Proc. Natl. Acad. Sci. USA
Vol. 77, No. 10, pp. 6207–6210, October 1980
Neurobiology

Immunoreactive dynorphin in pituitary and brain

(endorphin/neuropeptide/radioimmunoassay)

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Contributed by Avram Goldstein, June 16, 1980

ABSTRACT Distribution of the potent opioid peptide dynorphin has been determined in pituitary gland (pig, beef, rat), in the various regions of rat brain, and in rat spinal cord, by using a highly specific antiserum. By gel permeation chromatography in 4 M guanidine, the porcine pituitary immunoreactivity is found in a major peak of apparent molecular weight about 1700 and a minor peak of about 3400. Similar peaks are found in rat pituitary extracts, whereas rat brain contains, in addition, two peaks of larger apparent molecular weight. In the pituitary, immunoreactive dynorphin is found predominantly in pars nervosa. In the central nervous system, it is distributed widely, with highest concentrations in hypothalamus, medulla-pons, midbrain, and spinal cord. Although dynorphin contains leucine-enkephalin, the regional distribution of dynorphin is different from that of enkephalin or of any other known opioid peptide.

Dynorphin is an opioid peptide, recently isolated from porcine pituitary extract, which contains leucine-enkephalin at its NH_2 terminus. It is distinguished by its extraordinary potency in the guinea pig myenteric plexus–longitudinal muscle bioassay (1). Its first 13 residues are Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys. Dynorphin-(1–13) was as potent as natural dynorphin in the bioassay. Antisera were raised against thyroglobulin-conjugated dynorphin-(1–13), and one of them ("Lucia 9/14") was used as the basis for a sensitive and highly specific radioimmunoassay (RIA) (2). For significant immunoreactivity, this antiserum requires residues in addition to those of the leucine-enkephalin portion of the peptide; crossreactivity of leucine-enkephalin itself is less than 10^{-6}%. Moreover, no other opioid peptide crossreacts significantly. The free carboxyl group of Lys-13 is not essential for immunoreactivity, because both dynorphin-(1–12) and the methyl ester of dynorphin-(1–13) are fully immunoreactive. Therefore, naturally occurring dynorphin, which is thought to have at least four additional residues at the COOH terminus (3), should be measurable. Dynorphin-(2–13) is also highly immunoreactive, indicating that a free amino group at Tyr-1 is not essential for immunoreactivity, and therefore that precursor peptides with NH_2-terminal extensions should also be measurable. We now report the distribution of immunoreactive (ir-) dynorphin in porcine, bovine, and rat pituitary, in various regions of rat brain, and in rat spinal cord.

MATERIALS AND METHODS

Porcine pituitary glands were received, frozen, from Pel-Freez. They were dissected into anterior and neurointermediate lobes, weighed, and extracted immediately. For the experiment with gel permeation chromatography, lyophilized porcine neurointermediate lobes were obtained from the same source. Bovine glands were obtained fresh at a nearby slaughterhouse and kept in ice until dissection and extraction a few hours later. Neurointermediate lobes from a few glands were further divided into three portions—an anterior-facing slice, including the cone of Wulzen, which should be enriched in intermediate lobe tissue; a posterior slice, which should be enriched in neural lobe tissue; and the remaining central portion. Rat pituitary was obtained immediately upon decapitation and separated under a dissecting microscope into anterior and neurointermediate lobes. Intermediate-lobe tissue (loosely adherent pale-yellow cells) was removed by vigorous stirring of anterior and neurointermediate lobes in 0.9% NaCl in a Corex centrifuge tube, using a Vortex stirrer. The completeness of removal of pars intermedia cells was checked once by histologic examination and was confirmed by stereomicroscopy of every preparation. Pooled intermediate cells from six glands were centrifuged. The pellet was extracted, and the supernatant solution was lyophilized and also extracted.

