BIOLOGY OF REPRODUCTION 27, 755–764 (1982)

β-Endorphin is Present in the Male Reproductive Tract of Five Species[1]

TSONG SHU-DONG,[2,3] DAVID M. PHILLIPS,[3] NICHOLAS HALMI,[5] DOROTHY KRIEGER[4] and C. WAYNE BARDIN[3,6]

The Population Council[3]
New York, New York 10021
and
Departments of Medicine[4] *and Anatomy*[5]
Mt. Sinai School of Medicine
New York, New York 10029

ABSTRACT

Previous studies from this laboratory have demonstrated immunostainable β-endorphin-like material (β-EP) in Leydig cells and epithelia of the epididymis, seminal vesicle and vas deferens of the rat. These observations would be strengthened if it could be demonstrated that they were not a peculiarity of the rat. Accordingly, we now present immunocytochemical evidence for the presence of β-EP in the Leydig cells of mouse, hamster, guinea pig and rabbit. No immunoreactive material was identified in Sertoli, myoid, endothelial or germ cells of any of the species examined. Immunostainable β-EP was also demonstrated in the epididymides of mouse, guinea pig, rabbit, and rat, but not hamster. Immunostainable material was also present in the epithelia of the vas deferens and seminal vesicles of mouse and rat, the only two species thus far examined. Since β-EP was present in Leydig cells, we wondered whether this peptide could be identified in other steroid-producing tissues. When rat ovaries and adrenals were reacted wtih anti-β endorphin, staining was demonstrated in corpus luteum and adrenal cortex. No staining was observed in the adrenal medulla or other portions of the ovary. In order to determine whether the β-EP detected in the testis and epididymis was derived from a pituitary source, animals were hypophysectomized and tissues examined 2 weeks later. Both the Leydig cells and the epididymal epithelium remained immunostainable. In summary, immunostainable β-EP has been identified in Leydig cells of five species. Stainable material is also present in the epithelium of other portions of the male reproductive tract and in steroid-secreting cells of the ovary and the adrenal. Such β-EP may have a paracrine function in the testis and other portions of the male reproductive tract.

INTRODUCTION

β-Endorphin, a peptide which is derived from the processing of its precursor molecule, pro-opiomelanocortin (Mains et al., 1977; Roberts and Herbert, 1977), is present in many tissues. It has been identified in the anterior and intermediate lobes of the pituitary (Li and Chung, 1976), brain (Bradbury et al., 1976, placenta (Liotta and Krieger, 1980), and possibly the pancreas (Bruni et al., 1979). β-Endorphin and other opiates have been implicated in the regulation of reproductive function (Quigley and Yen, 1980; Blankstein et al., 1981). Recently, this peptide has been identified in crude testicular extracts (Sharp et al., 1980) and in human semen (Sharp and Pekary, 1981). Our laboratory confirmed this observation for rabbit semen and initiated a study to determine the localization of this peptide in the male reproductive tract. Immunostainable β-endorphin-like material (β-EP) was identified in Leydig cells and the epithelia of epididymis, seminal vesicles, and vas deferens of the rat (Tsong et al., 1982). Additional studies using standard radioimmunoassay, SDS gel electrophoresis, and high-pressure liquid chromatography identified α-, β- and γ-endorphin along with ACTH-like material in these tissues. Based on these findings, we speculated that β-endorphin and other

Accepted May 17, 1982.
Received February 22, 1982.
[1] This work was supported by NIH Grants HD-13541, NS-02893, AM-30687 and the Lita Annenberg Hazen Charitable Trust.
[2] Dr. Tsong was the recipient of a fellowship from the Rockefeller Foundation. Present address: Dept. of Family Planning, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing, China.
[6] Reprint requests: Dr. C. Wayne Bardin, The Population Council, 1230 York Ave., New York, 10021.

Downloaded from https://academic.oup.com/biolreprod/article/27/3/755/2767437 by guest on 20 August 2022

pro-opiomelanocortin-derived peptides may have a paracrine function in the testis and other portions of the male reproductive tract. This postulate would be strengthened if it could be demonstrated that immunostainable β-EP was not a peculiarity of the rat, in which the initial studies were performed. Accordingly, experiments were initiated to identify this peptide in the testis and male reproductive tract of additional species. This is the subject of the present report.

MATERIALS AND METHODS

Animals

Swiss Webster mice were obtained from Taconic Farms (Germantown, NY). Golden hamsters and New Zealand white rabbits were from Dutchland Laboratory Animals, Inc. (Denver, PA); Hartley Guinea Pigs, intact adult rats (200–300 g, 3–4 months of age) and hypophysectomized rats (90 days of age) were from Charles River Breeding Lab. (Wilmington, MA).

Tissue Preparation

Tissues were fixed in freshly prepared Bouin's solution at 4°C for 6 h, cut into small pieces, and fixed for an additional 12 h in the same solution. The blocks were then washed, dehydrated in ethanol, and embedded in paraplast (Sherwood Medical Ind., St. Louis, MO). Sections 7–10 μm were cut and mounted on glass slides.

Antisera

One β-endorphin antiserum was raised against camel β-endorphin. The structure of this peptide is identical with that of β-endorphin from the rat. In radioimmunoassay (RIA) this antiserum cross-reacts on a nearly equimolar basis with β-lipotropin (LPH) and the pro-opiomelanocortin molecule, and exhibits no cross-reactivity with α- and γ-endorphin (Tsong, 1982). Antiserum was also prepared against porcine β-endorphin. In RIA this cross-reacts with lesser affinity with β-LPH and the pro-opiomelanocortin molecule. Antiserum against androgen binding protein (ABP) was prepared with the use of a protein isolated from rat epididymides (Musto et al., 1977; Musto et al., 1980). Anti-ABP antibodies were monospecific when assayed by crossed immunoelectrophoresis against both homogeneous ABP and cytosols prepared from normal testes and epididymides (Larrea et al., 1981). Rabbit peroxidase, antiperoxidase (PAP) and goat anti-rabbit immunoglobulin G were purchased from Polyscience Inc. (Warrington, PA). 3,3′-Diaminobenzidine tetrahydrochloride (DAB) was from Sigma Chemical Co. (St. Louis, MO).

