Vol. 147, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 31, 1987 Pages 416-421

THE ACTION OF NEUTROPHIL ELASTASE ON INTACT AND OXIDIZED (MET)-ENKEPHALIN-ARG^{6}-GLY^{7}-LEU^{8} PEPTIDE

Wieslaw Watorek[1] and James Travis[2]

[1]Institute of Biochemistry, University of Wroclaw, Wroclaw, Poland

[2]Dept. of Biochemistry, University of Georgia, Athens, Georgia 30602

Received July 17, 1987

SUMMARY: Neutrophil elastase has been found to cleave met-enkephalin-arg^{6}-gly^{7}-leu^{8} between met^{5} and arg^{6} thereby releasing the active opiate met-enkephalin. Oxidized met-enkephalin octapeptide is not attacked by this enzyme. These data suggest a potential role for neutrophil proteinases and oxidases in the regulation of opiate production in tissues. © 1987 Academic Press, Inc.

The enkephalins are a group of opioid-like peptides derived by processing of a series of precursor proteins which include pro-opiomelanocortin, prodynorphin, and proenkephalin (1). Such proteins are apparently synthesized in a number of tissues, including the adrenal medulla, brain, and intestinal mucosa and processes either completely into the met-enkephalin or leu-enkephali pentapeptides or, alternatively, into intermediate fragments (2). These peptides have been reported to function not only as analgesics in the central nervous system (3) but also in immunoregulatory processes in plasma (4) where they bind to specific receptors on immune-competent cells (5). At the moment it is unclear as to how these peptides are processed from precursors to the final form (6). However, it has been suggested that a carboxypeptidase B-like enzyme (enkephalin convertase) (6) and/or cathepsin B (7) might be involved.

0006-291X/87 $1.50
Copyright © 1987 by Academic Press, Inc.
All rights of reproduction in any form reserved.

In this laboratory we have been investigating the function of two major proteinases from human neutrophils, elastase and cathepsin G (8,9). The former has been found to rapidly cleave peptide bonds after methionyl residues (10); however, if the methionine residue is oxidized no hydrolysis is observed (10,11). In the processing scheme for enkephalin production specific cleavage at the met^{5}-arg^{6} peptide bond is required in order to obtain the met-enkephalin pentapeptide, while oxidation of the methionine residue markedly reduces opiate activity (12). Because neutrophils are found virtually everywhere in human tissues but especially at inflammatory sites where they readily degranulate to release both hydrolytic and oxidative enzymes, and because enkephalin precursor peptides have been found to circulate in plasma apparently destined for these same sites (13), we began a study to determine whether such cells might play a role in modulating the conversion of met-enkephalin precursors to their final form.

MATERIALS AND METHODS

(Met)-enkephalin-arg^{6}-gly^{7}-leu^{8} was obtained from Sigma and re-purified by reverse phase HPLC on a Bio-Rad RP 318 column. Human neutrophil elastase was purified according to the procedure of Baugh and Travis (14).

Enkephalin digestion: (met)-enkephalin-arg^{6}-gly^{7}-leu^{8} or its oxidized form (440 nanomoles) was digested at 37^{o} with neutrophil elastase (1 nanomole) in a final volume of 250 ul of 0.1M sodium phosphate buffer, pH 8.0, 0.1M NaCl. Forty ul aliquots were removed both prior to addition of enzyme and after 10, 30, 90, and 120 min, with the reaction being stopped by adjustment of the pH to 4.0 with 10 ul of 3.0M sodium acetate buffer, pH 4.0. The products of digestion were identified by reverse phase HPLC on a Bio-Rad RP 318 column (250 x 4.6 mm) equilibrated with 0.1% TFA, using a linear gradient from zero to 45% acetonitrile (v/v) in 0.1% TFA at a flow rate of 1.0 ml/min. All peptide containing fractions were lyophilized and subsequently analyzed after acid hydrolysis (6.0N HCl, 0.1% thioglycollic acid, 0.05% phenol, 100^{o}, in vacuo, 24 hr), using a Beckman 119CL amino acid analyzer.

