Neuropeptide Y (NPY) Synthesis in Lymphoblasts and Increased Plasma NPY in Pediatric B-Cell Precursor Leukemia

By Per Kogner, Anders Ericsson, Gisela Barbany, Håkan Persson, Elvar Theodorsson, and Olle Björk

Neuropeptide Y (NPY), a regulatory peptide in both the central and peripheral nervous systems, has recently been found in neuroendocrine tumors as well as in the bone marrow of rat and certain autoimmune mice, but not in human bone marrow. To investigate a possible role for NPY in the human hematopoietic system, we have prospectively studied NPY-like immunoreactivity in plasma (P-NPY-LI) and NPY mRNA in bone marrow from children with acute leukemia. Northern blot showed high levels of NPY mRNA in bone marrow and peripheral lymphoblasts from children with B-cell precursor leukemia. In situ hybridization showed NPY mRNA in malignant B-cell precursor lymphoblasts. No NPY mRNA was detected in the bone marrow of children with T-cell leukemia. P-NPY-LI was higher (P < .001) in 51 children with leukemia (200: 50 to 385 pmol/L, median: interquartile range) compared to 51 age-matched healthy controls (37: 20 to 52 pmol/L). P-NPY-LI was higher (P < .001) in those with favorable clinical risk classification. Elevated P-NPY-LI, compared with the upper age-adjusted reference limit, was only found in children with B-cell precursor leukemia (31 of 40), whereas all children with B-cell, T-cell, or myeloid leukemia (n = 11) had normal P-NPY-LI (P < .001). During the 2- to 46-month follow-up, children with elevated P-NPY-LI had better (P < .001) outcome compared to those with normal P-NPY-LI (79.4% v 34.6% probability for event-free survival).

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Material and Methods

Patient Material. A series of 51 consecutive children with acute leukemia was investigated for NPY in plasma (Table 1). They were diagnosed using morphologic criteria of the French-American-British (FAB) Cooperative Group and classified using established risk criteria (Table 1) into acute lymphoblastic leukemia of standard risk (ALL-SR), intermediate risk (ALL-IR), high risk (ALL-HR), or acute nonlymphocytic leukemia (ANLL). Uniform treatment was given according to national protocols (ALL-SR: Swedish Pediatric Leukemia Group), international protocols (ALL-IR and ALL-HR: German Cooperative Study, BFM-83), and Nordic protocols (ANLL: Nordic Society of Paediatric Haematology and Oncology). Fifty-one healthy children admitted for elective surgery matched for age and sex served as controls (Table 1). P-NPY-LI was compared with the upper age-adjusted reference limit obtained from a study comprising 112 healthy children (P.K., O.B., E.T.: unpublished observations, 1992). Children with leukemia were observed for 2 to 46 months from diagnosis. Bone marrow or peripheral lymphoblasts from eight children with acute leukemia were studied for NPY mRNA (Table 2).

Sample Handling. Venous blood was collected from children with acute leukemia at diagnosis and matched healthy controls in prechilled heparinized tubes, transported in ice bath, and centrifuged (1,500g, 10 minutes, +4°C) within 30 minutes. Plasma was stored at −20°C until analysis.

Bone marrow aspirates were obtained from the posterior iliac crest and immediately fresh frozen at −70°C. Peripheral malignant lymphoblasts were obtained from venous blood of one child and quick frozen (−70°C) for subsequent extraction of RNA.

Radioimmunoassay for NPY. P-NPY-LI was analyzed in extracted plasma by a competitive radioimmunoassay using an antiseraum raised in a rabbit as previously described.

Northern Blot Analysis of NPY mRNA. Total cellular RNA was prepared from bone marrow and peripheral lymphoblasts, fractionated in parallel on a denaturing agarose gel, blotted onto nitrocellulose membranes, and hybridized to a 32P-labeled human NPY cDNA probe as previously described. Polyadenylated RNA from rat cerebral cortex was run in parallel as a positive hybridization control.

