Intracerebroventricular injection of cerebrospinal fluid (CSF) from a patient with congenital indifference to pain induces analgesia in rats

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Summary. CSF from a patient with congenital indifference to pain was found to produce analgesia in the rat following intracerebroventricular injections. The analgesic effect was attenuated by pretreatment with naloxone suggesting the involvement of hyperactive endogenous opiate mechanisms in this patient.
Key words. Rat; cerebrospinal fluid, human; analgesia; naloxone; pain indifference, congenital; opiates, endogenous.

Congenital indifference to pain is a rare disease in which analgesia is present without demonstrable abnormalities in the peripheral and central pain transmission pathways[2]. The patients perceive and correctly describe nociceptive input but fail to appreciate its painful nature and do not react with defense or flight behavior.

We have recently described a 19-year-old boy suffering from this disease presenting no neurologic defects except universal analgesia[3]. Briefly, the patient did not react to routine painful maneuvers, nor to high-intensity stimulation of skin, nerve trunks, dorsal roots, tooth pulp and corneal mucosa. He had normal unmyelinated and myelinated fibers in skin nerve branches, the corneal reflex latency was in the normal range, and stimulation of tooth pulp with electrical pulses evoked large cortical potentials in vertex recordings. On psychometric testing he was classified as dull normal and his emotional reactions to psychological distress were adequate.

So far, few cases suffering from this disease have been described in the literature and no well defined cause has been proven even though, recently, it has been hypothesized that hyperactivity of endogenous pain modulating systems may mediate the abnormality[4-6]. In the patient we studied, high levels of analgesic peptides, i.e., opioids and calcitonin, were present in the CSF[7,8], suggesting their possible involvement in the analgesic state of the subject. In order to validate this hypothesis and further evaluate the biological significance of these findings, we injected reconstituted CSF from this patient intracerebroventricularly i.c.v. into unanesthetized rats and tested their subsequent nociceptive reactions.

Material and methods. 6 ml of CSF of the patient were obtained via indwelling catheter introduced into the lumbar subarachinoid space with a lumbar puncture needle. The CSF was collected in a plastic tube, centrifuged, frozen, liophylized, stored at −70 °C and reconstituted with 300 µl of saline before testing. For the control, 3 ml of CSF were collected from each of 2 patients undergoing rachianesthesia for a minor surgical operation (hernia inguinalis and appendycectomia), pooled, and processed in the same way. The chemical analysis of the CSF of these patients was normal.

21 Sprague Dawley male rats (300 g), anesthetized with chloral hydrate, were stereotaxically implanted with chronic indwelling cannulae guides constructed from 23-gauge TW stainless steel tubing and fitted with stainless steel stilettes of corresponding length to prevent occlusion between injections. The cannulae guides were aimed for the lateral ventricle (AP+ 7.8, LAT+ 1.2, DV+ 1.8)[9].

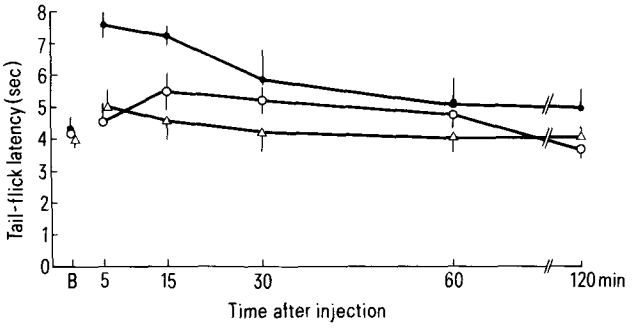
One week following surgery the rats were divided into 3 equal groups (A, B, C) and injected i.c.v. with 10 µl of the reconstituted CSF of the patient (groups A and B) or of the control (group C). Immediately prior to the i.c.v. injection the 3 groups were i.p. injected, respectively, with 5 mg/kg of naloxone (A) or saline (B and C). All rats were tested as previously described with the tail flick and hot-plate tests at 5, 15, 30, 60 and 120 min after the injection of the substance.[10].

