Life Sciences, Vol. 26, pp. 851-859 Pergamon Press
Printed in the U.S.A.

α-ENDORPHIN, γ-ENDORPHIN AND THEIR DES-TYROSINE FRAGMENTS IN RAT PITUITARY AND BRAIN TISSUE

J. Verhoef, J.G. Loeber*, J.P.H. Burbach, W.H. Gispen**, A. Witter and D. de Wied

Rudolf Magnus Institute for Pharmacology, Medical Faculty, University of Utrecht, Vondellaan 6, 3521 GD Utrecht, The Netherlands

(Received in final form January 15, 1980)

Summary

Enkephalins, endorphins and related peptides were determined in pituitary and brain tissue of rats which were killed by decapitation or microwave irradiation. The tissues were heated in 1M acetic acid prior to homogenization and the levels of the various peptides were measured by means of a combination of HPLC and radioimmunoassays. Enkephalin levels in pituitary and brain of irradiation-killed rats were much higher as compared to those in tissue of rats sacrificed by decapitation. Similar data were obtained with respect to pituitary levels of γ-endorphin, des-Tyr-γ-endorphin and des-Tyr-α-endorphin. However, brain levels of α- and γ-endorphin and their respective des-Tyr-fragments were not different with the two methods of sacrifice used. The concentrations of β-endorphin in the pituitary gland were similar in rats killed by microwave irradiation and decapitation, but irradiation showed higher β-endorphin levels in the brain than decapitation. These results suggest that β-endorphin fragments like α- and γ-endorphin and des-Tyr-α- and des-Tyr-γ-endorphin are endogenous peptides in the rat pituitary gland and the brain.

Opioid peptides derived from the C-terminal part of β-lipotropin (β-LPH), e.g. β-endorphin (β-LPH$_{61-91}$), γ-endorphin (β-LPH$_{61-77}$), α-endorphin (β-LPH$_{61-76}$) and methionine-enkephalin (β-LPH$_{61-65}$), have been isolated from the pituitary gland and the brain of various species (1-6). In addition to their morphinomimetic activities, these peptides have been implicated in the control of adaptive behavior (7,8) and several investigators have suggested an involvement of endogenous opioid peptides in the etiology of mental illness (9-11). Recently, the non-opioid peptide des-Tyr-γ-endorphin (β-LPH$_{62-77}$) was found to elicit neuroleptic-like effects with probably a more specific profile than that of currently used neuroleptic drugs (12,13). This peptide has also proved to possess strong antipsychotic activities in schizophrenic patients (14). These findings have led to the hypothesis that schizophrenia might be related to a reduced availability of des-Tyr-γ-endorphin as a result of a disturbed or inborn error of enzymatic degradation of β-endorphin (15). Evidence is available that β-endorphin can be degraded differentially into α-endorphin, γ-endorphin and their des-Tyr-analogues by enzymes associated with a rat brain fraction

*Present address: National Institute of Public Health, P.O.B. 1, Bilthoven, The Netherlands.
**Division of Molecular Neurobiology, Institute of Molecular Biology, University of Utrecht, Padualaan 8, Utrecht, The Netherlands.

0024-3205/80/110851-09$02.00/0

enriched in synaptosomal membranes (16). Although brain and pituitary levels of β-endorphin and enkephalins have been reported, up to the present very few data exist regarding the occurrence in vivo of α-endorphin, γ-endorphin and their respective des-tyrosine compounds. Therefore we decided to analyse the levels of β-endorphin, β-endorphin fragments and enkephalins in rat pituitary and brain tissue. We have previously reported that a combined application of the high resolving power of high-pressure liquid chromatography (HPLC) and the sensitivity of specific radioimmunoassay systems for Met-enkephalin and peptides of the α-, β- and γ-endorphin type is very useful for this purpose (17). The known susceptibility of endorphins and enkephalins to degradation by peptidases (18-20) prompted us to compare tissue levels of rats killed by decapitation with those of animals killed by focussed microwave irradiation, a method of sacrifice providing a procedure to destroy enzymatic activities very rapidly (21,22).

