Changes in Neuropeptide Levels after Brain Damage in Rats

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Abstract — The physiological and pathophysiological roles of neuropeptides are still not clear. The aim of our study was to detect long lasting changes of vasoactive-intestinal peptide (VIP), somatostatin (SOM) and substance P (SP) contents in the rat cerebral cortex and hippocampus after brain lesion. The experiments were performed on groups of adult male Wistar rats. The first group consisted of animals with unilateral ablation of the sensorimotor cortex performed at the age of 60 days. The second group was a control one (rats of the same age but with an intact brain). Both groups of animals were sacrificed at the age of 90–105 days and radioimmunoassay was used to determine amounts of VIP, SOM and SP. The mean values of VIP levels were decreased significantly only in contralateral cortical areas, while there was an increase of SP in lesioned animals. Our results suggest that discrete changes in neuropeptide levels occur during restorative processes after brain lesion.

Introduction

Numerous peptide molecules are involved as neuromodulators in neuroendocrine, restorative and behavioral processes. Thus, changes in somatostatin (SOM) levels in the central nervous system have been extensively studied in Alzheimer's disease1-3 while significant alterations in radioimmunologically and immunohistochemically detectable SOM and neuropeptide Y (NPY) were demonstrated with aging.4-5 Peripheral nerve injuries evoke alterations in levels of vasoactive intestinal peptide (VIP) and other neuropeptides in the spinal cord.6 Further, long-term moderate hypoxia induces an increase in substance P (SP) immunoreactivity in the peripheral nervous system,7 as well as in VIP-like immunoreactivity in discrete
brain areas and peripheral structures. The aim of our study was to detect changes of VIP, SOM and SP contents in rat cerebral cortex and hippocampus after unilateral cortical lesion. A preliminary account of this study has already appeared.

Materials and methods
Experiments were performed on adult male Wistar albino rats. The experimental animals were subjected to a 12 h light-dark cycle and were housed with free access to food and water. The animals were handled and their behavior, such as walking, feeding and drinking was monitored daily for at least 15 days before preparing the brains for biochemical analysis. At the beginning of the experiment the rats were 60 days old and their weight was 250-300 g. One group of experimental animals (N = 5) was subjected to brain lesion. The surgical procedures were performed under nembutal (pentobarbitone-Na) anaesthesia (40 mg/kg, i.p.) and the rats were fixed in a stereotaxic apparatus. The sensorimotor cortex was unilaterally removed on the right side to the depth of the white matter, by gentle aspiration (suction ablation) through a polypropylene tip. The stereotaxic coordinates for craniotomy were as follows: 4 mm lateral from the midline, 2 mm anterior to bregma and 4 mm posterior to bregma. The partially removed part of the skull was replaced in position with bone wax, after cortical ablation. In the control group of experimental animals (N = 5) were rats of the same age but without brain injury. More than 30 days after cortical lesion, the overall motor behavior of investigated rats was normal in the cage, the increase of body weight was the same in the group of control and lesioned animals and there were no spontaneous behavioral epileptic signs. Both groups of animals were sacrificed by decapitation between 11-13 h to avoid circadian fluctuations, under deep nembutal anaesthesia at the age of 3-3.5 months (i.e. 30-45 days after the cortical injury). The brains were quickly removed, the hemispheric regions were dissected as follows: right (ipsilateral to the lesion side) and left (contralateral to the lesion side) parietal cortex and right and left hippocampal regions. The extracted tissues were frozen, weighed, immersed in 10 volumes of 0.5 M acetic acid and then placed in a boiling water bath for 10 min. The extracts were allowed to cool and stored at -20°C until radioimmunoassay. The VIP, SOM and SP assays were performed as described in detail. Specific antisera and standards were from Hammersmith Hospital, London. Care and use of the animals were in accordance with protocols approved by US National Institutes of Health and also by our institutional guidelines. Statistical evaluation of the data was performed by analysis of variance (ANOVA) followed by Student's t-test and p values <0.05 were considered to be significant.

Results
Vasoactive intestinal peptide
The mean concentrations of VIP in regions of the parietal cerebral cortex and hippocampus are shown in Figure 1. Mean VIP levels were greater in cerebral cortex than in hippocampus, as confirmed by ANOVA. There were no significant differences in the VIP contents between left and right brain regions in the controls. However, there was a tendency for the mean value of VIP content to be decreased in lesioned animals, as compared to the controls and a significant decrease of more than 30% was found in the contralateral parietal cortex, even 30 (or more) days after ablation.

Somatostatin
No difference in mean values for SOM in different brain areas was found either in the control or in the
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Lesioned group of animals. SOM values showed great variations after cortical lesion and therefore the preliminary observed decrease in SOM values in certain brain regions were not maintained. The changes were not statistically significant in this study, as shown in Figure 2.

Substance P

SP levels did not differ greatly in the investigated brain regions either in control animals or in lesioned ones. There was a tendency for an increase of SP level to occur in each examined structure, which was especially marked and statistically significant in the left—contralateral cerebral cortex in lesioned animals (Fig. 3).

Discussion

Although neuronal plasticity restores and maintains a certain level of cortical stability, the present results show that there are long lasting region-specific neuropeptide changes, even 30–45 days after unilateral lesion of the sensorimotor cortex. Therefore, VIP and SP (but not SOM) may be supposed to have specific roles in recovery processes after brain damage, conversely to the down regulation of SP and VIP after peripheral nerve injuries. Our biochemical results are in accordance with electrophysiological studies on the same model of brain injury which predominantly affects the intrinsic machinery of inhibition in the cerebral cortex. It cannot be suggested which neuropeptide change is the best indicator of cortical lesion. Moreover, it is not clear why there is no significant change in SOM which is present in high concentrations in the cerebral cortex. In Alzheimer’s disease there are no changes in VIP and SP levels while a marked decrease of SOM is well documented. The high concentrations of immunoreactive VIP found in the cerebral cortex of our control group confirm other studies in rodents. For continuation of our electrophysiological study of the acute model of epilepsy the proposed role of neuropeptides in regulation of seizure threshold and interictal behaviour is relevant. Pentylentetrazol-induced kindling elevates the levels of SOM. Generalized seizures induced by kainic acid caused a loss of SOM-immunoreactive neurons in the hippocampus and an increase in mRNA concentrations of neuropeptide Y, cholecystokinin and neurokinin B in the hilus of the dentate gyrus. From the point of view of plastic changes in the time domain, the acute changes of more pronounced and/or diverse region-specific alterations of certain neuropeptides after brain lesion could be very important as recently shown for acute stress, which led to rapid and selective changes of SP, SOM and cholecystokinin levels. More studies are needed to further investigate the functional significance of certain neuropeptide changes after different brain lesions.

Acknowledgements

The authors are grateful to Prof. S. R. Bloom and Dr. M. Ghatei, Hammersmith Hospital, London, for the gift of peptide standards and antisera necessary for this work. This work was supported by the Serbian Ministry of Science and Technology.

References

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