Rat brains were dissected immediately after decapitation, on a chilled aluminum plate covered with Parafilm. Tissues were kept moist with chilled 0.9% NaCl solution, then weighed after careful removal of excess fluid and extracted. The dissection was as described by Glowinski and Iversen (4), but with the following modifications. Hypothalamus was dissected out as a uniform rectangular block approximately 4.0 mm wide, 5.5 mm long, and 2.5 mm thick, weighing about 55 mg. The lateral bounds were straight cuts in the sulcus between hypothalamus and adjacent cortex, and the dorsal cut was just ventral to anterior commissure. Although the resulting block of tissue was smaller than that obtained by Glowinski and Iversen, it had the dimensions of hypothalamus as described by Craigie (5). Hypothalamus was divided into anterior and posterior halves by a perpendicular transverse cut through the median eminence as the block of tissue rested ventral side up. Finally, instead of recombining rostral and caudal parts of cortex, we extracted them separately.

Rat spinal cord mid-thoracic transections were carried out while the animals were under pentobarbital anesthesia, and sham-operated controls were included. Access to the cord was by a burr hole at T7 (Wistar females, 200 g) or laminectomy at T4 (Sprague–Dawley males, 375 g). The same procedures were carried out in sham-operated controls, except that the dura was not opened. Transected (but not sham-operated) animals displayed complete paralysis of hind limbs, with insensibility to noxious stimuli (vocalization test). Twelve to 32 days postoperatively, the rats were sacrificed by decapitation. A 1-cm segment of spinal cord at the site of operation was discarded, then adjacent segments, cephalad and caudad, weighing approximately 200 mg each, were extracted, and ir-dynorphin was determined by RIA.

Tissues were extracted by homogenizing with a Tissuemizer in 10 vol (but not less than 1 ml) of MeOH/0.1 M HCl (1:1, vol/vol) at 70°C, followed by 10-min incubation at 70°C before chilling on ice. The RIA procedure, using antiserum Lucia 9/14, was exactly as described (2). For gel permeation chromatog-

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Abbreviations: RIA, radioimmunoassay; ir-, immunoreactive; IC_{50}, concentration of competing peptide that reduces binding of ^{125}I-labeled dynorphin-(1–13) by 50% in the RIA.

raphy, a tissue extract was applied to a Bio-Gel P-60 column and eluted with 4 M guanidine hydrochloride, essentially as described by Rossier *et al.* (6).

In our previous publication (2), we demonstrated the validity and reliability of the tissue extraction and RIA procedures for rat pituitary. Identical experiments, carried out with whole rat brain, are summarized briefly below (see ref. 2 for experimental details).

(*a*) *Homogenization and extraction conditions.* In two experiments, brains were divided into right and left halves. One half was homogenized in Tris·HCl buffer (50 mM, pH 7.4) and allowed to stand 10 min at 23°C before MeOH and HCl were added to the usual final concentration. The other half brain was homogenized in MeOH/HCl at 70°C and held at that temperature for 10 min, in the usual manner. The ir-dynorphin content was 25–28% lower in the halves homogenized in Tris. In the same experiments, ^{125}I-labeled dynorphin-(1–13) was added at the time of homogenization. Virtually all the radiolabeled peptide was degraded after homogenization in Tris buffer, as determined by thin-layer chromatography, whereas almost no degradation could be detected after homogenization in MeOH/HCl.

(*b*) *Stability of ir-dynorphin in intact brain.* Whole rat brains, allowed to stand for 30 min at 23°C before extraction, contained the same amount of ir-dynorphin as brains homogenized in hot MeOH/HCl immediately after decapitation. Thus, during the dissections on a chilled platform, as described above, there should not have been significant loss of immunoreactivity.

(*c*) *Stability of extracts on storage.* There was no loss of immunoreactivity in MeOH/HCl extracts of brain stored at −20°C for 30 days.

(*d*) *Destruction of ir-dynorphin by protease action.* Incubation with papain, as described for pituitary (2), destroyed the immunoreactivity present in brain extracts, whereas incubation with papain inactivated by heating did not.

(*e*) *Parallel dilution curves of brain extracts and standards.* Dilutions of extracts from all brain regions yielded competition curves in the RIA that were parallel to the dynorphin-(1–13) standard curve, with midpoint slope in agreement with that predicted by the mass law for simple competition at a binding site (7).