Radioimmunoassays

Immunoreactive β-EP was measured in plasma as previously described in our laboratory (Yamaguchi et al., 1980).

Immunoperoxidase Staining

The unlabeled antibody PAP method of Sternberger (1970) was used, with a primary antiserum dilution of 1:300– 1:500. The sites of antigen antibody binding were demonstrated by the peroxidase reaction with a diaminobenzidine/H_2O_2 substrate. The sections were mounted without counterstain for examination by light microscopy.

Controls of the specificity of the immunoperoxidase reactions involved replacement of the primary antiserum with preimmune serum, unrelated hyperimmune serum, antiserum absorbed with excess antigen (both in liquid phase and attached to Sepharose), and replacement of one or more of the reagents of the immunoperoxidase reaction with buffer.

RESULTS

Testis

Immunoreactive β-EP was localized in the Leydig cells of five species. There was strong immunostaining of mouse Leydig cells when sections of testis were incubated with camel β-endorphin antiserum (Fig. 1a). There was effectively no staining in control sections reacted with antisera absorbed with an excess of β-endorphin (Fig. 1b). Figures 1c-1f show immunostaining for β-EP-like material in the Leydig cells of rat, hamster, guinea pig, and rabbit, respectively. Testicular sections reacted with absorbed antisera and hyperimmune sera were nonreactive and comparable to the Leydig cells shown in Fig. 1b. Leydig cells of the rat and mouse were also stained after incubation with anti-porcine β-endorphin; no staining was observed with absorbed antiserum. In intact animals no immunoreactive material was detected in Sertoli, myoid, epithelial or germ cells in any of the species examined.

Epididymis

Immunoreactive β-EP was identified in the epididymides of four species. Immunoreactive material was demonstrated in the caput epididymidis of the mouse (Fig. 2a), and was not seen in the same tissue stained with absorbed antiserum (Fig. 2b). In this portion of the epididymis there was uniform staining of the cytoplasm of the epithelium. By contrast, in the body and cauda epididymides not all cells contained immunoreactive material (Fig. 2c). Figures 2d-2f demonstrate immunoreactive material in the body of the epididymis from rat, guinea pig and rabbit, respectively, following incubation with anti-camel β-endorphin. The patterns of staining in both the body (Figs. 2d-2f) and caput (not shown) of these species

Downloaded from https://academic.oup.com/biolreprod/article/27/3/755/2767437 by guest on 20 August 2022

