SUBCELLULAR LOCALIZATION OF ENDORPHINE ACTIVITY IN BOVINE PITUITARY AND BRAIN[1]

Gary Queen, Carl Pinsky, and Frank LaBella[2]

Department of Pharmacology and Therapeutics
University of Manitoba, Faculty of Medicine
Winnipeg, Manitoba, Canada
R3E 0W3

Received August 13,1976

SUMMARY

The endorphine content of bovine anterior (AP), posterior (PP), intermediate (IP) pituitary, brain, and subcellular fractions of these tissues was estimated in an opiate radioreceptor assay. IP was most concentrated in activity, containing 7, 15, 780, and 9,100 times as much as PP, AP, midbrain, and cortex, respectively. More than 90 per cent of total endorphine activity in all lobes of the pituitary sedimented during centrifugation at 12,000 x g for 10 min. Density-gradient centrifugation gave almost identical sedimentation patterns for all pituitary lobes. In brain, endorphine activity sediments similarly but not identically to that of pituitary. Endorphine activity in the pituitary is localized to a secretory granule presumably derived from a cell type common to all lobes. Brain endorphine is localized to a similar granule, but derived at least in part from nerve endings.

The development of a sensitive and specific radioreceptor assay for opiate drugs led to the identification of endogenous peptides which exert morphine-like actions both in vitro and in vivo. Methionine-enkephalin (tyr-gly-gly-phe-met-OH) was identified in brain (1) and a hexadecapeptide (α-endorphine), with this same N-terminal sequence, from hypothalamus-neurohypophysis (2). The sequence of peptides is contained within a 91 amino acid residue protein, beta-lipotropic hormone (LPH), isolated from whole pituitaries (3). Continuation of the sequence to the C-terminus yields a potent opiate-like peptide, C-fragment (4). Thus, LPH may serve as a prohormone for endorphine (a generic term for all opiate

[1]Supported by the Medical Research Council and NMUD/Health and Welfare Canada
[2]Associate of the MRC.

peptides: enkephalin, α-endorphine, C-fragment), as well as for β-MSH as suggested earlier (5).

Elucidation of the tissue and intracellular localization of LPH and endorphines should contribute to an understanding of the origin, function, and fate of both the brain and pituitary opiate-like peptides. In this report we present results on the distribution of endorphine activity as estimated by an opiate radioreceptor assay.

EXPERIMENTAL

Seventeen per cent homogenates of fresh, bovine tissues in water were adjusted to pH 2.0 with 0.1 N HCl, placed in a boiling water bath for 15 min, cooled and TRIS added in a final concentration of 0.15 M, pH 7.7. The neutralized suspension was centrifuged at 900,000 g-min, and the supernatant decanted and stored in the cold for subsequent assay. For pituitary tissue, a thin central wedge of the intermediate lobe was dissected out, as was an interior portion of the anterior or posterior lobe, well away from the intermediate zone. Brain tissues included a portion of the cortex and one from the "midbrain" (cortex and cerebellum removed). The radioreceptor assay was based on the method of Pert and Snyder (6) and Pasternak et al. (7), but centrifugation rather than filtration was used to isolate brain membranes. Brains (cerebellum removed) from male, Sprague-Dawley rats were homogenized in 30 volumes of 0.05 M TRIS, pH 7.7 and centrifuged for 750,000 g-min. The pellet was resuspended in an equal volume of TRIS, incubated at 37° for 30 min, and recentrifuged. The pellet was resuspended in TRIS to yield 60 mg brain equivalent per ml. One-half ml aliquots were combined with 0.3 ml TRIS and 0.1 ml of competing extract or standard opiate drug in TRIS and incubated for 30 min at 0°. ^{3}H-naloxone (2.6×10^{-9} M; 33 Ci/mmol, New England Nuclear) in 0.1 ml TRIS was added, and the tubes incubated for 30 min at 0°. The tubes were centrifuged to sediment membranes, and the supernatant aspirated and discarded. The pellet was dissolved in 0.3 ml of 2N KOH at 70° for 10 min, and a 0.2 ml aliquot added to a scintillation counting medium contained methylcellosolve. Non-radioactive levorphanol, 10^{-6} M, depleted the counts by 60-70%, whereas its pharmacologically inactive isomer, dextrorphan, caused no depletion. For differential centrifugation, a 10% homogenate of pituitary or whole brain tissue in 0.25 M sucrose was prepared with a teflon pestle fitted to a glass vessel. Nuclei and debris were sedimented at 7,000 g-min and discarded. All procedures were carried out at room temperature to avoid depletion of stored hormones (8). Pellets obtained at 30 thousand, 120 thousand, and 480 thousand g-min and the resulting supernatant were extracted with HCl for endorphine as described for whole tissue. For density-gradient centrifugation, the 7,000 g-min supernatant was applied to the top of a 35 ml plastic tube, containing sucrose or a sucrose-ficoll gradient, and centrifuged in a Spinco model L at 4°. The sedimentation bands were isolated by means of a Spinco tube-slicing device. Each particulate band was diluted with 10 volumes of 0.25 M sucrose and centrifuged at 12000 thousand g-min to pellet suspended material. The pellets were extracted with HCl for endorphine estimation. Methionine- and leucine-enkephalin were purchased from Peninsula Co., and α-endorphine was provided by Dr. R. Guillemin to Dr. V. Havlicek, Dept. of Physiology. Levorphanol and dextrorphan were provided by Dr. G. Krip, Hoffmann-LaRoche Ltd., Montreal, and naloxone by Mr. J. Beaulac, Endo Laboratories, Montreal.

