The chromogranins comprise a class of acidic proteins that are secreted from large dense core vesicles and expressed in neuronal and endocrine tissues. We describe here the molecular characterization of NESP55 (neuroendocrine secretory protein of Mr 55,000), a novel member of the chromogranins. Several NESP55 cDNA clones were isolated from bovine chromaffin cell libraries. The cDNA sequence of NESP55 totals 1499 nucleotides. All of the clones that were isolated contained in their 3′-untranslated mRNA a sequence that was homologous to exon 2 of the G-protein Gs. The open reading frame encodes for an acidic and hydrophilic protein of 241 amino acids with a predicted molecular mass of 27,494 Da. An antiserum directed against the C terminus of NESP55 labeled a band of Mr 55,000 with an acidic pl ranging from 4.4 to 5.2 in one- and two-dimensional immunoblots of secretory proteins from chromaffin granules. NESP55 is localized within the cell to the large dense secretory vesicles and is expressed, apart from the adrenal medulla, in the anterior and posterior pituitary and various regions of the brain. For the physiological function, one interesting factor has emerged. NESP55 is proteolytically processed within the chromaffin granule to smaller peptides that might be physiologically active. One tetrapeptide, Leu-Ser-Ala-Leu (LSAL), present in the NESP55 sequence and flanked by arginine residues suitable for cleavage by prohormone convertases, has been identified by the NESP55 antibody as an endogenous antagonist of the serotonic 5-HT1B receptor subtype. Alterations in the serotonergic system are thought to play an important role in mental disorders, especially depression, and might be related to abnormal ethanol consumption. It is tempting to speculate that increased expression of NESP55 or its proteolytically derived peptide LSAL might contribute to the pathophysiology of the serotonergic transmission.

Synaptic transmission is mediated via exocytotic release of neurotransmitters from presynaptic terminals followed by binding of these substances to specific receptors located on the postsynaptic plasma membrane. In addition to classical neurotransmitters like glutamate, γ-aminobutyric acid, acetylcholine, noradrenaline, dopamine, or serotonin, several peptides are secreted. These peptides are stored together with neurotransmitters in specialized vesicular containers, i.e. large dense core vesicles. In recent years we characterized the content of large dense core vesicles to a great extent with the intention of identifying novel peptidergic substances involved in chemical signaling across neurons (1, 2). The majority of the proteins found in the content of large dense core vesicles have an acidic pl of 4–5 and were collectively named chromogranins (3). These proteins typically consist of 200–700 amino acids with glutamic acid as the most abundant individual amino acid. In their primary amino acid sequence, multiple pairs of consecutive basic amino acid residues known as cleavage sites for trypsin-like endoproteases are present. The family is composed of several members: the structurally related chromogranins A and B, secretogranins II and III, and vgf (1, 2, 4–6). The chromogranins are widely expressed in neuronal and endocrine tissues, which has made them very useful in identifying elements of the diffuse neuroendocrine system. Both intracellular (involvement in sorting of peptidergic components to the large dense core vesicles (5)) as well as extracellular functions (representing precursors of small biologically active neuropeptides like pancreastatin, vasostatin, or secretoneurin) have been proposed for chromogranins (1, 2, 4, 7).

We report here the molecular and cellular characterization of NESP55 (neuroendocrine secretory protein with a Mr of 55,000), a novel member of this class of proteins. NESP55 is expressed in the adrenal medulla, pituitary, and brain. It is an acidic protein that is processed within the secretory vesicles to smaller peptides. A tetrapeptide (present in the primary amino acid sequence flanked by Arg residues) suitable for endoproteolytic processing has been identified recently as an endogenous inhibitor of the serotonergic 5-HT1B receptor.