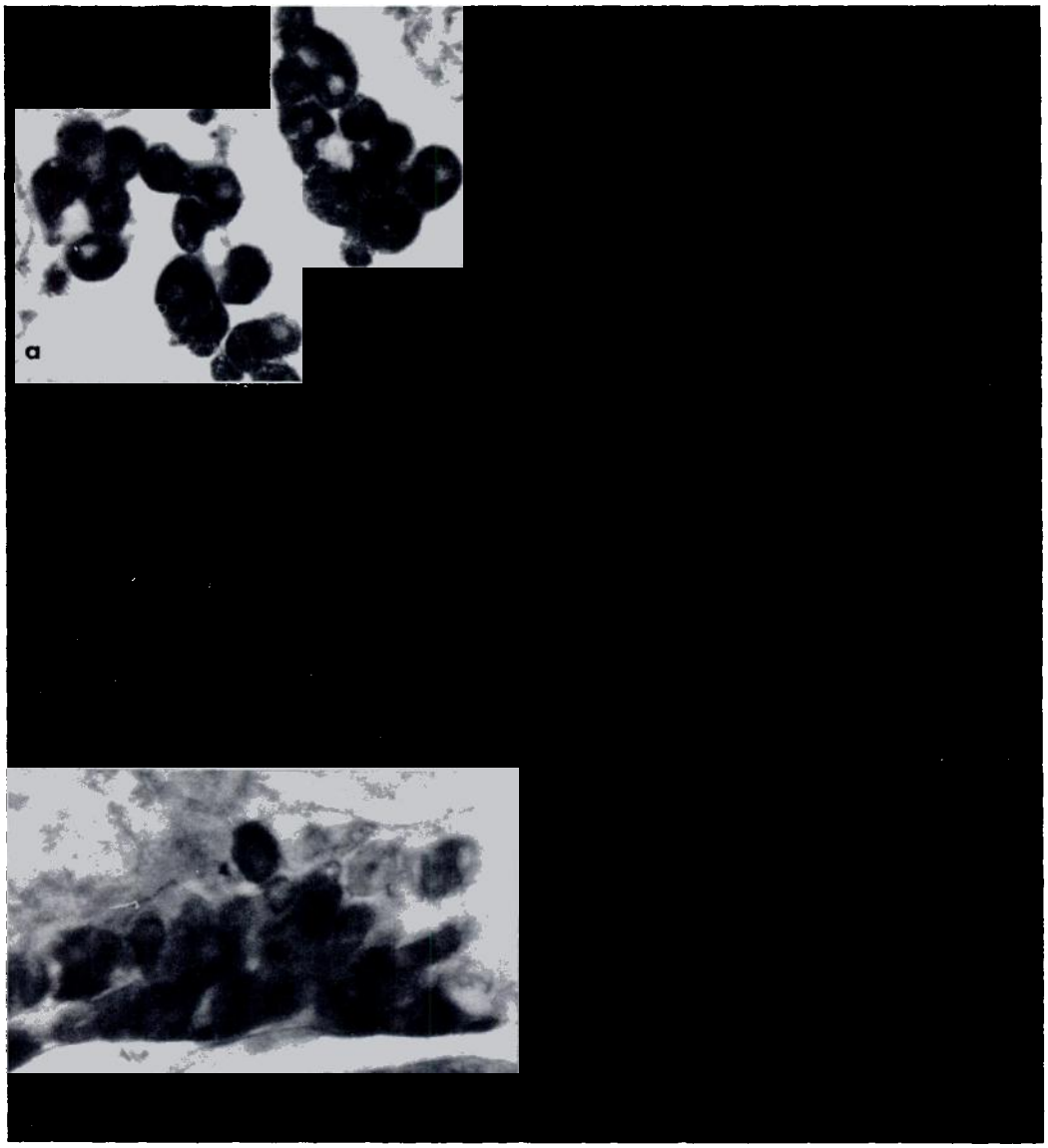

FIG. 1. Immunostainable β-endorphin in Leydig cells of various species demonstrated with anti-camel β-endorphin. *1a*) Mouse Leydig cells showing immunostainable β-endorphin. *1b*) Mouse Leydig cell control in which staining was carried out with antiserum absorbed with β-endorphin. *1c*) Immunostainable β-endorphin in rat Leydig cells. *1d*) Immunostainable β-endorphin in hamster Leydig cells. *1e*) Immunostainable β-endorphin in guinea pig Leydig cells. *1f*) Immunostainable β-endorphin in rabbit Leydig cells. The control sections for the Leydig cells in *1c-1f* prepared with both hyperimmune and absorbed antisera were comparable to *1b*. X291

were similar to that in the mouse. Control sections stained with absorbed or hyperimmune antisera were negative and comparable to that shown in Fig. 2b. Interestingly, no immunostainable β-EP was demonstrated in the epithelium of hamster epididymis, even though the Leydig cells of this species were positive when reacted on the same slide. The epithelia of the epididymides from rat and mouse were also stained with anti-porcine β-EP but not with absorbed antiserum.

Vas Deferens, Seminal Vesicle and Prostate

The epithelium of the vas deferens (Figs. 3a and b) and seminal vesicle in both the rat and the mouse (Figs. 3c and d) were strongly

Downloaded from https://academic.oup.com/biolreprod/article/27/3/755/2767437 by guest on 20 August 2022