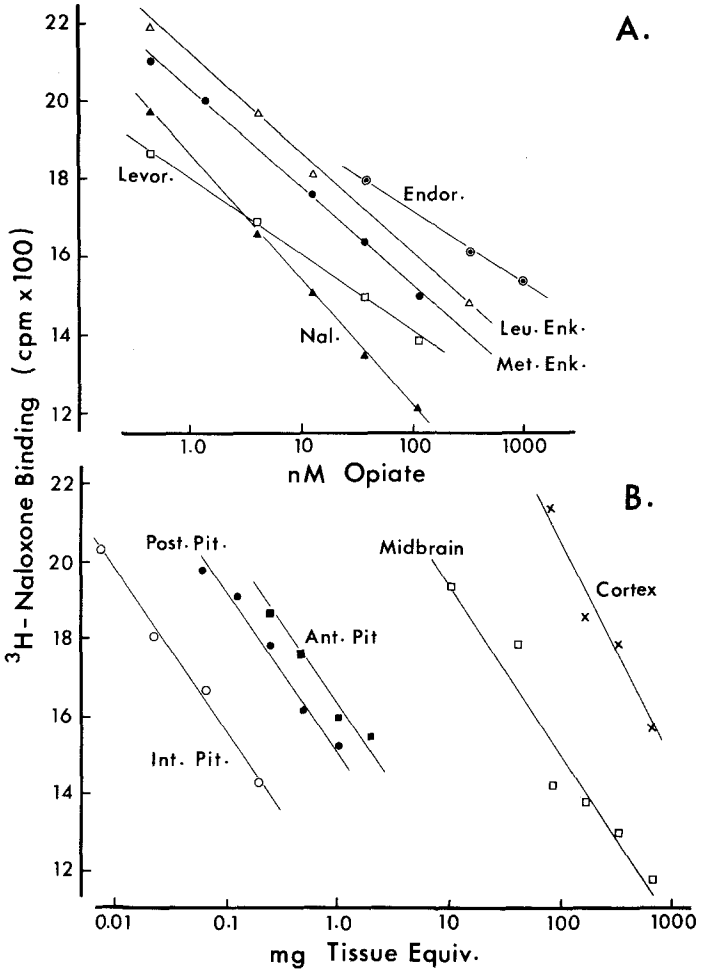

FIGURE 1. (A) Dose-response curves for synthetic peptides and opiate drugs. (B) Dose-response curves of acid extracts of beef pituitary and brain in the opiate radioreceptor assay.

RESULTS AND DISCUSSION

Opiate peptides of varying size may be potentially derived from cleavage of the putative prohormone, LPH. However, only a 16-residue and a 31-residue peptide have been reported in pituitary (2,4) and a pentapeptide in brain (1). Until the number and relative amounts of pituitary opiate peptides in these tissues are determined, only total "endorphine" activity can be estimated. The relative potencies of several synthetic peptides in the

opiate radioreceptor assay are shown in Figure 1A. All three peptides are less potent than the opiate drugs, and α-endorphine considerably less potent than the enkephalins. The deviation from parallelism of the dose-response curves for levorphanol and α-endorphine is a consistent observation in assays performed in the absence of sodium.

