IDENTIFICATION OF PRO-OPIOMELANOCORTIN AND SECRETION OF ITS PEPTIDE FRAGMENTS IN BOVINE ADRENALS

A. V. Tennov, A. D. Dmitriev,
E. A. Kizim, and E. E. Ustinova

UDC 612.451/.453 : 612.018

KEY WORDS: pro-opiomelanocortin; endorphins; immunoblotting; radioimmunoassay; adrenals.

Corticosteroid production in mammalian adrenals is controlled by the hypothalamo-hypophyseal system. Important elements of this system were pro-opiomelanocortin (POMC), a pituitary polypeptide and precursor of ACTH, and several other hormones (α-, β-, and γ- melanocyte-stimulating hormones, β- and γ-lipotrophins), and α-, β-, and γ-endorphins [3, 11]. Peptide hormones contained in the structure of POMC are considered to be purely pituitary in origin, but it has recently been found that some POMC fragments are present in organs such as the hypothalamus, thyroid gland, lungs, gastrointestinal tract, reproductive organs, and so on [2, 8, 9, 12, 13]. Despite the widespread occurrence of peptide fragments of POMC in the tissues of certain endocrine glands and other organs, it seems unlikely that the POMC gene could be expressed in the adrenals, for the adrenals are the target organ for pituitary ACTH. The absence of peptide fragments of POMC, such as β-endorphin, for example [6], has been definitely observed in a number of investigations.

Meanwhile facts have been discovered to contradict the view that pituitary ACTH plays an exclusive role in the regulation of corticosteroid production in the adrenals, and pointing indirectly to the possibility of formation of POMC and its biologically active fragments in these glands. For example, it has been shown that after destruction of the suprachiasmatic nuclei of the rat hypothalamus the blood ACTH level falls, and this is accompanied by complete disappearance of the circadian rhythm of fluctuations in the blood ACTH level, whereas the plasma corticosterone concentration is virtually unchanged, and the amplitude and frequency of circadian fluctuations in the plasma corticosterone level are indistinguishable from normal [14]. It has also been found that hypophysectomy causes no changes in the circadian rhythm of cyclic nucleotide production in cells of the adrenal cortex [5]. It has also been reported that some peptide fragments of POMC (β-endorphin, ACTH, and α-melanotrophin) [4] have been found in extracts of human adrenal tissues post mortem. However, the authors cited did not identify POMC itself in the adrenal tissues and were unable to demonstrate the presence of its fragments in adrenals of other species of mammals (ox, sheep, rabbit, rat, dog, or guinea pig).

This paper describes the results of an investigation to show that biosynthesis of POMC, its proteolytic processing, and secretion of the peptide products of that processing take place in the bovine adrenals.

EXPERIMENTAL METHOD

The method of obtaining rabbit antisera against endorphins and of using them for radioimmunoassay of these peptides was described previously [1]. Antisera against β-lipotrophin were obtained in the same way (bovine β-lipotrophin was generously presented by Professor Yu. A. Pankov, Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Medical Sciences of the USSR). ^{125}I-Labeled peptides were obtained by the chloramine method [1] and purified from free ^{125}I on Sephadex G-10 (0.7 × 5 cm, centrifugation for 10 min at 1500 g). To obtain acid extracts the minced tissue was heated for 20 min at 95°C in 7 volumes of 1 M CH_3COOH, pH 2.0 (HCl), then homogenized and treated with an equal volume of redistilled chloroform. The mixture was centrifuged for 40 min at 15,000 g, and the aqueous phase was lyophilized, redissolved in RIA buffer (0.05 M Na phosphate, pH 7.5, 0.15 M NaCl, 0.2% bovine serum albumin, 0.02% NaN_3), and neutralized with 1 M NaOH to pH 7.0. To obtain extracts for immunoblotting the tissue was homogenized in 5

Laboratory of Biochemistry, Institute of Clinical Psychiatry, All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR. Laboratory of Pathophysiology of the Heart, Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 8, pp. 181-184, August, 1985. Original article submitted July 18, 1984.

