ENKEPHALINASE A ACTIVITY IN DIFFERENT REGIONS OF BRAIN AND SPINAL CORD OF NORMAL AND CHRONIC ARTHRITIC RATS

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1. Introduction

Several reports suggest that enkephalins are neuromodulators involved in the control of acute pain [1]. Their role in the control of chronic pain has been less extensively studied. Increased levels of Met-enkephalin (ME) were found in both dorsal and ventral halfs of the spinal cord of polyarthritic rats [2]. The origin of the elevated ME levels, either increased synthesis, decreased degradation or both is not known. Recent studies indicate that enkephalinase A (enkephalinase), a membrane-bound carboxydipeptidease [3], is selectively involved in the biological degradation of the enkephalins [4,5]. Hence changes in enkephalinase activity could play a role in the control of chronic pain. The finding that chronic morphine treatment has an effect on the enkephalinase activity, at least in the striatum, cortex and hypothalamus of mice, is consistent with this hypothesis [3,4]. To test a possible role of enkephalinase in the control of chronic pain we measured the ME-degrading activity of enkephalinase in different regions of the brain and spinal cord of chronic arthritic rats and control animals.

The specific enkephalinase activity in the spinal cord was ~40% of the activity in the striatum, the region presenting the highest activity in the central nervous system (CNS). The specific activity was higher in the dorsal half than in the anterior half of the spinal cord in correspondence with the known ME distribution [2]. Chronic morphine treatment slightly increased the enkephalinase activity in several brain regions. However in chronic arthritic rats no difference in enkephalinase activity could be detected in any of the regions examined.

2. Materials and methods

Puromycin and *p*-chloromercuribenzoate (PCMB) were purchased from Aldrich, Tyr–Gly–Gly (TGG) from Serva, ME from UCB and [*Tyr*-3,5-^{3}H]ME (36 Ci/mmol) from Radiochemical Centre (Amersham). Captopril was a gift from Squibb SA (Brussels). Porapak Q beads (100–120 mesh) were obtained from Waters Associates and TLC Kieselgel 60 plastic sheets (0.2 mm) from Merck.

2.1. *Arthritis induction*

The preparation and selection of arthritic rats were done at the Janssen Pharmaceutica Center (Beerse). Polyarthritis induction and housing conditions were as in [6].

2.2. *Morphine implantation*

A morphine pellet (morphine–HCl 75 mg, chlorbutol 1%, Mg–stearate 1% and lactose q.s. ad 100 mg) was implanted subcutaneously on the back of male Wistar rats (200 g body wt) under light ether anesthesia. Control rats received a placebo pellet. All operations were carried out between 9:00 and 10:00 a.m. The absorption rate of morphine from the pellets was linear for at least 8 days (6.25%/day), as determined by absorption at 285 nm, after extraction of the morphine from the pellet with 0.1 N HCl.

2.3. *Brain and spinal cord dissection*

All animals were killed by decapitation between 10:00 and 11.00 a.m. and their brains and spinal cords were rapidly removed and placed on an ice-cold plate. The brain was dissected according to [7]. The lumbo–thoracal enlargement of the spinal cord was

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cut in 1 mm slices on a glass plate cooled above N_2 vapour and divided into a dorsal and ventral half. The cervical enlargement was kept intact.

2.4. *Preparation of a particulate fraction*

Tissues obtained from each rat were homogenized separately in 10 vol. (v/w) of 25 mM Tris–HCl buffer (pH 7.4 at 4°C) with a motor-driven Teflon pestle (2000 rev./min; Janke-Kunkel, Ika-Werk) in glass Potter homogenizers (Thomas AA) at 4°C. The homogenate was centrifuged at 10 000 × *g* for 20 min at 4°C. The supernatant was discarded and the pellet resuspended in buffer and left for 1 h at 4°C. After a second centrifugation the final pellet was resuspended in 20–30 vol. (v/w) of buffer and kept frozen until use. Protein content of the particulate fractions was determined by the method [8].