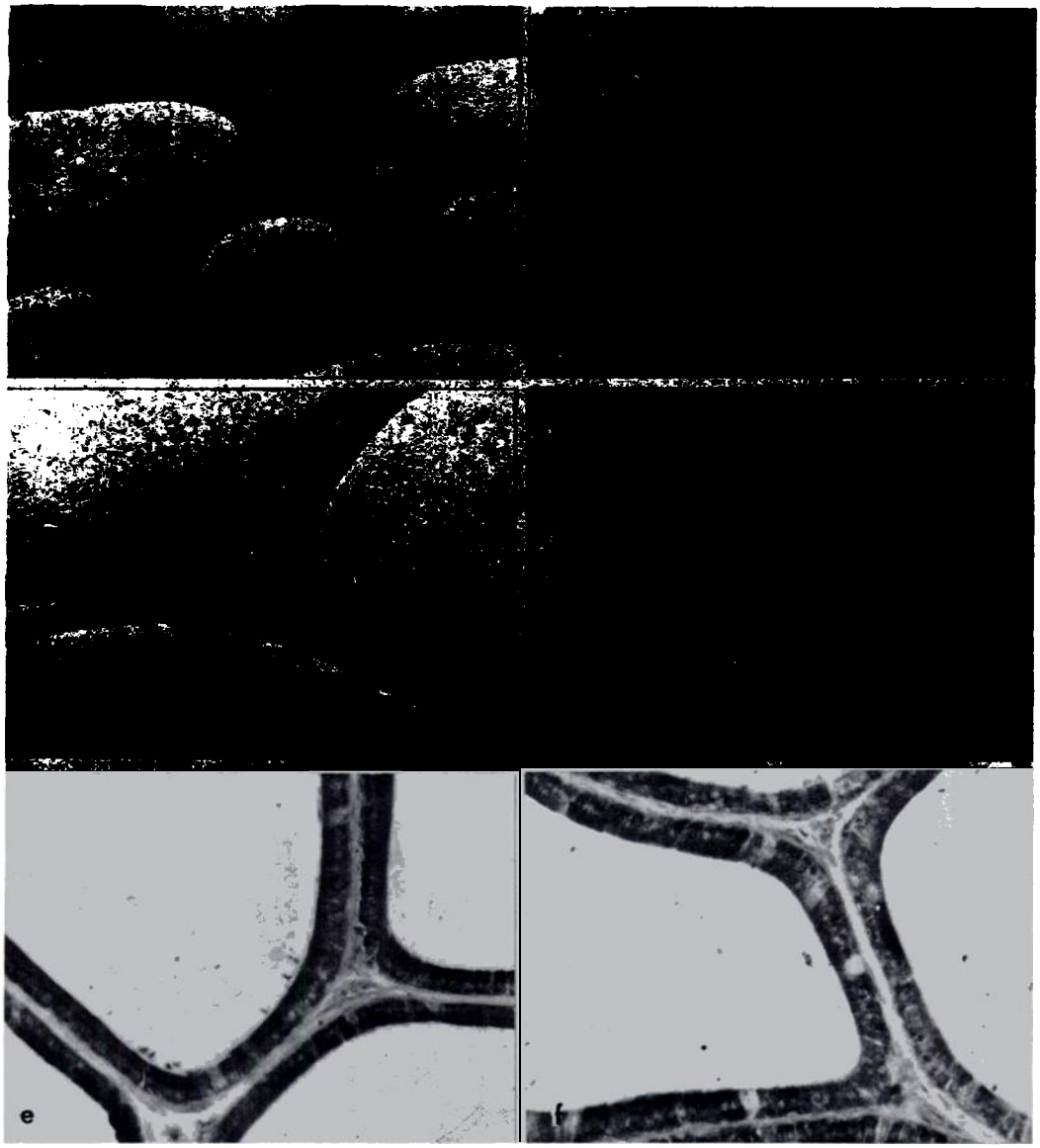

FIG. 2. Immunostainable β-endorphin in the epididymides from several species demonstrated following incubation with camel β-endorphin antiserum. *2a*) Immunostainable β-endorphin in the caput epididymis from the mouse. Uniform staining of all epithelial cells is evident. *2b*) Caput epididymidis of the mouse incubated with antiserum absorbed with β-endorphin. *2c*) Immunostainable β-endorphin in the body of the epididymis from a mouse. Unlike the caput, only selective cells showed a positive reaction, a pattern observed in the epididymides of all species. *2d*) Immunostainable β-endorphin in the body of the epididymis from a rat. *2e*) Immunostainable β-endorphin in the body of the epididymis from a guinea pig. *2f* Immunostainable β-endorphin in the body of the epididymis from a rabbit. The control sections for the epididymides in *2c-2f* prepared with both hyperimmune and absorbed antisera were comparable to *2b*. X121

stained with β-endorphin antisera. The prostate of neither of these rodents was positive. These organs were not examined in the other species. When secretion was retained in the lumen of the seminal vesicle, strong immunostaining was observed in both the rat and the mouse following incubation with anti-β-endorphin (not shown).

Downloaded from https://academic.oup.com/biolreprod/article/27/3/755/2767437 by guest on 20 August 2022

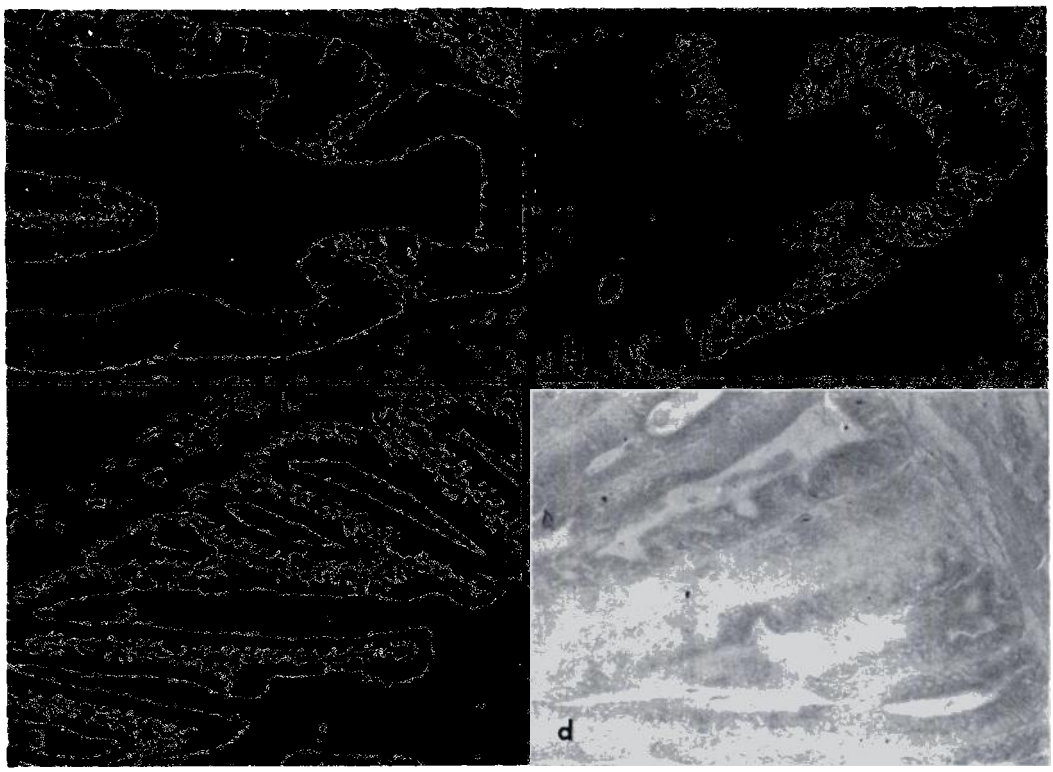

FIG. 3. *3a*) Immunostainable β-endorphin in the epithelium of the vas deferens. *3b*) Vas deferens control. *3c*) Immunostainable β-endorphin in the epithelium of the seminal vesicle. *3d*) Seminal vesicle, control. X73

Ovary and Adrenal

Since immunoassayable β-EP has been demonstrated in human placenta (Liotta and Krieger, 1980) and immunostainable β-EP was identified in Leydig cells, we wondered whether these peptides could be identified in other steroid-producing tissues. Accordingly, ovary and adrenal were examined. Immunostainable β-EP was found in the corpus luteum of the rat ovary (Figs. 4a and b). Similar staining was demonstrated in the mouse ovary (not shown). The mouse adrenal cortex also contained immunostainable β-EP (Figs. 4c and d). The zona reticularis showed a stronger reaction than the zona glomerulosa. Comparable staining was also observed in the rat adrenal cortex (not shown). The adrenal medulla of neither species reacted.

The immunostaining of the rat corpus luteum as compared to that of the oviducts is shown in Figs. 4e and f. Specific immunostaining was demonstrated in the epithelium of the oviduct, but the reaction was weak compared with staining of the corpus luteum of the same slide (Figs. 4e and f) and of the epithelium in the vas deferens (Figs. 3e and f).

