Altered levels of neuropeptides characterize the brain of lupus prone mice

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Abstract

It has been reported that more than 50% of lupus patients show various forms of neurological deficits including impaired cognitive functions and psychiatric disorders. Using an animal model of lupus we investigated the production of neuropeptides in the brain of NZB/W F1 female hybrid mice and its parental strain NZB and NZW. Our results indicate that the alteration in learning and memory described in lupus mice are paralleled by a decrease in calcitonin gene-related peptide, substance P and neuropeptide Y (NPY) levels in the hippocampus and a significant decrease of NPY in the cortex. These findings are interesting in the light of previously reported results suggesting that these neuropeptides can play an important role in cognitive functions. We also observed a decrease of NPY and vasoactive intestinal polypeptide levels in the hypothalamus of lupus prone mice and these changes may be related to the disregulation of the hypothalamus-pituitary-adrenal axis observed in lupus prone mice. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

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Systemic lupus erythematosus (SLE), a multi-systemic autoimmune disease primarily affecting women, is characterized by a hyperactivation of B-cells, increased production of autoantibody, autoimmune complexes and inflammatory cytokines. Effects on the central nervous system (CNS) have been reported in more than 50% of SLE patients showing neurologic deficits such as cognitive dysfunction, seizures, and psychiatric disorders including psychosis, depression and anxiety [5,8]. Although the abnormal production of antineuronal and antiphospholipid antibodies [3,16] seems to be partly responsible for some of the neurological symptoms in SLE, the mechanisms leading to altered nervous system functions in SLE are still unclear. The pathogenesis of cerebral lupus may be studied using murine models such as NZB/W F1, obtained from the mating of NZB mice, the first described model of autoimmune disease [9], with the phenotypically normal NZW. The NZB/W F1 female hybrids spontaneously develop an autoimmune disease closely resembling human SLE [22]. As observed also in SLE patients, SLE prone mice present neurological impairments such as abnormal postural responses and persistent tremors [11], and changes in cognitive functions [25], which become more severe during the progress of the disease. In a previous study we described a significant decrease in the levels of nerve growth factor (NGF) in different areas of the brain of SLE mice at 8 months of age. Since NGF regulates neuropeptide expression [14,24], we hypothesized that a modification in NGF production can affect neuropeptide expression in SLE mice. Neuropeptides are widely distributed through the brain and numerous studies indicate that they may participate in several physiological processes including pain sensation, memory, regulation of mood and neuroendocrine functions.

It is therefore possible that modifications in the baseline concentration of neuropeptides in the brain can contribute to the behavioural alterations that characterize lupus prone mice.

In order to evaluate whether the progression of SLE can be related to variation of neuropeptide synthesis in CNS, we analyzed the levels of substance P (SP), calcitonin gene-related peptide (CGRP), and neuropeptide Y (NPY) in the brain of NZB/W F1 mice.
related peptide (CGRP), neuropeptide Y (NPY) and vasoactive intestinal polypeptide (VIP) in hippocampus, hypothalamus and cortex of NZB/W F1 mice and its parental strains, NZB and NZW at 5 and 8 months of age.

Female NZB, NZW and NZB/W F1 mice were purchased from Harlan Olac, UK, and kept under standard conditions of temperature and light. The activity of the mice was assessed by visual inspection at 5 and 8 months. The mice \( (n = 10 \text{ for each group and each age}) \) were sacrificed by cervical dislocation and the kidneys, thymus and spleen were dissected, weighed and frozen immediately in liquid nitrogen. All samples were stored at \(-70^\circ\text{C}\) until analysis.

The samples were cut into small pieces, boiled for 10 min in 1 M acetic acid, homogenized using a Polytron and boiled again in distilled water for 10 min. After centrifugation at 1000 \( \times \text{g} \) for 10 min, the supernatants were collected, lyophilized and then stored at \(-70^\circ\text{C}\).

SP was analyzed using antisera SP2 raised in rabbit against conjugated rat SP. The antisera reacts with SP and SP sulfoxide but not with other tachykinins. The detection limit of the assay was 3 pmol/l. Intra- and inter-assay coefficients of variation were 7 and 11%, respectively.