(*f*) *Assay replicability.* For repeated assays of a brain extract in six experiments, the coefficient of variation was 13%, in good agreement with the value obtained for pituitary extracts.

(*g*) *Recovery of added peptide.* When dynorphin-(1–13) was added to brain extracts in an amount that increased total ir-dynorphin by only 90%, there was a parallel shift of the extract dilution curve. Recovery of added peptide, computed from the change in the 50%-inhibitory concentration, IC_{50}, was 106% ± 18% (SEM) in six independent experiments.

(*h*) *Stability of radiolabeled dynorphin-(1–13) in the RIA incubation.* A diluted extract of brain was incubated in the usual manner with antiserum and ^{125}I-dynorphin-(1–13). After 48 hr at 0–4°C the same inhibition of binding (44%) was observed as after 24 hr. Moreover, delayed addition of ^{125}I-dynorphin-(1–13) and antiserum to diluted brain extract that had been preincubated for 24 hr also resulted in the same inhibition. As with pituitary extracts (2), these results show that the apparent immunoreactivity is not due to degradation of radiolabeled dynorphin-(1–13) by brain extract during the RIA incubation.

(*i*) *Lack of crossreactivity of statherin and a porcine neurophysin in the RIA.* Among polypeptides with known primary structure (8), only one besides leucine-enkephalin (see *Introduction*) contains a sequence of five or more residues identical to any sequence in dynorphin-(1–13). This is statherin, a 43-residue salivary protein, which contains -Phe-Leu-Arg-Arg-Ile- as residues 7–11 (9). Another peptide of interest is porcine MSEL neurophysin (neurophysin III), a 95-residue hypothalamic-pituitary peptide, which terminates in -Phe-Leu-Arg-Arg-Ala-OH (10, 11). Human statherin was kindly furnished by D. H. Schlesinger (Univ. of Illinois, Chicago) and porcine neurophysin III, by T. C. Wuu (Medical College of Ohio, Toledo). These peptides were dissolved and diluted in 50 mM sodium phosphate buffer (pH 7.4) containing 0.1% bovine serum albumin and 0.1% Triton X-100, and also in MeOH/HCl (see below). Neither peptide displayed any immunoreactivity at more than 200,000 times the IC_{50} of dynorphin-(1–13). The lack of crossreactivity is consistent with information already published about the characteristics of the antiserum (2).

RESULTS AND DISCUSSION

Pituitary. Table 1 shows that, in pig, beef, and rat pituitary, ir-dynorphin is found predominantly in posterior lobe. Of the total ir-dynorphin in pituitary, posterior lobe contained 97% in pig, 96% in beef, and 78% in rat. The distribution in the bovine gland is in agreement with our previous report that "slow reversing endorphin" (as dynorphin was then called), measured by bioassay, was largely in posterior lobe (12). Localization to pars nervosa in the rat was demonstrated by removal of intermediate lobe cells by vigorous stirring. We found no decrease in immunoreactivity of neurointermediate lobe, and only negligible immunoreactivity in the intermediate cells themselves (harvested by centrifugation) or in the saline solution in which they had been stirred. In the bovine gland, the highest concentration of ir-dynorphin was in the posterior part of the neurointermediate lobe, which contains the least amount of pars intermedia tissue. Anterior lobe ir-dynorphin in pig and beef, as well as posterior lobe in all three species, yielded dilution curves that were parallel to the standard curve and gave com-

Table 1. Immunoreactive dynorphin in pituitary of three species

Measurement	Pig (n = 5)	Beef (n = 6)	Rat (n = 5)*
Anterior			
Tissue wet weight, mg	158 ± 32	1390 ± 131	9.61 ± 0.75
Total ir-dynorphin, pmol	1.10 ± 0.43	2.29 ± 0.26	0.40 ± 0.03
Concentration, pmol/g	6.14 ± 1.44	1.69 ± 0.20	42.4 ± 5.4
Posterior (neurointermediate)			
Tissue wet weight, mg	51.6 ± 10.3	346 ± 22	1.40 ± 0.08
Total ir-dynorphin, pmol	36.6 ± 14.5	60.4 ± 8.8	1.46 ± 0.08†
Concentration, pmol/g	636 ± 196	177 ± 26‡	1060 ± 80

