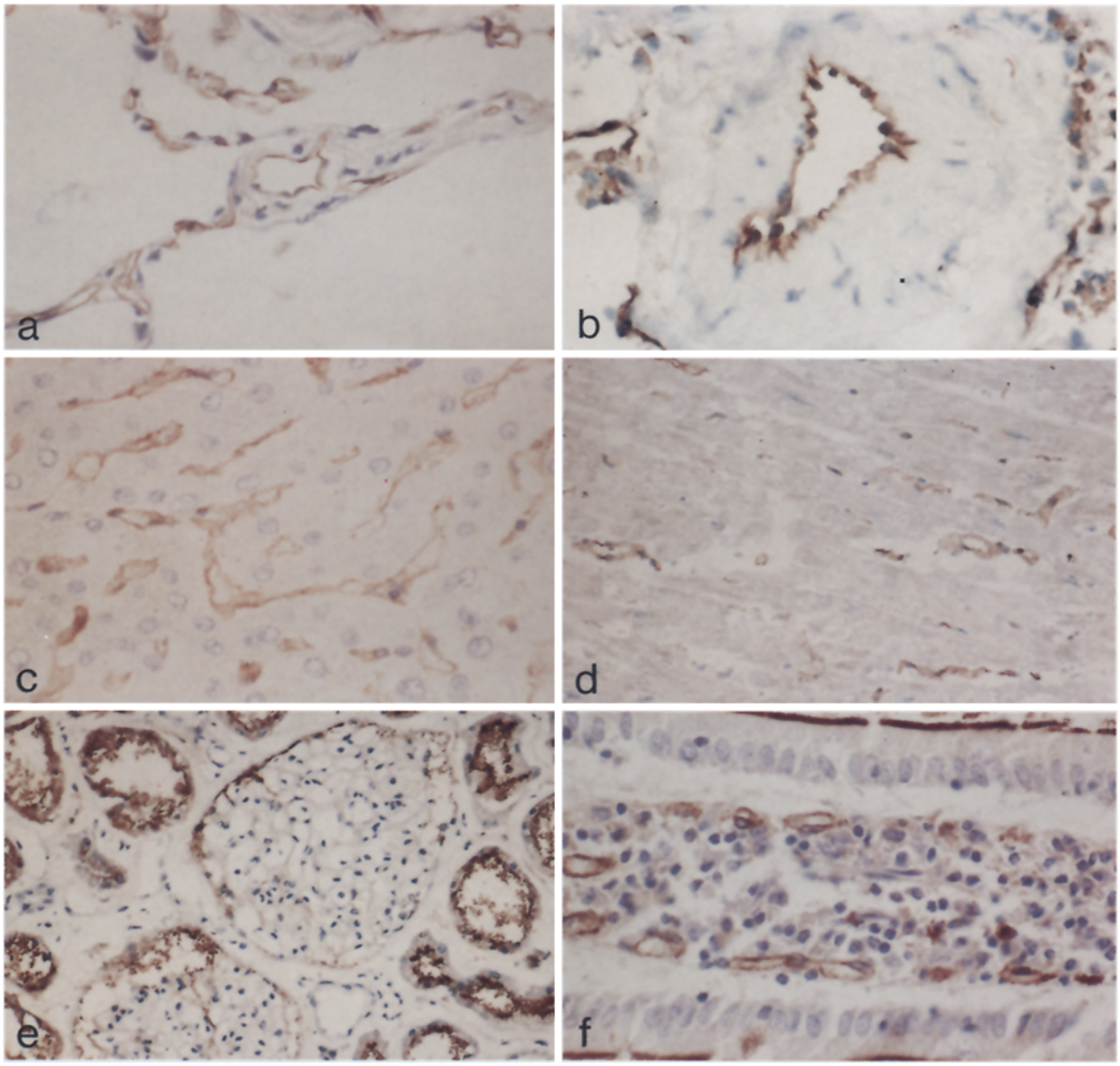

Fig. 1a–f. Immunohistochemical staining of ACE with monoclonal antibody 9B9 in human tissues. In the lung the endothelial cells are stained in arterioles and capillaries (**a**) and in larger vessels (**b**). In the liver (**c**) and myocardium (**d**) only endothelial cells are stained. In the kidney (**e**) only slight staining is seen in some endothelial cells but the epithelium of proximal tubules is markedly stained. In the intestine (**f**) there is strong staining in the brush border of epithelial cells and in the endothelial cells of capillaries. Magnification: ×700 (**a**); ×800 (**c, d, f**); ×400 (**b**); ×200 (**e**)

tested further for antigen binding with soluble ACE by an immunoadsorbtion test (Danilov et al. 1987). The primary population of cells producing antibody 9B9 was selected for incubation, cloning, subcloning, and ascites production. The cloned hybridoma line was designated as anti-ACE 9B9 F1. MAb separated from the ascitic fluid showed a high antigen binding activity both in ELISA and immunoadsorbtion tests, but had no effect on the enzymatic activity of soluble or immobilized ACE preparations.

