Neural Expression of a Novel Alternatively Spliced and Polyadenylated Gsa Transcript*

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We have isolated an alternative transcript of the rat Gsa signal transduction protein gene, referred to as GsaN1. GsaN1 was isolated by differential hybridization screening of genes induced upon dexamethasone treatment of the neuronal-like CA77 rat thyroid C-cell line. The 1-kilobase GsaN1 transcript is generated by alternative splicing and polyadenylation of a novel terminal exon. This exon lies 800 base pairs downstream of exon 3 in the Gsa gene. Dexamethasone differentially induced GsaN1 severalfold relative to Gsa mRNA in the CA77 cells, similar to the bias seen with alternative processing of the calcitonin/calcitonin gene-related peptide transcript. In addition to the differential regulation by dexamethasone, the expression pattern of GsaN1 in rat tissues differed markedly from Gsa. GsaN1 mRNA was much more abundant in the brain, with intermediate levels in skeletal muscle and very low levels in other tissues. This was in contrast to the more ubiquitously expressed Gsa mRNA. Within the brain, GsaN1 was particularly abundant in discrete regions of the brainstem and hypothalamus that modulate autonomic functions. Examination of rat embryos demonstrated that Gsa is expressed in both brain and nonneural tissue at least 1 day before GsaN1 mRNA could be detected in the embryonic brain. Based on the regulated expression of the GsaN1 transcript and previous studies on Gsa proteins, the prirt is generated by alternative splicing of the GsaN1 protein may potentially modulate several heterotrimeric G protein functions in the nervous system.

The neural crest is a transitory structure during embryogenesis that gives rise to a wide variety of cell types in response to environmental cues, including thyroid C-cells and peripheral neurons (LeDouarin, 1982). While thyroid C-cells are normally calcitonin-producing endocrine cells, it has been shown that C-cells can acquire some neuronal features in primary cultures and as tumor cell lines (Barasch et al., 1987; Nishiyama and Fujii, 1992; Russo et al., 1992). We have demonstrated that the CA77 thyroid C-cell line has a neuronal phenotype and that the synthetic glucocorticoid dexamethasone can partly repress these properties to induce features more characteristic of the parental C-cells (Russo et al., 1992). The morphological changes are an increased cell roundness and neurite remodeling, seen as a partial retraction and thinning with an increased number of varicosities. Dexamethasone treatment also increases the number of dense core secretory vesicles and calcitonin peptide secretion, decreases cellular proliferation, and biases the alternative splicing pattern of the calcitonin/calcitonin gene-related peptide (CGRP) transcript to favor calcitonin mRNA. These studies suggest that there is a population of neural crest cells with neurogenic potential that undergo a late and reversible commitment to the C-cell phenotype. This hypothesis is consistent with the actions of glucocorticoids on the sympathoadrenal neural crest (Anderson, 1989) and suggests that common differentiation mechanisms may be shared between the sympathoadrenal and vagal crest lineages. Differential cDNA hybridization screening has been a powerful tool for identifying developmentally regulated genes, and several laboratories have successfully applied this technique to sympathoadrenal neural crest-derived cells and cell lines (Anderson and Axel, 1985; Helman et al., 1987; Leonard et al., 1987; Milbrandt, 1987). In this report we describe the use of a PCR-based plus/minus screening strategy with the CA77 cells to reveal a novel alternatively spliced and polyadenylated Gsa transcript. Gsa is a member of the heterotrimeric guanine nucleotide-binding protein family that was initially identified as a critical link in signal transduction from transmembrane receptors to intracellular targets (Gilman, 1987; Johnson et al., 1989; Birmbaum et al., 1990). In recent years there has been a striking increase in the diversity of G proteins and the number of cellular activities involving G proteins, including cellular differentiation (Simon et al., 1991; Helper and Gilman, 1992; Spiegel et al., 1992). In addition to multiple genes, there are now several examples of alternatively processed transcripts. In the case of Gsa, diversity has been shown to be generated by alternative splicing and promoter usage. The first examples were alternative inclusion/exclusion of exon 3 and use of an alternative acceptor at exon 4 to generate short and long isoforms (Kozasa et al., 1988). These isoforms appear to have very similar activities in stimulating adenylyl cyclase and calcium channels (Mattera et al., 1989). Both the short and long forms are expressed in a variety of tissues, although the relative expression differs among different tissues and brain regions (Mumby et al., 1986; Cooper et al., 1990; Granneman and Bannom, 1991) and during development (Rius et al., 1991). More recently, novel 5' exons that are defined by alternative promoter and splicing events have been shown to be linked.
to exon 2 and the remaining Gsa exons (Ishikawa et al., 1990; Swaroop et al., 1991). In addition, aberrant splicing events involving internal deletions at nonsense splice sites have been described in glial cell lines (Ali et al., 1992). The alternative GsaN1 transcript described in this report differs from previously described isoforms in its structure and unique tissue and developmental expression pattern.