Oxidation of (met)-enkephalin-arg^{6}-gly^{7}-leu^{8} was obtained by incubation in hydrogen peroxide. Specifically, 440 nanomoles of peptide was dissolved in 400 ul of water and 800 ul of 3% hydrogen peroxide added. After incubation at 4^{o} overnight, the oxidized peptide was lyophilized and subjected to amino acid analysis to insure complete oxidation of the methionine residue (15).

RESULTS AND DISCUSSION

The effect of neutrophil elastase on the conversion of (met)-enkephalin-arg^{6}-gly^{7}-leu^{8} to (met)-enkephalin and the tripeptide arg-gly-leu is readily seen in the results given in both Figure 1 and Table 1. HPLC separation readily documents the decrease in levels of the starting peptide (C) and the appearance of two other components (A and B). Amino acid analysis of each of these peptides indicates that they are as follows:

peak (A): arg-gly-leu

peak (B): tyr-gly-gly-phe-met

peak (C): tyr-gly-gly-phe-met-arg-gly-leu

The yields of each component after specific digestion times are given in Table 1.

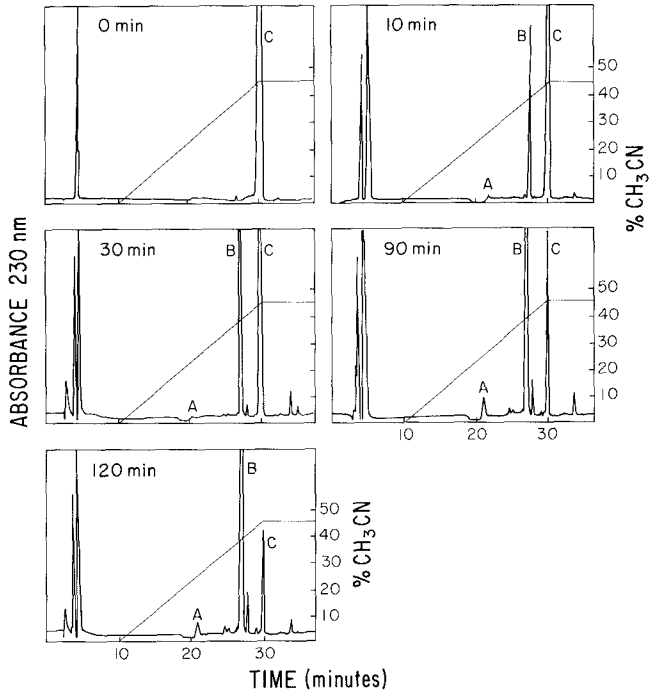

Figure 1. Reverse-phase high pressure liquid chromatography separation of human neutrophil elastase digestion products of (met)-enkephalin-arg^{6}-gly^{7}-leu^{8} peptide at various time intervals. Peak A: arg-gly-leu; Peak B: tyr-gly-gly-phe-met; Peak C: tyr-gly-gly-phe-met-arg-gly-leu.

Table 1. Recovery of peptides produced by digestion of (met)-enkephalin-arg^6-gly^7-leu^8 with neutrophil elastase

Peak Number	Time of Incubation			
	10 min	30 min	90 min	120 min
A	6.0	12.0	40.0	55.0
B	3.5	15.0	27.0	35.0
C	46.0	30.0	6.0	4.0

Recoveries are expressed as nanomoles of peptide recovered per 70 nanomoles of digest analyzed.

In contrast to this rapid conversion no hydrolysis was obtained with the oxidized (met)-enkephalin octapeptide even after the longest incubation time period (120 min).

It is known that (met)-enkephalin-arg^6-gly^7-leu^8 has only 35% of the opiate receptor binding activity of (met)-enkephalin (16). Furthermore, sinced increased enkephalin release occurs under stress conditions (17), at a least a part of which is in the form of incompletely converted polypeptide precursors (18), speculation can be made for the involvement of neutrophil enzymes in controlling both the processing and activity of all of these peptides. One possibile role is for maximum production of the most functional opiate in this series, the met-enkephalin pentapeptide, while a second and possibly more important function might involve the enhanced recruitment of leukocytes to inflammatory sites since met-enkephalin is known to promote this effect (19). As noted, the oxidized octapeptide cannot be converted into (met)-enkephalin by neutrophil elastase. This suggests a third, regulatory role for oxidizing enzymes such as neutrophil myeloperoxidase which would limit the quantity and functional activity of both (met)-enkephalin precursors and met-enkephalin at inflammatory sites, in effect increasing pain in such areas.