Data are means ± SEM expressed in terms of dynorphin-(1–13), determined from IC_{50} values obtained in tissue extract dilution curves. Dilution curves were parallel to the dynorphin-(1–13) standard curve, except for rat anterior pituitary, which had an unusually shallow slope with maximum inhibition approximately 80%.

* Males, Sprague–Dawley. In one experiment with pooled tissues from six females, concentrations of ir-dynorphin were: anterior pituitary, 13.5 pmol/g; pars nervosa, 1820 pmol/g.

† In rat posterior lobe, removal of pars intermedia (see text) had no effect on content of ir-dynorphin. Content of ir-dynorphin in pars intermedia cells harvested from six posterior lobes in two experiments (see text) was 0.02 and 0.01 pmol per gland; content in supernatant solutions was 0.07 and <0.01 pmol per gland.

‡ In beef neurointermediate lobes sliced into three segments—anterior (including cone of Wulzen), middle, and posterior—the lowest concentration of ir-dynorphin was in the anterior segment, which is richest in pars intermedia tissue.

plete inhibition at high extract concentrations. Anterior lobe extracts from rats, however, gave curves with more shallow slopes and incomplete inhibition at high concentrations. This might indicate greater heterogeneity of the ir-material in rat anterior lobe.

The distribution of ir-dynorphin in pituitary is different from that of ir-β-endorphin, because the latter is found in anterior lobe, is very concentrated in pars intermedia, and is absent from pars nervosa. However, the distribution of ir-dynorphin is somewhat similar to that of ir-enkephalin, which is also found predominantly in pars nervosa. The possibility that some or all of the ir-enkephalin is really dynorphin was ruled out by the following observations. With an enkephalin antiserum ("DBD-3," kindly furnished by June Dahl and Iris Lindberg), dynorphin-(1-13) crossreacted only 1.3% (on a molar basis) as compared with leucine-enkephalin. With this antiserum, extracts of rat pituitary pars nervosa were found to contain about 1.8 pmol of ir-leucine-enkephalin per gland, slightly more than the amount of ir-dynorphin determined with antiserum Lucia 9/14 (cf. Table 1). A similar amount of ir-leucine-enkephalin had been reported by Rossier *et al.* (6). With their antiserum ("RB-92," kindly furnished by F. Bloom), we found that dynorphin-(1-13) crossreacted only 0.2% as compared with leucine-enkephalin. Thus, ir-dynorphin and ir-enkephalin are both present, and in similar amounts, in pars nervosa. It remains an open question whether or not leucine-enkephalin is derived from dynorphin in this tissue.

Fig. 1 shows the result of gel permeation chromatography with an extract of porcine neurointermediate lobe. Two peaks of immunoreactivity are evident. The major one elutes at the position of an α-endorphin marker (M_r 1700), the lesser one coincides with a β-endorphin marker (M_r 3400). An identical pattern was observed when the tissue was extracted with 1 M acetic acid at 90°C instead of with MeOH/HCl (not shown). The material in the M_r 1700 peak has about the same activity in the myenteric plexus bioassay as would be expected from its immunoreactivity (unpublished observations); thus, natural porcine dynorphin has about the same immunoreactivity as dynorphin-(1-13) on a molar basis.