Materials and Methods

Sacrifice of the animals: Experiments were carried out with male Wistar rats weighing 210 - 240 g at the time of sacrifice. One group of animals was killed by exposing their heads for 3 sec to a focussed beam of microwave irradiation (160 W/cm^2), generated in a specially designed device by a Philips YT magnetron (2.45 GHz, 6 kW; ref. 21). To minimize possible stress induced by the immobilisation of the animals in the applicator of the microwave device that could lead to variation in pituitary and brain endorphin and enkephalin levels, the animals had been habituated to the procedure by a 7 day training period. The second group of rats was killed by decapitation. These animals had been handled and trained for 7 days to walk through a subway resembling the applicator of the microwave device.

Extraction from pituitary and brain tissue: Immediately after sacrifice of the animals the skull was opened and the brain and the pituitary gland were rapidly removed from the cranium. The brain (minus cerebellum) and the pituitary gland were transferred to polypropylene tubes containing 8 ml and 250 µl 1M acetic acid respectively, which were preheated to 95° C in a boiling water bath. After 15 min the solutions were chilled in ice. Brain tissue was homogenized with a Brinkman Polytron (setting 6 for 30 sec). Homogenization of pituitary tissue was achieved by sonification with a Branson sonifier (20 kHz for 30 sec). The homogenates were centrifuged 20 min at 30.000 g_{av} and the supernatants were lyophilized. The dry extracts were dissolved in 0.01 M ammonium acetate pH 4.15 (brain, 1.0 ml; pituitary, 200 µl) and, after removal of unsoluble residues by centrifugation, fractionated by high-pressure liquid chromatography (HPLC).

Fractionation by HPLC: The procedure of fractionation of β-endorphin fragments and enkephalins by HPLC and the quantitation of the peptides in the various fractions by appropriate radioimmunoassay systems has been described in detail elsewhere (17). The pituitary and brain extracts were fractionated on a reversed-phase µBondapak C18 column (0.39 x 30 cm; Waters Ass.) and UV absorbance was measured continuously at 210 nm. Because the endogenous levels of endorphins and enkephalins were too low to detect directly by UV absorbance, fractions with retention times corresponding to a number of synthetic β-LPH$_{61-91}$ fragments were collected.

Briefly the procedure was as follows: To the tissue extracts 10 µg ACTH$_{4-10}$ and 10 µg α-MSH were added as internal standards. The extracts were fractionated via gradient elution with 0.01 M ammonium acetate (pH 4.15) and methanol at a flow rate of 2 ml/min and fractions of 1 ml were collected in polystyrene tubes. Finally the column was calibrated with a mixture of synthetic β-endorphin, β-endorphin fragments and enkephalins. After removal of methanol in vacuo (Büchler vortex-evaporator, 60° C), fractions of the tissue extracts were pooled on guidance of the retention times of the internal standards (ACTH4-10 and α-MSH) and the reference chromatogram obtained with the synthetic endorphins and