The endorphine content of various lobes of the bovine pituitary and brain, as estimated by the competitive radioassay, is shown in Figure 1B. It can be seen that the intermediate lobe is most concentrated in endorphine activity, and contains about 7, 15, 780 and 9,100 times as much as the posterior pituitary, anterior pituitary, midbrain, and cortex, respectively. A direct comparison of potency between brain and pituitary may not be valid, since only the pentapeptide, enkephalin, has been identified in the former tissue and only α-endorphine and C-fragment in the latter. Dose-response

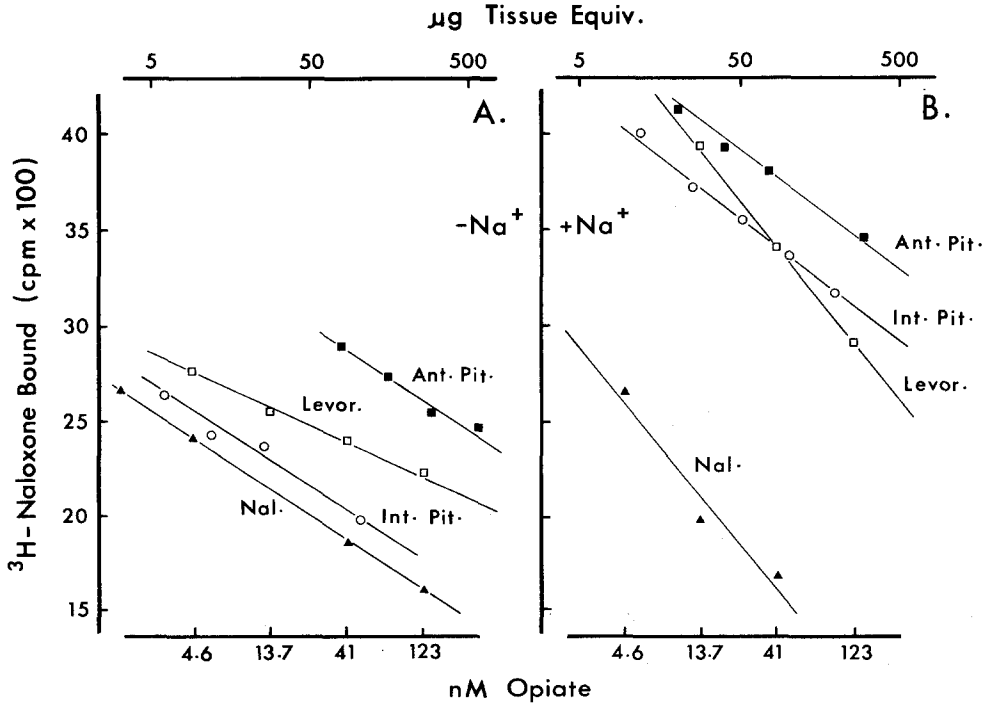

FIGURE 2. Dose-response curves for endorphine and opiate drugs in the radioreceptor assay in the absence (A) and presence (B) of 100 mM NaCl.

curves for extracts of pituitary parallel one another, but curves for pituitary and brains did not consistently parallel one another. The opiate-agonist character of the receptor binding substance in the tissues was indicated by the effect of sodium on binding (Figure 2): with the addition of NaCl the ED_{50} for naloxone was decreased but increased for levorphanol and tissue extracts. Sodium apparently promotes the antagonist (naloxone) conformation of the opiate receptor, thereby diminishing the number of sites in the agonist (levorphanol, endorphines) conformation (9).

For all pituitary lobes more than 90 per cent of endorphine activity is sedimented during centrifugation at 12,000 g for 10 min, indicating the active substance to be localized to a subcellular organelle, presumably a secretory granule. The distribution of endorphine activity among particulate fractions from brain differs somewhat from that in pituitary, although again, most sediments in the low speed pellet. By density-gradient centrifugation in two different systems, the similarity in endorphine sedimentation for all three pituitary lobes is apparent, as is the distinctive pattern for brain (Figure 3). The endorphine peptide(s) is strongly bound to the granules, because only 1 to 3 per cent of the total is recovered in the supernatant fraction after removal of particulates. Also, manipulation of the isolated granules, e.g. during density-gradient centrifugation, liberates only negligible amounts of activity.

