Neuropeptide-converting enzymes in cerebrospinal fluid

Activities increased in pain from herniated lumbar disc, but not from coxarthrosis

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We measured activities of dynorphin-converting enzyme (DCE), substance P endopeptidase (SPE) and angiotensin-converting enzyme (ACE) in cerebrospinal fluid (CSF) in 13 patients with rhizopathic pain from an herniated lumbar disc, in 9 patients with pain from coxarthrosis and in 11 control patients without pain. In the patients with disc hernia and coxarthrosis, another sample of CSF was analyzed 3–12 months after treatment, when pain had subsided.

The DCE activity in the patients was higher than that in both the control patients and the patients with pain from coxarthrosis (nociceptive pain). Similarly, the activity of SPE was lower in the patients with herniated lumbar disc than in controls and in the patients with coxarthrosis. After treatment, the difference in activity compared to controls was lower, but still significant in patients with herniated discs. The ACE activity did not differ from controls in patients with ischialgia, while it was increased in patients with coxarthrosis. This increase also remained after arthroplasty with pain relief.

In conclusion, measurements of neuropeptides may be useful for evaluating neuropathic pain.

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Neuropeptides play an important role in the propagation and modulation of pain impulses in the central nervous system (Fields and Basbaum 1989, Yaksh and Aimone 1989). Extensive studies have been done on the quantification of these compounds, especially on opioid peptides and substance P in nervous tissue or in body fluids from subjects with pain disorders. Opioid peptides and substance P have been evaluated in the cerebrospinal fluid (CSF) collected from patients with depression and with different types of chronic pain (for review, see Nyberg et al. 1988). Variations in the levels of CSF endorphins and/or substance P have been reported in many conditions, such as chronic lumbar disc pain, chronic organic neurogenic pain and migraine (Nyberg 1993).

The activity and concentration of opioid peptides in neuronal tissue is under enzymatic control. In human CSF, a dynorphin-converting enzyme (DCE) (Nyberg et al. 1985) has been identified, as well as an endoprotease with high specificity towards substance P (Nyberg et al.1984), named substance P endopeptidase (SPE). In addition to DCE, angiotensin-converting enzyme (ACE) also can hydrolyze opiopeptides (Terenius and Nyberg 1991). Because of their role in hydrolyzing amino acid bonds of their substrates, the specific neuropeptide convertases are believed to be markers for synaptic activity in pain fibers in the CNS (Terenius and Nyberg 1991).

We investigated whether these enzymes differed in patients with rhizopathic pain (from herniated lumbar disc), compared to nociceptive pain (coxarthrosis) and to patients with no pain.

Patients and methods

Patients and sampling procedure

Patients in group A (5 women, 8 men; mean age 38 (23–54) years) for an average of 6 (1–13) months had symptoms of ischialgia caused by a herniated lumbar disc,verified at myelography and/or at operation. 12 mL of CSF were collected, and divided into 4 aliquots of 3 mL and kept in MiniSorb tubes (NUNC, Denmark). The CSF was frozen immediately at –70 °C. Samples were taken at lumbar myelography and at repeated lumbar puncture (LP) 3-11 months after opera-

tive removal of a herniated disc or spontaneous subsidence of symptoms.

Patients in group B (6 women, 3 men; mean age 73 (53–95) years) all had pain due to coxarthrosis and were candidates for total hip replacement. CSF was obtained in the same manner as in group A—i.e., immediately before operation during spinal anesthesia and 4–6 months after the operation

All patients graded their pain on a visual analogue scale immediately before the myelography and spinal anesthesia, and also before the lumbar control puncture.

Controls (group C) were patients (3 women, 8 men; mean age 45 (19–71) years) with healed fractures without pain, admitted for removal of osteosynthesis material. CSF was taken in the same manner as in groups A and B, but only one sample was taken from each patient.

The study was approved by the local ethics committee at University Hospital, Uppsala, Sweden and consent was given by all patients taking part in the study.