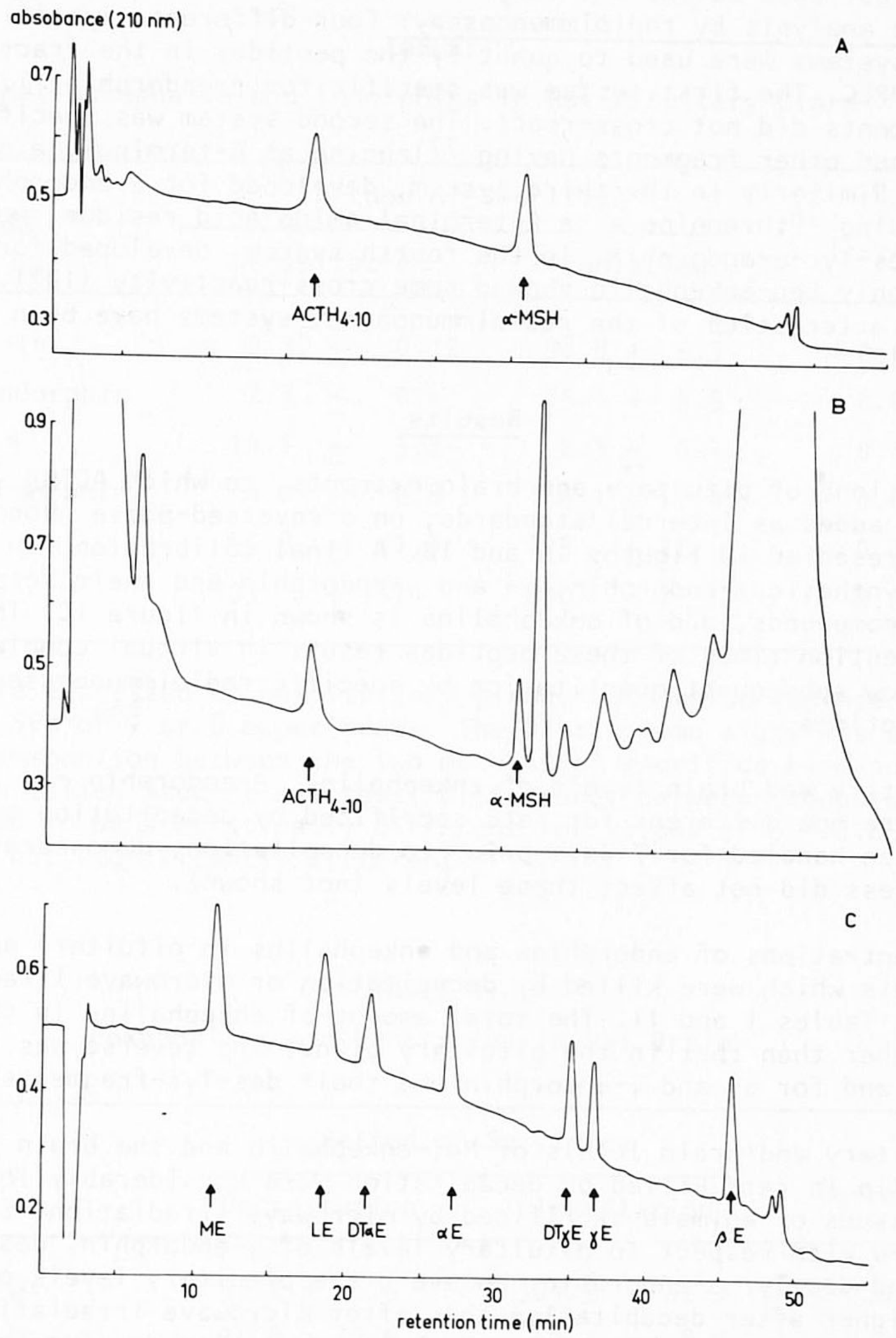

FIG. 1

HPLC chromatogram of a pituitary extract (A), a brain extract (B) and a mixture of synthetic endorphins and enkephalins (C) on a μBondapak C_{18} colum (0.39 x 30 cm). To the pituitary and brain extracts 10 μg $ACTH_{4-10}$ and 10 μg α-MSH were added as internal standards. The calibration run with synthetic peptides was done with a mixture of 10 μg of each peptide, except for β-endorphin (20 μg). Elution was performed with a 45 min concave gradient (program 7) of 0.01 M ammonium acetate, pH 4.15 (X) and methanol (Y): initial conditions X : Y = 7 : 3, final conditions X : Y = 1 : 3. The flow rate was 2 ml/min. Abbreviations used: DTαE = des-Tyr-α-endorphin, DTγE = des-Tyr-γ-endorphin, αE =α-endorphin, βE = β-endorphin, γE = γ-endorphin, LE = Leu-enkephalin, ME = Met-enkephalin.

enkephalins. The pooled fractions were lyophilized, the residues dissolved in phosphate buffered saline and subjected to radioimmunoassay.

Quantitave analysis by radioimmunoassay: Four different, specific radioimmunoassay systems were used to quantify the peptides in the fractions obtained by HPLC. The first system was specific for β-endorphin, i.e. smaller peptide fragments did not cross-react. The second system was specific for γ-endorphin and other fragments having ^{77}leucine as C-terminus, e.g. des-Tyr-γ-endorphin. Similarly in the third system, developed for α-endorphin, only fragments having ^{76}threonine as a C-terminal amino acid residue, were recognized, e.g. des-Tyr-α-endorphin. In the fourth system, developed for Met-enkephalin, only Leu-enkephalin showed some cross-reactivity (10%). More detailed characteristics of the radioimmunoassay systems have been described previously (17).

Results

Fractionations of pituitary and brain extracts, to which $ACTH_{4-10}$ and α-MSH had been added as internal standards, on a reversed-phase μBondapak C18 column are presented in Figures 1A and 1B. A final calibration run with a mixture of synthetic β-endorphin, α- and γ-endorphin and their respective des-tyrosyl compounds, and of enkephalins is shown in figure 1C. The differences in retention times of these peptides result in virtual complete separation and allow subsequent quantitation by specific radioimmunoassay systems for these peptides.