Immunocytochemical procedures. Prior to staining the slides were allowed to attain room temperature and were washed in phosphate-buffered saline (PBS) for 10 min. The tissue sections were then incubated with monoclonal antibody 9B9 for 30 min (hybridoma culture supernate or antibody purified from ascitic fluid and diluted in PBS to a concentration 10 µg/ml). After three washings in PBS for 10 min the sections were incubated for 30 min with goat antiserum to mouse immunoglobulines ("Miles") in PBS at a dilution 1:40. The sections were washed again in PBS and were incubated for 30 min with a monoclonal PAP-reagent. This reagent was prepared by adding horseradish peroxidase ("Sigma", type YI, 50 µg/ml) to the supernate fluid from the culture of hybridoma clone AP-FC-2B4 producing monoclonal antibody (IgG_1) to horseradish peroxidase (Faerman et al. 1987). The sections were washed again, and peroxidase activity was revealed by 5 min incubation with 0.05 *M Tris* buffer (pH 7.4) containing 0.05 *M* imidazole, 0.05% of 3,3′- diaminobenzidine ("Sigma") and 0.01% of hydrogen peroxide ("Merck") (Straus 1982). After final washing in PBS the sections were counterstained with hematoxiline, cleared, and mounted in balsam. As controls we used sections treated only with the second antibody and PAP, or with nonspecific antibodies taken as first antibodies. In some cases, prior to immunohistochemical staining, formalin fixed tissues were incubated in methanol with 0.3% of hydrogen peroxide in order to inhibit endogenous peroxidase activity.

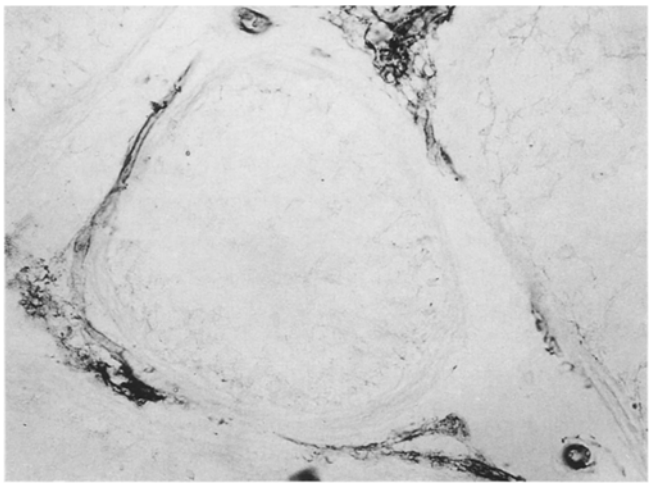

Fig. 2. Immunohistochemical staining of testis section with monoclonal antibody 9B9: vascular endothelial cells are stained (×180)

Results

The results of staining with MAb 9B9 were similar with fresh frozen and formalin-fixed tissues. This indicates preservation of antigenic determinants and their recognition by the antibody, in spite of formalin fixation. Treatment of sections with methanol and hydrogen peroxide did not affect the binding of MAb 9B9 to ACE either, although the staining intensity was somewhat decreased.

In lung sections, staining was observed in the endothelial cells of capillaries (Fig. 1a) as well as in those of larger vessels (Fig. 1b). A strong specific staining was also seen in vascular and capillary endothelial cells of the liver (Fig. 1c) and the myocardium (Fig. 1d). Hepatocytes and cardiomyocytes were not stained. Unlike the endothelium of these organs, the endothelium of kidney vessels was almost free of staining of MAb 9B9; there was only a slight staining in some endothelial cells of intertubular capillary network. In kidney sections, the most intense staining was observed in the apical region of epithelial cells, both in convoluted and straight segments of the proximal tubules. The epithelial cells in other parts of the nephron did not show staining (Fig. 1e). We cannot exclude the possibility that the staining observed in the glomeruli is an artifact of diffusion of proximal tubule contents into a glomerulus occuring during tissue dissection and freezing (Defendini et al. 1983). Non-vascular localization of ACE was also found in the small intestine where the brush border of the epithelial cells was stained markedly. The endothelial cells of vessels in the intestinal mucosa and submucosa were also stained (Fig. 1f). In sections of the testis, MAb 9B9 binding was observed perhaps only in vessels of interstitial tissue between seminiferous tubules, whereas the cells of the tubules were not stained (Fig. 2).

