6. I. S. Freidlin, N. K. Artemenko, V. D. Kravtsov, et al., Phagocytosis and Immunity [in Russian], Moscow (1983), pp. 226-227.
7. A. A. Yushchenko, Current Problems in Leprology [in Russian], Astrakhan' (1984), pp. 40-42.
8. A. A. Yushchenko and F. E. Vishnevetskii, Byull. Éksp. Biol. Med., No. 3, 376 (1987).
9. J. Convit, N. Aranzazu, and M. E. Pinardi, The Armadillo as an Experimental Model in Biomedical Research, Washington (1976), pp. 41-46.
10. C. K. Job, W. F. Kirchheimer, and R. M. Sanchez, Int. J. Leprosy, 50, 177 (1982).
11. W. F. Kirchheimer and E. E. Storrs, Int. J. Leprosy, 39, 693 (1971).
12. K. J. W. Rees, Nature, 211, 557 (1966).
13. K. J. W. Rees, Bull. Wld. Hlth. Org., 40, 785 (1969).
14. C. C. Shepard, J. Exp. Med., 112, 445 (1960).
15. C. C. Shepard and D. H. McRae, Int. J. Leprosy, 36, 78 (1968).

MONOCLONAL ANTIBODIES TO α-ENDORPHIN EFFECTIVE IN IMMUNOHISTOCHEMISTRY AND IMMUNOBLOTTING

Yu. S. Massino, V. V. Tsibezov, A. D. Dmitriev, V. M. Vostrikov, I. A. Soldatova, and G. I. Kolyaskina

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The endorphins include groups of endogenous opioid peptides, which are synthesized mainly in the anterior and intermediate lobes of the pituitary [1, 2, 11]. α-, β-, and γ-endorphins are known: β-endorphin consists of 31 amino-acid residues, and α- and γ- endorphins are fragments 1-17 and 1-16 of β-endorphin [1, 2, 11]. The concentration of endorphins in the pituitary does not exceed 1 μg/mg wet weight of tissue. To identify such quantities of biologically active substances in tissues, sensitive immunochemical methods based on the use of highly specific antibodies to the substances assayed are nowadays widely used.

In the investigation described below monoclonal antibodies to α-endorphin were obtained and characterized, for subsequent use in immunochemical methods of α-endorphin assay in tissues and culture fluids.

EXPERIMENTAL METHOD

BALB/c mice were immunized by repeated subcutaneous injections of α-endorphin (synthesized and generously provided by Professor M. I. Titov, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR), conjugated with bovine serum albumin with the aid of bis-diazotized benzidine [5]. Splenic lymphocytes from immune mice (with a titer of not less than 1:200) were fused with mouse myeloma X63-Ag8.653 cells [8], using the fusion technique described in detail in [10]. The clones were tested for the presence of antibodies by radioimmunoassay [3]. Positive hybridomas were cloned by the limiting dilutions method, using mouse peritoneal or splenic cells as the nurse layer. To obtain ascites fluid, 10^6 hybridoma cells were injected intraperitoneally into young male BALB/c mice, into which Pristane had been injected 2 weeks previously, or Freund's incomplete adjuvant on the previous day. Cross reactivity of the monoclonal antibodies to α-endorphin relative to β- and γ-endorphins was determined by studying the ability of the antibodies to bind equivalent quantities of ^{125}I-β- and γ-endorphins, with specific activity equal to that of ^{125}I-α-endorphin. Radioimmunoassay

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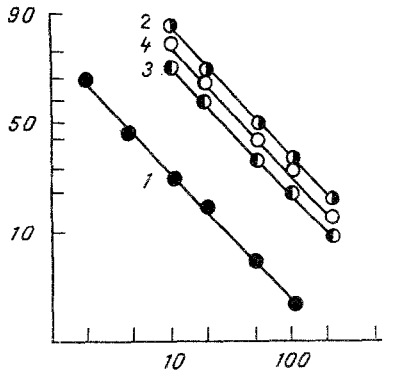