**EXPERIMENTAL PROCEDURES**

**cdNA Screening and Sequencing**—A agt11 expression library was constructed according to the manufacturer’s (Invitrogen) protocol using mRNA isolated from cultured bovine chromaffin cells with the guanidinium thiocyanate method. The library was screened with an antibody directed against secretogranin II. One positive clone was plaque-purified, subcloned into pGEM-SZ(+) and then sequenced into M13mp19 (Pharmacia Biotech, Uppsala, Sweden) with the dideoxy chain termination method. Based on the sequence, two oligonucleotides were synthesized. These oligonucleotides were 5′ end-labeled and used to screen two different Okayama-Berg libraries by colony hybridization. Both libraries were prepared from cultured bovine chromaffin cells and were a generous gift from Drs. Jeff Erickson, Lee Eiden, and Anna...
Molecular Cloning of a Novel Neuroendocrine Precursor

**FIG. 1. Schematic representation of NESP55 cDNA clones.** The 5′- and 3′-untranslated RNA is indicated by **solid lines.** The sequence in the 3′-untranslated mRNA corresponding to Gα is represented by a **broken line.** G2-G7 are the sequences corresponding to exons 2-7 of Gα. The open reading frame (ORF) is boxed. Four different species of clones (a, L311; b, 81, 82; c, 14, 51; and d, 31) were isolated from three independent libraries. Their sequence is identical in the 5′-untranslated mRNA and the ORF but differs in the 3′-untranslated RNA. The positions of two homologous partial clones found in the Expressed Sequence Tag database of GenBank™ are given below in relation to the NESP55 sequence.

---

Iacangelo (Laboratory of Cell Biology, National Institute of Mental Health). Thirty positive clones of each library, identified by autosequencing of filters, were rescreened twice. The five clones containing the largest inserts were sequenced with the Sequenase kit. Sequences were assembled and analyzed using the Genetics Computer Group software package of the University of Wisconsin, release 8.1. The signal peptide cleavage site was predicted by computer analysis (8).

**Antiserum Preparation and Radioimmunoassay**—The peptides GAIPRRH and GAIP were synthesized by standard Fmoc (N-(9-fluorenylmethoxycarbonyl) chemistry and purified by reversed phase high performance liquid chromatography. The peptide structure was verified by amino acid analysis and mass spectrometry. Both peptides were coupled via an additional N-terminal cysteine to maleimide-activated keyhole limpet hemocyanin (Pierce), and these conjugates were used to generate peptide antisera in chinchilla bastard rabbits (Ivano-vas, Küsslegg, Germany) with a standard immunization protocol (9). YGAIPRRH was iodinated by the chloramine-T oxidation method and purified by reversed phase high performance liquid chromatography. The antiserum was produced by Cappel (Downingtown, PA) using standard procedures.

**Isolation of Subcellular Fractions**—Bovine tissues were homogenized in 0.3 M sucrose, and subcellular organelles were isolated by differential centrifugation. A large granule fraction was centrifuged (130,000 × g for 90 min at 4 °C) over a sucrose gradient ranging from 1.3 to 2.0 M sucrose as described in detail (9). After centrifugation, different fractions were collected, diluted four times with 0.3 M sucrose (20%, v/v), and recentrifuged at 110,000 × g for 1 h at 4 °C. The pellets were resuspended in lysis buffer (5 mM Tris/sodium succinate, pH 5.9), and soluble proteins were analyzed by RIA and Western blotting.

**Immunoblotting**—Chromaffin granules were isolated over 1.8 M sucrose as described (9). Soluble proteins were isolated by hypotonic lysis of chromaffin granules (9). One-dimensional electrophoresis was performed on 12% polyacrylamide slab gels under reducing conditions (10). Molecular weight standards were obtained from Novex (San Diego, CA).

Two-dimensional electrophoresis was performed as described (11) with minor modifications. In all solutions Nonidet P-40 was replaced by Triton X-100 (Serva, Heidelberg, Germany). Mercaptoethanol was added to the sample buffer at a concentration of 0.33%. Proteins were dissolved in sample buffer (11) and applied to the focusing system at the anodic (acidic) end of the gels. Gels (ampholines, pH 3–10, Bio-Rad) were electrophoresed at 15 °C for 10 min at constant voltage of 100 V, then from 250 to 800 V at constant current, and finally at constant voltage of 800 V for another 3 h.