**EXPERIMENTAL PROCEDURES**

**Differential Screening**—cDNAs probes for plus/minus hybridizations were synthesized from control (minus) and dexamethasone-treated (plus) cell RNA. Heat denatured poly(A)+ RNA (2 μg, 10 min 65°C) was incubated in 50 mM Tris-Cl, pH 8.3, 40 mM KCl, 7 mM MgCl₂, 1 mM dithiothreitol, 1 mM each of dCTP, dGTP, TTP, 5 μM dATP, 70 μCi [32P]dATP (3,000 Ci/mmol, Amersham Corp.), 1 μg oligo(dT)₁₅ primer, 20 units of RNasein (Promega), and 20 units of avian myeloblastosis virus reverse transcriptase (Promega) in 20 μl at 37°C for 60 min, followed by an additional 10 units of enzyme for 30 min. In some experiments the RNA was also denatured in MeHgOH, although this did not appear to affect the size or quantity of probe synthesized. The RNA was then base-hydrolyzed and the incorporation of radioactivity was measured on DE81 paper (Whatman). Unincorporated nucleotides were removed by two sequential ethanol precipitations.

The CA77 cDNA library was prepared from cells that had been treated with 0.5 μM dexamethasone for 6 days and constructed in Lambda4 phage vector using the Riboclonc cDNA Synthesis System following the manufacturer's recommendations (Promega). The packaged phage were plated at 2,000-4,000 plaques/150-mm plate on Escherichia coli LE392 cells. Duplicate plaque lifts on nitrocellulose filters were hybridized with the plus and minus cDNA probes at 10⁶ counts/minute/ml 50% formamide hybridization buffer (Sambrook et al., 1989) for 72 h at 42°C. About 3,000 out of 12,000 plaques showed a significant hybridization signal with either probe, and 56 of these plaques showed a differential plus/minus hybridization signal. These plaques were eluted from the agar plug and the inserts amplified by PCR using vector primers as previously described (Mutchler et al., 1992). The inserts were then labeled by random priming for sequences across the splice sites was determined on one strand, using the U3 and D2 primers with the Cyclist PCR sequencing reagents (Stratagene).

**Northern, Southern, and in Situ Hybridizations**—RNA isolation and Northern blot analysis were performed as previously described (Russo et al., 1992). Southern blots of genomic DNA (10 μg digested overnight with restriction enzymes) and PCR products were transferred under alkaline conditions to Zetabind filters (Cuno Inc.) and hybridized using the same conditions used for Northern blots. The probes were: a GsaN1-specific probe prepared as an EcoRI-XbaI fragment of pGEMdes25 cDNA containing only the 3′-untranslated region of GsaN1 (nucleotides 441-743); a Gsa common region probe prepared from a BamHI fragment of pBK315LT cDNA common to both GsaN1 and Gsa (nucleotides 1-200); a probe for genomic PCR blots prepared from a PCR product using the U3-D2 primers and pBK315LT cDNA (nucleotides 251-335). The Northern and Southern blots were exposed to autoradiographic film with intensifying screens at -70°C, otherwise indicated in the figure legends as room temperature exposures, which were used for better resolution or for very strong signals. For comparison, exposures with intensifying screens had comparable signals in one-fifth the time. The sizes were determined from ribosomal RNAs, an RNA ladder standard (Bethesda Research Laboratories), and comparison with the 1-kb calctatin mRNA. The signal intensities were measured with an Ultrascan XL densitometer (LKB, Bromma, Sweden). The *in situ* hybridizations were performed using paraformaldehyde-fixed and frozen adult rat tissue as previously described (Russo et al., 1988). The hybridized RNA probes were hybridized to the RNA isolated from the PC8 neural tissue and thionin counterstaining to confirm the localization of hybridization signals.