Results. The figure shows the tail flick latencies before and after the injection of the reconstituted CSF. The CSF from the patient with congenital indifference to pain induced a significant increase in the tail flick latencies that was maximal at 5 min after the injection and returned to baseline in 60 min. I.p. administered naloxone (5 mg/kg) significantly attenuated this effect, suggesting an action mediated at least in part by opiate receptor activation. Control CSF did not induce any statistically significant changes in the reaction latencies. The hot-plate test showed a similar pattern of response but of a lesser magnitude (data not shown). The only comparison among hot-plate latencies that proved to be statistically significant ($p < 0.05$) was between group A and B 15 min following the i.c.v. injection.

Discussion. The high levels of opioid radioreceptor activity and calcitonin (CT), a centrally analgesic peptide[11,12], in the CSF of a patient with congenital absence of pain[7,8] prompted us to investigate their biological significance. We have found that the i.c.v. injection of the CSF from the patient into rats induced a prompt analgesic effect that could be attenuated by pretreatment with the specific opioid antagonist naloxone.

Dehen et al.[4] observed that in a patient insensitive to pain, naloxone at the dose of 1.2 mg induced a significant augmentation of the cutaneous avoidance reflex. Yanagida et al.[5], in another analgesic patient, showed that naloxone at a slightly higher dose (2 mg) caused the appearance of previously unelicitable tooth pulp evoked potential. More recently, Dunger et al.[6] have described a 3rd case with insensitivity to pain and an endocrine disorder, both of which were reversed by prolonged naloxone therapy. However, a failure of naloxone treatment in reversing the analgesic state of 2 patients with congenital indifference to pain has also been reported, suggesting the existence of 2 forms of this disease – one naloxone antagonizable and the other not[12].

In our patient i.v. naloxone, up to 12 mg, was ineffective in restoring pain sensations and in modifying the corneal reflex[2]. We attempted to account for this observation by assuming that



Antinociceptive effects of CSF from patient with congenital indifference to pain. B, Baseline tail-flick latencies; vertical lines indicate SEM. ●—●, CSF from patient with congenital indifference to pain; ○—○, naloxone (5 mg/kg i.p) + CSF from patient with congenital indifference to pain; △—△, CSF from 2 control patients. *$p < 0.05$ for comparisons of post CSF treatment means with control means at each time point using the Dunnet's test. **$p < 0.05$ for comparisons of treatment means following active CSF with treatment means following active CSF + naloxone.

the major contributing factor in CSF was calcitonin[7], a naloxone-resistant analgesic peptide[10]. The present findings, however, suggest that opiate substances, which were also high in the CSF of this patient, may play an important role in determining pain insensitively as well. It is possible that we and the others[12] could have observed a naloxone attenuation of pain indifference if higher doses or chronic drug regimen had been employed. Further experiments are in progress to evaluate the possible benefits to our patients of a more prolonged naloxone therapy.

While some of the analgesic substances in the CSF of our patient as well as in the patient described by Dunger et al[6] are opiate-like, they are apparently neither beta-endorphin nor met-enkephalin[6,8]. Instead, they are probably one or more of the numerous endogenous opioid peptides so far described[14]. These findings offer further support to the idea that congenital indiffernece to pain might be considered to be an endogenous analgesic peptide disorder in which a patient is excreting abnormally high levels of opiate as well as non-opiate analgesic substances.

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2 Comings, D.E., and Amromin, G.D., Neurology *24* (1974) 838.
3 Manfredi, M., Bini, G., Cruccu, G., Accornero, N., Berardelli, A., and Medolago, L., Archs Neurol. *38* (1981) 507.
4 Dehen, H., Willer, J.C., and Cambier, J., Pain *5* (1978) 351.
5 Yamagida, H., Lancet *2* (1978) 520.
6 Dunger, D.B., Wolff, O.H., Leonard, J.V., and Reece, M.A., Lancet *1* (1980) 1277.
7 Fraioli, F., Panerai, A.E., Santoro, C., Fabbri, A., Santoro, F., and Isidori, A., Horm. Metab. Res. *14* (1982) 312.
8 Fabbri, A., Fraioli, F., Gnessi, L., Moretti, C., Bini, G., Cruccu, G., and Manfredi, M., Archs Neurol. *40* (1983) 64.
9 König, J.F.R., and Klippel, R.A., The Rat Brain. Williams & Wilkins, Baltimore 1963.
10 Pert, A., Moody, T.W., Pert, C.B., DeWald, L.A., and Rivier, J., Brain Res. *193* (1980) 209.
11 Braga, P., Ferri, S., Santagostino, A., Olgiati, V.R., and Pecile, A., Life Sci. *22* (1978) 971.
12 Fraioli, F., Fabbri, A., Gnessi, L., Moretti, C., Santoro, C., and Felici, M., Eur. J. Pharmac. *78* (1982) 381.
13 Pari, A., Meyer, M., Boltshauser, E., Foletta, D., and Gramsch, C., Lancet *1* (1982) 622.
14 Olson, G., Olson, R., Kastin, A.J., and Coy, D.H., Peptides *3* (1982) 1039.