After electrophoresis, proteins were transferred electrophoretically to nitrocellulose sheets (Schleicher & Schuell) at 150 mA for 24 h as described (9, 12). After the transfer, blots were then incubated for 3 h with antiserum (1:200). After several washes, nitrocellulose sheets were treated with 125I-protein A (5 × 10^6 cpm/ml, DuPont NEN). Then the nitrocellulose sheets were washed to removed unbound 125I-protein A, blotted dry, and exposed to x-ray film (Cronex, DuPont) at −70 °C. Quantitation was done by a Fuji X Bas 1000 phosphorimager analyzer.

**RESULTS**

**Isolation and Characterization of cDNA Clones**—A λgt11 expression library prepared from cultured bovine chromaffin cells was screened with a polyclonal antiserum against secretogranin II. One immunoreactive clone (L311) was plaque-purified, subcloned into the pGEM-5Zf(+) vector, and then sequenced with the dideoxy chain termination method. It soon became clear that clone L311 was a false positive since it was different from other clones obtained by screening of an Okayama-Berg library with radioactively labeled degenerative oligonucleotides synthesized according to peptides obtained by CNBr cleavage of secretogranin II (13). In a study by Sigafos and colleagues (14) on peptides occurring in the secretory content of
chromaffin granules, five novel peptides were identified by Edman sequencing among several already known chromogranin-derived peptides. One of these putative novel peptides, GAIPIRRH, was encoded by clone L311. Thus, additional clones were isolated from two different Okayama-Berg libraries prepared from cultured bovine chromaffin cells by homologous hybridization with two oligonucleotides that were synthesized according to the sequence of clone L311. Sixty clones were isolated, and the five containing the longest inserts were further characterized. The overall structure of these clones is given in Fig. 1. All clones shared an identical sequence in the 5'-untranslated mRNA and the mRNA encoding for the open reading frame. In the 3'-untranslated mRNA, variations were found among the different clones. In all clones a sequence encoding for exon 2 of the G-protein Gsα was present (Fig. 1). Further downstream, clones L311, 81, and 82 shared a novel unique sequence, whereas in clones 14, 31, and 51 a sequence corresponding to exons 3–13 of Gsα was found. Further differences between clones resulted from deletions in the 3'-untranslated mRNA upstream or downstream of the sequence homologous to exon 2 of Gsα (see Fig. 1).

**Sequence Analysis and Protein Structure**—The nucleotide sequence of three clones (L311, 81, 51) was determined with the dideoxy chain termination method. The sequence (Fig. 2) totals 1499 nucleotides. An in-frame stop codon is located at position 178, and the first ATG codon is present 358 nucleotides downstream of the 5'-end. The open reading frame consists of 725 nucleotides and ends with a stop codon TAA at position 1081–1083. A search of the GenBank™ data base revealed no homology with other published sequences for nucleotides 1–1230 and 1303–1499. Nucleotides 1231–1302 located in the 3'-untranslated mRNA (Fig. 1) were 100% identical to the sequence of exon 2 of the bovine G-protein Gsα (15, 16). A search of the Expressed Sequence Tag division of GenBank™ revealed significant homologies with sequences W49084 and W16881. W49084, a 403-base pair mouse brain cDNA clone generated by the Washington University-HHMI mouse Expressed Sequence Tag project, is 79% identical to the nucleotide sequence of the mouse Gsα.

![Fig. 2. Nucleotide sequence and deduced amino acid composition of bovine NESP55. The translated sequence is given in the single letter code. The signal peptide represents the first 28 amino acids. Pairs of basic amino acids within the mature NESP55 are underlined. The two putative peptides (LSAL, GAIPIRRH) generated by endoproteolytic cleavage from NESP55 are indicated by bold letters.](image)