CGRP was analyzed using antisera CGPR8 raised in rabbit against conjugated rat CGRP. The detection limit of the assay was 9 pmol/l and the cross-reactivity with SP, neurokinin A, neurokinin B, neuropeptide K, gastrin, neurotensin, bombesin, NPY, and calcitonin was less than 0.01%. Intra- and inter-assay coefficients of variation were 8 and 14%, respectively.

NPY was analyzed using antisera N1 which cross-reacts 0.1% with avian pancreatic polypeptide, but not with other peptides. The detection limit of the assay was 11 pmol/l. Intra- and inter-assay coefficients of variation were 7 and 12%, respectively.

VIP was analyzed using antisera VIP2 raised against conjugated natural porcine VIP. The antisera does not cross-react with gastrin, pancreatic polypeptide, glucagon, NPY or neurotensin. The detection limit of the assay was 3 pmol/l. Intra- and inter-assay coefficients of variation were 9 and 13%, respectively.

All results are given as pmol/g of wet weight \( \pm \) standard error of the mean (SEM) and the multiple comparison between the groups was performed using ANOVA, followed by Kruskal–Wallis’ post-hoc test.

In the hippocampus at 5 months there was no significant difference between NZB/W F1 and NZW mice, while a significant decrease of SP, CGRP and NPY was measured in 8-month-old NZB/W F1 and NZB mice compared with NZW (\(*P < 0.05, **P < 0.01, \text{ANOVA Kruskal–Wallis’ post-hoc test}\).)

In the hypothalamus at 5 months there was no significant difference between NZB/W F1 and NZW mice, while at 8 months NZB and NZB/W F1 mice were found to have significantly diminished levels of SP, CGRP and NPY compared with NZW (Fig. 2A,B,D). A significant increase of VIP was detected in the hippocampus of 8-month-old NZB mice while no difference was found in NZB/W F1 mice (Fig. 1C).

As shown in Fig. 2, at 8 months of age the hypothalamus of NZB/W F1 mice displayed higher levels of SP and CGRP (Fig. 2A,B) than NZW mice, while the levels of VIP and NPY (Fig. 2C,D) were significantly decreased in 5- and 8-month-old NZB/W F1 and NZB mice.

No significant changes in SP, CGRP and VIP levels were seen in the cortex of lupus prone mice (Fig. 3A–C) while NPY levels were decreased in NZB/W F1 and NZB mice at both 5 and 8 months of age compared with NZW (Fig. 3D). It has recently been reported that lupus prone mice have decreased activity and show avoidance of open areas...
In an earlier study we have reported that NZB/W F1 are characterized by decreased levels of NGF in the hippocampus [2]. Since NGF plays an essential role in the development, differentiation and maintenance of brain cholinergic neurones [23], which are implicated in memory and learning performances, it is possible that the behaviour deficits observed in lupus mice are associated with an altered availability of NGF.

Lupus, like many other autoimmune diseases, is also characterized by a disorganization of the hypothalamus-pituitary-adrenal (HPA) axis. Interestingly our results show decreased levels of VIP and NPY in the hypothalamus of lupus prone mice. Endogenous VIP not only regulates HPA axis activity in normal conditions but also plays a pivotal role in the mechanisms involved in the stress-induced activation of the HPA [17]. The down-regulation of VIP and NPY might be crucial to triggering the disorganization of the HPA axis. This hypothesis is supported by the finding that intracerebroventricular administration of NPY caused an increase in CRF concentrations in the hypophyseal-portal vein and sustained increases in plasma ACTH and cortisol [15]. Thus activation of the central NPY pathway causes an acute and sustained stimulation of the HPA axis [13]. Decreased concentration of NPY in the hypothalamus may therefore represent one of the mechanisms involved in the dysfunction of the HPA axis seen in lupus.

Taken together our findings suggest that the altered distribution of neuropeptides in the brain of lupus mice might be involved in behavioural deficits and to the overall deterioration of the CNS functions described in human patients and animal lupus models.

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