Chemicals

Standard peptides used in this study were purchased from Bachem (Bubendorf, Switzerland) except for substance P_{1-7} and Tyr-substance P_{1-7}, which were prepared by Dr. Gunnar Lindeberg (Department of Immunology, University of Uppsala, Sweden). The protease inhibitors amastatin and phosphoramidon were obtained from Sigma Chemical Co. (St Louis, MO, USA) and captopril from Squibb (Princeton, NJ, USA). The ACE substrate hippuryl-His-Leu was purchased from CRB (Cambridge, UK) and its ^{14}C-labeled analogue from NEN Products (Stockholm, Sweden). All other chemicals and solvents were of analytical reagent grade and came from commercial sources. Labeling of peptides with radioactive iodine was carried out with the chloramine-T method and, before use in radioimmunoassay, the iodinated peptide was purified by HPLC (Persson et al. 1992).

Test procedures

To determine DCE activity, we used dynorphin A as substrate and the activity of dynorphin-converting enzyme was monitored by following the release of Leu-enkephalin-Arg_6 from synthetic dynorphin A by a radioimmunoassay (Persson et al. 1992). Further degradation was prevented by adding protease inhibitors to the reaction mixture. Details of incubations, dilutions and preparations before radioimmunoassay were the same as described by Persson et al. (1992).

The activity of substance P endopeptidase was monitored by following the conversion of substance P to its N-terminal fragment substance P_{1-7}. The formation of product was guided by a SP_{1-7} specific radioimmunoassay and preparation of mixtures before radioimmunoassay were the same as described by Persson et al. (1992). The ACE activity was monitored with hippuryl-His-Leu as substrate, as described by Lantz et al. (1992). The radioimmunoassays for Leu-enkephalin-Arg_6 and substance P_{1-7} were conducted as described in a previous paper (Persson et al. 1992).

Statistics

Statistical analyses of the recorded enzymatic activities were performed by means of duplicate determinations, using two-factor analysis of variance (ANOVA) with factor interaction, followed by the student-Newman-Kreul's multiple comparison test. All calculations were carried out on log-transformed data since, when tested for normality, data were found to be log-normally distributed.

Results (Table 1)

Before myelography, the patients with ischialgia from a herniated lumbar disc, graded their pain as 56 *SD 13* on a 100 mm visual analogue scale. At the control lumbar puncture after treatment, it was 13 *13* mm. The patients with coxarthrosis graded their pain as 49 *19* at their first lumbar puncture before operation, and as 7 *10* at follow-up. The reduction of pain was significant in both groups.

DCE activity in CSF from patients with a herniated disc was increased more than in the control samples. Postoperatively, the DCE activity showed a tendency to decline. However, it was still significantly elevated in comparison with that in the controls. The reduction in DCE activity seen in these patients was as common in patients who had only partial pain relief as in those with total pain relief, i.e., there was no correlation between a decline in DCE activity and relief of pain.

In contrast to DCE, the levels of SPE activity in CSF from patients with herniated disc was preoperatively significantly lower than in the controls. Following operation, the SPE activity increased to reach control levels. The ACE activity in CSF from these patients was the same both pre- and postoperatively.

In patients with coxarthrosis levels of both DCE and SPE, activity remained unchanged when compared to those of controls. Furthermore, no alteration in their activity was observed following operation. On the other hand, ACE activity in these patients was significantly increased both pre- and postoperatively.

