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Substance P-like Immunoreactivity and Somatostatin-like Immunoreactivity in the Ventricular Fluid of Patients with Chronic Pain Syndromes

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Summary. Substance P-like and somatostatin-like immunoreactivities (SPLI and SLI) were determined in ventricular fluid of patients with chronic pain syndromes and in a comparison group with multiple sclerosis, essential tremor, epilepsy and postanoxic myoclonus. Concentrations of SPLI and SLI were non-significantly decreased by 40% and 33% in chronic pain patients as compared with control patients without pain. There were no differences apparent between subgroups of pain patients (deafferentation pain, neoplasia-induced pain, thalamic pain). High pressure liquid chromatography combined with radioimmunoassay showed marked heterogeneity of SPLI and SLI.

Key words: Substance P – Somatostatin – Chronic pain – Cerebrospinal fluid

Introduction

The undecapeptide substance P (SP) is found in descending and ascending fibres of the spinal cord and is a putative neurotransmitter in nociceptive primary afferents (Otsuka and Takahashi 1977; Jessel et al. 1979; Lembeck et al. 1981; Pearson et al. 1982; Pernow 1983). The tetradecapeptide somatostatin (SST), occurs widespread in the central nervous system and in the spinal cord, where it probably functions as an inhibitory modulator of nociceptive input (Randic and Miletic 1978; Kuraishi et al. 1985; Wiesenfeld-Hallin 1986). Intrathecal application of SP elicits behaviour suggestive of pain (Moochala and Sawynok 1984; Sakurada et al. 1987) in animals, while SST shows analgesic effects on intrathecal (Chrubasik et al. 1984) and intracerebroventricular (Madrazo et al. 1987) application in man.

Cerebrospinal fluid (CSF) level of peptides in general appear to reflect peptide release in adjacent tissues. While lumbar CSF studies of substance P-like immunoreactivity (SPLI) and somatostatin-like immunoreactivity (SLI) have revealed reduced levels in chronic pain syndromes (Nutt et al. 1980; Almay et al. 1988; Urban et al. 1988), studies of cerebroventricular levels of SP and SST in pain patients have not been made. It is assumed that lumbar CSF neuropeptides largely arise from the spinal cord, nerve roots or dorsal root ganglia (Nutt et al. 1980). Because the pathophysiology of chronic pain disease appears to involve supraspinal systems of pain perception and pain control, we have studied SPLI and SLI in the ventricular fluid of patients with chronic pain syndromes. The findings were compared with those of patients with other nervous diseases which do not appear to influence CSF SLI levels (Cramer et al. 1984; Steardo et al. 1986).

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Patients and Methods

Forty-five patients admitted for therapeutic stereotactic surgery were examined. Sixteen patients suffered from chronic pain syndromes (Table 1). Of these, 4 had deafferentation pain, 4–41 years after amputation of a limb. Six patients had severe pain due to neoplasias. Five patients had thalamic pain following brain infarction, 1–6 years before. All pain syndromes were chronic and had resisted medical treatment. Most of the patients received medication (3 morphine, 3 neuroleptics, 1 thymoleptics, 3 benzodiazepines).

The comparison group was composed of 39 patients with other diseases (Table 1). Fourteen patients had chronic stable multiple sclerosis with myoclonia or intention tremor. Six patients had temporal lobe epilepsy. Thirteen patients had essential tremor without other signs of extrapyramidal disease. Six patients were admitted for action myoclonus following traumatic cerebral hypoxia. Patients with epilepsy were treated with phenobarbitone (3), phenytoin (3), valproic acid (2), carbamazepine (1) and clonazepam (1). Of the patients with essential tremor 1 recevied metixene chloride, 1 oxazepam.

Ventricular puncture targeted to the foramen of Monro was performed on one side with a stereotaxic technique (Mundinger 1975) under local anaesthesia between 9 and 11 a.m. Fluid obtained before ventriculography and stereotaxic lesioning was immediately frozen and stored at −40°C until analysis.

Table 1. Clinical data and CSF levels of total protein, substance P-like (SPLI) and somatostatin-like (SLI) immunoreactivity in patients with chronic pain, multiple sclerosis, essential tremor, epilepsy and postanoxic myoclonus

Patient group	Number	Sex ratio f/m	Mean age ± SEM	Total protein (g/l)	SPLI (fmol/ml)	SLI (fmol/ml)
I Pain syndromes	16	4/12	57.8 (2.5)	0.21 (0.05)	6.1 (1.1)	27.4 (4.2)
II Multiple sclerosis	14	9/ 5	38.4 (2.3)	0.20 (0.02)	10.7 (1.5)	47.4 (7.2)
III Essential tremor	13	5/ 8	56.8 (4.4)	0.12 (0.01)	8.2 (1.1)	35.4 (8.6)
IV Epilepsy	6	1/ 5	35.3 (4.6)	0.18 (0.05)	12.2 (3.8)	33.7 (8.1)
V Postanoxic myoclonus	6	1/ 5	21.2 (6.0)	0.19 (0.04)	11.6 (2.2)	46.4 (6.8)
Total II–V	39	16/23	41.4 (2.8)	0.17 (0.01)	10.3 (0.9)	41.1 (4.2)