Effects of Hypophysectomy on the Immunostainable β-EP in Rat Testis and Epididymis

In order to determine whether the β-EP in the testis and epididymis could arise from pituitary derived material, animals were hypophysectomized and tisssues examined 2 weeks later. At this time, testicular and epididymal weights were 30% and 50% of intact controls, respectively; serum testosterone levels were undetectable. In addition, serum β-EP concentrations were also undetectable [(immunoreactive β-EP: normal, 149 ± 21 pg/ml ± SEM (n=18); 2 week hypox, <10 pg/ml (n=16)]. The Leydig cells of such rats were nevertheless immunostainable with anti-β-endorphin antisera (Figs. 5a and b). Unexpectedly, some germ cells in the degenerating seminiferous tubular epithelium were also immunoreactive. The epithelium of the epididymis from hypophysectomized rats also remained immunostainable

Downloaded from https://academic.oup.com/biolreprod/article/27/3/755/2767437 by guest on 20 August 2022

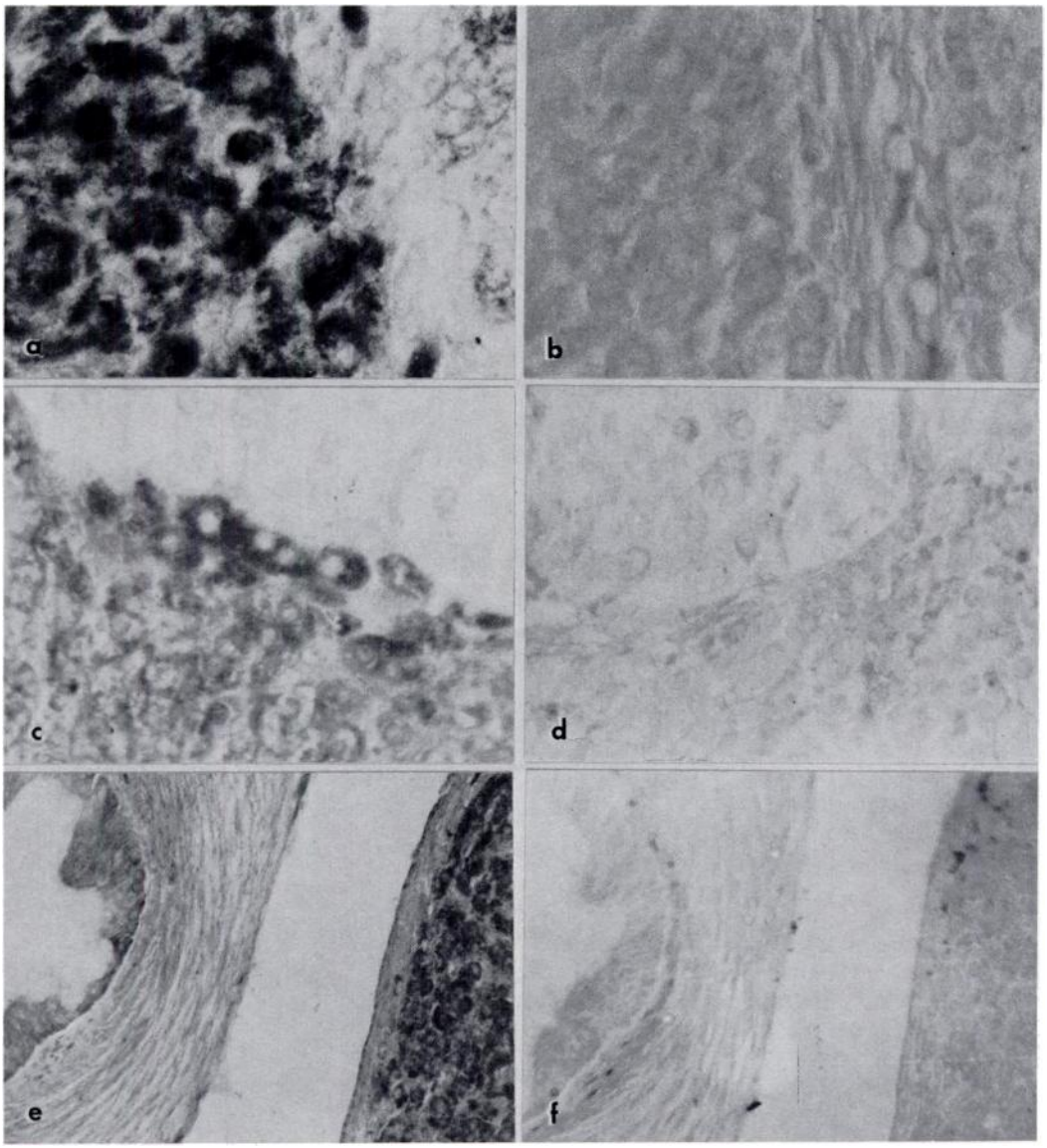

FIG. 4. Immunostainable β-endorphin in the corpus luteum, adrenal cortex and oviduct. Immunostainable material was demonstrated following incubation with camel β-endorphin antiserum, as described in *Materials and Methods*. The control sections were incubated with the same antiserum after it had been absorbed with β-endorphin. *4a*) Immunostainable β-endorphin in the corpus luteum of the rat ovary. The theca interna and externa in the right hand portion of the section are poorly stained. *4b*) Ovary control. *4c*) Immunostainable β-endorphin in the mouse adrenal cortex (*lower portion of the section*). The medulla, (*top of the section*), is unstained. *4d*) Mouse adrenal cortex and medulla, control. *4e*) Corpus luteum (*right*) and oviduct (*left*) of the rat. The epithelium of the oviduct shows moderate immunostaining compared to the corpus luteum, which was heavily stained. *4f*) Rat ovary and oviduct, control. Figs. 4a-d, ×400; Figs. 4e-f ×73

(Figs. 5c and d). It was not possible from these studies to determine whether there was a change in the tissue concentration of this immunoreactive material; however, independent assessment by radioimmunoassay suggests that testicular and epididymal concentrations of β-EP do not decline following hypophysectomy even though the total contents of both organs are reduced in parallel with weight (Tsong et al., 1982).