0007-4888/85/0008-1055 $09.50

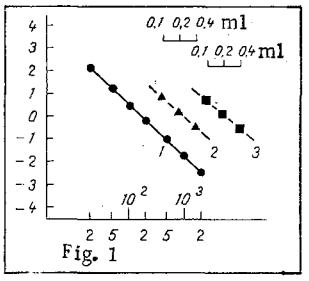

Fig. 1

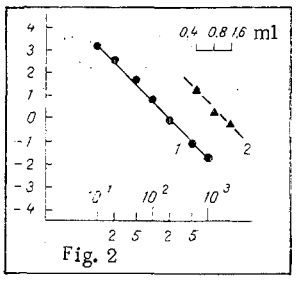

Fig. 2

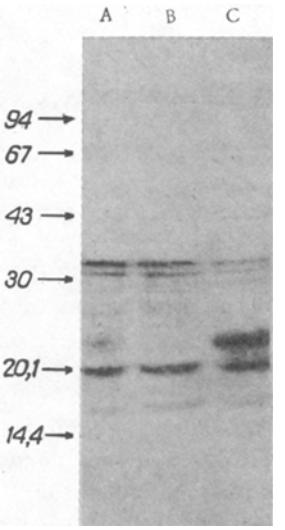

Fig. 3

Fig. 1. Displacement of ^{125}I-α-endorphin from complexes with specific antibodies by α-endorphin and by extracts of the bovine adrenal cortex and medulla: 1) α-endorphin; 2) adrenal cortex, 3) adrenal medulla.

Fig. 2. Displacement of ^{125}I-β-endorphin from complexes with specific antibodies by β-endorphin and by perfusate of bovine adrenals. Retrograde continuous perfusion through central adrenal vein with 60 ml of Locke's solution, containing 0.2% bovine serum albumin, at rate of 4.0 ml/min for 1 h. 1) β-Endorphin; 2) adrenal perfusate. Remainder of legend as to Fig. 1.

Fig. 3. Identification of POMC and its fragments in extracts of bovine pituitary and adrenal medulla and cortex. After fractionation in 16% polacrylamide gel, proteins were electrophoretically transferred onto nitrocellulose leaves and were subsequently incubated with antiserum to β-LPT and covalently bonded with horseradish peroxidase to Ig G rabbit antibodies. We induced peroxidase activity with the help of diaminobenzidene. In the role of molecular mass indicators we used the following proteins – phosphorylase (94 kD), bovine serum albumin (67 kD), egg albumin (43 kD), carbohydrase (30 kD), trypsin inhibitor (20 kD), and α-lactoalbumin (14.4 kD). a) Adrenal medulla; b) adrenal cortex; c) hypophysis.

volumes of 0.05 M Tris-HCl buffer, pH 6.8, at 0°C, heated for 5 min at 100°C in the presence of 5% sodium dodecylsulfate and 1% 2-mercaptoethanol, centrifuged for 60 min at 50,000 g and 20°C, and the supernatants were dialyzed against buffer consisting of 0.05 M Tris-HCl, pH 6.8, 0.1% sodium dodecylsulfate, and 0.1% 2-mercaptoethanol.

Retrograde perfusion of the bovine adrenals was carried out through the central adrenal vein at 30°C, and at the rate of 4.0 ml/min, with Locke's solution (154 mM NaCl, 5.6 mM KCl, 2.2 mM $CaCl_2$, 1 mM $MgCl_2$, 6 mM $NaHCO_3$ and 10 mM glucose), saturated with O_2/CO_2 (19 : 1) mixture. The perfusates were collected in flasks with 1/50 of their volume of 10 M HCl (final concentration 0.2 M), with constant mixing. After perfusion the samples were heated for 20 min to 90°C, frozen, and lyophilized. The lyophilized samples were redissolved in 1/15 volume of 0.005 M sodium phosphate, pH 7.5 with 0.02% NaN_3, neutralized with 1M NaOH to pH 7.0, and used for radioimmunoassy of peptides.