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Electroreception in the Turkistan catfish

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Summary. The Turkistan catfish, *Glyptosternum reticulatum*, has highly sensitive electroreceptors (threshold voltage gradient 1 μV/cm) that detect a voltage drop across the skin. These electroreceptors were found to be sensitive to magnetic stimulation.
Key words. Catfish; electroreceptors; skin; magnetic stimulation; siluriform fishes; lateral line organ.

The presence of specialized electroreceptors within the lateral-line organs of siluriform fishes (order Siluriformes) has been demonstrated in various kinds of experiments. Until recently the species of only 10 families of this order (Clariidae, Saccobranchidae, Siluridae, Malapteruridae, Ictaluridae, Pimelodidae, Doradidae, Loricaridae, Gallichthyidae, Plotosidae) were known to have the electroreceptor apparatus[1-4]. We believed that other siluriform fishes might also be electroreceptive, therefore we carried out experiments on the Turkistan catfish *Glyptosternum reticulatum* (Sisoridae, Siluriformes), which lives in the rivers of Soviet Central Asia.

In our experiments single unit activity was recorded from the lateral-line nerve innervating the receptors of the caudal part of the body. In every case, the peripheral portion of the cut nerve was placed on a dark Perspex dissecting plate which contained Ringer solution, and the nerve sheath was carefully removed. The nerve was then divided into very fine strands under a dissecting microscope. Nerve activity was recorded in filaments which contained only one active fiber with silver hook electrodes. The nerve impulses were amplified and displayed by conventional means[5,6]. To stimulate the sensory organs, homogenous electric fields were used. Direct current pulses were applied from DC stimulator via a high series resistance through 2 silver electrodes. In addition, electroreceptor responses to magnetic stimulation were investigated. Magnetic stimuli were produced by a constant bar magnet that moved horizontally in the air at various distances above the fish. To measure the skin electrical properties, the isolating ring with 15 mm in diameter was placed on the skin above the water level (fig. 2c). The skin around the ring was rinsed with distilled water and dried with filter paper to prevent electrical leakage from the ring. The skin potential was recorded by means of an electrolytically chlorided silver wire electrode (E_1), which was placed inside the ring. The ring was filled with water. The reference electrode (E_2) was a chlorided silver wire introduced into the body. DC current pulses (10^{-8}–10^{-6} A) were supplied from DC stimulator to the isolating ring through the silver electrode (E_3). the skin potential and its changes were recorded via an impedance of 10^8 Ω by a pen-recorder. The skin resistance was estimated by the voltage drop across the skin; the

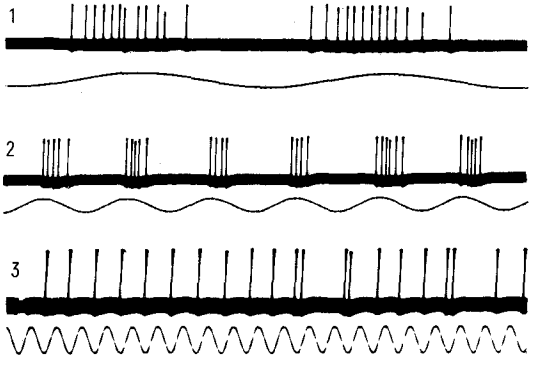

Figure 1. Responses of a single Turkistan catfish electroreceptor to application of a sinusoidal electric field.
Voltage gradient 15 μV/cm. Frequency of stimulation 1(1), 3(2) and 10(3) Hz.