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Immunological characterization of peptidyl-glycine-α-amidating monooxygenases

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Antibodies to the soluble form of the copper-containing enzyme, peptidyl-glycine-α-amidating monooxygenase isolated from secretory granules of bovine pituitary anterior lobes were found to belong to immunoglobulin G_1. The antibodies were used to study the subcellular distribution of the enzyme in this tissue, and positive tests were found only for granular and cytosol fractions. The antibodies do not crossreact with other copper-containing systems of secretory granules, such as neurocuprein and dopamine-β-monooxygenase. It was shown that the antibodies give the crossreaction with the enzyme isolated from secretory granules of bovine pituitary anterior lobes, cardiac atria, pancreas and adrenal medulla, indicating the antigenic identity of the enzyme from secretory granules of different glands.

Antibody to peptidyl-glycine-α-amidating monooxygenase; Copper-containing enzyme

1. INTRODUCTION

Peptidyl-glycine-α-amidating monooxygenase ('the amidating enzyme') is known to participate in the processing and the activation of many polypeptide hormones [1–5]. In different endocrine tissues, this enzymatic activity was detected to be associated with secretory granules [6–10]. Moreover, from pituitary and adrenomedullar (chromaffin) granules, the soluble form of the enzyme in its highly purified state was isolated, and some physico-chemical properties of the enzyme were elucidated [11–13]. It was shown, in particular, that the enzyme from these two sources consists of one polypeptide chain of 65 kDa and contains one EPR-detectable type II ('non-blue') copper atom per molecule [13].

At least some types of the secretory granules contain, besides the amidating enzyme, other copper enzymes and proteins, among them dopamine-β-monooxygenase and neurocuprein. The first of these is known to be a multicopper and multisubunit enzyme, whereas neurocuprein is an extremely acidic monocopper protein consisting of one polypeptide chain of 10 kDa. The apoform of neurocuprein was found to be the effective inhibitor of both the amidating enzyme and dopamine-β-monooxygenase [13–16]. It is evident that the further study of all copper-containing proteins of secretory granules and the interrelationship between these proteins still seems to be necessary.

In this communication we report on antigenic properties of the soluble forms of peptidyl-glycine-α-amidating monooxygenases isolated from secretory granules of different glands, consider the subcellular localization of this form of the enzyme, and compare antigenic properties of soluble forms of the amidating enzyme, dopamine-β-monooxygenase and neurocuprein, all isolated from the same gland.

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2. MATERIALS AND METHODS

Secretory granules from bovine adrenal medulla, pituitary anterior lobes and cardiac atria were prepared essentially by generally accepted methods [17–19]. According to electron-microscopic control measurements, contamination by other subcellular particles in granular preparations as 5–7% [12,13]. The big granular fraction from bovine pancreas was prepared according to [20]. Suspensions of granules in 30% sucrose were stored at −30°C before use. The lysis of granules was carried out by the freeze–thaw procedure repeated several times or by sonication for 1 min at 22 kHz. The supernatant obtained after centrifugation of lysates at 80 000 × *g* for 1 h was used as a starting material for the subsequent purification of peptidyl-glycine-α-amidating monooxygenase, dopamine-β-monooxygenase and neurocuprein [12,13].

Electrophoretically homogeneous preparations of peptidyl-glycine-α-amidating monooxygenase were used for immunization of rabbits. The enzyme emulsified with an equal volume of complete Freund's adjuvant was used for intradermal multiple injections (70 μg/kg). Booster injections of the enzyme in incomplete Freund's adjuvant were given three times at 2 week intervals after the primary injections. Portions of immune blood were collected from the ear vein at 1–2 weeks after the final booster injection; sera were separated by centrifugation, and the immunoglobulin G fractions were purified by ammonium sulphate fractionation, DEAE-cellulose chromatography followed by affinity chromatography on Protein A-Sepharose. The identification of immunoglobulin G subgroups was carried out by immunoenzyme assays using antibodies to μ-, α-, γ_1-, γ_{2a}-, γ_{2b}-peptide chains of immunoglobulins. Subcellular fractions of pituitaries were obtained by differential fractionation and by discontinuous sucrose gradient (0.3 M/0.2 M and 0.3 M/1.3 M/1.6 M) centrifugation [21]. The following fractions were isolated: cytosol, microsomes, mitochondria, granules, sinaptosomes and nuclei. To destroy subcellular particulate fractions, they were sonicated at 22 kHz for 2 min at 4°C. For immu-

nodiffusion assays, supernatants of each fraction, obtained after centrifugation of sonicates were used.