Finally, it is known that one of the seven potential forms of enkephalins which can be obtained from the precursor protein is the potent leu-enkephalin opiate (1). How the formation of this pentapeptide occurs is as yet unknown. It should be pointed out, however, that the other major proteinase in neutrophil granules, cathepsin G, can hydrolyze peptide bonds after leucyl residues (20) Whether this actually occurs in the formation of leu-enkephalin remains to be established. However, it is worth noting that in elastase, cathepsin G, and myeloperoxidase, one has all of the enzymes required for the final processing and regulation of the seven potential enkephalins present in proenkephalin (1).

REFERENCES

1. Lewis, R.V. (1984) Life Chem. Rep. 2, 237-256.
2. Herbert, E., Oates, E., Martens, G., Comb, M., Rosen, H., and Uhler, M. (1983) Cold Spring Harbor Symp. Quant. Biol. 48, 375-382.
3. Goodman, R.R., Fricker, L.D., and Snyder, S.H. (1983) in Brain Peptides, Krieger, D.T., Brownstein, M.J., and Martin, J.B. (eds.), pp. 827-849, J. Wiley, New York.
4. Evans, C.J., Erdelyi, E., and Barchas, J.D. (1986) in Enkephalins and Endorphins. Stress and Immune System, Plotnikoff, N.P., Faith, R.E., Murgo, A.J., and Good, R.A. (eds.), pp. 3-15, Plenum Press, New York.
5. Loper, A., Abood, L.G., Moss, W., and Lionetti, F.J., (1980) Biochem. Pharmacol. 29, 1361-1365.
6. Lynch, D.R. and Snyder, S.H. (1986) Annu. Rev. Biochem. 55, 773-779.
7. Marks, N., Berg, M.J., and Benuck, M. (1986) Arch. Biochem. Biophys. 249, 489-499.
8. Salvesen, G., Farley, D., Shuman, J., Przybyla, A., Reilly, C., and Travis, J. (1987) Biochemistry 26, 2289-2293.
9. Sinha, S., Watorek, W., Karr, S., Giles, J., Bode, W., and Travis, J. (1987) Proc. Natl. Acad. Sci. USA 84, 2228-2232.
10. Nakajima, K., Powers, J.C., Ashe, B.M., and Zimmerman, M. (1979) J. Biol. Chem. 254, 4027-4032.
11. Del Mar, E.G., Brodrick, J.W., Geokas, M.C., and Largman, C. (1979) Biochem. Biophys. Res. Commun. 88, 346-350.
12. Turkall, R.M., Denison, R.C., and Tsan, M-F. (1982) J. Lab. Clin. Med. 99, 418-427.
13. Brown, S.L., Tokuda, S., Saland, L.C., and Van Epps, D. (1986) in Enkephalins and Endorphins. Stress and Immune Systems, Plotnikoff, N.P., Faith, R.E., Murgo, A.J., and Good, R.A. (eds.), p.p. 367-386, Plenum Press, New York.
14. Baugh, R. and Travis, J. (1976) Biochemistry 15, 836-841.

15. Shechter, Y., Burstein, Y., and Patchornik, A. (1975) Biochemistry 14, 4497-4503.
16. Kilpatrick, D.L., Jones, B.N., Kojima, K., and Udenfriend, S. (1981) Biochem. Biophys. Res. Commun. 103, 698-705.
17. Terman, G.W., Shavit, Y., Lewis, J.W., Cannon, J.T., and Liebeskind, J.C. (1984) Science 226, 1270-1277.
18. Viveros, O.H., Diliberto, E.J., Jr., Hazum, E., and Chang, K.J. (1979) Molec. Pharmacol. 16, 1101-1108.
19. Van Epps, D.E., Saland, L., Taylor, C., and Williams, R.C., Jr. (1983) in Progress in Brain Res. 59, Behan, P.O., Mellen, V., and Rose, F.C. (eds.), pp. 361-374, Elsevier, Amsterdam.
20. Blow, A., and Barrett, A. (1979) Biochem. J. 161, 17-19.