**RESULTS**

A cDNA library was prepared from dexamethasone-treated CA77 cells and screened with radiolabeled cDNA probes prepared from either control or dexamethasone-treated cells. To facilitate the analysis of cDNA clones showing differential hybridization, we used primers based on the vector sequences for direct PCR amplification of the phage inserts from the primary screen (Mutchler et al., 1992). The PCR products were then used as probes to establish relationships by cross-hybridization on Southern blots and to confirm differential expression on Northern blots, and subsequently for sequence determinations. Among the differentially expressed cDNAs were 19 CGRP, nine calcitonin, two arginosuccinate synthase, and 10 unknown cDNAs. The relative abundance of calcitonin and CGRP cDNAs and detection of arginosuccinate synthase were measuring validations of the plus/minus screening technique since calcitonin/CGRP transcripts are abundant (about
1% of the cDNA library) and known to be induced by dexamethasone (Russo et al., 1992; Tverberg and Russo, 1992), and argininosuccinate synthase has been reported to be induced by dexamethasone in the liver (Jackson et al., 1986). Eight of the unknown clones were related by cross-hybridization, and three representative cDNAs from this group (dex25, dex12, dex30) are indicated in Fig. 1.

The two larger cDNAs had identical sequences at the 5' ends with rat Gsa (Itoh et al., 1986), while the 3' ends were unique in the databanks. Additional clones were obtained by screening a second CA77 cDNA library and by reverse transcription and PCR amplification (RT-PCR) of CA77 mRNA (see “Experimental Procedures”) (Fig. 1). These cDNA clones were designated GsaN1 to indicate that the mRNA contains a point of divergence corresponds to the exon-intron splice site previously been reported. In those cases, splicing occurs from either exon 3 or two alternate acceptors at exon 4 to yield short and long isoforms (Kozasa et al., 1988).

To establish the genomic localization of the novel GsaN1 sequences, PCR amplification of rat genomic DNA was done using a Gsa primer corresponding to exon 3 of the human gene (U3) and a GsaN1-specific primer near the junction with the Gsa transcript (Fig. 1). These cDNA clones were identified as GsaN1 clones (P15, I-I, and A3), and by reverse transcription and PCR amplification of CA77 mRNA (3157') are shown. The PCR product was 1.3 kb, which is the size predicted by the additional cDNA sequences between the splice junction and polyadenylation primers. The identification of a single exon is consistent with genomic Southern blot hybridizations with the GsaN1-specific probe. Single bands of 4.0, 4.4, and 9.8 kb were detected with genomic DNA digested with HindIII, BglII, and BamHI restriction enzymes, respectively. These results demonstrate that the novel GsaN1 sequences are contributed by a single exon about 800 bp downstream of exon 3 of the rat Gsa gene.

Dexamethasone regulation of GsaN1 mRNA levels was shown by using a fragment of the 3'-untranslated region as a specific probe on Northern blots (Fig. 3A). A major band of 1 kb was observed. Longer exposures also revealed a fainter 2-kb species, which may correspond to a precursor, or possibly another form of GsaN1. Dexamethasone increased GsaN1 levels about 6-fold. To compare the relative levels of Gsa and GsaN1, we used a probe containing the 5'-coding region that was common to both Gsa and GsaN1 (Fig. 3B). The probe