Electrophoresis of proteins was carried out in plates of 16% polyacrylamide gel [7]. Antiserum against β-lipotrophin and donkey antiserum against rabbit IgG, covalently bound with horseradish peroxidase, were used for immunoblotting by the method in [15].

TABLE 1. Content (in picomoles/g tissue) of Immunoreactive α-, β-, and γ-Endorphins (α-E, β-E, and γ-E) and of β-LPT in Bovine Adrenal Medulla and Cortex (M±m)

Test object	α-E	β-E	γ-E	β-LPT
Adrenal medulla ($n=5$)	2,13±0,22	0,41±0,03	0,07±0,01	1,04±0,30
Adrenal cortex ($n=3$)	3,42±0,49	0,35±0,07	0,10±0,01	0,64±0,31

EXPERIMENTAL RESULTS

When studying biosynthesis of POMC in the adrenals, as peptide markers of POMC we used its C-terminal fragments: α-, β-, and γ-endorphins and β-lipotrophin (β-LPT). Concentrations of immunoreactive endorphins and β-LPT in extracts of the bovine adrenal cortex and medulla showed that these structures contain appreciable quantities of endorphins and lipotrophin; increasing quantities of extracts displaced labeled peptides from complexes with specific antibodies, moreover, in the same way as did increasing quantities of α-, β-, and γ-endorphins and of β-LPT. This points to the close kinship or complete identity of the corresponding peptides and immunoreactive compounds contained in the tissue extracts. As an illustration, data obtained when determining the content of α-endorphin in extracts from the adrenal medulla and cortex are illustrated in Fig. 1. The quantitative results of estimation of endorphins and β-LPT in the bovine adrenal medulla and cortex are given in Table 1.

To detect secretion of peptide fragments of POMC in the adrenals experiments were undertaken to determine the β-endorphin content in perfusates obtained during retrograde perfusion of the bovine adrenals. It was found that immunoreactive compounds, indistinguishable in their immunochemical properties from β-endorphin, are present in the perfusates, just as in the tissue extracts. The concentration of immunoreactive β-endorphin in the perfusates was 1.4±0.4 femtomole/ml (M±m, n = 7).

To identify POMC itself in the adrenal tissue, a combination of techniques of electrophoretic protein fractionation based on molecular weight [7] and immunoblotting [15] was used. POMC and its fragments were detected with the aid of antiserum against β-LPT. For comparison, a protein extract of bovine pituitary gland, known to contain nonglycosylated and glycosylated forms of POMC [3], was analyzed. These forms of pituitary POMC (mol. wt. 30-35 kilodaltons) are clearly visible in Fig. 3 (band C). Similar forms of high-molecular-weight polypeptides (30-35 kilodaltons) also were found in extracts of bovine adrenal medulla and cortex (Fig. 3, bands A and B), so that it can be definitely concluded that endogenous POMC is present in bovine adrenal glands. Incidentally, similar results were obtained during analysis of tissue extracts from rat adrenals (data not given).

After fractionation in 16% polyacrylamide gel plates, proteins were transferred electrophoretically from the gels to sheets of nitrocellulose and then incubated with antiserum against β-LPT and with donkey antibodies against rabbit IgG covalently bound with horseradish peroxidase. Peroxidase activity was demonstrated with the aid of diaminobenzidine. The following proteins were used as markers of molecular weight (in kilodaltons): phosphorylase (94), bovine serum albumin (67), ovalbumin (43), carbonic anhydrase (30), trypsin inhibitor (20), and α-lactalbumin (14.4). It can thus be concluded from these results that the POMC gene is expressed in the medulla and cortex of the bovine adrenal gland, and also that as a result of processing of POMC in the adrenal cells, appreciable quantities of its peptide fragments are formed and secreted. Since under these circumstances high local concentrations of POMC fragments must be observed, it can also be postulated that pathways of regulation of adrenal function independent of the pituitary, involving the participation of peptide fragments of POMC, formed in the adrenal, must exist.