![Fig. 3. Schematic representation of bovine NESP55. The arrows indicate pairs of basic amino acids suitable for cleavage by kex-like prohormone convertases, and the numbers are their positions in the sequence. Two putative peptides generated proteolytically from NESP55, GAIPRRH and LSAL, which is a 5-HT1B receptor antagonist, are indicated. SP, signal peptide.](image)
24–519 and most likely comprises a partial mouse homologue of NESP55 (Fig. 1). W16881 comprises a 421-base pair human fetal lung mRNA sequence derived from the Washington University-Merck Expressed Sequence Tag project. Nucleotides 41–118 of this clone are 91% homologous to nucleotides 119–194 of the 5' untranslated mRNA of NESP55 (see Fig. 1 for a schematic representation). Further downstream (nucleotides 119–421), the sequence of this clone is identical to exons 2 and 4–6 of human \( G_{\alpha 1} \) (16).

The open reading frame encodes for a hydrophilic and acidic protein of 241 amino acids with a predicted molecular mass of 27,494 Da. The calculated pI of the protein is 4.8 and reflects the high abundance of acidic amino acids (15.8% Glu, 5.4% Asp). The first 28 amino acids have characteristic features of a signal peptide. Several sites in the primary amino acid sequence meet the criteria as acceptor sites of phosphorylation by casein kinase II (Ser-81, Thr-86, Ser-91, Ser-107, Thr-109, Ser-116, Ser-126), casein kinase C (Ser-49, Thr-156, Ser-183, Thr-221, Ser-231), and cAMP-dependent protein kinase (Thr-150, Thr-221), protein kinase C (Ser-49, Thr-156, Ser-183, Thr-221, Ser-231), and casein kinase II (Ser-81, Thr-86, Ser-91, Ser-107, Thr-109, Ser-116, Ser-126, Thr-119, Thr-123, Thr-131, Ser-154, Ser-177, Thr-221). The primary amino acid sequence does not contain a potential motif for N-glycosylation (Asn-X-Ser) but does contain five pairs of basic amino acids (Fig. 3) that could be used for endoproteolytic cleavage. Two peptides found in the sequence have been described previously, GAIPIRRH and LSAL.

The octapeptide GAIPIRRH (Fig. 3, 234–241, NESP55) has been purified by HPLC from extracts of chromaffin granules, and its sequence was established by the Edman degradation (14). This peptide is located at the C terminus of NESP55 and is preceded by a KR (Lys-Arg) cleavage site at its N terminus. The tetrapeptide LSAL (Fig. 3, 159–162, NESP55) has recently been isolated from cow and rat brain as an endogenous inhibitor of the 5-HT\(_{1B}\) receptor (17). This peptide is flanked at its N and C termini by an Arg residue suitable for endoproteolytic cleavage. Thus, NESP55 is a likely candidate for the precursor of this novel endogenous serotonergic receptor antagonist. The N-terminal amino acid sequence of bovine NESP55 is highly conserved (Fig. 4, 91% similarity) to the mouse brain homologue deduced from clone W49084.

Molecular Forms of NESP55 in Bovine Adrenal Medulla—To further characterize the NESP55 protein, antiserum directed against sequences corresponding to the C terminus of this protein were generated. In one- and two-dimensional blots of the soluble content of bovine chromaffin granules, an antiserum directed against a peptide (Fig. 5) and the shorter peptide GAIP (not shown) labeled one prominent broad band corresponding to a protein of \( M_\text{r} \) 55,000. In two-dimensional blots, a tilted band with an acidic pi between 4.4 and 5.2 was labeled (Fig. 5). The appearance of this band in Western blots resembles that of the chromogranin A proteoglycan but is different from that of N-glycosidically linked glycoproteins (dopamine \( \beta \)-hydroxydase and glycoproteins II and III), which is consistent with the lack of a N-glycosylation signal in the primary amino acid sequence. Thus, it is most likely that glycosaminoglycosylation together with the high amounts of acidic residues (21%) lead to the retardation of this protein on SDS-polyacrylamide gels that has already been established for the other chromogranins (1, 2).