Downloaded from https://academic.oup.com/biolreprod/article/27/3/755/2767437 by guest on 20 August 2022

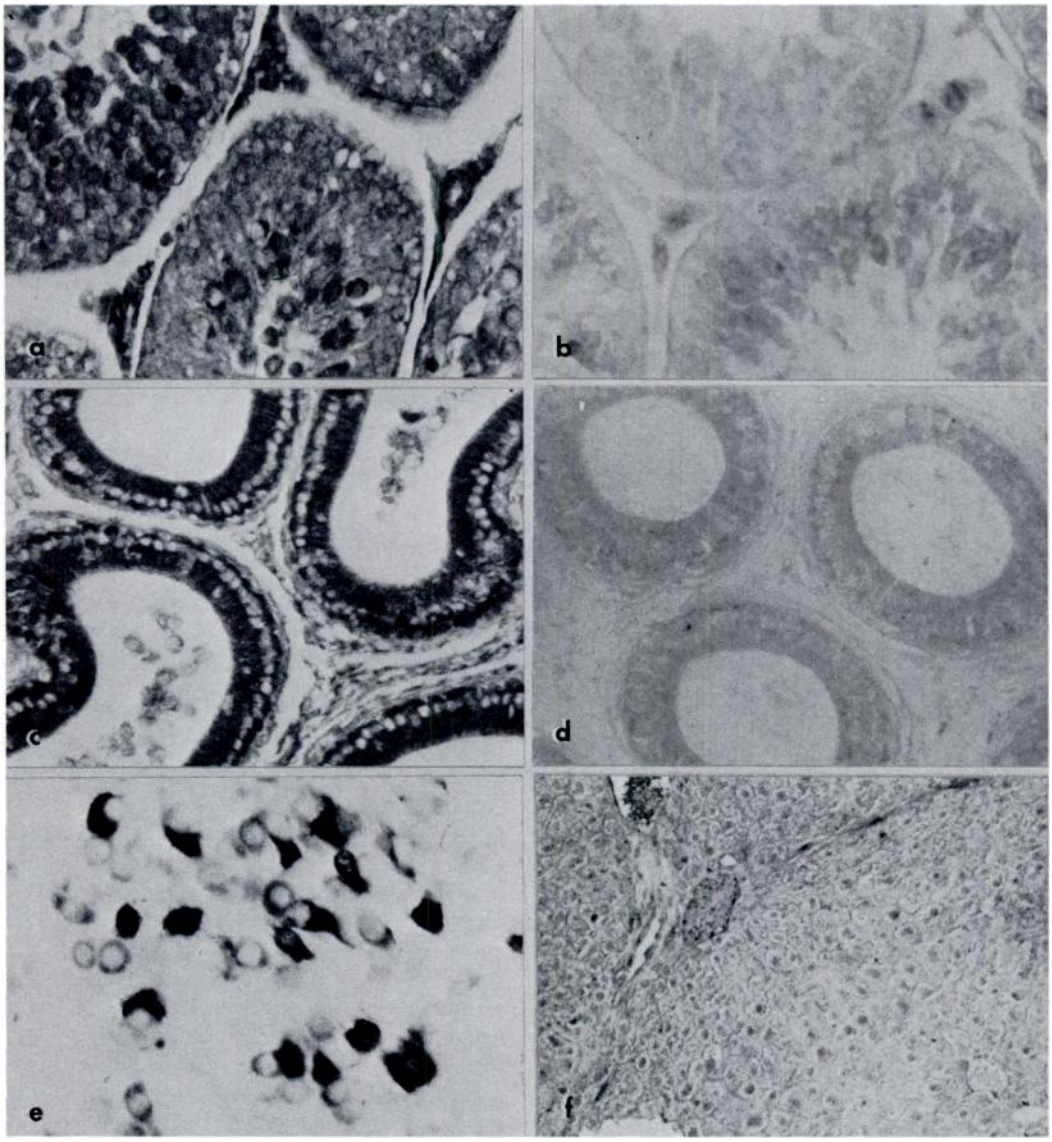

FIG.5. *5a*) Immunostainable β-endorphin in the testis from a rat 2 weeks after hypophysectomy. Leydig cells and some degenerating germ cells adjacent to the seminiferous tubular lumen are strongly reactive. *5b*) Testis from a hypophysectomized rat, control incubated with absorbed antiserum. *5c*) Immunostainable β-endorphin in the caput epididymides from a rat 2 weeks after hypophysectomy. The immunoreactive material is similar to that of the intact animal. *5d*) Caput epididymides from a rat 2 weeks after hypophysectomy, control. *5e*) Immunostainable β-endorphin in the ACTH cells of the anterior pituitary of the rat (mouse). Growth hormone cells were also weakly immunoreactive. *5f*) Rat liver showing no immunoreactive material following incubation with β-endorphin antiserum. Figs. 5a-d X125; Fig. 5e X600; Fig. 5f X73

Tissue Controls

Figure 5e shows the immunostaining of rat pituitary. As expected, with camel β-endorphin antiserum the ACTH cells were strongly positive and growth hormone cells were weakly positive (Halmi and Krieger, 1982). Tissues such as liver (Fig. 5f), muscle, and connective tissue were immunonegative. Anti-rat ABP was used as a positive control for the testes and epididymides from rat and mouse. This antibody stained Sertoli cells, but not Leydig cells. In the epididymis, ABP antiserum stained primarily the

Downloaded from https://academic.oup.com/biolreprod/article/27/3/755/2767437 by guest on 20 August 2022

epithelium in the proximal portion of the caput with the body and cauda showing little, if any, immunoreactive material (Pelliniemi et al., 1981; Attramadal et al., 1981). This pattern of epididymal staining is markedly different from that observed with the β-endorphin antisera.