Discussion

Monoclonal antibodies against ACE show high specificity of binding, in vitro, to ACE preparations obtained from different human organs (lung, kidney, liver, myocardium, and brain) (Danilov et al. 1986). The results presented in the present paper show that monoclonal antibody 9B9 reveals the antigenic determinant localized on the surface of vascular endothelial cells in the human organs studied (lung, liver, myocardium, intestine, testis). A similar localization of ACE was observed in rabbit, rat, mouse and human tissues by Caldwell et al. (1976) and Defendini et al. (1982) by using polyclonal antibodies and by Auerbach et al. (1982) by using monoclonal antibody. Thus, the monoclonal antibody against ACE which we obtained can be used as a specific marker of human vascular endothelium. This is important for investigating the origin and development of endothelial cells in different human tumors (hemangioma, for example) and other kinds of vascular pathology. For example, an immunocytochemical study of some cases of nonspecific human Takayasu disease which we conducted with the use of monoclonal antibody 9B9, showed that the pathology was followed by ingrowth of vasa vasorum into the medial layer of the adventicia (paper in preparation).

In sections of the human renal cortex, we observed intense staining in the apical region of the proximal tubule epithelium, in contrast to the week staining in endothelial cells of the intertubular network. A high ACE activity of the proximal tubule epithelium was also observed in the mouse and rabbit kidneys (Auerbach et al. 1982; Wigger and Stalcup 1978). Defendini and co-authors (1983) have shown that ACE activity in rat kidney is much lower then in the kidneys of other animals. Immunocytochemically, with the use of polyclonal antibodies, the same authors observed only slight staining in the proximal tubules of rat kidney. We also revealed slight staining of rat kidney sections with monoclonal antibody 9B9, though staining at the rat lung sections was very strong (unpublished).

We found non-vascular localization of ACE in the intestinal epithelium, among other organs being studied. It is also known that a certain amount of ACE is concentrated in the epithelial cells of the brain ventricle (Defendini et al. 1983).

Different opinions are expressed in the literature as to the physiological role of ACE localization on the surface of epithelium in the kidney, brain vascular plexus and intestine i.e., in the cells which form a barrier between different biological fluids and the blood (Defendini et al. 1983). However, the exact function of ACE at these epithelial locations is not known. ACE may be a necessary component of the glycocalix (Ryan 1986) of the surface of barrier-forming epithelia and endothelia. The low ACE content in the kidney of rats, already observed by Defendini et al. (1983) and confirmed by us, suggested that in the rat the function of the kidney ACE is fulfilled by ACE localized in another organ (in the intestine, for example) or by some other kidney enzyme.

The following observation is of particular interest. Whereas the antibody 9B9 binds to ACE on the surface of vascular endothelial cells in lung, liver, myocardium, intestine and testis and also binds to the epithelial surface of kidney proximal tubules and those of small intestine, the antibody does not interact with ACE in the seminiferous tubules of the testis where large amount of ACE are known to be localized (Stritmatter and Snyder 1984; Velletri et al. 1985; Brentjens et al. 1986). The organ specificity of ACE has been studied by several authors. However convincing proof for differences in the structure and function of ACE at different ACE locations has been obtained only in the case of the testis (Soffer and El-Dorry 1983; Berg et al. 1986; Stritmatter and Snyder 1984; Velletri 1985). Our data

suggest that MAb 9B9 interact with epitope on ACE molecule of vascular and epithelial origin which is not expressed in testicular ACE. Another explanation of this result is that 9B9 epitope is masked and therefore unavailable for MAb 9B9. We consider these observation to be an immunological proof for the difference between testicular ACE and ACE from other human organs. Similar results have been obtained by Stritmatter and Snyder (1984) in their study of rat tissue with monoclonal antibodies against rat lung ACE.

A more detailed study of MAb 9B9 binding to sections of the human male reproductive system has to be conducted because Stritmatter and Snyder (1984) have discovered serological differences between ACE localized in the testis, epididymis and prostate of the rat.

Currently, we attempt to use these antibodies for immunochemical revealing of sarcoidosis, a disease involving the formation of granulomas consisting of ACE-expressing cells (Pertschuk et al. 1981).

Acknowledgements. We wish to express our gratitude to Dr. E. Allikmets (USSR Cardiology Research Center) for her help in obtaining monoclonal antibodies to ACE.