Putative proteolytic processing to smaller peptides was investigated by separation of adrenal medullary extracts by gel filtration HPLC followed by analysis of the individual fractions by RIA. In the adrenal medulla, two main immunoreactive peaks were found (Fig. 6). One main peak corresponds to the uncleaved NESP55 eluting at a molecular weight of 55,000 (fractions 29–33), and a second peak (fraction 46) elutes in the position corresponding to the C-terminal octapeptide GAIPIRRH (Fig. 6).

Subcellular Distribution of NESP55—The subcellular distribution of NESP55 in the bovine adrenal medulla was investigated after separation of subcellular elements by differential centrifugation. In the large granule fraction, 74.5 ± 1.6% of the total immunoreactivity was present, comparable to that of the established secretory protein secretogranin II (64.8 ± 2.6%). The microsomal fraction contained only 5.8 ± 0.7% of the total immunoreactivity. The large granule fraction containing mainly chromaffin granules, lysosomes, and mitochondria was
further separated by equilibrium centrifugation on sucrose gradients ranging from 1.3 to 2.0 M. NEsp55 immunoreactivity as measured by RIA was found in fractions 3–5 of the gradient corresponding to chromaffin granules as shown by a distribution comparable to that of the established granule marker secretogranin II (Fig. 7). An identical distribution of NEsp55 in the gradient was found with immunoblots as with the RIA (not shown). A lysosomal enzyme marker (cathepsin D) peaked in fractions 6–8, whereas mitochondria and microsomes equilibrated in fraction 9 (not shown).

Tissue Distribution of NEsp55—Several bovine tissues were analyzed for the presence of NEsp55 with our specific RIA directed against the C terminus of this protein. High amounts of NEsp55 immunoreactivity were detected in the adrenal medulla (5029 ± 338 fmol/mg (w/w., ± S.E., n = 4), the anterior (204 ± 35 fmol), and posterior (69 ± 25 fmol) pituitary. In the bovine brain, NEsp55 immunoreactivity was detected in the hypothalamus (54 ± 7 fmol), hippocampus (27 ± 3 fmol), caudate nucleus (23 ± 0.6 fmol), thalamus (21 ± 3 fmol), and in significantly lower amounts in the cerebellum (5 ± 0.5 fmol).

**DISCUSSION**

Characterization of NEsp55—We describe here the molecular characterization of the cDNA encoding for NEsp55, a novel chromogranin-like protein. The primary amino acid sequence of NEsp55 was established by sequencing six independent clones obtained from three different libraries. NEsp55 comprises a 241-amino acid-long protein with a primary amino acid sequence unrelated to any other protein deposited previously in the GenBank™ data base. It resembles in several aspects, however, the class of proteins called chromogranins. (i) Like other chromogranins, i.e. chromogranins A and B and secretogranin II, NEsp55 is characterized by a high abundance of acidic amino acids (21%) in its primary amino acid sequence. (ii) NEsp55 is heat-stable (results not shown). (iii) It is localized to the soluble content of the large dense core vesicles of the adrenal medulla, the chromaffin granules. (iv) Like other chromogranins, NEsp55 is synthesized not only in the adrenal medulla, but also it is expressed in certain regions of the brain and other endocrine tissues (anterior and posterior pituitary). Preliminary immunohistochemical experiments demonstrate a strong reactivity in the raphe and locus coeruleus of the rat brain, but in general a much lower abundance of NEsp55 is found in the rat brain when compared with chromogranins A or B (not shown). (v) In the primary amino acid sequence, several pairs of basic amino acids are found that are used as cleavage sites by endopeptidases.

After translation, the protein is modified post-translationally as judged by the difference in molecular weight calculated from the primary sequence and that obtained from immunoblots on SDS-polyacrylamide gels. The continuous tilted pattern occurring in the two-dimensional gel is reminiscent of the proteoglycan of chromogranin A (18), indicating a putative glycosaminoglycan of NEsp55. Further work is required on this point. The N terminus of NEsp55 was deduced from the sequence assuming that the first in-frame methionine located 180 nucleotides downstream from the bordering stop codon is used. It is corroborated further by comparison with the partial mouse sequence obtained from the translation of clone W49084. The first in-frame methionine is located in this cDNA only 15 nucleotides downstream of the bordering stop codon, and the amino acid sequence starting at this methionine, but not preceding it, is highly conserved between both cow and mouse.

