Neuropeptide Y Expression in the Developing Adrenal Gland and in Childhood Neuroblastoma Tumors

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ABSTRACT

Neuropeptide Y (NPY) expression is limited to tissues of the central and peripheral nervous system. In the adrenal gland, NPY is found in a subset of cells of the adrenal medulla. Using in situ hybridization analysis, NPY mRNA expression was characterized during human fetal adrenal medullary development. We found a biphasic pattern of NPY mRNA expression during the development of the human adrenal medulla. NPY mRNA is detectable at the earliest evaluable time point (7.5 weeks of gestational age) through 18 weeks of gestational age, and is then not detectable until 8 months after birth. We also analyzed NPY mRNA expression in neuroblastoma tumors, which often arise in the adrenal medulla. Thirty-eight neuroblastoma tumors were analyzed for NPY mRNA expression using in situ hybridization. We found NPY mRNA expression in 30 of 38 tumors; 15 of 15 Stage IVS tumors from children under 1 year of age at diagnosis expressed NPY mRNA, whereas 0 of 4 Stage IV tumors from children less than 1 year of age at diagnosis expressed NPY mRNA. These data suggest that in children under 1 year of age at diagnosis, Stage IVS and Stage IV neuroblastoma may be marked by the presence or absence, respectively, of NPY mRNA expression. Moreover, since NPY is expressed only for a short period of time during embryogenesis, these tumors may arise from different neural crest populations occurring during the course of adrenal medullary development.

INTRODUCTION

Pediatric tumors frequently have morphological and biochemical characteristics that reflect the embryonic tissues from which they originate. Neuroblastoma, a tumor of sympathetic nervous system tissue, is derived from embryonic neural crest cells that form the adrenal medulla and sympathetic ganglia (1), and often contains evidence of neuronal differentiation. Mature ganglion cells, neurepil, and such ultrastructural features as neurosecretory granules and neurofilaments can be seen in neuroblastoma, although sheets of undifferentiated small, round, blue tumor cells resembling embryonal neural crest cells are also observed (2). The presence of such differentiated features suggests that neuroblastoma tumor cells retain the molecular mechanisms that mediate differentiation of neural crest cells.

An examination of neuroblastoma tumors for the expression of genes regulated during the course of neural crest cell maturation may provide insight into the biological relationship between this tumor and the embryonal cells from which it arises. NPY (3) is a 36-amino acid peptide whose expression is restricted to the adrenal medulla and some neurons of the central (3) and peripheral nervous system (4). We characterized its expression during human adrenal medullary development using in situ hybridization analysis, and surveyed neuroblastoma tumor tissues and cell lines for NPY mRNA expression. We found evidence suggesting that NPY expression may identify tumors that arise from cells occurring at recognizable stages of adrenal medullary maturation.

MATERIALS AND METHODS

Case Material. Thirty-eight samples from Evans Stage III, IV, and IVS human neuroblastoma tumors were obtained from the Children's Cancer Study Group. All tumor samples were obtained at diagnosis from the pituitary tumor site. The primary sites varied, including the adrenal gland as well as other sites. These tumors and a human adrenal pheochromocytoma tumor were obtained by surgical excision at diagnosis, and a portion was snap-frozen at the time of surgery to −70°C. Frozen human fetal adrenal glands were obtained from Dr. Thomas Shepard, Central Laboratory for Human Embryology, University of Washington, Seattle, WA, and human pheochromocytoma tumor tissue was obtained from Dr. W. Marsden Linehan, National Cancer Institute, NIH. Paraffin sections of normal human fetal, newborn, and adult adrenal glands were obtained from Dr. Grover Hutchins, Department of Pathology, The Johns Hopkins Hospital, Baltimore, MD. Additional normal human adrenal glands in paraffin were obtained from Dr. Robert J. Bolande, Department of Pathology, East Carolina University, Greenville, NC.

Cell Lines. Cell lines derived from human neuroblastoma tumors were examined by Northern blot analysis. These include CHP-134, CHP-404, CHP-234, and CHP-126 (Dr. Audrey Evans, Children's Hospital of Philadelphia, PA) (5, 6); NLF, NGP, and NMB (Dr. Garret Brodeur, Washington University, St. Louis, MO) (7, 8); SMS-KAN, SMS-KCN, and SMS-KCNR (Dr. Patrick Reynolds, Children's Hospital of Los Angeles, Los Angeles, CA) (9); and SK-N-LE (Dr. Larry Henson, ICI Americas, Wilmington, DE) (10). Cells grown in RPMI 1640 medium containing 15% fetal calf serum, 2 mm glutamine, 2 mm sodium pyruvate, 100 units/ml penicillin, and 50 μg/ml streptomycin were harvested when 90% confluent for RNA isolation. RA treatment of cells was performed as previously described (11, 12) in medium containing 5 μM all-trans RA (Sigma) or equivalent concentrations of solvent control (ethanol) and harvested after incubation for varying durations of time up to 11 days.