References

Auerbach R, Alby L, Grieves J, Joseph J, Lingren C, Morrissey LW, Sidky YA, Tu M, Watt SL (1982) Monoclonal antibody against angiotensin-converting enzyme: its use as a marker for murine, bovine and human endothelial cells. Proc Natl Acad Sci USA 79:7891–7895

Barba L, Caldwell P, Downie G, Camussi G, Brentjens L, Andres G (1983) Lung injury mediated by antibodies to endothelium. J Exp Med 158:2141–2158

Berg T, Sulner J, Lai CY, Soffer RL (1986) Immunohistochemical localization of two angiotensin I-converting isoenzymes in the reproductive tract of male rabbit. J Histochem Cytochem 34:753–760

Brentjens JR, Matsuo S, Andres GA, Caldwell PR, Zamboni L (1986) Gametes contain angiotensin-converting enzyme (kininase II). Experientia 42:399–401

Bunning P, Riordan JF (1981) Physiological function and catalytic metabolism of angiotensin-converting enzyme. In: Holzer H (ed) Metabolic interconversion of enzymes 1980. Springer, Berlin Heidelberg New York, pp 248–256

Caldwell P, Seegal B, Hsu K, Dos M, Soffer R (1976) Angiotensin-converting enzyme: Vascular endothelial localization. Science 191:1050–1051

Cushman DW, Ondetti MA (1980) Inhibitors of angiotensin-converting enzyme. In: Ellis SG, West G (eds) Progress in medicinal chemistry, vol 17. Elsevier/North Holland, Amsterdam, pp 42–104

Danilov S, Sakharov I, Allikmets E, Dukhanina E, Trakht I (1986) Organ-specificity of angiotensin-converting enzyme revealed by monoclonal antibody. Proc 4th International Symposium of Biology of the Vascular Endothelial Cells, Noordwijkerhout, The Netherlands

Danilov SM, Allikmets EY, Sakharov IY, Dukhanina EA, Trakht IN (1987) Monoclonal antibody to human lung angiotensin-converting enzyme. Biotechnol Appl Biochem (in press)

Defendini R, Zimmerman E, Weare J, Alhenc-Gelas F, Erdos E (1983) Angiotensin-converting enzyme in epithelial and neuroepithelial cells. Neuroendocrinology 37:32–40

Erdos EG, Skidgel RA (1985) Structure and function of angiotensin-converting enzyme (kininase II). Biochem Soc Trans 13:42–44

Faerman A, Chervonsky A, Chipysheva T, Gilstain B, Kolayda A (1987) Monoclonal antibody to horseradish peroxidase. Obtaining and using in immunohistochemistry and immunoblotting. Bull Exp Biol Med (Russian) (in press)

Harris RB, Wilson IB (1985) Comparison of hydrolysis of atriapeptin II stand-in substrate by atrial dipeptidyl carboxy hydrolase on angiotensin I-converting enzyme. Int J Pept Prot Res 26:78–82

Kohler G, Milstein C (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256:495–497

Lanzillo J, Fanburg B (1982) Development of competitive enzyme immunoassay for human serum angiotensin-I-converting enzyme: A comparison of four assay configurations. Anal Biochem 126:156–164

Moore M, Chrzanovski R, McCormick J, Cieplinski W, Schwin KA (1984) Production of monoclonal antibodies to rat lung ACE. Clin Immunol Immunopathol 33:301–312

Pertschuk LP, Silverstein E, Friedland J (1981) Immunohistologic diagnosis of sarcoidosis: detection of angiotensin-converting enzyme in sarcoid granulomas. Am J Clin Pathol 75:350–354

Polsky-Cynkin R, Fanburg BL (1979) Immunochemical comparison of angiotensin I-converting enzyme from different rat organs. Int J Biochem 10:669–674

Ryan U (1986) The endothelial surface and responses to injury. Fed Proc 45:101–108

Sakharov IY, Danilov SM, Dukhanina EA (1987) Affinity chromatography and some properties of angiotensin-converting enzyme from human heart. Biochem Biophys Acta 923:143–149

Soffer RL, El-Dorry HA (1983) Angiotensin-converting enzyme: immunologic, structural, and developmental aspects. Fed Proc 42:2735–2740

Straus W (1982) Imidasol increases the sensitivity of the cytochemical reaction for peroxidase with diaminobenzidine at neutral pH. J Histochem Cytochem 30:491–493

Stritmatter S, Snyder S (1984) Angiotensin-converting enzyme in male rat reproductive system: Autoradiographic visualisation with ^{3}H-captopril. Endocrinology 115:2332–2341

Velletri PA, Aquilano DR, Bruckwick E, Tsai-Morris CH, Dufau ML, Lovenberg W (1985) Endocrinological controle and cellular localization of rat testicular angiotensin-converting enzyme. Endocrinology 116:2516–2522

Velletri PA (1985) Angiotensin-I-converting enzyme. Life Sci 36:1597–1608

Wigger HY, Stalcup SA (1978) Distribution and development of angiotensin-converting enzyme in the fetal and newborn rabbit. Lab Invest 38:581–585