Functional Aspects of NEsp55—For the physiological function of NEsp55, at least one interesting factor has already emerged. In principle, both an intracellular function within the secretory vesicle or an extracellular one after secretion into circulation and the synaptic cleft seem conceivable. The amino acid sequence of NEsp55 contains several pairs of basic amino acids that can be used for endoproteolytic cleavage by the subtilisin-like prohormone convertases. In fact, our data demonstrate a significant amount of proteolytic processing of NEsp55 within the chromaffin granule. Thus, proteolytic processing of NEsp55 might yield smaller physiologically active peptides. GAIPIRRH, one of these putative peptides, is located at the C terminus of NEsp55. It was isolated in 1993 in a random search for novel peptides secreted from chromaffin granules (14) and has not yet been assigned with a specific function.

The tetrapeptide LSAL is located in the center of the NEsp55 sequence. This peptide has been identified as an endogenous peptidergic inhibitor of the serotonergic 1B receptor subtype in a recent study (17). LSAL is flanked on both sides by Arg residues; the C-terminal Arg is followed by another Arg in position P2’ (Arg-Leu-Arg) that is compatible with a type IV precursor protein cleavage site recognized by the subtilisin/kex-like proprotein convertases (19). Thus, NEsp55 is most likely the protein precursor of this novel serotonergic receptor antagonist. The 5-HT1B receptor is localized mainly presynaptically and controls serotonin release from serotonergic nerve terminals (20). 5-HT1B receptors are also found on non-serotonergic terminals where they inhibit the release of, for example, acetylcholine from the hippocampus or noradrenaline from peripheral tissues (20). Alterations in the serotonergic system are thought to play an important role in mental disorders, especially in depression (21, 22). Specific 5-HT1B agonistic...
drugs have been used clinically with aggressive psychiatric patients (23). 5-HT<sub>1B</sub> receptor knock-out mice drink twice as much ethanol compared with wild-type mice (24) and show an altered release of serotonin from in vitro slice preparations (25). The characterization of the precursor of the novel peptidergic 5-HT<sub>1B</sub> receptor antagonist LSAL now facilitates co-localization experiments with the 5-HT<sub>1B</sub> receptor in vivo. The molecular nature of the perturbations of the serotonergic transmission leading to mental disorders or increased ethanol consumption are as yet still unknown. It is tempting to speculate that an increased expression of NESP55 or its proteolytically derived peptide LSAL might contribute to the pathophysiology of the serotonergic transmission.

Presence of G<sub>a</sub> Transcripts in the 5'-Untranslated mRNA—In the 5'-untranslated mRNA of all of the NESP55 clones isolated, a sequence corresponding 100% to exon 2 of the bovine G<sub>a</sub> is present. Since our clones have been isolated from three independent libraries, a cloning artifact can be excluded. For G<sub>a</sub>, several different mRNAs exist. Originally, four forms of G<sub>a</sub> generated by alternative splicing of exons 3 and 4 were described (26, 27). In addition, mRNAs resulting from the use of alternative promoters and alternative first exons were reported (28–30). There is no in-frame AUG located between the AUG in exon 2 as described previously (29). The significance of alternative promoters and alternative first exons were described (26, 27). In addition, mRNAs resulting from the usage of common upstream promoter elements or whether they share a mutual function.

It is worth emphasizing that probes generated according to the sequence of exon 2 will not specifically hybridize to G<sub>a</sub> mRNA but will also detect NESP55 mRNA. Furthermore, probes corresponding to exons 3–13 might as well detect both messages due to the presence of these exons in cDNA clones NESP55 c and d (Fig. 1). Thus, the detection of G<sub>a</sub> protein by immunological methods is necessary to establish the expression of this G-protein in the various tissues.

Acknowledgment—We wish to thank Dr. R. Schneider for fruitful discussions.