The pituitary and brain levels of enkephalins, β-endorphin and β-endorphin fragments were not different for rats sacrificed by decapitation and rats which had been handled for 7 days prior to decapitation, demonstrating that possible stress did not affect these levels (not shown).

The concentrations of endorphins and enkephalins in pituitary and brain tissue of rats which were killed by decapitation or microwave irradiation are given in Tables I and II. The total amount of enkephalins in the brain was much higher than that in the pituitary gland. The reverse was found for β-endorphin and for α- and γ-endorphin and their des-Tyr-fragments.

The pituitary and brain levels of Met-enkephalin and the brain levels of Leu-enkephalin in rats killed by decapitation were considerably lower than those in tissues of animals sacrificed by microwave irradiation. Similar data were obtained with respect to pituitary levels of γ-endorphin, des-Tyr-γ-endorphin and des-Tyr-α-endorphin. However, the pituitary levels of α-endorphin were higher after decapitation than after microwave irradiation. Brain levels of α-endorphin, γ-endorphin and their respective des-Tyr-fragments were not significantly different with the two methods of sacrifice used.

The amount of β-endorphin in the pituitary gland was similar in rats killed by decapitation and microwave irradiation, but in brain tissue lower β-endorphin levels were found after decapitation than after microwave irradiation.

Discussion

The high resolving power of reversed-phase HPLC allows separation of peptides that differ in only one amino acid residue, e.g. des-Tyr-α-endorphin from α-endorphin, des-Tyr-γ-endorphin from γ-endorphin and α-endorphin from γ-endorphin. Since the des-tyrosine peptide fragments cross-react completely in the radioimmunoassay systems developed for the parent peptides (17), it is possible to measure these peptides in biological tissue and fluid compartments. The present study provides the first evidence that des-Tyr-α-endorphin

TABLE I

Met-enkephalin and Endorphins in Rat Pituitary Gland

Peptide	Method of Sacrifice		P (t-test)
	Decapitation	Irradiation	
Met-enkephalin	0.30 ± 0.12	38.8 ± 5.3	< 0.001
des-Tyr-α-endorphin	2.7 ± 0.6	25.1 ± 4.5	< 0.001
α-endorphin	14.1 ± 3.2	2.3 ± 0.3	< 0.001
des-Tyr-γ-endorphin	4.7 ± 0.8	66.1 ± 17.4	< 0.005
γ-endorphin	45.8 ± 11.0	123 ± 31	< 0.05
β-endorphin	1103 ± 151	800 ± 71	N.S.

Data are expressed as ng/pituitary gland. Each value represents the mean ± SEM of 7 or 8 experiments. The last column signifies statistical comparison between the two methods of sacrifice by means of the two-tailed Student's t-test; differences between groups were assigned to be statistically different for values of $p < 0.05$. N.S. = not significantly different.

TABLE II

Enkephalins and Endorphins in Rat Brain

Peptide	Method of Sacrifice		P (t-test)
	Decapitation	Irradiation	
Met-enkephalin	90.2 ± 11.5	304.9 ± 62.7	< 0.005
Leu-enkephalin	34.8 ± 2.6	62.7 ± 8.0	< 0.005
des-Tyr-α-endorphin	2.6 ± 0.2	2.4 ± 0.1	N.S.
α-endorphin	1.5 ± 0.2	1.04 ± 0.1	N.S.
des-Tyr-γ-endorphin	2.3 ± 0.2	2.2 ± 0.1	N.S.
γ-endorphin	10.7 ± 0.3	15.5 ± 2.7	N.S.
β-endorphin	58.7 ± 7.0	171.4 ± 23.0	< 0.001

Data are expressed as ng/brain (minus cerebellum) and each value is given as the mean ± SEM of 9 or 10 experiments. For statistical evaluation see Table I.

and des-Tyr-γ-endorphin, which represent non-opiate-like β-endorphin fragments with profound effects in behavioral paradigms (15), might be formed in the pituitary gland and the brain. This opens the possibility to investigate the physiological role of these peptides in the control of adaptive behavior as well as in psychopathology.