Brain. Table 2 gives the distribution of ir-dynorphin in nine regions of rat brain. The comparisons of tissue weights show that our regions are comparable with those of Glowinski and Iversen

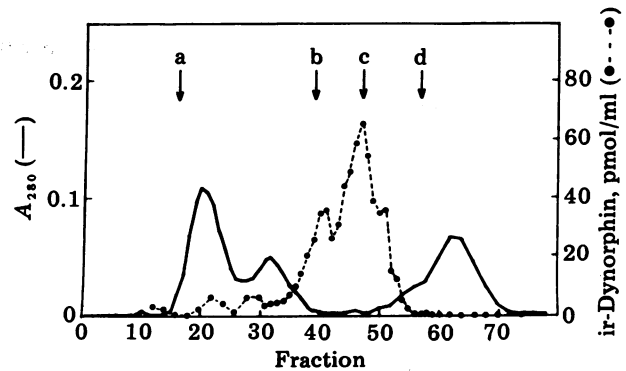

FIG. 1. Gel permeation chromatography of porcine pituitary posterior lobe extract. One lyophilized porcine posterior lobe (12.6 mg) was extracted in 1 ml of MeOH/0.1 M HCl (1:1, vol/vol) at 70°C, incubated 10 min, then chilled and centrifuged (10,000 × g, 10 min). The supernatant (0.6 ml) was applied to a Bio-Gel P-60 column (50 × 1.2 cm, equilibrated with 4 M guanidine·HCl) (6). The column was eluted with 4 M guanidine·HCl at 4°C, 5 ml/hr, and 1-ml fractions were collected. Dynorphin immunoreactivity is expressed in terms of dynorphin-(1-13) standard. Markers indicated by arrows: a, blue dextran; b, ^{125}I-labeled human [Leu^5]-β-endorphin, M_r 3400; c, ^{125}I-labeled α-endorphin, M_r 1700; d, ^{14}C-labeled glycine, M_r 75. Total ir-dynorphin applied to column, 63 pmol; sum of fractions eluted from column, 63 pmol.

(4), with the exceptions already noted, and that the variability between rats in our dissections is also comparable with theirs. Of the total ir-dynorphin in brain, 32% is in cortex, 21% is in midbrain, and 20% is in medulla-pons. The rank order of ir-dynorphin concentrations is: anterior hypothalamus > posterior hypothalamus ≫ medulla-pons = midbrain > hippocampus > striatum > cortex ≫ cerebellum. This order is different from that reported for enkephalin, which is most concentrated in striatum and least concentrated in hippocampus, cortex, and cerebellum (6, 13-16). It is also different from that reported for β-endorphin, which is virtually absent from striatum, hippocampus, cortex, and cerebellum (6, 17, 18).

Gel permeation chromatography (unpublished observations) yields two major peaks of ir-dynorphin in rat pituitary posterior lobe extracts, comparable with those shown for porcine extracts in Fig. 1. Rat brain extracts contain two peaks that elute in the same positions as the peaks of ir-dynorphin from pituitary, and

Table 2. Immunoreactive dynorphin in rat brain

Region	Present data		Glowinski–Iversen data (4)		Total	Concentration of
	Wet wt, mg	*CV*, %	Wet wt, mg	*CV*, %	ir-dynorphin, pmol	ir-dynorphin, pmol/g
Cerebellum	275	4	267	8	0.36 ± 0.06	1.34 ± 0.23
Medulla-pons	221	3	252	10	1.88 ± 0.36	8.45 ± 1.62
Striatum	96	19	102	17	0.40 ± 0.04	4.25 ± 0.40
Midbrain	233	7	146	7	1.97 ± 0.44	8.45 ± 1.81
Hippocampus	157	25	151	15	0.93 ± 0.17	5.97 ± 0.81
Cortex C*	868	6	804	7	1.30 ± 0.16	3.08 ± 0.28
Cortex B*					1.74 ± 0.23	3.87 ± 0.46
Hypothalamus, anterior†	54	17	110	13	0.51 ± 0.12	16.36 ± 4.00
Hypothalamus, posterior†					0.26 ± 0.10	10.48 ± 2.32
Whole brain	1900	6	1830	Not given	9.36 ± 0.93	4.94 ± 0.54

Data are means ± SEM expressed as dynorphin-(1-13) equivalents, for nine brain regions in six male Sprague-Dawley rats (mean body weight 364 ± 10 g). Dissections were according to Glowinski and Iversen (4), with modifications described in the text. Tissue weights and coefficients of variation (*CV*) are shown for comparison.