Fig. 1. Sequence and schematic representation of GsaN1. A, the composite nucleotide GsaN1 cDNA sequence and predicted protein sequence are shown. The polyadenylation signal is underlined. B, the GsaN1 and Gsa cDNAs are represented to scale with the point of divergence indicated by an arrow. The filled regions to the left of the arrow are identical between Gsa and GsaN1, while the remaining coding (open and hatched boxes) and 3'-untranslated regions are not homologous. Representative GsaN1 clones identified by the differential screening (dex25, dex12, dex30), by screening a second library by partial DNA sequence of the intron across the splice sites revealed consensus sequences at the junction points for a splice donor and acceptor. Attempts to generate a PCR product using primers from Gsa exons 3 and 4 were unsuccessful, perhaps because of the predicted large intron size (about 5 kilobase pairs based on the human Gsa gene). As a control, the exon 3 and 4 primers could amplify the correct fragment from a Gsa cDNA clone (data not shown). To determine whether the GsaN1 sequences were contributed by a single exon or multiple exons, the PCR amplification was repeated using a GsaN1-specific primer located near the polyadenylation site (D2) (Fig. 2D). The product was 800 bp downstream of exon 3 of the rat Gsa gene.

The PCR product size predicts an approximately 800-bp intron between exon 3 and the N1 exon. Partial DNA sequence of the intron across the splice sites revealed consensus sequences at the junction points for a splice donor and acceptor. Attempts to generate a PCR product using primers from Gsa exons 3 and 4 were unsuccessful, perhaps because of the predicted large intron size (about 5 kilobase pairs based on the human Gsa gene). As a control, the exon 3 and 4 primers could amplify the correct fragment from a Gsa cDNA clone (data not shown). To determine whether the GsaN1 sequences were contributed by a single exon or multiple exons, the PCR amplification was repeated using a GsaN1-specific primer located near the polyadenylation site (D2) (Fig. 2D). The product was 1.3 kb, which is the size predicted by the additional cDNA sequences between the splice junction and polyadenylation primers. The identification of a single exon is consistent with genomic Southern blot hybridizations with the GsaN1-specific probe. Single bands of 4.0, 4.4, and 9.8 kb were detected with genomic DNA digested with HindIII, BglII, and BamHI restriction enzymes, respectively. These results demonstrate that the novel GsaN1 sequences are contributed by a single exon about 800 bp downstream of exon 3 of the rat Gsa gene.

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Fig. 2. Genomic localization of GsaN1. A, Southern blot of PCR products from rat genomic DNA amplified with the U3 and D2 primers (lane 1) and U3 and D3 primers (lane 2). As controls, parallel PCR reactions containing the same reaction mixtures as in lanes 1 and 2, except without genomic DNA, are shown in lanes 3 and 4, respectively. The filter was hybridized with a probe generated by PCR amplification of the GsaN1 cDNA using the U3 and D2 primers and exposed for 6 min at room temperature. The 1.3- and 0.9-kb products are indicated. B, schematic representation of the N1 exon with the Gsa gene and the observed genomic PCR products. The distance from exon N1 to exon 4 is not known. C, sequence across the splice junctions of Gsa exons 3 and N1. The sequence was determined on only one strand from the 900-bp U3-D2 PCR product. The canonical GT and AG nucleotides at the splice sites are underlined, and a potential branchpoint upstream of the polyadenylidne tract is indicated by an asterisk. D, Southern blot of rat genomic DNA digested with HindIII (lane 1), BglII (lane 2), and BamHI (lane 3). The filter was hybridized with the GsaN1-specific probe and exposed for 20 h. The size standards (kb) are indicated.
detected both the 2-kb Gsa and 1-kb GsaN1 mRNAs. GsaN1 mRNA levels were 18% of the Gsa mRNA levels in the control CA77 cells, and the relative levels increased to about 45% in the dexamethasone-treated cells (Fig. 3D). In contrast to a 6.1-fold induction of GsaN1 mRNA, dexamethasone increased Gsa expression only 2.4-fold. This observation is consistent with the fact that we did not obtain any Gsa cDNA clones in the differential screens, despite the relative abundance of Gsa to GsaN1. Consequently, dexamethasone caused a severalfold differential induction of GsaN1 relative to Gsa in the CA77 cells.

