Immunocytochemical staining of hypothalamic hypocretin neurons in Prader-Willi patients

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Narcolepsy is a human sleep disorder characterized by excessive daytime sleepiness and cataplexy. Recently it has been discovered that narcoleptic patients have undetectable levels of the neuropeptide hypocretin (orexin) in their cerebrospinal fluid. Immunocytochemistry and in situ hybridization of hypocretin mRNA in the perifornical area of the hypothalamus of narcoleptic patients in postmortem material indicated global loss of hypocretin in all cases examined. It is hypothesized that this deficiency is caused by an auto-immune mediated degeneration of hypothalamic hypocretin neurons. Prader-Willi syndrome is a genetic disorder characterized by mental retardation, hypogonadism, obesitas, dysmorphic features and behavioral dysfunctions. Most notably are food-related problems such as hyperphagia and food seeking. Excessive daytime sleepiness is a symptom that has only recently gained attention, because it was first thought to be caused by the obesity that is common in Prader-Willi patients. There have been some reports, however, that Prader-Willi patients may show narcolepsy-like symptoms, such as sleep onset with REM and cataplexy, independent of their obesity related sleep problems such as sleep apnea. Hypocretin neurons project throughout the central nervous system to nuclei known to be important in the control of feeding and sleep-wakefulness. We hypothesize that there is a reduction of hypothalamic hypocretin neurons in Prader-Willi patients, causing narcolepsy-like symptoms. We immunocytochemically stained the hypocretin neurons in the hypothalami of eight Prader-Willi patients and eight controls matched for age, sex, post-mortem delay and fixation time, using polyclonal rabbit anti-human hypocretin A antibodies (batch# R2626, Phoenix Pharmaceuticals Arizona). Hypothalami were obtained from the Netherlands Brain Bank. We compared the area in which staining was found in patients and controls. The area (number of slides) in which hypocretin neurons were found, was found to be significantly smaller in the Prader-Willi hypothalami vs the control hypothalami (Mann-Whitney rank-sum test, p=0,019). Currently the total number of hypocretin containing neurons is determined in both groups using a computerized image analysis system. The results will be presented.

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