* Cortex C = cortex rostral to optic chiasma, mean weight 420 ± 26 mg. Cortex B = cortex caudal to optic chiasma, mean weight 449 ± 21 mg.

† Hypothalamus, anterior = portion rostral to central pore of median eminence, mean weight 31 ± 2 mg. Hypothalamus, posterior = portion caudal to central pore of median eminence, mean weight 23 ± 3 mg.

Table 3. Effect of transection on ir-dynorphin content of rat spinal cord

Treatment	ir-Dynorphin, pmol/g	
	Cephalad segment	Caudad segment
Wistar females		
Unoperated	17.9, 16.6	21.0, 15.8
Sham-operated	22.9, 16.5	15.8, 17.5
Transected	20.0, 20.9	20.5, 21.7
Sprague-Dawley males		
Sham-operated	16.1, 17.2	23.8, 18.1
Transected	16.4, 15.6	22.1, 31.2

Spinal transection or sham operation was carried out as described under *Materials and Methods*. Cephalad and caudad are relative to the site of operation. Wistar females were allowed access to food and water ad libitum. To avoid differential access to food and water between transected and sham-operated Sprague-Dawley males, these animals were maintained on 30 ml of 10% glucose as sole nutrient, in 0.9% saline daily in divided subcutaneous injections, resulting in loss of about one-quarter of body weight over the 2-week period. Except for the first-listed transected Wistar female, which was sacrificed on postoperative day 32, all animals were sacrificed at day 12–14. Data in each row are means of duplicate or triplicate determinations for two rats.

in addition contain peaks of higher apparent molecular weight. It may be, therefore, that dynorphin of posterior lobe arises from a larger precursor, which is synthesized in hypothalamus, then processed as it is transported down the pituitary stalk, in the manner established for vasopressin and oxytocin (19). This is indicated also by the recent finding, by immunohistochemistry, of a cluster of large cells containing ir-dynorphin in rat supraoptic nucleus (S. J. Watson and H. Akil, personal communication).

These findings suggest a possible relationship of dynorphin to vasopressin or oxytocin, because these peptides also occur in supraoptic nucleus and pars nervosa. Their concentrations in hypothalamus and pars nervosa are about 1000 times higher than that of ir-dynorphin (20, 21). It should be noted, however, that our data are expressed in terms of dynorphin-(1–13) (porcine sequence) standard, whereas the true crossreactivity of rat dynorphin in this RIA is unknown.

Cox *et al.* (22) have shown recently that homozygous Brattleboro rats, which do not produce either vasopressin or its specific neurophysin (19), nevertheless have normal levels of ir-dynorphin in pars nervosa and in a small wedge of tissue containing supraoptic nucleus. Dynorphin-containing neurons originating in supraoptic nucleus might impinge on terminals of vasopressin or oxytocin neurons in pars nervosa and thus regulate the release of one or the other hormone, as proposed for enkephalin by Rossier *et al.* (23). The presence of opiate receptors in pars nervosa (24) is consistent with this hypothesis. Alternatively, dynorphin could be a third neurohypophyseal hormone, secreted to act on distant peripheral or central targets.

Table 3 shows that the ir-dynorphin content in spinal cord is very high—at least as high as in hypothalamus—and, moreover, that it remains unchanged below the level of spinal transection. This suggests that spinal cord dynorphin does not have a supraspinal origin. Rhizotomy experiments in rats should be informative. ir-Dynorphin has recently been found in dorsal root ganglia of rabbits (B. M. Cox, personal communication).

In summary, dynorphin is a neuropeptide that is present in pituitary gland and throughout the brain and spinal cord. Opiate receptors in the guinea pig myenteric plexus had already been found to have greater affinity for dynorphin than for any known opioid peptide. It seems possible, therefore, that there are similar receptors, with high affinity for dynorphin, in various parts of the central and peripheral nervous systems, upon which the terminals of dynorphin neurons impinge. The physiological functions of dynorphin remain to be elucidated.