2.5. *Enkephalinase A assay*

Assays were performed in duplicate at 30°C for 15 min in Eppendorf tubes, containing Tris–HCl buffer 25 mM (pH 7.4), PCMB 0.2 mM, puromycin 0.2 mM, [*Tyr*-^{3}H]ME 10 nM and a sample of the particulate fraction (containing 100–200 μg protein) in 0.25 ml final vol. The reaction was started by adding [^{3}H]ME and stopped with 0.1 ml 15%. After deproteinisation the labeled metabolites were separated in duplicate by chromatography on Porapak Q beads columns (80 mg) as in [3,9]. Values were corrected by subtracting blanks being obtained by adding trichloroacetic acid before the ME solution. More than 90% of the labeled metabolites, formed during the incubation, consisted of TGG as revealed by TLC (R_F 0.36 in butanol:acetic acid:water, 4:1:1) (not shown). By cutting the entire TLC plate in fractions of 0.5 cm no other radioactive peaks than Tyr (R_F 0.51), TGG, ME (R_F 0.75) and ME-oxide (R_F 0.27) could be detected, excluding the action of enkephalinase B [10] (not shown). The involvement of angiotensin-converting enzyme in the formation of TGG can be ruled out by working with ME at < 100 nM [4]. Furthermore Captopril (SQ 14 225), a selective inhibitor of angiotensin-converting enzyme [11], added at 10^{-8}–10^{-4} M did not decrease the formation of ME metabolites (not shown).

The Tyr release during the assay is presumably caused by the contamination of the particulate fraction with nuclei. When a striatal membrane fraction, purified by using a discontinuous sucrose gradient [12], was tested in our assay only TGG was revealed. However, in the nuclear fraction a very high, partly inhibited by PCMB and puromycin, Tyr-releasing activity was found (not shown).

3. Results and discussion

3.1. *Regional distribution of enkephalinase A*

In agreement with the known distribution of ME [13] and of opiate receptors [14] in rat brain, the highest enkephalinase A activity was found in the striatum. In the spinal cord the activity was only slightly lower than in the cortex or the brain stem and markedly higher in the dorsal than in the ventral part (table 1). This corresponds to the distribution of ME in the spinal cord, which is also higher in the dorsal part [2].

Only a few reports, which are difficult to compare

Table 1
Enkephalinase activity in various brain parts of arthritic and control rats at day 21 and 31 of the disease

Brain area	Day 21		Day 31	
	Arthritis	Control	Arthritis	Control
Cortex	145.68 ± 8.04 (5)	149.44 ± 16.35 (5)	132.64 ± 12.11 (6)	128.06 ± 11.63 (6)
Striatum	352.68 ± 12.52 (4)	362.92 ± 9.30 (5)	334.94 ± 25.63 (6)	333.66 ± 32.86 (6)
Brainstem	192.88 ± 14.36 (5)	176.67 ± 10.53 (5)	154.32 ± 9.78 (6)	141.21 ± 4.73 (6)
Cervical spinal cord	143.58 ± 4.86 (5)	142.14 ± 7.25 (5)	99.12 ± 7.59 (6)	98.98 ± 7.62 (5)
Lumbo–thoracal spinal cord				
dorsal	148.25 ± 10.67 (5)	141.53 ± 12.32 (5)	121.93 ± 11.13 (6)	124.43 ± 4.68 (5)
ventral	103.01 ± 5.40 (5)	98.53 ± 4.72 (5)	85.60 ± 5.87 (6)	84.60 ± 3.80 (5)

Specific activity expressed as fmol ME degraded . mg protein^{-1} . min^{-1}; means ± SEM (no. rats)

with our results, have described a regional distribution of enkephalinase activity in rat brain. Data on the spinal cord are not available. In a first report [15] the enzymatic activities were extremely low, probably due to proteolytic processes during the preparation of their particulate fraction and the absence of inhibitors in their assay. In a second report [16] very high values were obtained due to the purification of the enzyme source and the high enkephalin concentration in the assay. However, the activities in various brain structures of the rat found here are very similar to those observed in mice and rats [4], if one takes into account the difference in incubation temperature.