Fig. 1. Displacement of ^{125}I-α-endorphin from its complex with monoclonal antibodies of hybridomas E11 (1), A8 (2), F8 (3), and H5 (4) by unlabeled α-endorphin. Abscissa, logarithm of α-endorphin concentration in sample (in nM); ordinate, logit (B/B_0, where B_0 is the quantity of ^{125}I-α-endorphin bound with antibodies in the absence of unlabeled α-endorphin; B is the same, in the presence of the corresponding quantity of α-endorphin).

of α-endorphin was carried out and ^{125}I-derivatives of α-, β-, and γ-endorphins were obtained as described previously [3]. During the immunohistochemical investigation of the pituitary by the indirect immunofluorescence method [7], monoclonal antibodies to α-endorphin from the culture medium were used as the first antibodies, and rabbit antimouse immunoglobulin, conjugated with fluorescein (Dakopatts) as the second antibodies. Electrophoresis of bovine pituitary extracts in polyacrylamide gel in the presence of sodium dodecylsulfate (SDS) was carried out by the method in [9]. Proteins were transferred from the polyacrylamide gel to nitrocellulose and subsequently treated (immunoblotting) by the method in [14].

EXPERIMENTAL RESULTS

As a result of two consecutive fusions of splenic lymphocytes of immune mice and myeloma cells 625 hybrid cultures were obtained, of which five produced antibodies to α-endorphin. Four hybridomas (E11, A8, F8, and H5) were successfully cloned and recloned, after which the positive clones were frozen in liquid nitrogen. One clone, actively growing and producing antibodies, was selected from each of the four hybridomas, and subsequently used in the experiments. To study specificity of antibodies produced by hybridomas E11, A8, F8, and H5, the culture medium was used. Antibodies of hybridomas E11, A8, F8, and H5 bound 12.5, 20.6, 9.6, and 6.6% of ^{125}I-β-endorphin and 35.5, 15.1, 12.8, and 12.2% of ^{125}I-γ-endorphin.

Antibodies of hybridomas E11, A8, F8, and H5 bound half of the indicator dose of ^{125}I-α-endorphin with a final dilution of the culture medium of 1:500, 1:50, 1:100, and 1:50 respectively. This value for ascites fluid reached 1:32,000. Unlabeled α-endorphin inhibited binding of ^{125}I-α-endorphin with antibodies of hybridomas E11, A8, F8, and H5 by half in concentrations of 5, 50, 30, and 35 nM respectively (Fig. 1). It follows from these data that the monoclonal antibodies obtained were unsuitable for radioimmunoassay of physiological amounts of α-endorphin: the sensitivity of the method is on average 100-1000 times less than that obtained with polyclonal antibodies [3]. Low sensitivity of radioimmunoassay with the use of monoclonal antibodies is also characteristic of other peptides: enkephalins [6], β-endorphin [13], and ACTH [15] and it is evidently connected with the low affinity constant of monoclonal antibodies for peptides. It must be emphasized that this state of affairs is no obstacle for the practical use of monoclonal antibodies as affinity agents for peptide purification. Thorpe et al. [13], for instance, successfully purified β-endorphin from hog pituitary by the use of a column with monoclonal antibodies to the peptide, although these antibodies were characterized by low affinity for β-endorphin. Thus with respect to sensitivity in radioimmunoassay, monoclonal antibodies to α-endorphin resemble the monoclonal antibodies to compounds of peptide nature usually obtained.

We know that in mammals endorphins are synthesized mainly in the anterior (about 20% of endorphin-producing cells) and intermediate lobes (about 90% of endorphin-producing cells) of