Preparation of RNA and Northern Blot Analysis. Total cellular RNA was isolated from tissue culture cells and pheochromocytoma tissue by the guanidine thiocyanate/CsCl method (13). RNA was size-fractionated on 1% agarose formaldehyde gels, transferred to Nytran membranes (Schleicher and Schuell), and hybridized to [32P]dCTP-labeled DNA at 42°C for 16 h. After hybridization, membranes were washed as described previously (14). The final wash was 0.1 × (×1 standard saline citrate is 0.15 m NaCl plus 0.015 m sodium citrate) and 1% sodium dodecyl sulfate at 65°C for 30 min. Membranes were then exposed to XAR5 film (Kodak) using an intensifying screen at −70°C for 7 days.

In Situ Hybridization. In situ hybridization analysis of frozen and paraffin-embedded normal tissues and frozen tumor specimens was performed with [35S]UTP-labeled antisense cRNA as described previously (15). Control analyses in every experiment included hybridization of normal adult adrenal glands with sense cRNA probe to detect NPY-positive medullary cells and NPY-negative cortical cells, and hybridization with a sense cRNA probe to determine the level of nonspecific background signal. Samples were scored as positive for NPY expression if the average number of grains over cells (at least 50...
cells (small arrows) are shown. Cells (large arrows) and scattered chromaffin cells (Fig. 1b, small arrows). Chromaffin cells were expressed detectable NPY mRNA. No adult adrenal gland indicated that approximately 1 of 250 micrographs of a bright-field, low-power view of the adrenal cortex (C) and medulla (M) is shown (× 20). b, the boxed region of the medulla in a is shown at × 100 in b. Ganglion cells (large arrows) and scattered chromaffin cells (small arrows) are shown.

Preparation of Radiolabeled Molecular Probes. Northern blot hybridization analysis for NPY mRNA expression was performed using radiolabeled DNA from pE9, a complementary DNA clone encoding human NPY obtained from a pheochromocytoma complementary DNA library (14). A 533-base pair PstI fragment radiolabeled with 32P-dCTP by nick translation (16) to a specific activity of approximately 2 × 10^6 cpm/μg was prepared from pE9 DNA. This 533-base pair PstI DNA fragment was also subcloned into the plasmid vector pGEM3 (pG3NPY; Promega Biotech, Madison, WI) for use in the preparation of RNA probes for in situ hybridization experiments. pG3NPY plasmid DNA was linearized by HindIII digestion and used as a template for T7 RNA polymerase (Promega Biotech) to synthesize a [35S]UTP-radiolabeled 533-base pair antisense cRNA (specific activity, approximately 5 × 10^6 cpm/μg), as described previously (15, 17, 18). Negative control probes complementary to the noncoding strand of NPY DNA, i.e., sense cRNA probes, were synthesized following linearization of DNA from this recombinant plasmid with EcoRI and in vitro transcription using SP6 RNA polymerase. pG21 DNA (19) radiolabeled by nick translation was also used for Northern blot hybridization analysis.

Statistical Methods. All P values were determined by 2-sided Fisher’s exact test.

RESULTS

In situ hybridization was initially performed on human adult adrenal glands to identify cells expressing NPY mRNA. Fig. 1a is a cross-section of an adrenal gland showing both cortical and medullary regions. Fig. 1b is an enlargement of the boxed area outlined in Fig. 1a. Grain formation over some cells in the adult adrenal gland indicated that approximately 1 of 250 medullary cells expressed detectable NPY mRNA. No NPY mRNA was detected in any cortical layer of the gland. NPY mRNA is most highly expressed by the larger ganglion cells (Fig. 1b, large arrows), but can also be seen in some smaller chromaffin cells (Fig. 1b, small arrow). Chromaffin cells were identified by the detection of immunoreactive chromogranin A in adjacent sections (data not shown).