The yield of endorphins and enkephalins in biological material varies with the extraction methodology. Smyth et al. (23) have suggested that the occurrence of α- and γ-endorphin and Met-enkephalin in brain tissue might be due to the degradation of β-endorphin during extraction under acidic conditions (0.1 M HCl, 0.1 - 2 M acetic acid) and they have found that extraction with acid acetone was the most effective procedure to suppress the enzymatic conversion of β-endorphin. However, our preliminary studies have shown lower levels of both β-endorphin and α- and γ-endorphin in rat pituitary (17) and brain tissue (unpublished results) after HCl-acetone extraction as compared to extraction with 1 M acetic acid. Rossier et al. (5) have reported that β-endorphin was not appreciably degraded as long as cell structures remained intact and they proposed to inactivate peptidases by heating tissue in 1 M acetic acid prior to homogenization. In addition, it has been shown by Childers and Snyder (24) that degradation of enkephalins did not occur in brain tissue of mice remaining undisturbed up to 6 hours post-mortem. Therefore, in order to minimize post-mortem breakdown by proteolytic enzymes and to obtain high levels of β-endorphin, the probable precursor of α- and γ-endorphin and their respective des-Tyr-analogues (16), we have used two different methods: One group of rats was killed by decapitation and the dissected tissues were heated in 1 M acetic acid before homogenization, according to Rossier et al. (5). A second group of animals was sacrificed by microwave irradiation, which is a well known method to inactivate enzymatic activities very rapidly (21, 22), and the dissected tissues were treated as those of the decapitated animals.

Significantly lower pituitary levels of Met-enkephalin and brain levels of both Met- and Leu-enkephalin were found in rats killed by decapitation than in rats sacrificed by microwave irradiation. It thus appears that a rapid post-mortem degradation of enkephalins occurs, in particular in the pituitary gland, and that boiling is not effective to inhibit this process. Our results with respect to the levels of Met- and Leu-enkephalin in whole rat brain as well as to the observed differences between the two methods of sacrifice, are in accordance with those reported by Yang et al. (25) who have also used tissue extraction with acetic acid. The brain levels of Met-enkephalin in decapitated animals are similar to those found by Hughes et al. (26) in rats which were killed by exsanguination and which brains were subsequently extracted with 0.1 M HCl. Regarding the dramatic differences found for the pituitary levels of Met-enkephalin between decapitation and microwave irradiation, comparable values have been published (27, irradiation; 28, decapitation and extraction with 0.1 M HCl). Thus, although several investigators (29, 30) have emphasized that microwave irradiation should not be a prerequisite to inactivate enkephalin breakdown unless using tissue homogenization in 0.05 - 0.1 M HCl, our study and the reported literature data seem to contradict this.

Pituitary β-endorphin levels after sacrifice by decapitation or microwave irradiation were identical. So it is likely that pituitary β-endorphin is relatively resistant against rapid post-mortem proteolysis and/or that heating of pituitary tissue is very effective in inhibiting this process. Inconsistent data are available regarding the levels of β-endorphin for whole rat pituitary gland, ranging from a few hundred ng (31) to approximately 3 μg(32) per pituitary gland, presumably due to differences in extraction and assay procedures. Nevertheless, the total amounts found by us are in excellent agreement with those reported by Höllt et al. (33) and Akil et al. (34). The brain levels of

β-endorphin were found to increase thrice when the animals were killed by microwave irradiation, which implies that heat inactivation was - at least in our hands - not effective enough to protect brain β-endorphin against post-mortem degradation. The brain β-endorphin levels of microwave irradiation killed rats were in the same order of magnitude as those found by others (5, 32, 35). However, Rossier et al. (5) have reported similar brain β-endorphin levels after decapitation and microwave irradiation, which is in contrast to our results and those of Ogawa et al. (35).

