Changes of neuropeptide concentrations in the brain following experimentally induced mononeuropathy in Wistar Kyoto and spontaneously hypertensive rats

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Abstract

The effect of unilateral, experimentally induced, mononeuropathy on concentrations of neuropeptide Y (NPY), neurokinin A (NKA), substance P (SP), calcitonin gene-related peptide (CGRP) and galanin (GAL)-like immunoreactivities (LI) was studied in Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rat brains. Two weeks following ligation of the sciatic nerve, significantly higher concentrations of NPY-LI were found in the hippocampus, striatum and occipital cortex of both rat strains. CGRP-LI and GAL-LI were increased in the hippocampus of WKY rats. NKA-LI and SP-LI were decreased to different degrees in the pituitary of the WKY and SHR rats, indicating that the changes of the tachykinins, CGRP and GAL were selectively associated with the basal level of sympathetic tone. The increased concentrations of NPY-LI in the brain, not influenced by sympathetic tone, may be part of a general defense reaction in response to trauma.

Keywords: Mononeuropathy; Neuropeptide Y; Tachykinins; Galanin; Calcitonin gene-related peptide; Wistar Kyoto rat; Spontaneously hypertensive rat

Recent evidence indicates that changes in behavior, including changes in withdrawal reflexes after nerve injury, are related to changes in neuropeptide concentrations in various parts of the central nervous system (CNS). This is supported by studies showing decreased concentrations of substance P (SP) and calcitonin gene-related peptide (CGRP) [2,3,7,19,25], and increased concentrations of neuropeptide Y (NPY) and galanin (GAL) [17,30,32,33], in the spinal cord after peripheral nerve injury (axotomy and/or ligation). Increased concentrations of NPY have also been reported in sensory neurons [21].

In order to investigate the mechanisms of neuropathic pain, Bennett and Xie [4] developed a rat model of peripheral unilateral mononeuropathy. The mononeuropathy is induced by loosely tying 4 ligatures around the common sciatic nerve. The rats subsequently develop a behavioral pattern, which reaches its maximum during the second postoperative week [1,4,13], and which, hypothetically, is reminiscent of the clinical signs of hyperalgesia and allodynia commonly seen in humans suffering from peripheral neuropathic pain. Muscle atrophy [4] and loss of myelinated and unmyelinated fibres are reported in the ligated nerve [3,4,13]. At the supraspinal level, abnormal neuronal activity (increased responses to mechanical and thermal stimulation) is exhibited in the thalamus and cortex of the mononeuropathic rats 2 weeks after surgery [5,12,22].

The aim of the present work was to investigate, using the model, if unilateral mononeuropathy alters concentrations of neuropeptides in different regions of the rat brain. The neuropeptides co-exist and are co-released with monoamine transmitters [10,23]. The contribution of the sympathetic nervous system was investigated, using...
Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats.

Male SHR and WKY rats (Mollegaard, Denmark), weighing 200–220 g at the beginning of the experiments, were housed 2/cage at 21°C with water and food ad lib, and at a 12 h light/dark cycle.

Sixteen animals were anesthetized with chloralhydrate (0.4 g/kg) intraperitonally. The left common sciatic nerve was exposed and 4 ligatures (non-chronic silk) were tied loosely around it. An identical dissection was performed without ligation in 20 control animals. After surgery, the wound was closed in layers and the animals were left to recover for 2 weeks. Following this, all the ligated animals demonstrated foot ventroflexion, limping and hind paw guarding on the lesioned side. None of the rats in the control group displayed this behavior. The rats were sacrificed by focused microwave irradiation (MWI), using a microwave system (Metabostat, Gerling Moore, CA; maximal power 5 KW, 2450 MHz). The focused energy exposure time was 2 s. [27].

The brains were quickly removed, dissected on dry ice [11] and the frontal cortex, occipital cortex, hippocampus, striatum, hypothalamus and pituitary were weighed and stored at −80°C until extraction.

The samples were cut into small pieces while frozen, boiled for 10 min in 1 mol/l acetic acid and homogenized. After centrifugation 1000 X g for 10 min, the supernatants were lyophilized and stored at −20°C before analysis.