DISCUSSION

It is now recognized that a given peptide may be present in multiple tissues (Krieger and Martin, 1981). ACTH and β-endorphin, which are derived from the precursor molecule, pro-opiomelanocortin (Mains et al., 1977; Roberts and Herbert, 1977), are present in the pituitary, brain, placenta and pancreas (Li and Chung, 1976; Bradbury et al., 1976; Liotta and Krieger, 1980; Bruni et al., 1979). Evidence for synthesis of pro-opiomelanocortin has been presented for pituitary, brain (Liotta et al., 1979; Liotta et al., 1980) and placenta (Liotta and Krieger, 1980). In addition to their numerous other postulated functions (Krieger and Martin, 1981), β-endorphin and other opiates have been implicated in the regulation of reproductive functions (Quigley and Yen, 1980; Cicero et al., 1976). It has been suggested that such actions are mediated via the central nervous system (Rotsztein et al., 1978). By contrast, the effect of ACTH in decreasing the secretion of testosterone in males may occur via a direct action on the testis since it is not associated with a decline in luteinizing hormone concentrations (Rivarola et al., 1966; Beitins et al., 1973; Cowley et al., 1976). Current observations indicate that there may be multiple sites of action of a given peptide, such as is the case for luteinizing hormone releasing hormone, which acts on the brain, pituitary, gonads and portions of the reproductive tract such as uterus, prostate and seminal vesicles (Hsueh and Jones, 1981).

Following the identification of immunoreactive β-endorphin in extracts of rat testes (Sharp et al., 1980) and in human semen (Sharp and Pekary, 1981), we initiated studies to determine the localization of this and related peptides in the rat male reproductive tract. Immunostainable ACTH and β-endorphin-like materials were identified in Leydig cells and epithelia of the epididymis, seminal vesicles and vas deferens. In addition, characterization of immunoassayable peptide was performed on extracts of these tissues. Immunoreactive α-, β- and γ-endorphin, along with N- and C-terminal immunoreactive ACTH, were identified in testis, seminal vesicles, epididymis and prostate, with concentrations within the range of those previously reported for brain (Tsong et al., 1982). The present report extends our previous immunocytochemical observations in the rat to additional species. Immunostainable β-endorphin has been identified in the Leydig cells of five species and in the epididymis of four.

The unexpected finding of ACTH- and β-endorphin-like material in the male reproductive tract raises questions as to the origin of these peptides. It was considered possible that they could be from the pituitary. If this were the case, then the testis and the epididymis would have to possess unique mechanisms for selectively sequestering these peptides, since their concentrations in these tissues are 10–100 times those in the blood. The fact that immunostainable β-endorphin was still detectable in the testis and the epididymis of hypophysectomized animals with low blood levels suggests that these peptides may be locally produced. Proof of this latter hypothesis must await demonstration of synthesis directly in these tissues.

The presence of β-endorphin and other products that may be derived from the pro-opiomelanocortin molecule in Leydig cells and in selected portions of the reproductive tract of multiple species suggests that these peptides may have some functional significance. Their low concentration in these tissues argues that they might have a local paracrine function. For example, opiates may act via local opiate receptors. It is of interest that one of the classic assays for opiate receptors is based on contraction of the mouse vas deferens (Lord et al., 1977). In this organ it is possible that endorphin could be secreted into the lumen from the apical portion of the epithelium, contributing to the β-endorphin present in semen. In addition, endorphin could also be released from the basal portion of the epithelium so as to affect the neighboring musculature. A similar mode of apical and basal secretion has been suggested for ABP (Gunsalus et al., 1980). Another possible site of paracrine function is the testis. Several investigators have suggested that there is intratesticular communication between Leydig cells and Sertoli cells. The molecular basis of such communication, however, is yet to be identified. That at least one product of the pro-opiomelanocortin molecule can stimulate Sertoli cells was suggested by the studies of Mather (1980) who showed that ACTH can

Downloaded from https://academic.oup.com/biolreprod/article/27/3/755/2767437 by guest on 20 August 2022

increase the multiplication of these cells in culture.

REFERENCES

Attramadal, A., Bardin, C. W., Gunsalus, G. L., Musto, N. A. and Hansson, V. (1981). Immunocytochemical localization of androgen binding protein in rat Sertoli and epididymal cells. Biol. Reprod. 25:983–988.

Beitins, I. Z., Bayard, F., Kowarski, A. and Migeon, C. J. (1973). The effect of ACTH administration on plasma testosterone, dihydrotestosterone and serum LH concentrations in normal men. Steroids 21:553–563.

Blankstein, J., Reyes, R. I., Winter, J.S.D. and Faiman, C. (1981). Endorphins and the regulation of the human menstrual cycle. Clin. Endocrinol. 14: 287–294.

Bradbury, A. F., Smyth, D. G. and Snell, C. R. (1976). Lipotropin: Precursor to two biologically active peptides. Biochem. Biophys. Res. Commun. 69:950–956.

Bruni, J. F., Watkins, W. B. and Yen, S.S.C. (1979). β-Endorphin in the human pancreas. J. Clin. Endocrinol. Metab. 49:651–699.

Cicero, T. J., Meyer, E. R., Bell, R. D. and Koch, G. A. (1976). Effects of morphine and methadone on serum testosterone and luteinizing hormone levels and on the secondary sex oragns of the male rat. Endocrinology 98:367–372.

Crowley, T. H., Brownsey, B. G., Harper, M. E., Peeling,W. B. and Griffiths, K. (1976). The effect of ACTH on plasma testosterone and androstenedione concentrations in patients with prostatic carcinoma. Acta Endocrinol. 81: 310–320.

Gunsalus, G. L., Musto, N. A. and Bardin, C. W. (1980). Bidirectional release of a Sertoli cell product, androgen binding protein, into the blood and seminiferous tubule. In: Testicular Development, Structure and Function (A. Steinberger, and E. Steinberger, eds.). Raven Press, New York, pp. 291–297.