To gain some insight into the possible physiological significance of GsaN1, we asked whether it was expressed in normal rat tissues, and if so, whether the expression pattern paralleled Gsa mRNA expression. Gsa mRNA and protein is widely expressed in both neuronal and nonneuronal tissues (Mumby et al., 1986; Jones and Reed, 1987) (Fig. 4B). In contrast to Gsa mRNA, the 1-kb GsaN1 mRNA was found to be restricted primarily to the brain by Northern blots (Fig. 4A). Within the brain, GsaN1, as well as Gsa, mRNA is predominant in the hypothalamus. In addition to the brain expression, GsaN1 was found in skeletal muscle, and to a lesser extent in the thyroid and adrenal glands. The levels in skeletal muscle were estimated to be about 15% of the brain level. Interestingly, the major signal in adrenals and thyroids was a 2-kb GsaN1 species, the nature of which remains to be determined, but may correspond to the 2 kb band seen in CA77 cells and brain. Longer exposures of the Northern blots revealed low levels of GsaN1 mRNA in the liver, heart, lung, stomach, spleen, and kidney that were estimated to be about 100-fold lower than the brain expression (data not shown).

Low level expression of GsaN1 in the liver was also confirmed by RT-PCR reactions described below. Both the 1-kb GsaN1 and 2-kb Gsa mRNAs could be detected in brain regions by Northern blots using the common Gsa/GsaN1 probe (Fig. 4B). The GsaN1 mRNA levels in the brain, including the hypothalamus, were estimated to be approximately 10% of the Gsa mRNA levels. These results demonstrate that GsaN1 and Gsa mRNA levels are differentially expressed, with GsaN1 much more prevalent in the brain than in nonneuronal tissues.

To confirm the identification of GsaN1 mRNA in the tissues by Northern blots, we used RT-PCR with primers from the region in common with Gsa and the GsaN1-specific region (Fig. 5). For comparison, the primers were also used with CA77 cell RNA and the GsaN1 cDNA clone. The correct sized amplification product was identified by Southern blot hybridization from both brain and liver RNA. A much lower signal was seen from liver than from the same amount of brain RNA. Addition of 50-fold more liver RNA yielded a comparable signal to the brain RNA. While the RT-PCR was only semi-quantitative, the differential signals are consistent with the differential expression of GsaN1 shown by Northern blots. The detection of GsaN1 in liver by RT-PCR also confirms the presence of GsaN1 mRNA by the longer Northern blot exposures.

The expression in the brain was further investigated by in situ RNA hybridization to determine whether GsaN1 was expressed in discrete regions or uniformly throughout the brain (Fig. 6). The expression is clearly much greater in discrete regions of the brainstem and hypothalamus. To a first approximation, the pattern appears to overlap with the published localization of Gsa mRNA, which is also abundant in the hypothalamus and certain brainstem nuclei (Brann et
expression in localized regions, GsaN1 appears to be expressed throughout the brain since the brain sections showed a greater signal with the antisense probe than seen with a sense strand probe or with the antisense probe hybridized to liver sections (Fig. 6). This agrees with the Northern blots with different brain regions (Fig. 4).

We then asked when and where during rat development was GsaN1 expressed, and if it correlated with Gsa expression. Expression was monitored by Northern blots using GsaN1-specific and Gsa common region probes (Fig. 7). The 2-kb Gsa mRNA was detected at all embryonic stages at roughly equivalent levels in both the head region (mostly brain tissue, without the facial region) and the craniofacial region (mostly mesenchymal cells, cartilage, muscle, and bone). In contrast, GsaN1 was detected in the head regions, with little or no detectable expression in the craniofacial regions. As controls, adult rat brain and lung were included for comparison. The relative levels of GsaN1 and Gsa were consistent with those observed in the adult brains, with Gsa signals about 10-fold greater than GsaN1. Interestingly, GsaN1 expression was not clearly detected until day 14 embryos, while Gsa was detected as early as day 13 (Fig. 7). Differential expression of alternative Gsa transcripts has also been reported for the Gsa long and short isoforms during embryogenesis (Rius et al., 1991). These findings indicate that the GsaN1 alternative splicing event is developmentally regulated in both a temporal and spatial manner.

