Substance P-like Immunoreactivity and Analgesic Effects of Vibratory Stimulation on Patients Suffering From Chronic Pain

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ABSTRACT: By applying vibratory stimulation to patients suffering from pain, it is possible to set up an inhibitory control on the pain pathways which is based on the activation of large-sized afferent fibres. The exact mechanisms responsible for these analgesic effects still remain to be determined, however. For this purpose, we investigated in the present study whether or not the analgesic effects were accompanied by a decrease in the CSF substance P-like immunoreactivity levels (SPLI) of seven patients suffering from chronic pain, who were fitted with a ventriculo-peritoneal drain. The SPLI levels were determined before and after 30-min vibratory stimulation sessions. The results show that the SPLI levels decreased as the result of the vibration, but this decrease seems to be too slight to account for the pain relief obtained.

RÉSUMÉ: Immunoréactivité analogue à celle de la substance P et effets analgésiques de la stimulation vibratoire chez les patients souffrant de douleurs chroniques. Chez des patients souffrant de douleurs aiguës ou chroniques, l'application de stimulations vibratoires transcutanées est capable d'installer un contrôle inhibiteur sur les voies de la douleur par activation des fibres afférentes de gros diamètre. Les mécanismes responsables de cet effet antalgique restent cependant à préciser. Dans ce but nous avons examiné si cet effet s'accompagnait ou non d'une baisse de la substance P dans le LCR de sept patients souffrant de douleurs chroniques et porteurs d'une dérivation ventriculopéritonéale. Les dosages ont été effectués avant et après 30 minutes de vibrations. Les résultats obtenus montrent une baisse de la SP qui semble toutefois trop faible pour expliquer les effets antalgiques obtenus.

Can. J. Neurol. Sci. 1993; 20: 138-141

Cutaneous vibration, like transcutaneous electrical nerve stimulation (TENS), leads to the recruitment of large-sized afferent fibres and thus sets up an inhibitory control on the pain pathways. Vibratory stimulation has been found to have analgesic effects in patients with chronic[1-8] or acute pain.[9-12] The levels and duration of the pain relief obtained with this method are comparable to those reported using TENS.[1-5,9] In particular, a noticeable decrease in the patients' pain can occur sometimes as late as 24 hours after the vibration session. Although it is generally assumed that the analgesic effects of vibratory stimulation (VS) are probably due to the large-scale activation of the mechanoreceptors connected to the large-sized afferent fibres,[3,4,12] the exact mechanism underlying the inhibitory effects subsequently exerted on the pain pathways still remains to be elucidated.

It has been established for example that the endogenous morphines are probably not involved in this mechanism, since on the one hand the analgesic effects of vibration were not reversed by naloxone[13-15] and on the other hand, the beta-endorphin and met-enkephalin levels measured in the CSF of patients suffering from chronic pain showed no change when VS was applied.[13]

At the spinal level, the neurotransmitter adenosine is another candidate thought to possibly mediate the inhibitory messages conveyed by the large diameter afferent fibres to the spinal nociceptive neurones in response to cutaneous vibration.[16] The latter study was carried out on animals; however, the vibration was applied for only short periods, and the pain inhibition achieved was very short-lived.

Since it is likely that the analgesic effects of vibratory stimulation at least partly involve a spinal control, it was proposed to measure the SP levels in the CSF of patients with chronic pain which had been considerably alleviated by applying VS. This neuropeptide, which seems to be more of a neuromodulator[17-21] than a neurotransmitter[22] is released in the dorsal horns in

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Received July 23, 1992. Accepted in final form December 15, 1992

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response to a large variety of nociceptive stimuli.[23-25] Moreover, electrical stimulation of the small-sized afferent fibres is known to elicit the release of SP in the CSF.[26,27] Lastly, intra-thecal injections of SP resulted in hyperalgia[28] and conversely, an SP antagonist injected via the same pathway led to an increase in the pain threshold.[23-25]

On the basis of all these data, we adopted the following hypothesis: since nociceptive stimulation gives rise to the release of SP in the CSF, it is possible that the inhibitory effects induced in the spinal nociceptive neurones by vibrating the large-sized afferent fibres[16,29-31] may be accompanied by a parallel decrease in the SP levels released by the nociceptive fibres.

Patients and Methods

Patients

Seven patients (3 men and 4 women between 24 and 76 years of age; mean = 54) suffering from chronic pain (1 polyarthritis, 1 scapula bursitis, 1 spondylarthritis, 1 cervicobrachialgia, and 3 low back pain) were selected for this experiment (see below). All the patients had been admitted to the neurosurgery ward and had been fitted with a ventriculo-peritoneal drain to compensate for hydrocephaly, which made it possible to sample the cerebrospinal fluid *painlessly*. All possibly analgesic treatment was stopped 72 h before the beginning of the experiment, and no morphine like drugs had been administered for several weeks.

After being given a detailed description of the experiment, the patients agreed to participate as per the Helsinki convention. We also obtained permission to proceed from the Ethics Committee at the Timone Hospital.

Experimental procedure

Before the vibration session, 4-ml samples of CSF were collected by tapping the drainage tube or by drawing fluid from an external tap, the dead space in the drain was 0.26 ml, which amounted to 13% of the sample volume. Two ml of CSF were immediately placed in an acid medium before freezing prior to SP determination. Biochemical and cytobacteriological checks were performed on the remaining 2 ml.