To evaluate the expression of NPY mRNA during the course of adrenal medullary development, frozen and paraffin sections of 32 human adrenal glands obtained from fetuses and children whose ages span the period from 7.5 weeks of gestation through 5 years of age were examined in situ hybridization (Fig. 2). We detected NPY expression in primitive neuroblasts of all specimens from the earliest evaluable time period of 7.5 weeks until 18 weeks of gestational age. Representative examples of these data are shown in Fig. 3, a and b. The interference between invading neuroblasts and fetal cortex in this specimen can be seen at low and high magnification in Fig. 3, a and b, respectively. Most of the invading neuroblasts (which were immunoreactive for neuron-specific enolase by immunohistochemistry; data not shown), expressed NPY mRNA. This is seen as an increased hybridization signal when compared with the adjacent fetal cortical cells.

In 18 adrenal glands from fetuses aged 18 weeks of gestational age and older, we were unable to detect NPY mRNA expression until 8 months after birth. Representative examples of these data are shown in Fig. 3, c through f. At 24 weeks of gestational age, NPY mRNA is not found in the adrenal medulla (Fig. 3, c and d); however, NPY mRNA is again detectable in a subpopulation of adrenal medullary cells at 8 months after birth (Fig. 3, e and f) is indistinguishable from that seen in the adult adrenal medulla (Fig. 1, a and b). In summary, our data indicate that there is a biphasic pattern of NPY mRNA expression during development of the human adrenal medulla. NPY mRNA is detectable at the earliest evaluable time point (7.5 weeks of gestational age) through 18 weeks of gestational age, after which time it is not detectable again until 8 months after birth, when it remains expressed into adulthood.

The data illustrated in Fig. 3 also suggest that there are differences in the amount and cellular distribution of NPY mRNA during adrenal medullary development. The amount of NPY mRNA expression, as suggested by grain density, appears much less at 7.5 through 18 weeks of gestational age (Fig. 3, a...
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Fig. 2. Summary of in situ hybridization analysis of NPY mRNA expression during development of the human adrenal gland.

Fig. 3. Expression of NPY mRNA during development of the human adrenal medulla, determined by in situ hybridization. Adrenal glands from 3 representative time points in development are analyzed for NPY mRNA expression by in situ hybridization using [35S]UTP-labeled neuropeptide Y anti-sense cRNA. Fetal adrenal glands of 7.5 weeks' gestational age (a and b), 24 weeks' gestational age (c and d), and 8 months after birth (e and f) are shown. Boxed areas in a, c, and e, are enlarged in b, d, and f, respectively. 10× (a and c); × 20 (e); × 100 (b, d, and f).

and b) than in the postnatal period (Fig. 3, e and f). In addition most of the invading neuroblasts express NPY mRNA in the early time period, whereas only a small fraction of the adrenal medullary cells in the postnatal and adult adrenal medulla express NPY mRNA.

Distinct islets of neuroblasts have been described in the developing adrenal gland during the midtrimester of human fetal development (20). We found that from 10 to 18 weeks of gestational age, NPY mRNA expression marked the subpopulation of neuroblasts that formed distinct islets in the fetal adrenal gland (Fig. 4). Shortly after 18 weeks of gestational age, NPY mRNA is no longer detectable in these neuroblast islets. An example of an 18-week gestational age fetal adrenal gland containing neuroblast islets that express NPY mRNA is shown in Fig. 4. Fig. 4A is a low-power view of an adrenal gland that contains neuroblasts islets within the middle of the
NPY EXPRESSION IN CHILDHOOD NEUROBLASTOMA TUMORS

Fig. 4. In situ hybridization of 35S-UTP neuropeptide Y anti-sense cRNA to an adrenal gland of 18 weeks' gestational age. x 10 (A); x 100 (B).

gland. Fig. 4B is a higher-power view of one of the islets that expresses low levels of NPY mRNA. In contrast to the expression of NPY mRNA in the neuroblasts of these islets, NPY mRNA was not detected in neuroblasts outside these islets (data not shown), which are known to be scattered throughout the developing adrenal gland (21).

Evan's Stage IVS neuroblastoma is an intriguing form of neuroblastoma that typically regresses spontaneously (22). Because Stage IVS disease often occurs around the time of birth and almost all are diagnosed before 1 year of age, the malignant transformation of neuroblastomas in Stage IVS disease probably occurs prenatally. Since NPY is not expressed in the adrenal medulla between 18 weeks of gestation and 8 months after birth, the presence or absence of NPY expression in Stage IVS tumor cells may make the time during fetal adrenal development in which these tumors arise. Stage IVS neuroblastoma tumor tissue was therefore evaluated for NPY mRNA expression by in situ hybridization. For comparison, in situ hybridization analysis of Evan's Stage III and IV tumors was also performed.