**DISCUSSION**

We have identified an alternative splice and polyadenylation event that yields a novel Gsa transcript, termed GsaN1. GsaN1 mRNA is generated by splicing of the third exon of Gsa to an internal terminal exon containing consensus splice acceptor and polyadenylation sites. The alternative splice

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**Fig. 5. Reverse transcription and PCR amplification of GsaN1 from tissue RNA.** A, schematic of the expected product from RT-PCR using the U2 (Gsa common region) and D1 (GsaN1 specific) primers. B, Southern blot of PCR products from 50 ng of pBKS3LT cDNA plasmid (lane 1), and RT-PCR products from 5 ng of CA77 RNA (lane 2), 10 ng of liver RNA (lane 3), 10 ng of brain RNA (lane 4), and 500 ng of liver RNA (lane 5). The filter was hybridized with the Gsa common region probe and exposed for 1 h at room temperature. The size standards (bp) are indicated.

**Fig. 6. In situ hybridization of GsaN1 mRNA in the brain.** A–G, coronal sections of adult rat brain were hybridized to an antisense 35S-labeled GsaN1 RNA probe. H, as a control, a brainstem section was hybridized with a sense strand probe. I, a liver section hybridized with the antisense probe is shown for comparison. All sections were exposed to film for 3 days. Abbreviations of nuclei are: SO, supraoptic; DM, dorsal medial hypothalamic; Arc, arcuate; PVN, paraventricular; SO, supraoptic; MPO, medial preoptic area; Sch, suprachiasmatic.

al., 1987; Largent et al., 1987). In the brainstem, there was a striking amount of GsaN1 mRNA in the locus coeruleus, the major source of noradrenergic neurons in the brain. GsaN1 was also detected in other noradrenergic neurons in the brainstem, as well as in the serotonin-containing raphe neurons. GsaN1 mRNA levels were also relatively high in selected hypothalamic nuclei, perhaps most noticeably in the paraventricular nucleus and other neuropeptide-secreting nuclei. One functional theme among these regions is that they express biogenic amines and neuropeptides that modulate and control the autonomic nervous system. In addition to the strong

**Fig. 7. Expression of GsaN1 mRNA in rat embryos.** A, Northern blot of head (H) (lanes 1, 2, 4, 6, and 8) and craniofacial (F) (lanes 3, 5, 7, and 9) RNA (1 µg of poly(A)+) from the indicated rat embryonic days was hybridized with the GsaN1-specific probe. The sample in lane 7 was overloaded based on ethidium bromide staining of the gel. For comparison, adult rat brain (lane 10) and lung (lane 11) (1 µg of poly(A)+) were included. The filter had previously been hybridized with the Gsa probe, then stripped and rehybridized with the GsaN1-specific probe, and exposed for 3 days. Residual signal from the 2-kb Gsa can be seen in some lanes. B, the same filter as in A was hybridized with the Gsa common region probe and exposed for 17 h. The positions of residual 18 S ribosomal RNA and GsaN1 and Gsa are indicated.
choice appears to be regulated based on the differential expression pattern of GsaN1 in rat tissues and embryos. In addition, the steady state levels of GsaN1 are differentially regulated by dexamethasone treatment of the CA77 cells. Dexamethasone induced GsaN1 about 6-fold relative to a 2-3-fold increase in Gsa mRNA. Gsa mRNA levels have previously been reported to be induced by glucocorticoids (Rodan and Rodan, 1986; Chang and Bourne, 1987; Saito et al., 1989). However, the mechanism underlying the bias in steady state mRNA levels is not known. There is an intriguing correlation with calcitonin/CGRP RNA processing, which has the same splice/polyadenylation pattern and dexamethasone-induced bias in the CA77 cells (Russo et al., 1992). In both cases, the proximal splice acceptor and polyadenylation sites of internal terminal exons (calcitonin exon 4 and Gsa exon N1) are preferentially used relative to distal acceptor and polyadenylation sites (CGRP exon 5 and Gsa exon 4). A model to account for this apparent coregulation is that dexamethasone treatment regulates a general factor(s) involved in terminal exon definition. While this model is clearly speculative, there is increasing evidence that splicing involves definition of exon sequences (Niwa et al., 1992) and that the levels of general splice factors can determine alternative splicing pathways (Ge and Manley, 1990; Kramer et al., 1990).