Double immunodiffusion was carried out according to Ouchterlony [22] using 1.2% agarose suspension in 0.01 M phosphate buffer, pH 7.4 containing 0.1 M NaCl. Immunodiffusion was continued during 48–60 h at 22°C. Immunoelectrophoresis was carried out in 1.2% agarose suspended in 0.1 M barbital-acetate buffer, pH 8.6. Other conditions of electrophoresis were: voltage, 10 V/cm; current, 12 mA; duration, 4 h. The content of the amidating enzyme in diluted solutions was evaluated by solid-phase EIA using the convential method with peroxidase–antiperoxidase system [23].

3. RESULTS AND DISCUSSION

Rabbit antibodies against the amidating enzyme from bovine pituitary anerior lobes were established to belong to the subgroup G_1 of immunoglobulins. Fig. 1 shows results of Ouchterlony's double immunodiffusion titration experiments and immunoelectrophoretic studies of these antibodies. The same pattern was observed also with rabbit antibodies to the amidating enzyme purified from bovine atrial granules.

The subcellular localization of the enzyme in pituitaries was studied by both double immunodiffusion and EIA. From the six fractions considered, the enzyme was detected only in secretory granules and cytosol (Fig. 2). This conclusion was confirmed by detection of the enzymatic activity in the same subcellular fractions which gave positive immune tests. The identical result was obtained for the subcellular localization of the amidating enzyme in the adrenal medulla too. However, at this stage it cannot be excluded that the positive result with cytosolic fractions of both glands may be connected partially with the lysis of a small part of granules during the procedure of subcellular fractionation.

In further experiments the possibility of a crossreaction between antibodies to the amidating enzyme and other copper proteins and enzymes of secretory granules was tested. It was found, in particular, that antibodies produced against the amidating enzyme do not give immunoprecipitation bands with the soluble form of dopamine-β-monooxygenase isolated from chromaffin granules. Furthermore, no crossreactivity was observed between these antibodies and neurocuprein, and vice versa, antibodies to neurocuprein did not react with the amidating enzyme. Results of this study are shown in Fig. 3. This, it may be concluded that antigenic properties of the amidating enzyme are different to those of neurocuprein and dopamine-β-monooxygenase.

To consider the antigenic specificity of the amidating enzyme from different glands, the lysates of secretory granules obtained from bovine putuitary anterior lobs, adrenal medulla, cardiac atria and pancreas were studied using the double immunodiffusion technique. Results of these experiments when immune serum to the pituitary enzyme was tested, are shown in Fig. 4. The same pattern was observed when, instead of antibodies to the amidating enzyme from pituitaries, antibodies against the enzyme isolated from chromaffin granules were used. From these data, the similarity of antigenic properties of soluble forms of the enzyme isolated from secretory granules of different glands becomes evident.

