# DETECTION AND QUANTIFICATION OF ENDOGENOUS N/OFQ IN THE RAT CEREBROSPINAL FLUID, UNDER BASAL AND STIMULATED CONDITIONS

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The endogenous peptidergic system, represented by neuropeptide nociceptin/orphanin FQ (N/OFQ) and its receptor NOP, has been shown to be involved in a variety of functions including the modulation of nociceptive transmission, with different actions on the nociceptive threshold at the spinal and at the supraspinal level. To better understand the role of this peptidergic system in the nociceptive transmission at the spinal level, we investigated the presence of endogenous N/OFQ in the cerebrospinal fluid and its possible modifications after pharmacological and non-pharmacological manipulations, by means of a spinal perfusion procedure coupled with a sensitive radioimmunoassay (RIA), in anesthetized rats.
Under sodium pentobarbital/chloral hydrate (30/130 mg/kg i.p.) anesthesia, male Sprague-Dawley rats were tracheotomized and fixed in a stereotaxic apparatus. Then a first catheter, connected with the inlet of a perfusion pump, was inserted into the subarachnoid space through the atlanto-occipital membrane and lowered down about 8 cm toward the lumbar enlargement. A second catheter was lowered down 2 mm under the atlanto-occipital membrane and connected to the outlet of the perfusion pump. The subarachnoid space was then perfused with artificial cerebrospinal fluid (aCSF). Perfusion flow-rate was set to 100 µl/min and fractions of 15 minutes were collected, purified by C18 microcolumns, lyophilized and stored at –80 °C until the RIA.
The endogenous N/OFQ resulted detectable in the perfusate, and its recovery was also progressively increased by adding specific proteases inhibitors to the aCSF, using an high protein concentration in the aCSF to saturate aspecific binding sites in the perfusion apparatus, and by the immediate acidification of the collected perfusate. These precautions allowed us to recover a sixfold higher concentration of N/OFQ – like immunoreactivity (3.05 ± 2.07 fmol/tube vs 18.60 ± 2.01 fmol/tube, $P < 0.001$), in basal condition. The perfusion with a $K^+$ enriched aCSF (60mM) induced a significant increase of N/OFQ levels (+ 187.77 % ± 15.05 % vs basal values, $P < 0.001$). The application of two different exogenous stimuli represented by the subplantar administration of carrageenan (100 µl, 3%) or formalin (50 µl, 5%), caused different results. Carrageenan did not induce significant changes in N/OFQ levels within 4 hours after the administration, whereas formalin produced a significant increase (42% vs basal values, $P < 0.05$), starting about 30 min after its administration.
In conclusion, the spinal perfusion procedure allowed us to detect and quantify the endogenous N/OFQ in the rat subaracnhnoid space, under basal and stimulated conditions. The increase observed after spinal perfusion with a $K^+$-enriched aCSF suggested its neuronal origin. Data obtained after carrageenan or formalin administration suggested that N/OFQ release is more related to nociceptive stimuli rather than to inflammatory conditions. The delayed increase of N/OFQ observed after formalin lead to the hypothesis that this release could be more related to central sensitization rather than to nociception itself.