The expression pattern of GsaN1 mRNA was much more abundant in discrete regions of the brain than in nonneuronal tissue, which differs markedly from the ubiquitously expressed Gsa. Intermediate levels were observed in skeletal muscle and much lower levels could be detected in all tissues examined. Based on the preferential expression of GsaN1 mRNA in neurons, the predicted protein may be involved in a specialized neuronal function, such as signal transduction or regulated secretion. Since GsaN1 encodes only the amino-terminal region of Gsa, what might its activity be? Previous structure-function studies on Gsa and other Ga proteins have indicated that the amino-terminal region interacts with the βγ subunits, although the precise sequences are not yet clearly defined and βγ binding to this region remains to be directly established (Neer et al., 1988; Osawa et al., 1990; Journet et al., 1991; Denker et al., 1992). The effector- and receptor-binding domains and additional sequences needed for GTP binding are localized to the COOH-terminal region (see John-son et al., 1989; Birnbaumer et al., 1990). This suggests that GsaN1 itself will not be able to transmit a signal, but rather may act as a scavenger by sequestering free βγ subunits. While the Ga subunit is generally viewed as the signal transducer, Go and βγ subunit association is a dynamic interaction required for信号 transduction, and there is increasing evidence that βγ can also transmit information (Tang and Gilman, 1991; Federman et al., 1992; Helper and Gilman, 1992). One prediction is that GsaN1 would interfere with signal transduction by Go and/or free βγ subunits. In particular, GsaN1 sequestration of βγ subunits could repress signal transduction mediated by free βγ subunits, which might dampen “cross-talk” created by release of βγ subunits from different G protein-coupled receptors. This possibility is supported by recent studies in which transducin Go reduced activation of adenyl cyclase type II, presumably by acting as a βγ “scavenger” (Federman et al., 1992).

Should GsaN1 not bind βγ subunits, two alternative hypotheses are: (i) that GsaN1 is a nonproductive splicing product that yields a rapidly degraded or inactive protein, or (ii) that GsaN1 binds proteins other than βγ subunits. In the first alternative, the neural-specific expression pattern would suggest that production of GsaN1 mRNA may be a mechanism, albeit inefficient, that down-regulates Gsa gene expres-

sion in the brain. There is some precedence for regulated alternative splicing leading to a “dead-end” transcript, for example with c-Ha-ras (Cohen et al., 1989) and possibly the LH receptor (Wang et al., 1991). In this regard, the functional significances of the other known alternative Gsa transcripts remain to be fully determined. In the second alternative, GsaN1 might affect ion channel activity since G proteins have been shown to directly regulate Ca2+ and K+ channels (Birnbaumer et al., 1990). It is particularly intriguing that an amino-terminal peptide of Gsa present in the region encoded by GsaN1 has recently been reported to stimulate cardiac Na+ channel activity (Matsuda et al., 1992). In addition, as discussed below, calcium channels are affected by dexamethasone treatment of CA77 cells.

Finally, the question remains whether GsaN1 contributes to any of the phenotypic changes seen upon differentiation of CA77 cells. There is precedence for regulation and involvement of G proteins in other differentiation systems (Simon et al., 1991; Wang et al., 1992; Watkins et al., 1992). While the induction of GsaN1 upon repression of neuronal properties in CA77 cells appears to be contradictory to its neuronal expression pattern, there are several dexamethasone-induced activities in CA77 cells that are shown to be reduced by neurons and may potentially involve GsaN1, such as regulation of ion channels and secretion. In particular, we have recently found that chronic dexamethasone treatment reduces voltage-gated calcium currents. This decrease is apparently mediated by a posttranslational mechanism since the number of calcium channel proteins was not diminished as measured by anti- toxin and dihydropyridine binding. Since G proteins have been reported to regulate these types of calcium channels, one possibility is that GsaN1 may repress calcium currents. In addition, dexamethasone treatment causes an increased number of dense core secretory vesicles and neurite remodeling in the CA77 cells. Heterotrimeric G proteins have been implicated in vesicle trafficking (Barr et al., 1991; Aronin and DiFiglia, 1992; Colombo et al., 1992) and growth cone functions (Strittmatter et al., 1990). Future studies using specific antibodies should provide insight into the functional significance of GsaN1 in these and other cellular events.

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