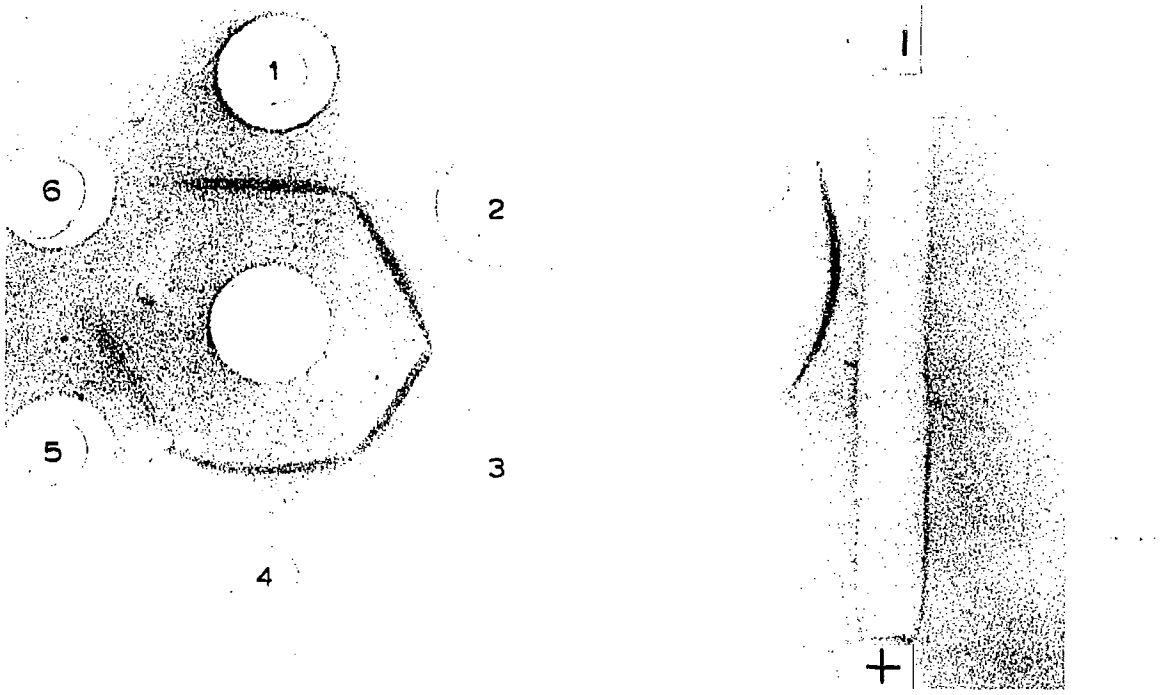

Fig. 1. Antigenic characteristics of peptidyl-glycine-α-amidating monooxygenase from bovine pituitaries. Left: the immunodiffusion titration of antibodies against the enzyme. The central well was filled with 1.5 μg of the enzyme, and rabbit serum diluted 2, 4, 8, 16, 32 and 50 times were filled into wells 1, 2, 3, 4, 5 and 6, respectively. Right: the immunoelectrophoresis pattern of the enzyme against the immune serum (central well).

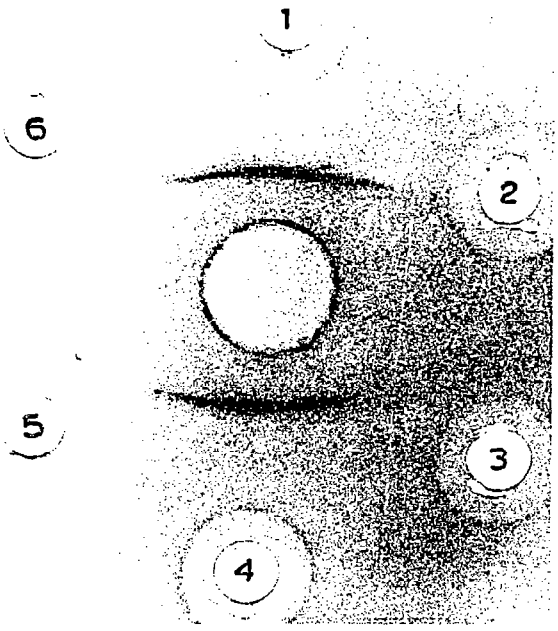

Fig. 2. The demonstration of the subcellular distribution of the amidating enzyme by immunodiffusion. The central well contain 10 μl of the immune serum. Aliquotes of centrifuged sonitates of cytosolic, microsomal, mitochondrial, granular, nuclear and sinaptosomal fractions of pituitaries were filled into wells 1, 2, 3, 4, 5 and 6, respectively.

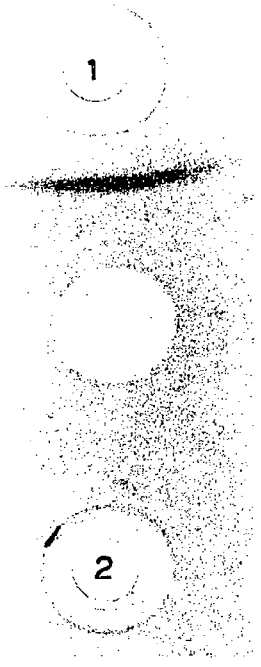

Fig. 3. The absence of the cross reactivity between antibodies against the amidating enzyme and other copper-containing proteins of granules. The central well contains 10 μl of immune serum. 1–1.5 μg of the amidating enzyme; 2–1.5 μg of dopamine-β-monooxygenase plus 1.5 μg of neurocuprein.

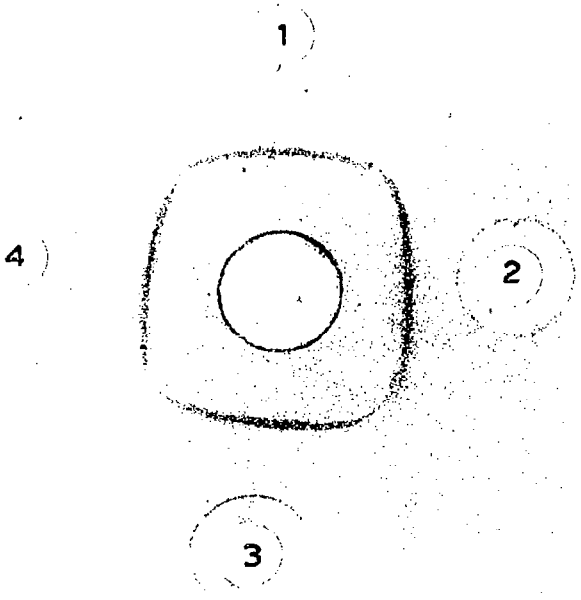

Fig. 4. Identity of antigenic properties of amidating enzymes from secretory granules of different glands. The central well contains antibodies against to the enzyme from pituitaries. 1, the enzyme (antigen) from anterior lobs of bovine pituitaries; 2, the enzyme from bovine chromaffin granules; 3, the enzyme from bovine atrial granules; 4, the lysate of granules from bovine pancreas.