Little or no information is available regarding the occurrence of α- and γ-endorphin and their des-Tyr-fragments in rat pituitary and brain tissue. Rubinstein et al. (31) have identified α-endorphin in rat pituitary gland. However, they were not able to detect γ-endorphin, in contrast to the results of Jegou et al. (36) who have found relatively high amounts of γ-endorphin in the neuro-intermediate lobe. Rossier et al. (5) have shown the existence of α-endorphin in rat brain and they suggested that α-endorphin might be an artifact derived from β-endorphin after disruption of brain tissue. We now present data that β-endorphin fragments like α-, γ-, des-Tyr-α- and des-Tyr-γ-endorphin might be formed in the pituitary gland and, to a lesser extend, in the brain of the rat. The pituitary levels of γ-endorphin, des-Tyr-γ-endorphin and des-Tyr-α-endorphin in decapitated animals were significantly lower than those in rats killed by microwave irradiation, and the reverse was found for α-endorphin. The differences in levels of β-endorphin fragments between the two methods of sacrifice must be due to different enzymatic conversion of pituitary β-endorphin. The absolute amounts of the individual products do not indicate whether formation and/or degradation is involved. In contrast to the pituitary gland, no significant differences could be demonstrated between the values of α-endorphin, γ-endorphin and their des-tyrosine compounds from brains after death by either decapitation or microwave irradiation. This observation might indicate that these peptides are not very susceptible to post-mortem degradation in the brain.

In conclusion, this study presents evidence that α-endorphin, γ-endorphin and their respective des-Tyr-fragments are found in the rat pituitary gland and the brain. In addition our results show that sacrifice by focussed microwave irradiation seems to be more effective to block rapid post-mortem proteolysis of endorphins and enkephalins, particularly in the pituitary gland, than decapitation although this was followed by heat inactivation prior to homogenization.

Increasing evidence is available that brain β-endorphin and enkephalins are independent of those from pituitary origin and that in both tissues these peptides occur in morphologically separated systems. Moreover, it seems likely that the enkephalins in vivo are not metabolic products of the endorphins (32, 37-39). Burbach et al. (16) have found that β-endorphin can be degraded differentially by rat brain synaptosomal membranes which might imply that the enzymatic conversion of brain β-endorphin can be modulated. The physiological significance of the presence of β-endorphin fragments, representing peptides with distinct behavioral activities (15), in pituitary and brain tissue is being further investigated.

Acknowledgements

Human β-endorphin and related peptides were generous donations of Dr. H.M. Greven and Dr. J. van Nispen (Organon International B.V., The Netherlands). The authors wish to thank Miss E.G. Vergeer and Mr. J.H. Brakkee for their skilful technical assistance.