3.2. *Effect of morphine and chronic pain on enkephalinase A activity*

The possibility exists that manipulations of the enkephalinergic system(s) such as chronic morphine treatment or chronic pain, might alter the enkephalinase activity. In [3] enkephalinase activity increased after morphine pellet implantation in mice. These changes are also detectable in rats. Eight days after implantation of the morphine pellet an increase in the activity of the enkephalinase was observed in cortex, striatum and brainstem (not shown). It is not easy to relate these changes during chronic morphine administration to alterations in the enkephalin levels because of the lack of congruent data. They range from a 2-fold increase [17], no changes [18] or a decrease only after a short regimen implantation [19].

To our knowledge only one report is available at the moment describing effects of chronic pain on the enkephalinergic system. In [2] no changes in ME levels were found in different brain areas of chronic arthritic rats at day 21 of the disease. We did not detect alterations of enkephalinase activity in cortex, striatum or brainstem (table 1). However increased ME levels in the spinal cord were observed [2]. Changes in enkephalinase activity in the cervical enlargement or in the dorsal or ventral halves of the lumbo–thoracal region could not be detected in our experiments at day 21. These observations are strengthenened by the fact that also in a later phase (day 31) no differences between arthritic and control rats were noticed (table 1).

These results do not sustain the hypothesis that a decreased enkephalinase activity is responsible for the increase of ME levels observed in the spinal cord of chronic pain animals. Increased synthesis and/or release of ME may be therefore more important mechanisms involved in the regulation of ME levels and the control of pain in the spinal cord.

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References

[1] Fields, H. L. and Basbaum, A. I. (1978) Annu. Rev. Physiol. 40, 217–248.
[2] Cesselin, F., Montastruc, J. L., Gros, C., Bourgoin, S. and Hamon, M. (1980) Brain Res. 191, 289–293.
[3] Malfroy, B., Swerts, J. P., Guyon, A., Roques, B. P. and Schwartz, J. C. (1978) Nature 276, 523–526.
[4] Schwartz, J. C., de la Baume, S., Malfroy, B., Patey, G., Perdrisot, R., Swerts, J. P., Fournié-Zaluski, M. C., Gacel, B. and Roques, B. P. (1980) in: Neural peptides and neuronal communication (Costa, E. and Trabucci, M. eds) pp. 219–235, Raven, New York.
[5] Snyder, S. H. (1980) Science 209, 976–983.
[6] De Castro Costa, M., De Sutter, P., Gybels, J. and Van Hees, J. (1981) Pain 10, 173–185.
[7] Gispen, W. H., Schotman, P. and De Kloet, E. R. (1972) Neuroendocrinology 9, 285–296.
[8] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[9] Vogel, Z. and Altstein, M. (1977) FEBS Lett. 80, 332–336.
[10] Gorenstein, C. and Snyder, S. H. (1979) Life Sci. 25, 2065–2070.
[11] Rubin, B., Laffan, R. J., Kotler, D. G., O'Keefe, E. H., Demaio, D. A. and Goldberg, M. E. (1968) J. Pharmacol. Exp. Therap. 204, 271–280.
[12] Whittaker, V. P. (1965) Prog. Biophys. Mol. Biol. 15, 39–96.
[13] Sar, M., Stumpf, W. E., Miller, R. J., Chang, K. J. and Cuatrecasas, P. (1978) J. Comp. Neurol. 182, 17–37.
[14] Pert, C. B., Kuhar, M. J. and Snyder, S. H. (1976) Proc. Natl. Acad. Sci. USA 73, 3729–3733.
[15] Sullivan, C., Akil, H. and Barchas, J. D. (1979) Commun. Psychopharmacol. 2, 525–531.
[16] Gorenstein, C. and Snyder, S. H. (1980) Proc. R. Soc. Lond. B 210, 123–132.
[17] Simantov, R. and Snyder, S. H. (1976) Nature 262, 505–507.
[18] Fratta, W., Yang, H.-Y. T., Hong, J. and Costa, E. (1977) Nature 268, 452–453.
[19] Shani, J., Azov, R. and Weismann, B. A. (1979) Neurosci. Lett. 12, 319–322.