LITERATURE CITED

1. A. D. Dmitriev, Yu. I. Golikova, A. G. Kobylyanskii, et al., Neirokhimiya, No. 1, 66 (1982).
2. J. A. Clements, J. W. Funder, K. Tracy, et al., Endocrinology, 111, 2097 (1982).
3. B. A. Eipper and R. E. Mains, Endocrinol. Rev., 1, 1 (1980).
4. C. J. Evans, E. Erdelyi, E. Weber, et al., Science, 221, 957 (1983).
5. J. Guillement, S. Guillement, and A. Rienberg, Experientia, 36, 367 (1980).
6. V. Höllt and M. Bergmann, Neuropharmacology, 21, 147 (1982).
7. U. K. Laemmli, Nature, 227, 680 (1970).
8. A.S. Liotta, D. Gildersleeve, M. N. Brownstein, et al., Proc. Natl. Acad. Sci. USA, 76, 1448 (1979).
9. A. Liotin, R. Houghten, and D. T. Krieger, Nature, 295, 593 (1982).
10. A. N. Margioris, A. S. Liotta, H. Vaudry, et al., Endocrinology, 113, 663 (1983).
11. S. Nakanishi, I. Akira, T. Kita, et al., Nature, 278, 423 (1979).
12. E. S. Orwoll and J. W. Kendall, Endocrinology, 107, 438 (1980).
13. E. M. Smith, W. J. Meyer, and J. E. Blalock, Science, 218, 1311 (1982).
14. A. Szafarczyk, G. Ixart, G. Alonso, et al., J. Steroid Biochem., 19, 1009 (1983).
15. H. Towbin, T. Staehelin, and J. Gordon, Proc. Natl. Acad. Sci. USA, 76, 4350 (1979).

FORMATION OF COMPLEXES OF α-TOCOPHEROL WITH PHOSPHATIDIC ACID

A. N. Erin, V. I. Skrypin, L. L. Prilipko, and V. E. Kagan

UDC 612.397.23.015.6:577.161.3

KEY WORDS: α-tocopherol; phosphatidic acid; complex formation.

Activation of phospholipases in the animal cell is an essential step, under certain conditions, in the course of several metabolic and functional processes [1]. In particular, phospholipase D takes part in the synthesis of ceramines – an important component of brain lipids [8, 5]. Meanwhile activation of phospholipases means not only guaranteeing the course of essential processes, but also modification of some of the physicochemical parameters of biological membranes, including brain synaptic membranes [5]. This state of affairs explains why protective mechanisms against the harmful action of phospholipases are essential in order to maintain cellular homeostasis.

It has recently been shown that α-tocopherol stabilizes synaptosomal membranes against the harmful action of phospholipase A_2 [5]. This stabilizing effect is due to the ability of α-tocopherol to form complexes with free fatty acids (FFA) [4, 7]. Investigation of the nature of complexes of α-tocopherol with FFA has shown that this process takes place through two types of interaction: polar interaction between the OH-group of the chromane ring of α-tocopherol and the carboxyl group of the fatty acid and hydrophobic interaction of cis-unsaturated double bonds of the fatty acid with methyl groups of the chromane ring of α-tocopherol [2].

Since complexes of this kind can potentially be formed with a fairly wide class of compounds [3, 6], the possibility of formation of complexes of α-tocopherol with phosphatidic acid (PA), the principal hydrolysis product of phospholipids by phospholipase D, was investigated.

EXPERIMENTAL METHOD

α-Tocopherol was obtained from Serva (West Germany), PA was obtained by phospholipid hydrolysis of ovolecithin (PAL), and dimyristoylphosphatidic acid (DMPA) were from Koch-Light (England), and deuterated chloroform from Merck (West Germany). Heptane was purified by redistillation.

All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Snezhnevskii.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 8, pp. 184-186, August, 1985. Original article submitted November 19, 1984.

0007-4888/85/0008-1058 $09.50