In situ Hybridization. Bone marrow smears were prepared at diagnosis on poly-lysin (50 μg/mL) coated slides, fixed in 4% paraformaldehyde, and hybridized to a 294-bp 

AvaI-EcoRI frag-
Table 1. Patient Material and Plasma NPY-LI at Diagnosis

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n</th>
<th>Age, mo</th>
<th>Sex</th>
<th>P-NPY-LI, pmol/L</th>
<th>P-NPY-LI</th>
<th>Median (range)</th>
<th>Interquartile range</th>
<th>Normal/ Elevated</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL-SR</td>
<td>15</td>
<td>39</td>
<td>M/F</td>
<td>200-266</td>
<td>0/15</td>
<td>290</td>
<td>6/9</td>
<td></td>
</tr>
<tr>
<td>ALL-IR</td>
<td>16</td>
<td>110</td>
<td>M/F</td>
<td>200-500</td>
<td>3/13</td>
<td>305</td>
<td>7/3</td>
<td></td>
</tr>
<tr>
<td>ALL-HR</td>
<td>16</td>
<td>66</td>
<td>M/F</td>
<td>28-92</td>
<td>12/3</td>
<td>36</td>
<td>9/6</td>
<td></td>
</tr>
<tr>
<td>ANLL</td>
<td>5</td>
<td>17</td>
<td>M/F</td>
<td>44-65</td>
<td>5/0</td>
<td>50</td>
<td>4/1</td>
<td></td>
</tr>
<tr>
<td>Leukemia</td>
<td>51</td>
<td>61</td>
<td>M/F</td>
<td>50-385</td>
<td>20/31</td>
<td>200</td>
<td>28/23</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>51</td>
<td>58</td>
<td>M/F</td>
<td>20-52</td>
<td>51/0</td>
<td>58</td>
<td>28/23</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ALL-SR, acute lymphoblastic leukemia-standard risk (without IR/HR criteria); IR, intermediate risk (leukocyte count 10 to 50 × 10⁹/L, or age 1 to 2 years or ≥10 years and no HR criteria); HR, high risk (leukocyte count >50 × 10⁹/L, central nervous system involvement, mediastinal mass, T-cell ALL, B-cell ALL, or chromosomal translocation); ANLL, acute nonlymphoblastic leukemia.

Table 2. Patients Examined for NPY mRNA

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age, mo</th>
<th>Diagnosis</th>
<th>State at Examination</th>
<th>P-NPY-LI pmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>JP M</td>
<td>26</td>
<td>BCP ALL-SR, CALLA++</td>
<td>DIA</td>
<td>128 (+)</td>
<td></td>
</tr>
<tr>
<td>NF F</td>
<td>68</td>
<td>BCP ALL-HR, CALLA++</td>
<td>DIA</td>
<td>100 (+)</td>
<td></td>
</tr>
<tr>
<td>MW F</td>
<td>82</td>
<td>BCP ALL-HR, CALLA+</td>
<td>DIA</td>
<td>28 (N)</td>
<td></td>
</tr>
<tr>
<td>SF F</td>
<td>32</td>
<td>BCP ALL-IR, CALLA++</td>
<td>DIA</td>
<td>200 (+)</td>
<td></td>
</tr>
<tr>
<td>VS M</td>
<td>72</td>
<td>T-cell ALL-AR</td>
<td>REL</td>
<td>95 (N)</td>
<td></td>
</tr>
<tr>
<td>FM M</td>
<td>157</td>
<td>BCP ALL-HR, CALLA-</td>
<td>PLB</td>
<td>310 (+)</td>
<td></td>
</tr>
<tr>
<td>MH M</td>
<td>122</td>
<td>T-cell ALL-AR</td>
<td>DIA</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>ML F</td>
<td>34</td>
<td>BCP ALL-SR, CALLA-R</td>
<td>REM</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BCP, B-cell precursor ALL; CALLA+, >20% CD10+ cells in bone marrow; CALLA++, >50% CD10+; DIA, diagnosis; REL, relapse; PLB, peripheral lymphoblasts investigated; REM, remission; (+), increased compared with healthy children; (N), normal plasma concentration; ND, not done.
with normal P-NPY-LI with elevated/normal age-adjusted P-NPY-LI. Diagnosis had better outcome compared to ALL (n = 31) compared to leukemic children with normal P-NPY-LI (NPY+, n = 20). EFS probability calculated according to Kaplan-Meier test (NPY+ 79.4% vs NPY− 51.9%, P = .09) and a tendency toward better survival (93.2% vs 32.1%, P < .05) and a tendency toward better survival (93.2% vs 32.1%, P < .05) in the only child investigated with B-cell ALL. A consistent and main in children with a favorable risk classification, and leukemic bone marrow possible. Furthermore, elevated P-NPY-LI was found mainly in children with a favorable risk classification, and only in those with B-cell precursor ALL. A consistent and rapid normalization of P-NPY-LI after initiation of chemotherapy implied tumoral origin of increased systemic NPY-LI, which was supported by the evidence for NPY synthesis in leukemic bone marrow and peripheral lymphoblasts of B-cell precursor ALL.