References

1. J. HUGHES, T.W. SMITH, H.W. KOSTERLITZ, L.A. FOTHERGILL, B.A. MORGAN and H.R. MORRIS, Nature 258 577-579 (1975).
2. R. SIMANTOV and S.H. SNYDER, Proc. Natl. Acad. Sci. USA 73 2515-2519 (1976).
3. C.H. LI, D. CHUNG and B.A. DONEEN, Biochem. Biophys. Res. Comm. 72 1542-1547 (1976).
4. N. LING, R. BURGUS and R. GUILLEMIN, Proc. Natl. Acad. Sci. USA 73, 3942-3946 (1976).
5. J. ROSSIER, A. BAYON, T.M. VARGO, N. LING, R. GUILLEMIN and F. BLOOM, Life Sci. 21 847-852 (1977).
6. D.G. SMYTH, C.R. SNELL and D.E. MASSEY, Biochem. J. 175 261-270 (1978).
7. D. DE WIED, B. BOHUS, J.M. VAN REE and I. URBAN, J. Pharmacol. Exp. Ther. 204 570-580 (1978).
8. M. LE MOAL, G.F. KOOB and F.E. BLOOM, Life Sci. 24 1631-1636 (1979).
9. F. BLOOM, D. SEGAL, N. LING and R. GUILLEMIN, Science 194 360-362 (1976).
10. Y.F. JACQUET and N. MARKS, Science 194 632-634 (1976).
11. L. TERENIUS and A. WAHLSTRÖM, Centrally Acting Peptides, ed. J. Hughes, pp. 161-178, MacMillan, London (1978).
12. D. DE WIED, G.L. KOVACS, B. BOHUS, J.M. VAN REE and H.M. GREVEN, Eur. J. Pharmacol. 49 427-436 (1978).
13. S.B. WEINBERGER, A. ARNSTEN and D.S. SEGAL, Life Sci. 24 1637-1644 (1979).
14. W.M.A. VERHOEVEN, H.M. VAN PRAAG, J.M. VAN REE and D. DE WIED, Arch. Gen. Psychiatry 36 294-298 (1979).
15. D. DE WIED, Characteristics and Function of Opioids, eds. J.M. van Ree and L. Terenius, pp. 113-122, Elsevier, Amsterdam (1978).
16. J.P.H. BURBACH, J.G. LOEBER, J. VERHOEF, E.R. DE KLOET and D. DE WIED, Nature, in press (1979).
17. J.G. LOEBER, J. VERHOEF, J.P.H. BURBACH and A. WITTER, Biochem. Biophys. Res. Comm. 86 1288-1295 (1979).
18. N. MARKS, A. GRYNBAUM and A. NEIDLE, Biochem. Biophys. Res. Comm. 74 1552-1559 (1977).
19. B.M. AUSTEN and D.G. SMYTH, Biochem. Biophys. Res. Comm. 77 86-94 (1977).
20. J.P.H. BURBACH, J.G. LOEBER, J. VERHOEF, E.R. DE KLOET and D. DE WIED, Biochem. Biophys. Res. Comm. 86, 1296-1303 (1979).
21. V.M. WIEGANT, A.J. DUNN, P. SCHOTMAN and W.H. GISPEN, Brain Res. 168 565-584 (1979).
22. R.H. LENOX, P.V. BROWN and J.L. MEYERHOFF, Trends in Neurosciences 2 106-109 (1979).
23. D.G. SMYTH, B.M. AUSTEN, M.J. GEISOW and C.R. SNELL, Molecular Endocrinology, eds. Macintyre and Szelke, pp. 327-336, Elsevier, Amsterdam (1977).
24. S.R. CHILDERS and S.H. SNYDER, Endorphins in Mental Health Research, eds. E. Usdin, W.E. Bunney and N.S. Kline, pp. 181-188, MacMillan Press, London (1979).
25. H.-Y. YANG, J.S. HONG and E. COSTA, Neuropharmacology 16 303-307 (1977).
26. J. HUGHES, H.W. KOSTERLITZ and T.W. SMITH, Br. J. Pharmacol. 61 639-647 (1977).
27. J.S. HONG. H.-Y. YANG, W. FRATTA and E. COSTA, J. Pharmacol. exp. Ther. 205 141-147 (1978).
28. R.J. MILLER, K.-J. CHANG, B. COOPER and P. QUATRECASAS, J. Biol. Chem. 253 531-538 (1978).
29. D. WESCHE, V. HÖLLT and A. HERZ, Naunyn-Schmiedeberg's Arch. Pharmacol. 301 79-82 (1977).
30. C. GROS, P. PRADELLES, C. ROUGET, O. BEPOLDIN, F. DRAY, M.C. FOURNIE-ZALUSKI, B.P. ROQUES, H. POLLARD, C. LLORENZ-CORTES and J.C. SCHWARTZ, J. Neurochem. 31, 29-39 (1978).
31. M. RUBINSTEIN, S. STEIN and S. UDENFRIEND, Proc. Natl. Acad. Sci. USA 74, 4969-4972 (1977).
32. J. ROSSIER, T.M. VARGO, S. MINICK, N. LING, F.E. BLOOM and R. GUILLEMIN,

Proc. Natl. Acad. Sci. USA 74 5162-5165 (1977).
33. V. HÖLLT, R. PRZEWLOCKI and A. HERZ, Life Sci. 23 1057-1066 (1978).
34. H. AKIL, S.J. WATSON, R.M. LEVY and J.D. BARCHAS, Characteristics and Function of Opioids, eds. J.M. van Ree and L. Terenius, pp. 123-134, Elsevier, Amsterdam (1978).
35. M. OGAWA, A.E. PANERAI, S. LEE, G. FORSBACH, V. HAVLICEK and H.G. FRIESEN, Life Sci. 25, 317-325 (1979).
36. S. JEGOU, M.C. TONON, F. LEBOULENGER, C. DELARUE, H. VAUDRY and N. LING, Biochem. Biophys. Res. Comm. 83 201-208 (1978).
37. S.J. WATSON, H. AKIL, C.W. RICHARD and J.D. BARCHAS, Nature 275, 226-228 (1978).
38. E. WEBER, K.H. VOIGT and R. MARTIN, Proc. Natl. Acad. Sci. USA 75, 6134-6138 (1978)
39. K. KANGAWA, H. MATSUO and M. IGARASHI, Biochem. Biophys. Res. Comm. 86 153-160 (1979).