Results are shown as mean, (SEM)

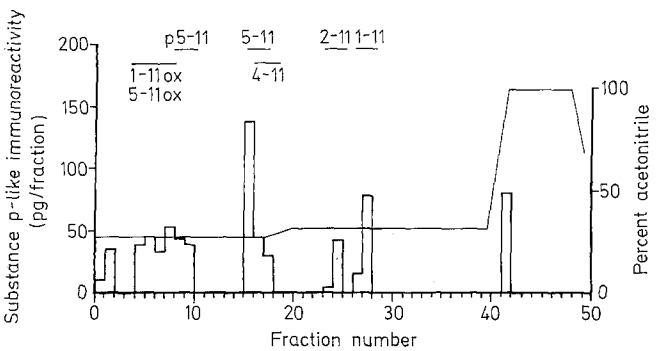

Fig. 1. High performance liquid chromatography of cerebrospinal fluid (CSF) coupled with radioimmunoassay of substance P-like immuno-reactivity. Pools of CSF from two to three patients with painful radiculopathy were lyophilized, resuspended in elution buffer and chromatographed. Mean values of three different pools. *Horizontal bars* indicate elution volumes of synthetic SP1-11, SP2-11, SP4-11, SP5-11, pyroglutamyl-SP5-11, sulphoxidated SP1-11 and SP5-11

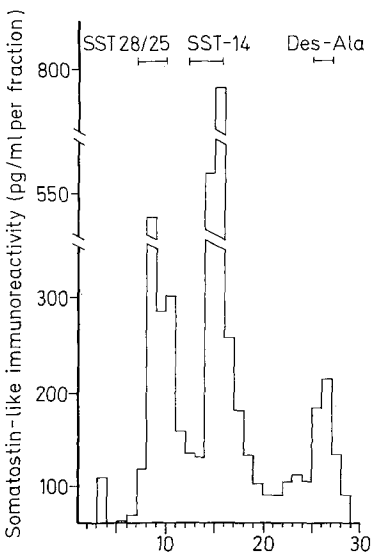

Fig. 2. High performance liquid chromatography (HPLC) of cerebrospinal fluid (CSF) of a control patient. Somatostatin-like immuno-reactivity (SLI) was determined by radioimmunoassay in each fraction of HPLC. Total SLI was 44 fmol/ml. *Horizontal bars* indicate elution volumes of synthetic SST-14, SST-25, SST-28 and des-Ala^1-Gly^2-desamino-Cys^3-SST-14 (GP10941)

Determination of SPLI. SPLI was determined as previously described (Cramer et al. 1985a, b, 1989) using an antiserum raised in rabbits which shows little cross-reaction with the closely related undecapeptides physalaemin (15%), substance K (0.1%) and cassinin (< 3%) while cross-reactions with eledoisine, bombesin and somatostatin can be disregarded (< 1%). The antibody has high affinity for SP5-11 (103% cross-reaction) and does not detect SP1-4 and SP1-7, which implies that it is directed exclusively against the C-terminal portion of the SP molecule. As a tracer we used 8-tyrosyl-SP (Peninsula Laboratories, Belmont, USA) which was iodinated by the chloramin T method and purified by ion-exchange chromatography on a 6 × 1 cm carboxymethylcellulose column (Whatman) using gradient elution starting with 10 ml of 0.002 *M* ammonium acetate solution (pH 4.6) followed by 0.2 *M* ammonium acetate solution (pH 4.6). The immunological identity between synthetic SP and SPLI from CSF was ascertained by preparing dilution curves of concentrated CSF and synthetic SP, both curves proving to be parallel. The detection limit of the assay was 15 pg/ml. Intra-assay and inter-assay variation coefficients were 5% and 11% respectively. The mean recovery of synthetic SP added to CSF was 98%.

The chemical nature of SPLI was examined in pools of CSF by reverse-phase high-performance liquid chromatography (HPLC) on a Spherisorb ODS-II column with gradient elution (0.1% trifluoroacetic acid in water and 0.1% trifluoroacetic acid in acetonitrile). Figure 1 shows that about 13% of SPLI co-eluted with SP and 13% with the fragment SP5-11, which on intrathecal administration appears to be as active as SP (Sakurada and Kisara 1987). Other fragments (SP4-11, SP5-11 and pyroglutamyl-5-11) accounted for about 30% and sulphoxidated species about 20% of the total immunoreactivity and higher molecular weight forms accounted for only a small amount of immunoreactivity.