Table 1. Neuropeptide-converting enzyme activities (mean *SEM*) in patients with chronic pain and in controls

Enzyme	Control			Ischialgia					Coxarthrosis				
				Preoperative			Postoperative		Preoperative			Postoperative	
DCE (pmol/h*mL) [a]	(n 11)	13	*1.2*	(n 13)	25	*2.2***	22	*2.7***	(n 9)	13	*1.3*	13	*1.6*
SPE (pmol/h*mL) [b]	(n 11)	12	*1.6*	(n 11)	7.0	*1.6**	10	*2.7*	(n 9)	13	*3.2*	11	*2.4*
ACE (nmol/h*mL) [c]	(n 11)	1.6	*0.14*	(n 13)	2.0	*0.21*	1.8	*0.20*	(n 8)	2.8	*0.24*	3.2	*0.38*

[a] DCE activity expressed as rate of formation of Leu-enk-Arg^6 (pmol/h*mL) from dynorphin A.
[b] SPE activity expressed as rate of formation of SP_{1-7} (pmol/h*mL) from substance P.
[c] ACE activity expressed as rate of formation of hippuric acid (nmol/h*mL) from hippuryl-His-Leu.
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Discussion

Various categories of pain, such as nociceptive, neuropathic or idiopathic pain, are recognized by the underlying disorder or by the character and distribution of the sensation of pain. Different forms of pain are sensitive to various treatment modalities which may reflect different pain-generating mechanisms (Arnér 1991). The differences in underlying neurochemical events may—at least theoretically—produce different patterns of pain-related substances in the CSF. Our study focused on converting enzymes acting on peptides known to be involved in pain transmission and/or pain modulation. These enzymes have previously been found in human CSF and they have been considered useful as markers of activity in the dynorphin and substance P systems (Terenius and Nyberg 1991). Furthermore, with regard to DCE a negative correlation between DCE activity and CSF levels of prodynorphin-related peptides has recently been observed (Thörnwall et al. 1994).

A correlation between enzyme activity (DCE) and peptide level was also suggested in animal studies (Silberring et al. 1992). In arthritic rats, the CSF levels of both DCE and SPE seem to be affected (Persson et al. 1992) during the acute phase of arthritis. Therefore, it is likely that the levels of the enzyme activities relate not only to the peptide concentrations, but also to disturbances in the pain-processing systems.

Hyyppä et al. (1990), using another method of analysis, registered a somewhat higher DCE activity in men than in women, and a slight increase with age, while the SPE activity did not differ with sex or age. In our study, the sex distribution was almost equal in the patients with ischialgia and in the controls, while the controls were older than the patients with ischialgia.

Several studies have indicated disturbances of CSF opiopeptides in neurogenic pain (Almay et al. 1985, Nyberg et al. 1988, Grond 1992). Thus, patients suffering from neurogenic pain have decreased levels of so-called fraction I endorphins (Almay et al. 1985). Fraction I represents a pool of opioid-active CSF peptides, most of which seem to be derived from the prodynorphin system (Nyberg et al. 1983). Therefore, the observed decrease in fraction I endorphins should be consistent with an increase in DCE activity, as we found in the CSF from patients with neuropathic pain.

The decrease in SPE activity seen before treatment of the patients with herniated lumbar discs might correspond to an increased level of substance P in the CSF, since this relation between SPE and substance P has been observed in animal experiments (Persson et al. 1992). On the other hand, Almay et al. (1985) reported a decline in the CSF levels of substance P in patients with neuropathic pain.

The pain in coxarthrosis, regarded as nociceptive, did not affect the activity either of DCE or of SPE in our patients. However, the CSF activity of ACE was higher than in the controls. As ACE is known to metabolize opiopeptides (Spillantini et al. 1986, Lantz et al. 1992), this increase may reflect changes in neuropeptide concentration, although the mechanisms are not known.

In rat experiments, decreased DCE activity has been reported in animals with adjuvant-induced arthritis (Silberring et al. 1992), and decreased DCE and SPE activities in collagen-induced arthritis (Persson et al. 1992). The latter type of arthritis is also known to be associated with increased levels of dynorphin in the spinal cord (Iadorala et al. 1988). These findings suggest that the CSF convertases DCE and SPE probably mirror events that are related to disturbances in pain-processing pathways.

The enzymatic changes we found may provide insight in the neural mechanisms underlying pain disorders and perhaps recognize different kinds of chronic pain.

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