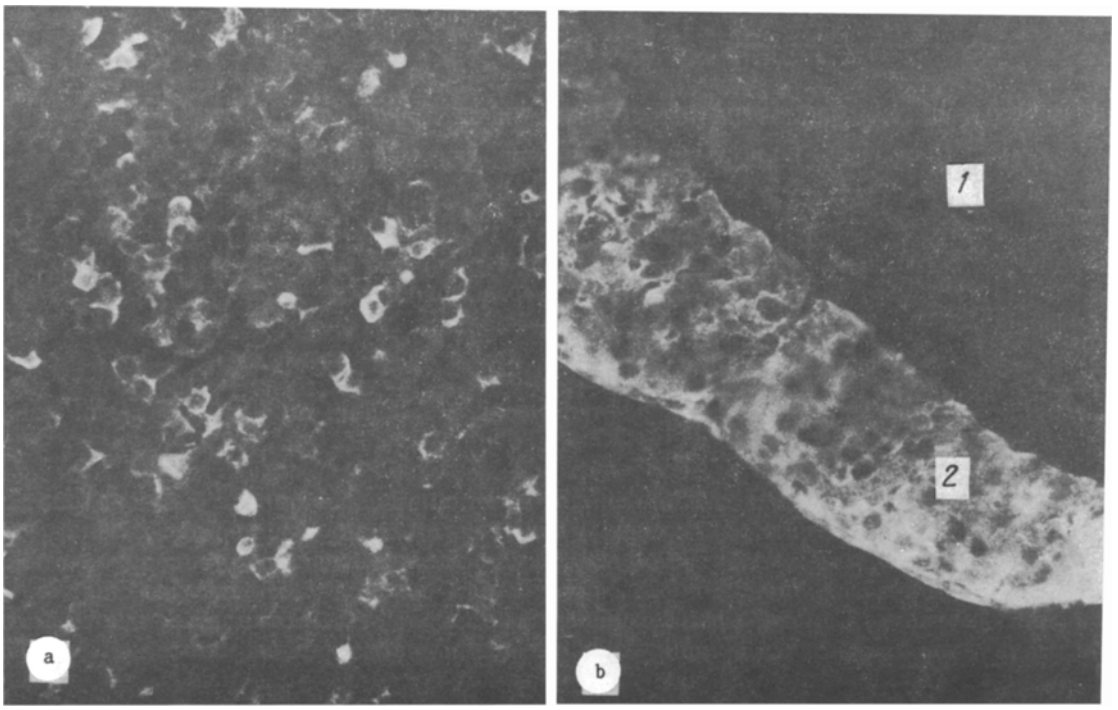

Fig. 2. Immunohistochemical localization of α-endorphin in rat pituitary: a) anterior lobe, b) posterior lobe (1) and intermediate lobe (2) of pituitary. 400 ×.

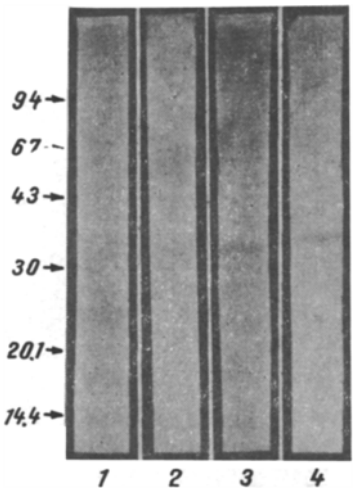

Fig. 3. Identification of POMC in pituitary extracts by immunoblotting method. After electrophoretic transport of proteins from PAG plates to nitrocellulose paper, bands corresponding to electrophoretograms were incubated with culture medium of hybridomas E11 (1), A8 (2), F8 (3), and H5 (4), and then with sheep antibodies to mouse IgG, conjugated with peroxide. Peroxidase activity detected with the aid of diaminobenzidine. Phosphorylase (mol. wt. 94,000), bovine serum albumin (mol. wt. 67,000), ovalbumin (mol. wt. 43,000), carbonic anhydrase (mol. wt. 30,000), trypsin inhibitor (mol. wt. 20,100), and β-lactalbumin (mol. wt. 14,400) were used as molecular weight markers.

the pituitary [1, 2, 11]. Antibodies of one of the hybridomas (E11) which exhibited maximal sensitivity in radioimmunoassay, were tested for their ability to detect endorphins immunocytochemically. Antibodies of hybridoma E11 were highly effective for immunofluorescence detection of pituitary endorphin-producing cells. Specific fluorescence was observed in the anterior and intermediate lobes of the pituitary (Fig. 2); the intensity of fluorescence in the

intermediate lobe, moreover, was several times higher than in the anterior lobe. In the posterior lobe of the pituitary, where there are no endorphin-producing cells, no fluorescence was observed (Fig. 2).