The tissue concentrations of NPY-, NKA-, SP-, CGRP- and GAL-LI were analyzed by competitive radioimmunoassays. NPY-LI was analyzed using antiserum N1 which cross-reacts 0.1% with avian pancreatic polypeptide, but not with other peptides [28]. The detection limit of the assay was 11 pmol/l. Intra- and interassay coefficients of variation of were 7 and 12%, respectively. NKA-LI was analyzed using antiserum K12 which reacts with NKA (100%), NKA (3–10) (48%), neurokinin B (26%), neuropeptide K (61%) and edeloin (30%), but not with SP [29]. SP-LI was analyzed using antiserum SP2 which reacts with SP and SP sulfoxide, but not with other tachykinins [6]. CGRP-LI was analyzed using antiserum CGPRP8 raised in a rabbit against conjugated rat CGRP. High performance liquid chromatography (HPLC)-purified [125I]-histidyl rat CGRP was used as radioligand, and rat CGRP as standard. The detection limit of the assay for rat CGRP was 9 pmol/l and the cross-reactivity of the assay to SP, NKA, neurokinin B, neuropeptide K, gastrin, neurotensin, bombesin, NPY and calcitonin was less than 0.01%. Cross-reactivity toward human alpha and beta CGRP was 93 and 24%, respectively, and toward rat alpha and beta CGRP, 100 and 120%, respectively. Intra- and interassay coefficients of variation were 8% and 14%, respectively. GAL-LI was analyzed using antiserum Rat-Gala4 raised against conjugated synthetic rat GAL. The antiserum does not cross-react with NKA, neuropeptide K, SP, NKB, NPY, gastrin, pancreatic polypeptide, gluca
cagon or neurotensin. HPLC-purified [125I] rat galanin was used as radioligand and rat galanin as a standard. The detection limit of the assay was 5 pmol/l. Intra- and interassay coefficients of variation were 6 and 10%, respectively.

Medians and interquartile range were used as measures of central tendency and variation, respectively. Skewed data were log-transformed toward normality. Concentrations of neuropeptides were analyzed using multivariate analysis of variance, with treatment and time as independent variables. When a significant group effect was found, the significance of the difference in neuropeptide concentrations was tested using Tukey’s test. \( P < 0.05 \) was considered significant.

In the control animals, the concentrations of tachykinins (both SP and NKA) were significantly higher \( (P < 0.001) \) in the pituitary of the SHR rats compared to the WKY rats (Table 1). Significantly higher concentrations of GAL-LI were found in the occipital cortex \( (P < 0.001) \) and hippocampus \( (P < 0.001) \) of SHR than in the WKY rats. A significantly \( (P < 0.05) \) higher concentration of NPY-LI was also detected in the striatum of the SHR rats.

Two weeks after ligation, NPY-LI was found in a significantly \( (P < 0.001) \) higher concentration in the hippocampus, striatum and occipital cortex of the mononeuropathic WKY and SHR rats compared to their respective controls. Higher concentrations of CGRP-LI \( (P < 0.05) \) and GAL-LI \( (P < 0.01) \) were found in the hippocampus of WKY rats, with no changes in SHR rats. In the pituitary, NKA-LI and SP-LI were decreased both in the WKY \( (P < 0.001) \) and \( P < 0.01 \), respectively and in the SHR \( (P < 0.05 \) and \( P < 0.01 \), respectively) rats (Table 1).

No changes in the neuropeptide concentrations analyzed were found in the frontal cortex and hypothalamus of either strain.

The data of the control groups were consistent with previous observations [24] that NPY levels in the striatum are significantly higher in SHR than in WKY rats. In contrast, NPY did not differ in the other brain regions analyzed, although significantly lower NPY concentration in cortex of SHR rats is reported earlier [24]. The higher concentrations of tachykinins found in the pituitary of SHR rats are in line with the study of Kamiya et al. [20] who showed a general tendency of lower levels of SP and neurokinin B in the brain of WKY compared to SHR rats.

Recent reports indicate that NPY-LI and GAL-LI are increased ipsilaterally in rat dorsal root ganglia 14 days after unilateral sciatic nerve transaction \( [17,30,32,33] \) or ligation [32]. Also, a dramatic increase of the NPY-receptor mRNA in large neurons was found after sciatic nerve lesion [35]. The results of the present study showed that many of the changes in peptide concentrations in the peripheral nervous system were paralleled by similar changes in different brain regions.

NPY may play an important role in the adaptive responses of the organism to chronic irritation. Spinal ad-
Table 1
Concentrations of neuropeptides (pmol/g wet weight) in brain regions of WKY and SHR rats 2 weeks following ligation of the left sciatic nerve using the model of Bennett and Xie compared to controls.