Elevated P-NPY-LI was closely related to clinical classification, differentiation, and outcome in 51 children monitored for 2 to 46 months. Elevated P-NPY-LI was only found in children with BCP ALL. The highest concentrations of P-NPY-LI were found in children with a high percentage of CALLA-positive cells in the bone marrow (Fig 1), but some children with earlier B-cell precursor differentiation (HLA-DR+, CD19+, and CD10- also showed elevated P-NPY-LI. P-NPY-LI was, on the other hand, normal in the only child investigated with B-cell ALL. Elevated P-NPY-LI was significantly associated with a favorable outcome in children with leukemia (Fig 2).

During the last few decades, introduction of effective multidrug chemotherapy has made the cure of childhood leukemia possible. Furthermore, identification of prognostic factors, including the immunophenotype, has made it possible to prospectively predict risk of relapse and, therefore, to design risk-based therapy to optimize the risk: benefit ratio of treatment for children with ALL. The identification of additional prognostic factors may further optimize therapy in these children, aiming both at increased survival for those with leukemia of poor risk as well
NEUROPEPTIDE Y IN PEDIATRIC LEUKEMIA

Fig 3. Detection of NPY mRNA in bone marrow and peripheral lymphoblasts (FM) from children with ALL. Polyadenylated or total cellular RNA (25 μg/slot), prepared from rat cerebral cortex (Rat ctx, polyadenylated RNA), bone marrow and peripheral lymphoblasts from children with leukemia (total cellular RNA), were electrophoresed in 1% agarose gels containing 0.7% formaldehyde and 0.1 μg ethidium bromide. After electrophoresis gels were examined under UV light to ensure equal amounts of RNA in each sample. RNA was transferred to nitrocellulose filters and hybridized to a human NPY cDNA probe labeled with α-32P-dCTP. Details on children studied in Table 2.

NPY synthesis was demonstrated in human leukemic bone marrow and peripheral malignant lymphoblasts of B-cell precursor ALL by RNA blot analysis (Fig 3). Furthermore, in situ hybridization showed NPY synthesis in leukemic lymphoblasts (Fig 4). NPY mRNA or NPY-LI have not previously been detected in human bone marrow, but was recently found in rat bone marrow and in a preparation of rat mononuclear cells. The highest concentrations of NPY-LI and NPY mRNA in these studies were found in megakaryocytes and platelets. However, no correlation between P-NPY-LI and platelet count was found in the present study, indicating that platelets were not the source of increased P-NPY-LI in children with leukemia. Interestingly, high levels of NPY mRNA and peptide were also found in bone marrow of autoimmune mice with B-cell lymphoproliferative disorders. These results, in conjunction with our present finding of stage-specific expression of NPY in malignant B-cell precursors (Figs 1 and 3), imply a role of NPY during normal B-cell development and/or pathologic disorders of B-line cells. Recently, NPY was shown to increase the adhesiveness of leukocytes to endothelial cells, suggesting a role of this peptide in cell adhesion in the hematopoietic system.

