EFFECT OF ADRENAL GLAND HORMONES ON NGF LEVEL AND CHOLINE ACETYLTRANSFERASE ACTIVITY IN RAT BRAIN

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Abstract. The effect of adrenal gland hormones on brain NGF-producing cells and on NGF target cells was studied in young adult rats. The results showed that adrenalectomy causes a decrease in the NGF level in the hippocampus and of choline acetyltransferase in the septum. These studies suggest that adrenal gland hormones are involved in the regulation of NGF in specific brain structures.

Nerve growth factor (NGF) is a well-characterized neurotrophic factor present in large amounts in snake venom (5), mouse submaxillary gland (23), prostate glands (14) and in a much lesser amount in several peripheral tissues (for a comprehensive review, see 21, 22, 35). Moreover, recent studies have shown that NGF is differentially expressed in mammalian brain tissues (19, 20, 31, 37), taken up by cholinergic nerve terminals projecting to the hippocampus (HI) and retrogradely axonally transported in the medial septal cholinergic neurons (11, 30). It has also been shown that the degeneration of basal forebrain neurons (BFN) that occurs following transection of the septo-hippocampal pathways is markedly reduced by NGF administration (13). Other studies have also indicated that NGF causes an increase in choline acetyltransferase (ChAT) in cortical and subcortical cholinergic cells, both in vivo and in vitro (9, 11, 15, 30). Furthermore, evidence has been provided that the ability of neurons to respond to NGF depends on the presence of
cell surface receptors, which in the central nervous system (CNS) are highly co-localized on the NGF-responsive cholinergic neurons (10, 35).

Previous studies from our laboratory demonstrated that injection of testosterone or thyroxine in young and adult mice causes an increase in NGF level in the submaxillary salivary glands (2, 22). Recently some evidence has been provided showing that thyroid hormones may be involved in NGF synthesis and NGF-receptor expression in the CNS (18, 35, 37). However, while the role of thyroid hormones in salivary NGF synthesis appears well established (2, 22) much less is known about the correlation between circulating hormones and brain NGF level. Since the HI, which contains the greatest amount of NGF in the brain (19, 31) is also a target structure for corticosteroid (26), it was hypothesized that this brain structure would represent a good model for studying to what extent the level of circulating adrenal gland hormones affects hippocampal NGF content. Furthermore, since the NGF synthesized by forebrain neurons seems to provide critical support for cholinergic neurons of the basal forebrain (29, 31, 34), the ChAT enzymatic activity and immunohistochemistry of forebrain cholinergic neurons was also examined.

In our studies, forty-day-old (100-120 g) female Sprague-Dawley rats (Nossan, Calco, Italy) were used. They were either sham operated (laparotomized, n = 52) or adrenalectomized (ADX, n = 58) and allowed free access to food and a 0.9% NaCl solution. NGF (2.5S) was purified from male mouse salivary glands (4). Polyclonal antibodies were prepared and purified as previously described (33). Corticosterone (Sigma, St. Louis USA) was dissolved in ethanol/saline, 1 : 9 (vol/vol), and injected intradermally into the adrenalectomized (ADX) rats at doses of 2 μg every other day for 2 weeks. Three weeks after operation, rats were sacrificed and brains used for NGF-receptor distribution ChAT immunohistochemistry, NGF biological assays, and ChAT enzymatic activity.

For NGF immunohistochemistry, brains were fixed by immersion and tissues processed as described (1, 37). For NGF receptor and ChAT immunohistochemistry, rats were perfused with 100 ml of saline followed by 300 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) and brain sections stained either with monoclonal antibodies against NGF receptors (192-IgG; a generous gift from E. M. Johnson, Jr., Washington University, St. Louis, MO, USA) or with ChAT monoclonal antibodies (a generous gift from C. Cozzari, Institute of Cellular Biology CNR, Rome, Italy). ChAT enzymatic activity was determined according to the method by Fonnum (8), and protein determined following the procedure reported by Lowry (24).
NGF receptor antibodies were iodinated with $^{125}$I by the chloramine-T method (17) and labeled antibodies were purified by Sephadex G-25 column chromatography. Control and ADX rats were injected with 4 μl of $^{125}$I-192-IgG (10^7 cpm) in the anterior HI. Twenty-four hours later, animals were anesthetized and perfused through the heart. Brains were removed and 40 μm thick coronal sections cut. Coded sections containing the septum were washed and iodinated antibodies were bound to the tissues and measured with a γ counter. They were then mounted on glass slides and processed for autoradiography (1). Statistical evaluations were performed by analysis of variance. Groups were compared by the two-tailed Student’s t-test.