After the sampling, in order to assess the pain levels involved, each patient was asked to rate his or her pain on a 6-point verbal intensity scale (no pain, mild pain, discomforting pain, distressing pain, horrible pain, excruciating pain), as well as on a visual analogue scale (or VAS) consisting of a 10-cm line.

Vibratory stimulation

The vibratory stimulation was applied to the painful region by means of a prototype, consisting of a D.C. motor equipped with an eccentric mass with a plastic base encased in a fine layer of rubber, which served as the applicator (surface area 6 × 10 cm). The vibration frequency was 100 Hz and the amplitude 1 mm peak to peak. Vibration was then applied to the painful area for 30 min. Further 2 ml samples were taken from patients whose pain was relieved by at least 40% on both scales in comparison with the initial pain rating (see Table 1), and used to perform SP determinations.

Determination methods

CSF sample collection and extraction. One-ml CSF samples, collected from the ventricular drains, were acidified with 1 volume of 10 N hydrochloric acid and frozen at –20°C until assay. To halt the action of degradative enzymes, samples were immersed in a boiling bath at 95°C for 10 minutes and then evaporated under a vacuum (in a Savant "speed vac" apparatus). The dried pellets were resolubilized in 8 ml of phosphate buffered saline (P.B.S), pH = 7.4, containing 0.1% bovine serum albumin.

Radioimmunoassay

SP-like immunoreactivity was assayed using an antibody provided by Dr C. Oliver and M. Hey (INSERM, U 297). SP antibody was obtained from Dr C. Oliver and Dr. F. Boudouresque (INSERM, U 297). Tracer was purchased from New England Nuclear and the assay protocol was similar to that previously described.[32] The ED50 was 50pg/tube.

Statistical analysis

Statistical analysis was performed using Wicoxon test.

Results

In 5 out of the 7 patients, complete pain relief was achieved after 30 min of VS, while 2 patients continued to feel some residual pain, amounting to less than 25% of the initial pain level (see Table 1). The levels of SP-like immunoreactivity were found to have decreased after VS in 6 cases, and showed no change in the seventh patient (see Table 2).The differences observed were significant ($p < 0.01$).

Discussion

Although the decrease in SPLI after VS is significant, the difference remains too small to explain the pain relief. For example, the analgesic effect produced by morphine injection, involved a decrease of SPLI in CSF of patients with low-back pain from 50% to 100%.[33] It will therefore be necessary to look for other mechanisms.

Little mention has been made so far of a decrease occurring in the CSF substance P levels of human patients with chronic pain undergoing analgesic treatment. A marked decrease in the SP levels has been reported however during morphine treament.[33,34] Almay et al.[35] on the contrary observed an increase in the SP cerebro-spinal fluid levels in patients treated with TENS using the high-frequency, low intensity mode. Although this increase was significant, it was fairly slight, and

Table 1. Pain levels as assessed on a visual analogue scale and a verbal scale, before and after applying VS to 7 patients suffering from chronic pain. The pain intensity is expressed as a percentage of the maximum pain level experienced (100%), before (T0) and after a 30-minute VS session (T30)

	Pain intensity rating (%)			
	T0		T30	
Patients	Verbal Scale	Visual Scale	Verbal Scale	Visual Scale
1	60	85	0	20
2	40	62	0	0
3	60	40	0	0
4	80	75	0	0
5	80	82	0	0
6	60	41	0	0
7	60	43	0	10

https://doi.org/10.1017/S0317167100047703 Published online by Cambridge University Press

Table 2. Results of Substance P-like immunoreactivity determinations in 7 patients suffering from chronic pain. Painless sampling was carried out before and after 30-min vibratory stimulation sessions. The SPLI levels are expressed in picogrammes per milliliter

Patient	Substance P - like Immunoreactivity (pg/ml) Before VS	After VS
1	0.508	0.46
2	0.69	0.58
3	0.71	0.66
4	0.56	0.46
5	0.43	0.39
6	0.49	0.22
7	0.42	0.43
Mean	0.54	0.45
SD	0.12	0.14

may have resulted from the painful lumbar sampling method used. In patients suffering from acute post-operative pain, the SP levels measured in the CSF seemed to be correlated with the intensity of the pain,[36] and yet when the patients were anaesthetized, no significant changes in the SP levels were observed.

In this study, SPLI is present in low concentration in CSF of patients with chronic pain. Similar values were reported by several authors.[36,37]

A few reservations require to be made, however, in connection with our data. First, the SP levels determined in the CSF do not reflect spinal activity alone: many brain regions contain considerable amounts of this neuropeptide, but not all of them are involved in the nociceptive pathways. This is so in the case of the nigro-striatal loop, for instance.[38,39] It is therefore possible that if an analgesic mechanism of spinal origin is indeed triggered by vibration, the intraventricular site is not the most appropriate site for CSF sampling. We nevertheless decided to proceed in this way for both ethical and methodological reasons, and particularly because we were thus able to sample the CSF with no pain to the patients.

Acknowledgements

This research was supported by CNRS and Ministère de la Recherche et de l'Industrie grants. The manuscript was translated into English by Dr. Jessica Blanc.

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https://doi.org/10.1017/S0317167100047703 Published online by Cambridge University Press