These observations indicate that endogenous opiate substances in pituitary and brain are virtually entirely contained within rapidly sedimenting cytoplasmic granules. Our results show that the intermediate lobe, which is a fairly distinct region of the beef pituitary, contains the highest concentration of endogenous opiate. That the activity sediments from homgenates practically identically in all three lobes indicates the presence of a common granule, and presumably a common cell type. The large proportion of the intermediate lobe is made up of parenchymal cells with numerous well defined secretory granules, and cords of these cells penetrate into

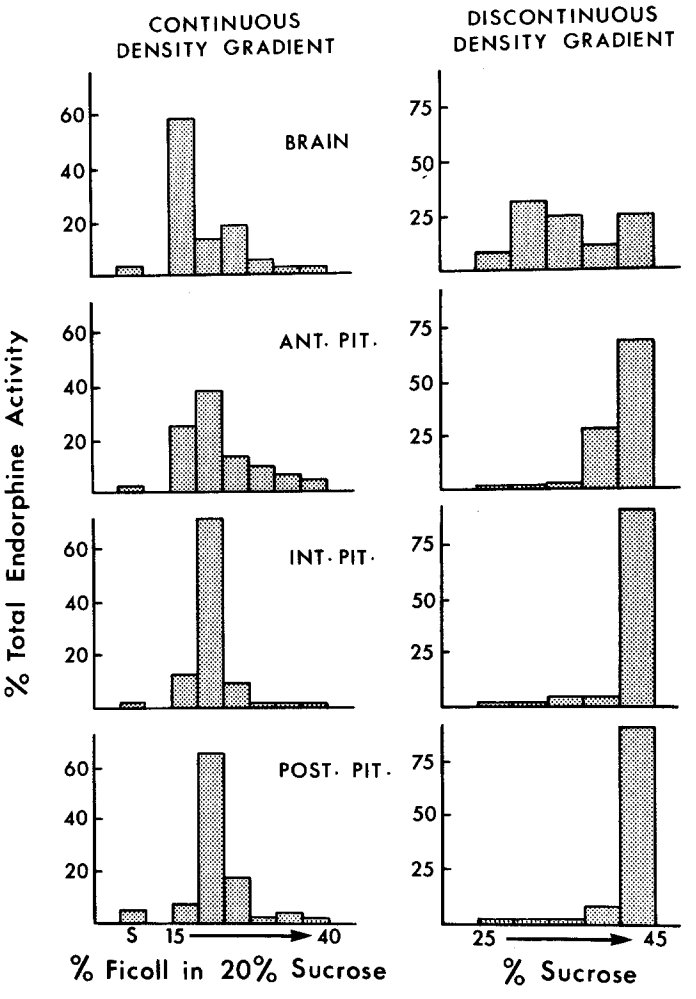

FIGURE 3. Centrifugation of pituitary and brain homogenates (7,000 g-min supernatant) in two density-gradient systems.

adjacent lobes (10). Although tissue samples of anterior and posterior pituitary were taken from the innermost portion of each lobe, intermediate lobe cells may be irregularly distributed throughout the pituitary gland.

There appear to be differences between brain and pituitary in the cell type in which the endorphine secretion granule is contained, in the products of LPH degradation, and in the cells which are targets of endorphine. Pituitary localization implies a systemic role for endorphines and brain localization a role in central synapses.

The subcellular distribution of LPH in both pituitary and brain parallels that of endorphine (unpublished). The paired basic amino acid residues in LPH (3), together with findings on the proteolytic specificity in the pituitary (4) suggest a secretory package containing prohormone, cleaving enzyme, and biologically active peptide product. This secretory mechanism is analogous to that of several other endocrine cells, except that several biologically active peptides, rather than a single one, are generated from putative prohormone, LPH.

REFERENCES

1. Hughes, J., Smith, T.W., Kosterlitz, H.W., Fothergill, L.A., Morgan, B.A. and Morris, H.R. (1975). Nature, 258, 577-579.
2. Guillemin, R., Ling, N. and Burgus, R. (1976). C.R. Acad. Sci. (Paris) ser. D., 282, 783-785.
3. Graf, L. and Li, C.H. (1973). Biochem. Biophys. Res. Commun., 53, 1304-1309.
4. Bradbury, A.F., Smyth, D.G. and Snell, C.R. (1976). Biochem. Biophys. Res. Commun., 69, 950-956.
5. Chretien, M., and Li, C.H. (1967). Can. J. Biochem., 45, 1163-1174.
6. Pert, C.B. and Snyder, S.H. (1973). Science, 179, 1011-1014.
7. Pasternak, G.W., Wilson, H.A. and Snyder, S.H. (1975). Molec. Pharmacol., 11, 340-351.
8. LaBella, F.S., Dular, R. and Vivian, S. (1973). Endocrinology, 92, 1571-1574.
9. Pert, C.B., Pasternak, G.W. and Snyder, S.H. (1973). Science, 182, 1359-1361.
10. Etkin, W., in Neuroendocrinology (1967) (eds., Martini, L. and Ganong, W.F.), 2, 261-282, Academic Press, New York.