Determination of SLI. SLI was determined by radioimmunoassay (RIA) (Cramer et al. 1985a, b) using a specific antibody (K18) raised in rabbits which recognizes the SST molecule at the ring structure. Cyclic synthetic SST-14 and synthetic SST-28 are detected on an equimolar basis by this antibody. Standard solutions were prepared with synthetic SST-14 (Serono, Freiburg, Germany). Free SLI was separated from the antibody-bound species by use of the charcoal separation technique.

The identity of SLI was studied by reverse-phase HPLC using a C18 column (LiChrosorb RP 18) and triethylammonium formiate 0.1 *M*, pH 3.5 + propanol 17% as solvent (Fig. 2). SLI was detected in fractions by RIA. HPLC revealed two major peaks of SLI, co-eluting with SST-14 and SST-28, respectively, a small peak eluted with the void volume (precursor) and a variable late peak was indicative of the presence of smaller fragments of SST-14 with preserved structure and recognition sites.

As statistical tests for significance, analysis of variance (ANOVA), the two-sided Student's *t*-test for independent samples and the Wilcoxon two-sample test were used.

Results

Patients with chronic pain syndromes showed a low mean SPLI level of 6.1 ± 1.1 fmol/ml which was 40% lower than the mean SPLI level of the comparison group. Analysis of variance (ANOVA) revealed no significant differences between the groups of pain patients and other patients (Table 1). A difference shown by the two-sided t-test and the Wilcoxon test, significant at the $P < 0.05$ level, suggested a trend towards lower levels in pain patients. In comparison to the controls, the mean SLI level of the pain patients was also low. However, levels varied largely (range 0.6–57 fmol/ml), and the difference between pain patients and controls was not significant in either test.

Between the subgroups of pain patients (deafferentation pain, peripheral pain due to neoplasia, central thalamic pain) there was no statistically significant difference for either neuropeptide.

To avoid a selection bias due to differences in sex distribution and age of the patient groups, correlations between sex or age and neuropeptide levels were tested. There were no significant correlations with either sex or age.

Discussion

Studies of neuropeptides in human CSF have been as yet largely restricted to lumbar fluid. However, in at least one instance it has been shown that ventricular peptide levels do not necessarily parallel lumbar changes. Thus, in Alzheimer's disease lumbar CSF SLI was markedly decreased (Oram et al. 1981; Cramer et al. 1985a; and others) but ventricular SLI was not different from that of control patients (Francis et al. 1987). Lumbar fluid SPLI levels were found increased in neurological patients with lumbar disc hernia (Masaki 1987) and with other acute painful peripheral neuropathy (Cramer et al. 1988) but decreased in patients with chronic polyneuropathy and autonomic dysfunction (Nutt et al. 1980) and with chronic pain syndromes of at least 6 months's duration (Almay et al. 1988). Also lumbar SLI was recently found markedly decreased in patients with chronic back pain (Urban et al. 1988). Levels of SLI did not correlate with clinical parameters such as pain scores and psychic disturbances.

This study, indicating overall low levels of SPLI and SLI in ventricular fluid, suggests decreased synthesis and release in central nervous structures proximal to the spinal cord in chronic pain disease. Decrease in lumbar CSF SPLI in chronic neuropathy might be caused by deafferentation. In animals, deafferentation was shown to decrease spinal SP levels (DiGiulio et al. 1985) and induce supersensitivity of SP receptors in the dorsal horn (Massari et al. 1985). In man, we observed particularly low lumbar levels of SPLI in subjects with leg amputation (Cramer et al. 1989). Low ventricular fluid peptide levels, on the other hand, could indicate failure of supraspinal SP neurons, which possibly are involved in pain perception and the reaction to pain in chronic pain disease (Menetrey and Basbaum 1987). In chronic pain disease central dysregulations are believed to take place, leading to an often progressive course independent of the original source of painful sensation. Alterations in forebrain neuropeptide synthesis and release may well induce, accompany or maintain this process. As yet, it is unclear whether the changes are specifically related to pain processing pathways and pain defence mechanisms or indicative of a "non-specific" depression of activity involving other systems and possibly related to chronic medical treatment of which little is known as yet (Steardo et al. 1986; Schalling et al. 1988; Doran et al. 1989). A serious weakness of ventricular CSF studies is that neurologically healthy controls are not available. An attempt to overcome this drawback in this study has been made by a comparison with painfree patients with mixed diagnosis, for which no alterations of these peptides have been observed. Clearly, because of the possible pathophysiological and therapeutic implication for chronic pain disease, further studies should be undertaken.

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