Systemic effects on the blood pressure could be expected because similar concentrations of P-NPY-LI to those reported here have been detected in pheochromocytoma patients during peroperative hypertension. NPY was suggested to play a role in hypertension found in those patients. However, in the present material only one infant, with normal P-NPY-LI, presented with hypertension. This child, with ANLL, was one of the three children with elevated catecholamine metabolites in urine (all with normal P-NPY-LI). These findings are in accordance with our previous findings in children with neuroblastoma that were normotensive despite elevated P-NPY-LI. In plasma and tumor tissue from neuroblastoma patients, high amounts of smaller NPY fragments were found that may, as previously shown, suppress the vasoconstrictive effect of intact NPY.

Combined with our previous findings on NPY in neuroblastoma, the results presented in this report warrant studies on other gene products expressed in common between pediatric neuroblastoma and B-cell precursor ALL as well as developing cells of the neural and hematopoietic system.
poietic systems. Recently, Ebener et al. found a consistent expression of several hematopoietic markers in a number of neuroblastoma cell lines. In addition, the proto-oncogene MYCN (N-myc), of importance for prognosis and tumor growth in neuroblastoma, appears to be expressed in cells of early B differentiation but not in mature B cells or plasma cells. Furthermore, it has been proposed that MYC activation may be of significance in the evolution of B-cell precursor leukemia. Nerve growth factor (NGF), inducing differentiation in neural cells and regulating NPY synthesis in PC12 cells, has been shown to stimulate DNA synthesis and Ig secretion in B cells. NGF receptors appear to be expressed both in leukocytes and several immune organs of different species including the chicken bursa of Fabricius, the primary tissue for B-cell production, as well as on human B cells of mature differentiation.

Hence, in addition to NPY, gene products important for cell growth and differentiation appear to be expressed in common between the hematopoietic and nervous systems. Future studies on these gene products may provide further insight into hematopoietic cell development and leukemic cell biology.

Our finding of a close relationship between NPY and expression of CALLA (CD10) motivates further studies on NPY-CALLA interactions. It was recently demonstrated that CALLA is identical to a zinc metalloprotease, neutral endopeptidase 24.11.37. CALLA hydrolyzes biologically active peptides inducing migration and aggregation of neutrophils expressing CALLA.

In summary, our study shows that NPY is synthesized by leukemic lymphoblasts of B-cell precursor differentiation, suggesting a role of NPY in the human hematopoietic system. Furthermore, a high concentration of plasma NPY-LI is associated with expression of CALLA (CD10) in a subset of B-cell precursor ALL with a favorable clinical risk classification. Elevated plasma NPY-LI at diagnosis appears to correlate with a favorable outcome in pediatric leukemia. Finally, we suggest that elevated P-NPY-LI could be used as an indicator of B-cell precursor differentiation in children with acute leukemia.

REFERENCES

25. Lundberg JM, Hökfelt T, Hems A, Theodorsson-Norheim E, Pernow J, Hamberger B, Goldstein M: Neuropeptide Y-like immunoreactivity in adrenaline cells of adrenal medulla and in...
38. Shipp MA, Stefano GB, Switzer SN, Griffin JD, Reinherz EL: CD10 (CALLA)/neutral endopeptidase 24.11 modulates inflammatory peptide-induced changes in neutrophil morphology, migration, and adhesion proteins and is itself regulated by neutrophil activation. Blood 78:1834, 1991
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