Thus, there are far-reaching analogies between the properties of the soluble amidating enzyme from different sources. In particular, the main physico-chemical properties (mol.wt., subunit composition, contents and surroundings of copper) and the antigenic characteristics of the enzyme are practically identical for preparations of the enzyme from secretory granules of different glands. Besides, the enzyme from all kinds of granules is inhibited by the apoform of neurocuprein, an extremely acidic copper protein, which was also detected in secretory granules of various endocrine tissues [15,16]. These data seem to suggest that in secretory granules, independent of their origin, there is a universal mechanism modulating the activity of granular copper-containing monooxygenases by changing the level of copper in these enzymes. As a result of changes in the copper content of monooxygenases their enzymatic activities are changed, and hence, the levels of processing and the activation of hormones may be also changed.

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REFERENCES

[1] Bradbury, A.F., Finnie, M.D.A. and Smith, D.G. (1982) Nature 298, 686–688.
[2] Landymore-Lim, A.E.N., Bradbury, A.F. and Smyth, D.G. (1983) Biochem. Biophys. Res. Commun. 117, 289–293.

[3] Yoo, O.J., Powell, C.T. and Agarwal, K.L. (1982) Proc. Natl. Acad. Sci. USA 79, 1049–1053.
[4] Seizinger, B.R., Liebisch, D.C., Gramsch, C., Hezz, A., Weber, E. and Evans Bohlen, P. (1985) Nature 313, 57–59.
[5] Tatemoto, K., Carlquist, M. and Mutt, V. (1982) Nature 296, 659–660.
[6] Glembotski, C.C. (1985) Arch. Biochem. Biophys. 241, 673–683.
[7] Eipper, B., Myers, A. and Mains, R. (1985) Endocrinology 116, 2497–2504.
[8] Whang, G., Ney, R., Mains, R. and Eipper, B. (1985) Neuroendocrinology 41, 482–489.
[9] Sakata, J., Mizuno, K. and Matsuo, H. (1986) Biochem. Biophys. Res. Commun. 140, 230–236.
[10] Valröy, H., Nyberg, F., Franzen, H. and Terenius, L. (1987) Biochem. Biophys. Res. Commun. 148, 24–30.
[11] Murthy, A.S.N., Mains, R.E. and Eipper, B.A. (1986) J. Biol. Chem. 261, 1815–1922.
[12] Sharoyan, S.G., Abramyan, K.S. and Nalbandyan, R.M. (1988) Neurokhimya (Russ.) 7, 389–396.
[13] Markossian, K.A., Paitian, N.A., Abramyan, K.S. and Nalbandyan, R.M. (1989) Biokhimya (Russ.) 54, 2046–2053.
[14] Markossian, K.A., Paitian, N.A., Mikaelyan, M.V. and Nalbandyan, R.M. (1986) Biochem. Biophys. Res. Commun. 138, 1–8.
[15] Mikaelyan, M.V., Markossian, K.A., Paitian, N.A. and Nalbandyan, R.M. (1988) Biochem. Biophys. Res. Commun. 155, 1430–1436.
[16] Mikaelyan, M.V., Grigorian, N.A. and Nalbandyan, R.M. (1987) Biochim. Biophys. Acta 924, 548–555.
[17] Helle, K.B., Flatmark, T., Serk-Hanssen, G. and Lonning, S. (1971) Biochem. Biophys. Acta 226, 1–8.
[18] Russell, J.T. (1981) Anal. Biochem. 113, 229–238.
[19] De Bold, A.J. (1982) Can. J. Physiol. Pharmacol. 60, 324–330.
[20] Hoffman, P.G., Linder, O., Bonner, W.M. and Polland, H.B. (1976) Arch. Biochem. Biophys. 176, 375–388.
[21] Dodd, T.R., Hardy, J.A., Oakly, A.E., Edwardson, J.A., Perry, E.A. and Delaunoy, J.P. (1981) Brain Res. 228, 107–118.
[22] Ouchterlony, O. (1967) Immunodiffusion and Immunoelectrophoresis Handbook of Experimental Immunology (Weir, ed.) Oxford-Edinburgh.
[23] Tijssen, P. (1985) Practice and theory of enzyme immunoassays. Elsevier, Amsterdam.