Our studies showed that adrenalectomy (ADX) carried out in young adult rats results in a decrease in the NGF level in the HI (1.7 ± 0.3 sham to 0.8 ± 0.2 ADX ng/g wet weight). Immunohistochemical examination showed that the NGF localized in the CA-2 area and in the dentate gyrus decreases significantly following ADX, while the distribution of NGF-receptors in the medial septum neurons increases after ADX. Furthermore, following injection of iodinated NGF-receptors into the HI of sham and ADX rats, the greatest accumulation of radioligands was observed in the medial septal neurons of the sham ADX rats (see Table II). Furthermore, ChAT enzymatic activity measured in five different brain regions showed that ADX caused a statistically significant decrease of this enzyme in the septum (Table I). Likewise immunohistochemical studies of serial brain sections indicated that the ChAT immunoreactive neurons in the basal forebrain decrease drastically after ADX. The total number of cholinergic neurons in the brain septal region, however, remains basically unchanged, as shown in Table II. Treat-

<p>| Table I |
|-----------------|----------------|----------------|-----------------|----------------|
| ChAT activity levels (nmole h⁻¹ mg protein/min) in different brain regions of sham and ADX rats |</p>
<table>
<thead>
<tr>
<th>Cortex</th>
<th>Hippocampus</th>
<th>Striatum</th>
<th>Septum</th>
<th>Cerebellum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>10.9±0.8</td>
<td>8.4±0.6</td>
<td>48.1±2.2</td>
<td>41.6±3.1</td>
</tr>
<tr>
<td>ADX</td>
<td>9.7±0.7 (a)</td>
<td>7.8±0.5</td>
<td>42.5±3.3</td>
<td>28.3±2.6</td>
</tr>
</tbody>
</table>

Each brain region was homogenated in (1:10) 50 mM tris buffer pH 7.3. The homogenate was then centrifuged and aliquots were taken for duplicate determinations of ChAT activity according to the method of Fonnum (8) and for protein determination (24). Values represent mean ± SEM n = 7. (a) p = 0.02; (b) p < 0.01; (c) non significant; (d) p < 0.005; (e) p < 0.03 compared to corresponding brain tissues of sham-operated rats (Student’s t-test). Highly significant differences were observed in the septum.
ChAT immunoreactivity and NGF receptors in septum of control (sham-operated) and ADX rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Total</th>
<th>Light</th>
<th>Dark</th>
<th>Total no. of cells labeled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1265 ± 213</td>
<td>36 ± 6</td>
<td>1228 ± 204</td>
<td>233 ± 38</td>
</tr>
<tr>
<td>ADX</td>
<td>1133 ± 65**</td>
<td>913 ± 122§</td>
<td>220 ± 39§</td>
<td>469 ± 27§</td>
</tr>
</tbody>
</table>

Data are mean ± SEM of five rats. * For quantitative analysis of ChAT-immunoreactive cells, rats were killed 2 weeks after operation, and brain tissue was processed for peroxidase-antiperoxidase immunohistochemistry. Immunostained cells of the SLB were analyzed at ×400 magnification in seven consecutive sections in a rostrocaudal direction. Cells were classified as darkly stained (cell body and neurites stained dark brown) and lightly stained (cell body weakly stained and neurite profile undefined). ** For detection of receptor-bearing cells, iodinated antibodies against NGF receptor were injected into the hippocampus 2 weeks after operation. After 24 h, rats were killed and brain tissues were processed. All labeled SLB cells (showing autoradiographic grain density at least 4 times as high as background) in seven consecutive sections were counted. ** p < 0.05 vs. control. § p < 0.001 vs. control.

Evidence published recently demonstrates that the NGF present in the HI provides critical support for forebrain cholinergic neurons, particularly for those located in the septal area (11, 19, 20, 29). This hypothesis has been further strengthened by the findings showing that NGF is retrogradely transported from the terminal field of the HI to cholinergic septal neurons (11), and that infusion of NGF can prevent the degeneration of these same cholinergic cells after transection of their connection with the HI (20, 37). Furthermore, the presence of NGF in the HI formation and the findings that this factor affects the level of brain cholinergic enzymes, have raised the intriguing possibility that the NGF synthesized in this brain region is involved in central cholinergic degenerative processes (10, 12), and in some geriatric and cognitive dysfunctions (3, 7, 25).

Furthermore, several studies published recently have described different types of structural and biochemical alterations in the hippocampal formation, following manipulation of circulating blood corticosteroid levels. These changes include: hippocampal cell protection (6, 26, 28), granule cell loss (32), reduction in gene expression (16), changes in lipid and protein metabolism (27). However, a link between these neurological
disturbances and impaired hippocampal NGF levels, resulting from changes in corticosteroid circulation has not been elucidated. Since it is well known that the HI is a target structure for adrenal hormones (26) and contains the highest amount of both NGF and mRNA-NGF, when compared to other brain areas (19, 31), it seemed worthwhile to examine the correlation between circulating adrenal gland hormones, HI NGF levels, and ChAT activity.

The results of these studies demonstrate that in young adult female rats, ADX causes a decrease in the NGF level in the HI and an enhanced expression of NGF receptor binding in the medial septal neurons projecting to the HI. It was also shown that ADX causes a decrease in ChAT immunoreactivity without neuronal cell loss, while enhancing ChAT enzymatic activity in the septum. It was also shown that corticosterone administration counteracted the alteration caused by ADX suggesting, therefore, that adrenal products may be specifically involved in the changes observed in the present studies.

Our previous (1) and present reports raise the possibility that such a link may exist. This hypothesis is also supported by the results reported in Tables I and II showing a clear correlation between a decrease of HI NGF and the concomitant reduction of ChAT enzymatic activity, particularly in the septal area, thus suggesting that this effect is rather specific.

These findings provide additional data on the physiological role(s) played by endogenous brain NGF on basal forebrain cholinergic neurons, and suggest the possibility that changes in circulating corticosteroid levels may affect the amount level of cholinergic activity in the basal forebrain nuclei. Further experiments focussing on the relationship between adrenal gland hormone circulation and specific cholinergic markers of CNS activity in both normal and aged animals, should provide useful information.

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