We acknowledge the outstanding technical assistance provided by Asha Naidu, Keiko Otsu, and Clarence Omoto. Louise I. Lowney and Ed Baer carried out experiments with gel permeation chromatography; Grace Pavlath carried out some of those with spinal cord. Dr. Frances Leslie obtained the results on degradation of ^{125}I-labeled dynorphin-(1–13) by brain homogenates. Dr. Ric Cone assisted in the brain dissections. We thank Drs. D. H. Schlesinger and T. C. Wuu, respectively, for gifts of statherin and neurophysin, Dr. F. Bloom for antiserum RB-92, Drs. June Dahl and Iris Lindberg for antiserum DBD-3, and Debbie Knapp for typing the manuscript. This investigation was supported by Grants DA-1199 and DA-7063 from the National Institute on Drug Abuse.

1. Goldstein, A., Tachibana, S., Lowney, L. I., Hunkapiller, M. & Hood, L. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 6666–6670.
2. Ghazarossian, V. E., Chavkin, C. & Goldstein, A. (1980) *Life Sci.* **27**, 75–86.
3. Hunkapiller, M. W. & Hood, L. E. (1980) *Science* **207**, 523–525.
4. Glowinski, J. & Iversen, L. L. (1966) *J. Neurochem.* **13**, 655–669.
5. Craigie, E. H. (1963) *Craigie's Neuroanatomy of the Rat*, revised and expanded by Zeman, W. & Innes, J. R. (Academic, New York).
6. Rossier, J., Vargo, T. M., Minick, S., Ling, N., Bloom, F. E. & Guillemin, R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5162–5165.
7. Goldstein, A., Aronow, L. & Kalman, S. M. (1974) *Principles of Drug Action* (Wiley, New York), 2nd Ed., pp. 89–96.
8. Dayhoff, M. O., Hunt, L. T., Barker, W. C., Schwartz, R. M. & Orcutt, B. C. (1978) *Protein Segment Dictionary* (National Biomedical Research Foundation, Washington, DC).
9. Schlesinger, D. H. & Hay, D. I. (1977) *J. Biol. Chem.* **252**, 1689–1695.
10. Wuu, T. C. & Crumm, S. E. (1976) *J. Biol. Chem.* **251**, 2735–2739.
11. Chauvet, M. T., Codogno, P., Chauvet, J. & Acher, R. (1979) *FEBS Lett.* **98**, 37–40.
12. Lowney, L. I., Gentleman, S. B. & Goldstein, A. (1979) *Life Sci.* **24**, 2377–2384.
13. Yang, H.-Y., Hong, J. S. & Costa, E. (1977) *Neuropharmacology* **16**, 303–307.
14. Hughes, J., Kosterlitz, H. W. & Smith, T. W. (1977) *Br. J. Pharmacol.* **61**, 639–647.
15. Hökfelt, T., Ljungdahl, A., Terenius, L., Elde, R. & Nilsson, G. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3081–3085.
16. Sar, M., Stumpf, W. E., Miller, R. J., Chang, K.-J. & Cuatrecasas, P. (1978) *J. Comp. Neurol.* **182**, 17–38.
17. Watson, S. J., Barchas, J. D. & Li, C. H. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5155–5158.
18. Bloom, F., Battenberg, E., Rossier, J., Ling, N. & Guillemin, R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1591–1595.
19. Brownstein, M. J., Russell, J. T. & Gainer, H. (1980) *Science* **207**, 373–378.
20. Jones, C. W. & Pickering, B. T. (1969) *J. Physiol.* **203**, 449–458.
21. George, J. M., Staples, S. & Marks, B. M. (1976) *Endocrinology* **98**, 1430–1433.
22. Cox, B. M., Ghazarossian, V. E. & Goldstein, A. (1980) *Neurosci. Lett.*, in press.
23. Rossier, J., Battenberg, E., Pittman, Q., Bayon, A., Koda, L., Miller, R., Guillemin, R. & Bloom, F. (1979) *Nature (London)* **277**, 653–655.
24. Simantov, R. & Snyder, S. H. (1977) *Brain Res.* **124**, 178–184.