<table>
<thead>
<tr>
<th>Neuropeptide</th>
<th>hippocampus</th>
<th>striatum</th>
<th>occipital cortex</th>
<th>pituitary</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
<td>SHR</td>
</tr>
<tr>
<td>Neuropeptide Y</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9.8 (7.9-11.5)</td>
<td>8.1 (7.5-9.8)</td>
<td>16.3 (12.9-19.8)</td>
<td>23.6 (18.1-24.7)</td>
</tr>
<tr>
<td>Mononeuropathy</td>
<td>47.6 (41.5-49.0)</td>
<td>38.8 (34.6-44.3)</td>
<td>43.8 (38.7-50.9)</td>
<td>43.7 (42.9-48.6)</td>
</tr>
<tr>
<td>Neurokinin A</td>
<td>9.0 (7.6-9.8)</td>
<td>9.1 (6.2-12.2)</td>
<td>65.4 (56.6-88.0)</td>
<td>83.3 (64.9-84.6)</td>
</tr>
<tr>
<td>Control</td>
<td>5.3 (4.4-6.4)</td>
<td>6.5 (4.1-6.8)</td>
<td>76.9 (71.2-78.2)</td>
<td>70.9 (60.4-80.6)</td>
</tr>
<tr>
<td>Mononeuropathy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CGRP</td>
<td>1.7 (1.1-2.1)</td>
<td>1.3 (1.1-1.8)</td>
<td>1.9 (1.8-3.0)</td>
<td>2.7 (2.4-4.9)</td>
</tr>
<tr>
<td>Control</td>
<td>2.5 (2.1-2.8)</td>
<td>1.3 (1.3-1.5)</td>
<td>1.5 (1.3-2.1)</td>
<td>2.0 (1.8-2.4)</td>
</tr>
<tr>
<td>Mononeuropathy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substance P</td>
<td>5.4 (5.1-8.4)</td>
<td>7.8 (4.4-8.9)</td>
<td>122.2 (99.0-136.2)</td>
<td>142.8 (138.4-166.9)</td>
</tr>
<tr>
<td>Control</td>
<td>6.1 (5.2-7.1)</td>
<td>4.9 (4.4-5.7)</td>
<td>108.3 (98.7-113.7)</td>
<td>114.2 (97.7-132.1)</td>
</tr>
<tr>
<td>Mononeuropathy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galanin</td>
<td>5.6 (4.4-7.0)</td>
<td>10.6 (9.4-16.4)</td>
<td>8.7 (5.1-10.1)</td>
<td>15.5 (11.7-16.7)</td>
</tr>
<tr>
<td>Control</td>
<td>11.6 (9.6-13.0)</td>
<td>13.6 (12.6-14.7)</td>
<td>5.2 (3.1-6.6)</td>
<td>7.2 (5.9-10.5)</td>
</tr>
<tr>
<td>Mononeuropathy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| The control groups consisted of 10 animals each; the operated groups of WKY, 9; SHR, 7. Values are expressed as medians with 95% confidence limits in parentheses, aP < 0.05; bP < 0.01; cP < 0.001.

Phenomenon of NPY reduces nerve stimulus-evoked release of SP-LI [9] and produces a powerful dose-dependent antinociceptive effect without motor dysfunction [16]. When applied to a hippocampal slice, NPY reduces the elicited synaptic excitation of pyramidal cells in areas CA1 and CA3 [8,14]. Taken together, these results suggest that NPY inhibits hyperexcitability in the central nervous system after peripheral nerve injury. In addition, NP Y may be involved in the complex interactions that exist between depression and pain [26], as NPY has been shown to take part in the mechanisms of anxiety [31], and have an anxiolytic effect after intracerebroventricular administration in rats [15].

Present results demonstrated increased concentrations of NPY in the hippocampus, striatum and occipital cortex, and a decrease of tachykinins in the pituitary, after an unilateral nerve lesion in rats. Högfelt et al. [18] suggest that adaptive responses to limit the consequences of the damage after axotomy take place in primary sensory neurons, thus attenuating the transmission in the dorsal horn. In addition, our data indicated that experimental unilateral mononeuropathy in the rat resulted in an up-regulation of NPY in widespread CNS areas, in rats with both normal (WKY) and increased (SHR) sympathetic tone, while the down-regulation of tachykinins in the pituitary differed between the strains. The decrease in NKA was more pronounced in the WKY whereas the decrease in SP was more prominent in SHR rats. Together with the increase of CGRP and GAL in the hippocampus of WKY, but not SHR rats, the results suggest that changes of tachykinins, CGRP and GAL in the rat brain, following experimental mononeuropathy, were selectively associated with the basal level of the sympathetic tone.
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