It was demonstrated previously [13] that a high-molecular-weight precursor of the endorphins, namely pro-opiomelanocortin (POMC, mol. wt. = 31,000) and its processing product β-lipotrophin (β-LPT, mol. wt. = 10,000) can be detected by immunoblotting with the aid of monoclonal antibodies to β-endorphin in extracts of hog pituitary gland. We determined the ability of monoclonal antibodies to α-endorphin to detect high-molecular-weight precursors of endorphins in extracts of bovine pituitary gland by the immunoblotting method. Antibodies of hybridomas F8 and H5 (Fig. 3) were found to be most able to detect POMC, whereas antibodies of hybridomas E11 and A8 were virtually ineffective. In no case were bands discovered with mol. wt. below 30,000, i.e., β-LPT and α-endorphin cannot be detected by the immunoblotting method. Absence of the α-endorphin band was evidently due to the low affinity of the nitrocellulose paper for peptides. For example, β-endorphin is not detected by the immunoblotting method no matter whether monoclonal [13] or polyclonal [4] antibodies to this peptide are used. Absence of the β-LPT band may be associated with specificity of the monoclonal antibodies for α-endorphin. For instance, according to data in [12], monoclonal antibodies to peptide Tyr—Gly—Gly—Phe, whose amino acid sequence occurs in the composition of all opioid peptides, virtually did not interact with β-LPT during radioimmunoassay, although they bound with β-endorphin, enkephalins, and $\beta\text{-LPT}^{60-65}$.

The monoclonal antibodies obtained to α-endorphin can thus be used for immunofluorescence identification of endorphin-producing cells in histochemical investigations, and also for the identification of POMC in extracts of endorphin-synthesizing tissues. The use of monoclonal antibodies to α-endorphin in order to prepare affinity immunosorbents for isolation of α-endorphin from some extracts and culture fluids also appears promising.

LITERATURE CITED

1. E. V. Golanov, The Present State of the Problem of Endogenous Morphine-like Substances [in Russian], Moscow (1986).
2. A. D. Dmitriev, Progress in Science and Technology. Series: Pharmacology, Chemotherapeutic Substances, Vol. 13, Opioid Peptides and Their Receptors [in Russian], Moscow (1982), pp. 7-49.
3. A. D. Dmitriev, A. V. Tennov, et al., Neirokhimiya, 4, No. 8, 48 (1985).
4. A. V. Tennov, A. D. Dmitriev, et al., Byull. Éksp. Biol. Med., 100, No. 8, 181 (1985).
5. R. M. Bassiri and R. D. Utiger, Endocrinology, 90, 722 (1972).
6. A. C. Cuello, C. Milstein, et al., J. Histochem. Cytochem., 32, 947 (1984).
7. W. G. Forssman, V. M. Pickel, et al., Techniques in Neuroanatomical Research, Berlin (1981), pp. 171-205.
8. G. Köhler and C. Milstein, Nature, 256, 495 (1975).
9. U. K. Laemmli, Nature, 227, 680 (1971).
10. R. D. Lane, J. Immunol. Meth., 81, 223 (1985).
11. N. Ling, R. Burgus, and R. Guillemin, Proc. Natl. Acad. Sci. USA, 73, 3492 (1976).
12. T. Meo, C. Gramsch, R. Inan, et al., Proc. Natl. Acad. Sci. USA, 80, 4084 (1983).
13. H. Thorpe, L. Spitz, M. Spitz, and B. M. Austen, FEBS Lett., 151, 105 (1983).
14. H. Towbin, T. Staehelin, and J. Gordon, Proc. Natl. Acad. Sci. USA, 76, 4350 (1979).
15. A. White, C. Cray, and J. Ratcliff, J. Immunol. Meth., 179, 185 (1985).