Representative examples of this analysis are shown in Fig. 5, a-f. Tumors 1 and 2 are from patients with Stage IVS disease and tumor 3 is from a patient with Stage IV disease. Both tumor 1 (Fig. 5, a and b) and tumor 2 (Fig. 5, c and d) expressed NPY mRNA. Tumor 3 does not have detectable NPY mRNA expression. Cell-to-cell heterogeneity of NPY expression, as marked by variations in grain density, can be seen in tumors 1 and 2, and was seen in all tumors that expressed NPY.

A total of 15 Stage IVS tumors and 23 Stage III and IV tumors were analyzed for NPY expression. The characteristics of patients from whom these specimens were taken are shown in Table 1. The results of this analysis are shown in Table 2. All Stage IVS patients were less than 1 year of age at diagnosis. All (15 of 15, 100%) Stage IVS tumors expressed NPY, whereas 15 of 23 (65%) Stage III and IV tumors expressed NPY. There was a significant difference in the expression of NPY between these groups (P = 0.01). We then stratified these data according to age at diagnosis, since most patients with Stage IVS disease present by 12 months of age (22); 15 of 19 Stage III and IV tumors from children older than 1 year at diagnosis expressed NPY mRNA (P = 0.08, when compared with Stage IVS), whereas 0 of 4 Stage IV tumors from children younger than 1 year expressed NPY mRNA (P = 0.0003, when compared with Stage IVS). No tumor samples from patients with Stage III disease under 1 year of age were available for study. These data suggest that the critically distinct entities of Stage IV and Stage IVS tumor from children under 1 year of age at diagnosis may be biologically distinguishable on the basis of NPY mRNA.

Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>Stage IVS</th>
<th>Stage III and IV (all)</th>
<th>Stage IV (&lt;1 yr. of age)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients studied</td>
<td>15</td>
<td>23</td>
</tr>
<tr>
<td>Mean age</td>
<td>3.8 mo</td>
<td>3.5 yr</td>
</tr>
<tr>
<td>Age range</td>
<td>0–12 mo</td>
<td>4 mo–11 yr</td>
</tr>
</tbody>
</table>

Fig. 5. In situ hybridization of 35S-UTP neuropeptide Y anti-sense cRNA to human neuroblastoma tumor tissues. Three representative neuroblastoma tumors were hybridized with 35S-labeled neuropeptide Y anti-sense cRNA. Pairs of bright-field (a, c, e) and dark-field (b, d, and f) photomicrographs are shown for each tumor (× 10).
expression. However, the sample size of this study was very small, and this hypothesis should be examined in a larger group of patients.

The availability of neuroblastoma tumor cell lines provided the opportunity to examine NPY mRNA expression in neuroblastoma in vitro. We evaluated 11 neuroblastoma cell lines for the expression of NPY mRNA by Northern blot hybridization analysis (Fig. 6). Only neuroblastoma cell lines derived from patients with adrenal primary tumors and advanced-stage disease were analyzed. We detected the expected 0.7-kilobase NPY mRNA in 4 of 11 cell lines and in pheochromocytoma tumor tissue that we examined previously (14). Since we had previously found that adrenal ganglion cells expressed NPY and RA treatment of neuroblastoma cell lines induces morphological and genetic changes consistent with the neuronal differentiation of these cells (11, 23), we then examined the effect of RA on NPY expression in the neuroblastoma cell line SMS-KCN (Fig. 7). RA treatment of SMS-KCNR cells for 5 days resulted in a significant increase in the steady-state level of NPY mRNA (Fig. 7A). This increase persisted when cells were treated for up to 11 days. Examination of this Northern blot with pG21, a single copy gene in SMS-KCNR cells that we have shown previously is not regulated by RA (Fig. 7B), indicates that equal amounts of intact RNA were loaded in each lane.