REFERENCES

1. Winkler, H., and Fischer-Colbrie, R. (1992) Neuroscience 49, 497–528
2. Fischer-Colbrie, R., Laslop, A., and Kirchmair, R. (1995) Prog. Neurobiol. (Oxf) 46, 49–70
3. Blaschko, H., Comline, R. S., Schneider, F. H., Silver, M., and Smith, A. D. (1967) Nature 215, 58–59
4. Iacangelo, A. L., and Eiden, L. E. (1995) Regul. Pept. 58, 65–88
5. Huttner, W. B., Gerdes, H. H., and Rosa, P. (1991) Trends Biochem. Sci. 16, 27–30
6. Simon, J.-P., and Aunis, D. (1989) Biochem. J. 262, 1–13
7. Natori, S., and Huttner, W. B. (1994) Biochimie (Paris) 76, 277–282
8. Nielsen, H., Engelbrecht, J., Brunak, S., and von Heijne, G. (1997) Protein Eng. 10, 1–6
9. Fischer-Colbrie, R., and Frischenschlager, I. (1985) J. Neurochem. 44, 1854–1861
10. Laemmli, U. K. (1970) Nature 227, 680–685
11. O’Farrell, P. H. (1975) J. Biol. Chem. 250, 4007–4021
12. Burnette, W. N. (1981) Anal. Biochem. 112, 195–203
13. Fischer-Colbrie, R., Gutierrez, J., Hsu, C. M., Iacangelo, A., and Eiden, L. E. (1990) J. Biol. Chem. 265, 9208–9213
14. Sigafos, J., Chestnut, W. G., Merrill, B. M., Taylor, L. C. E., Diliberto, E. J., Jr., and Viveros, O. H. (1993) J. Anat. 183, 253–264
15. Robishaw, J. D., Russell, D. W., Harris, B. A., Smigel, M. D., and Gilm, A. G. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 1251–1255
16. Kozasa, T., Itoh, H., Tsukamoto, T., and Kaziro, Y. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 2081–2085
17. Rousselle, J. C., Massot, O., Delepiere, M., Zifa, E., Rousseau, B., and Filion, G. (1996) J. Biol. Chem. 271, 726–735
18. Falkensammer, G., Fischer-Colbrie, R., and Winkler, H. (1985) J. Neurochem. 45, 1475–1480
19. Seidah, N. G. (1995) In Intramolecular Chaperones and Protein Folding (Shinde, U., and Inouye, M., eds) pp. 181–203, R. G. Landes Company, Austin, TX
20. Zifa, E., and Filion, G. (1992) Pharmacol. Res. 44, 401–458
21. Schildkraut, J. J. (1965) Am. J. Psychiatry 122, 509–522
22. Coppen, A. (1965) Br. J. Psychiatry 113, 1237–1264
23. Ratey, J. J., and Chandler, H. K. (1995) CNS Drugs 4, 256–260
24. Crabbe, J. C., Phillips, T. J., Feller, D. J., Hen, R., Weng, C. D., Lessov, C. N., and Schildkraut, J. J. (1965) Am. J. Psychiatry 122, 509–522
25. Pişeyro, G., Castanon, N., Hen, R., and Blier, P. (1995) Neuroreport 7, 353–359
26. Mattera, R., Graziario, M. P., Yatani, A., Zhou, Z., Graf, R., Cordina, J., Birnbaumer, L., Gilman, A. G., and Brown, A. M. (1989) Science 243, 804–807
27. Bray, P., Carter, A., Simons, C., Guv, V., Puckett, C., Kamholz, J., Spiegel, A., and Nirenberg, M. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 8885–8897
28. Habeker, B. A., Martin, J. M., and Nathanson, N. M. (1993) J. Neurochem. 61, 712–717
29. Ishikawa, Y., Biazinchi, C., Nadal-Ginard, B., and Homcy, C. J. (1996) J. Biol. Chem. 261, 8458–8462
30. Swaroop, A., Agarwal, N., Gruen, J. R., Bick, D., and Weissman, S. M. (1991) Nucleic Acids Res. 19, 4725–4729