Halmi, N. S. and Krieger, D. T. (1982). Immunocytochemistry of ACTH-related peptides in the hypophysis. In: The Anterior Pituitary (A. S., Bhatnagar, ed.). Raven Press, New York (in press).

Hsueh, A. J. and Jones, P.B.C. (1981). Extrapituitary actions of gonadotropin-releasing hormone. Endo. Rev. 2:437–461.

Krieger, D. T. and Martin, J. B. (1981). Brain peptides, N. Engl. J. Med. 304:876–885.

Larrea, F., Musto, N., Gunsalus, G. and Bardin, C. W. (1981). The microheterogeneity of rat androgen binding protein from the testis, rete testis fluid and epididymis, as demonstrated by immunoelectrophoresis and photoaffinity labeling. Endocrinology 109:1212–1220.

Li, C. H. and Chung, D. (1976). Isolation and structure of an untriakontapeptide with opiate activity from camel pituitary glands. Proc. Natl. Acad. Sci. USA 73:1145–1148.

Liotta, A. S., Gildersleeve, D., Brownstein, M. J. and Krieger, D. T. (1979). Biosynthesis *in vitro* of immunoreactive 31,000-dalton corticotropin/β-endorphin-like material by bovine hypothalamus. Proc. Natl. Acad. Sci. USA 76: 1448–1452.

Liotta, A. S. and Krieger, D. T. (1980). *In vitro* biosynthesis and comparative posttranslational processing of immunoreactive precursor corticotropin/β-endorphin by human placental and pituitary cells. Endocrinology 106:1504–1511.

Liotta, A. S., Loudes, C., McKelvy, J. F. and Krieger, D. T. (1980). Biosynthesis of precursor corticotropin/endorphin, corticotropin, α-melanotropin, β-lipotropin, and β-endorphin-like material by cultured neonatal rat hypothalamic neurons. Proc. Natl. Acad. Sci. USA 77: 1880–1884.

Lord, J.A.H., Wakefield, A. A., Hughes, J. and Kosterlitz, H. W. (1977). Endogenous opiod peptides: Multiple agonists and receptors. Nature 267:495–499.

Mains, R. E., Eipper, B. A. and Ling, N. (1977). Common precursor to corticotropins and endorphins. Proc. Natl. Acad. Sci. USA 74: 3014–3018.

Mather, J. P. (1980). Establishment and characterization of two distinct mouse testicular epithelial cell lines. Biol. Reprod. 23:243–252.

Musto, N. A., Gunsalus, G. L., Miljkovic, M. and Bardin, C. W. (1977). A novel affinity column for isolation of androgen binding protein from rat epididymis. Endocrinol. Res. Comm. 4:147–157.

Musto, N. A., Gunsalus, G. L. and Bardin, C. W. (1980). Purification and characterization of androgen binding protein from the rat epididymis. Biochemistry 19:2853–2860.

Pelliniemi, L. J., Dym, M., Gunsalus, G. L., Musto, N. A., Bardin, C. W. and Fawcett, D. W. (1981). Immunocytochemical localization of androgen binding protein in the male rate reproductive tract. Endocrinology 108:925–931.

Quigley, M. E. and Yen, S.S.C. (1980). The role of endogenous opiates on LH secretion during the menstrual cycle. J.s Clin. Endocrinol. Metab. 51:179–181.

Rivarola, M. A., Saez, J. M., Meyer, W. J., Jenkins, M. E. and Migeon, C. J. (1966) Metabolic clearance rate and blood production rate of testosterone and androst-4-ene-3,17-dione under basal conditions, ACTH and HCG stimulation. Comparison with urinary production rate of testosterone. J. Clin. Endocrinol. Metab. 26:1208–1218.

Roberts, J. L. and Herbert, E. (1977). Characterization of a common precursor to corticotropin and β-lipotropin: Identification of β-lipotropin peptides and their arrangement relative to corticotropin in the precursor synthesized in a cell-free system. Proc. Natl. Acad. Sci. USA 74:5300–5304.

Rotsztein, W. H., Drouva, S. C., Pattou, E. and Kordon, C. (1978). Metenkephalin inhibits *in vitro* dopamine-induced LHRH release from mediobasal hypothalamus of male rats. Nature 274: 281–282.

Sharp, B. and Pekary, A. E. (1981). β-Endorphin 61–91 and other β-endorphin immunoreactive peptides in human semen. J. Clin. Endocrinol. Metab. 52:586–588.

Sharp, B., Pekary, A. E., Meyer, N. V. and Hershman,

Downloaded from https://academic.oup.com/biolreprod/article/27/3/755/2767437 by guest on 20 August 2022

J. M. (1980). β-Endorphin in male rat reproductive organs. Biochem. Biophys. Res. Commun. 95:618–623.

Sternberger, L. A., Hardy, P. J., Cuculis, J. J. and Meyer, H. G. (1970). The unlabeled antibody-enzyme method of immunohistochemistry. Preparation and properties of a soluble antigen-antibody complex (horseradish peroxidase-anti-horseradish peroxidase) and its use in the identification of spirochetes. J. Histochem. Cytochem. 18:315–333.

Tsong, S. D., Phillips, D. M., Bardin, C. W., Halmi, N., Liotta, A. S., Margioris, A. and Krieger, D. T. (1982). ACTH and β-endorphin-related peptides are present in multiple sites in the reproductive tract of the rat. Endocrinology 110:2204–2206.

Yamaguchi, H., Liotta, A. S. and Krieger, T. D. (1980). Simultaneous determination of human plasma immunoreactive β-lipotropin, γ-lipotropin and β-endorphin utilizing immunoaffinity chromatography. J. Clin. Endocrinol. Metab. 51: 1002–1008.

Downloaded from https://academic.oup.com/biolreprod/article/27/3/755/2767437 by guest on 20 August 2022