**DISCUSSION**

The sequence of genetic and biochemical events that neural crest cells undergo during their differentiation to mature adrenal medullary cells is incompletely understood (21, 24–26). We have demonstrated here that the expression of NPY mRNA is developmentally regulated in the adrenal medulla, and that NPY mRNA expression is detectable in some (4 of 11) neuroblastoma cell lines derived from primary adrenal tumors. The observed heterogeneity of NPY expression in the cell lines may reflect the heterogeneity of NPY expression that we identified in neural crest-derived cells of the adrenal medulla. In addition, we have demonstrated that NPY mRNA expression can be regulated in vitro by RA-induced neuronal differentiation in the neuroblastoma cell line SMS-KCN. We have also found that most, but not all, neuroblastoma tumors express NPY mRNA, and in a subset of tumors from patients less than 1 year of age at diagnosis, NPY mRNA expression may help to distinguish Stage IVs from Stage IV patients.

NPY mRNA expression during development of the human adrenal medulla was evaluated in these studies by the technique of in situ hybridization. NPY mRNA expression was detected in a limited (1 of 250) proportion of adult adrenal medullary

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**Table 2** Comparison of neuropeptide Y mRNA expression in neuroblastoma tumors

<table>
<thead>
<tr>
<th>NPY mRNA expression</th>
<th>Stage IVs</th>
<th>Stage III and IV (all)</th>
<th>Stage IV (&lt;1 yr of age)</th>
<th>Stage III and IV (&lt;1 yr of age)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>0</td>
<td>8</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Positive</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>23</td>
<td>23</td>
<td>19</td>
</tr>
</tbody>
</table>

*By Fisher’s exact test.*

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Fig. 7. Northern blot hybridization analysis of neuropeptide Y mRNA expression in SMS-KCN neuroblastoma cell line treated with retinoic acid. A, the neuroblastoma cell line SMS-KCN was treated with solvent (KCNR C) or 5 µM RA for increasing periods of time up to 11 days as described in “Materials and Methods.” RNA was prepared from various time points, and 30 µg total RNA from each of these time points was analyzed for NPY mRNA expression by Northern blot analysis as described in “Materials and Methods.” B, the same blot was then stripped and reprobed with pG21 (19), to demonstrate equal loading of RNA in each lane.
cells, which were mostly ganglion cells (Fig. 1). We also found that, although NPY mRNA expression was highest in ganglion cells, a small fraction of chromaffin cells expressed NPY. These findings are similar to those reported in a study of rat adrenal glands that also utilized in situ hybridization (27). However, our findings and those of Schalling et al. (27), both done using in situ hybridization, contrast with the observations of Lundberg et al. (28), who found by immunohistochemical analysis that 20–30% of cells in the human adrenal medulla were NPY immunoreactive, and that these cells were exclusively chromaffin. Similar findings of cellular protein expression without detectable expression of corresponding mRNA have been reported in a study (29) characterizing the expression of a secreted pancreatic protein, pancreatic polypeptide, which is structurally related to NPY (30). In this study, the observed discrepancies between in situ hybridization and immunohistochemistry may have been due to the ability of in situ hybridization to detect solely steady-state levels of mRNA, whereas immunohistochemistry detects both protein synthesis and uptake/storage of exogenously made protein.

Solid tumors of childhood frequently contain morphological and biochemical characteristics of the tissues in which they arise (2), raising the possibility that such tumors may, like lymphoid malignancies (31, 32), express lineage-related genes in a pattern reflecting the developmental stage at which they underwent malignant transformation. Since NPY is a developmentally regulated gene, we sought to determine whether its expression in neuroblastoma tumors suggested a relationship between a subset of these tumors and cells that we could identify in the adrenal gland during the course of normal adrenal medullary development. Our data indicate that during normal development, NPY mRNA expression in the developing adrenal medulla is biphasic, i.e., NPY mRNA expression is detectable at 2 time periods during organogenesis of the adrenal medulla. It is expressed in invading neuroblasts of the fetal adrenal gland before 18 weeks of gestational age, and from 8 months after birth into adulthood. Our examination of 15 Stage IVS tumors revealed that all 15 tumors expressed NPY mRNA. Thus, Stage IV neuroblastoma, which most often appears around the time of birth, may arise from adrenal neuroblasts that express NPY in the first 18 weeks following conception. It is unlikely that Stage IVS tumors arise from adrenal medullary cells that occur in the medulla after 8 months of age and express NPY, since virtually all of the patients in this study (Table 1) were diagnosed before 8 months of age (mean, 3.8 months; median, 3 months).

Moreover, an analysis of Stage IV neuroblastoma tumors from patients under 1 year of age at diagnosis indicated that none, 0 or 4, expressed NPY mRNA. These findings suggest that such tumors may have arisen from a different population of non-NPY expressing neuroblasts that are rare in the first trimester, but predominant in the adrenal medulla between 18 weeks of gestation and 8 months after birth. Finally, 15 of 19 advanced-stage neuroblastoma tumors from patients older than 1 year of age at diagnosis expressed NPY mRNA, perhaps reflecting the fact that a fraction of postnatal adrenal medullary cells, which may give rise to these tumors, express NPY mRNA. The availability of additional markers expressed at very early or very late stages in adrenal medullary development will provide further insight into these possibilities.

An alternative explanation for the observation that some neuroblastoma tumors lack NPY mRNA expression could be that such tumors derive from neural crest precursors that previously expressed NPY but have lost their ability to do so. This explanation, though possible, is less likely in view of the fact that a majority of the tumors examined (30 of 38) in this study did express NPY mRNA, indicating that oncogenic transformation does not preclude NPY expression. Furthermore, there are numerous other examples in the literature of tumors that continue to synthesize tissue-specific products, for example, adrenocorticotropin hormone secretion of small-cell lung carcinoma (33), and immunoglobulin secretion in multiple myeloma (34).

Among the patients studied, all Stage IVS patients were alive without evidence of disease at 1 year after diagnosis, whereas none of the Stage IV patients under a year of age at diagnosis survived 1 year after diagnosis. This outcome differs somewhat from those of larger published studies (35, 36). Our small sample size may account for these differences and confounds the confident correlation of NPY expression with outcome. However, it is noteworthy in our study that all Stage IVS tumors examined expressed NPY mRNA, and none of the Stage IV tumors from patients under 1 year of age expressed NPY mRNA, suggesting that the expression of NPY mRNA in neuroblastoma tumors may be associated with a better prognosis among patients under 1 year of age at diagnosis. Furthermore, in a previous study we found that the expression of NPY mRNA in pheochromocytoma tumor is also associated with an improved prognosis (14). We found that 9 of 9 benign pheochromocytoma tumors, but only 4 of 11 malignant tumors (P = 0.0084), expressed NPY mRNA. These data suggested that benign and malignant pheochromocytomas may also arise from subpopulations of adrenal medullary cells that differ in their expression of NPY. In addition, other workers have demonstrated that neuropeptide was decreased in malignant pheochromocytomas and paragangliomas when examined for the expression of 11 different neuropeptides by immunohistochemistry (37). NPY was not included in this immunohistochemical study.

Recently, the NPY protein content of a series of human neuroblastoma tumors and cell lines has been analyzed (38, 39). Eight of 13 cell lines and 3 of 3 tumors had detectable NPY protein by radioimmunoassay. Three of the cell lines examined were also analyzed in our study. Although in general, the findings of the 2 studies complement one another, it is of interest that the cell line SMS-KAN made NPY mRNA but not detectable NPY protein. This discrepancy may be due to differential sensitivity of the techniques that were used, the inability of these cells to synthesize and secrete immunologically recognizable NPY, or an observed decline in protein production with increasing passage number (39) occurring perhaps at a posttranscriptional level. Furthermore, it has been suggested that NPY may be a growth-regulatory molecule (38) since several neuroblastoma cell lines are reported to express NPY receptors as well as biologically active NPY (40). Although activation of intracellular secondary messenger systems occurs when NPY is bound to its receptors in sympathetic nerves (41), the mitogenic potential of NPY for normal or tumor cells has not yet been examined.

Neuroblastic islets are found normally during fetal adrenal development. These islets increase in number and size with age until 17 to 20 weeks of gestation, and then decrease in number and size until they are no longer evident by birth (20). Our data indicate that all neuroblastoma tumors expressed NPY mRNA until 18 weeks of gestation (Fig. 4). Interestingly, all Stage IVS neuroblastoma tumors also express NPY mRNA (Table 2), and in most cases Stage IVS diseases undergoes what appears to be spontaneous regression as part of the natural history of this
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which support neural crest cell growth, or the loss of growth factors such as nerve growth factor receptors for these or other growth factors in the cells that express NPY. Future studies directed towards an understanding of the biological processes involved in the disappearance of these cells include the loss of growth factors such as nerve growth factor (42-44) and fibroblast growth factor (45, 46), which support neural crest cell growth, or the loss of growth factors such as nerve growth factor receptors in the brain. Science (Wash. DC), 237: 872-873, 1987.


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