by k_{cat}/K_m. Thus, any elevation in this parameter increases steady-state aminoacylation, accordingly improving growth under conditions in which the acylation of $tRNA^{Ala}$ is likely to be growth-limiting.

Our results suggest that when cell growth is limited or prevented by a specific catalytic activity, it is possible to select mutant forms of a catalytic unit having a higher intrinsic activity. The relative ease with which mutants were obtained, with a single mutagen that is restricted to G-to-A and C-to-T transitions, suggests that many more enhanced activity mutants could be selected by saturating the system with other mutagens.

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Molecular Cloning of a Gene Sequence Regulated by Nerve Growth Factor

Abstract. *Nerve growth factor (NGF) is essential for the development and differentiation of sympathetic or sensory neurons. A complementary DNA was cloned that corresponds to a gene sequence induced more than 50-fold in a cultured target cell line of pheochromocytoma cells (PC12 cells) 5 hours after the addition of NGF. The induced messenger RNA encodes a 90,000-dalton polypeptide that may represent one of the primary events in NGF-induced differentiation of neurons.*

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Nerve growth factor (NGF) is required for the development and maintenance of sympathetic and sensory neurons both in vivo and in vitro (*1*, *2*). In addition, it induces the differentiation of immature (*3*) and neoplastic chromaffin cells towards a neuronal phenotype, as exemplified by the PC12 cell line derived from a rat pheochromocytoma (*4*). While numerous biochemical effects of NGF on PC12 cells have been described, it has been difficult to correlate any of these effects with the induction of the neuronal phenotype. Even though induction of the neuronal phenotype requires RNA synthesis (*5*), Garrels and Schubert (*6*) and McGuire and Greene (*7*) reported no qualitative change in the protein pattern during induction in two-dimensional gel analyses of more than 800 proteins in PC12 cells. These authors suggested that NGF produced only quantitative modulations of protein synthesis rather than a qualitative change in the pattern of gene expression. We have investigated NGF modulation of gene expression in PC12 cells by identifying messenger RNA's (mRNA's) induced within 24 hours after exposure to NGF, a period that is 48 to 72 hours prior to the appearance of major morphological changes.

A complementary DNA (cDNA) library of approximately 5000 clones was constructed as described in the legend to Fig. 1 with mRNA isolated from PC12 cultures that had been treated with 2.5*S* NGF (100 ng/ml) for 24 hours (*8*). Those cDNA clones that gave a stronger hybridization signal with total cDNA probe from induced as compared to uninduced cells were selected and rescreened two additional times by the same procedure. Six clones were judged to represent NGF-induced RNA. This group of

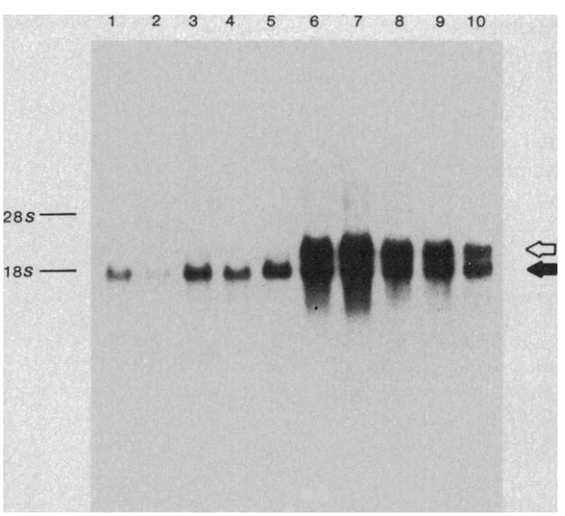

Fig. 1. Induction of the mRNA hybridizing to the VGF8a cDNA clone. Total RNA was prepared from cells treated as described (*16*). Approximately 10 μg of total RNA was subjected to electrophoresis in formaldehyde gels and blotted as described (*17*). The blot was hybridized with approximately 4×10^6 count/min per milliliter (10 ml) of the nick-translated VGF8a and rat actin cDNA clones. Equal amounts of plasmid DNA were mixed prior to labeling to obtain probes of comparable specific activity during nick translation. Conditions for the hybridization were as described (*18*). The cDNA library was prepared from 28 μg of oligodeoxythymidylate [oligo(dT)]–selected RNA (*19*) extracted from PC12 cells that had been treated with 2.5*S* NGF (100 ng/ml) for 24 hours. Hind III and Eco RI linkers were added to the ends of the double-stranded cDNA corresponding to the 3′ and 5′ ends of the RNA's, respectively, as described (*20*). *Escherichia coli* (DH1) were transformed with the recombinant DNA according to Hanahan (*21*). (Lane 1) RNA from FTRL5 cells; (lane 2) RNA from FTRL5 cells treated for 24 hours with 2.5*S* NGF (200 ng/ml); (lane 3) RNA from GH_3 cells; (lane 4) RNA from HTC cells; (lanes 5 to 10) RNA from PC12 cells treated with NGF (250 ng/ml) for 0, 3, 5, 9, 25, and 49 hours, respectively. The open arrow indicates the position of the RNA hybridizing with VGF8a cDNA clone. The solid arrow indicates the position of the actin RNA.

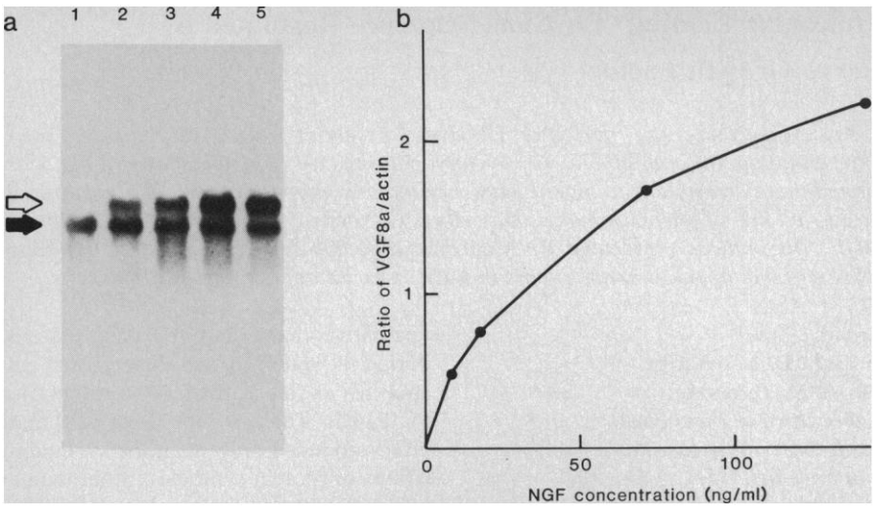

Fig. 2. The induction of VGF8a-related mRNA as a function of NGF concentration. (a) Total RNA was extracted from PC12 cells that had been treated for 6 hours with NGF. The RNA was subjected to electrophoresis in denaturing conditions and blotted on nitrocellulose paper as described in Fig. 1. (Lanes 1 to 5) RNA from cells treated with 2.5*S* NGF at 0, 8.7, 17.5, 70, and 140 ng/ml, respectively. (b) The ratio between the hybridization intensity (as measured by scanning densitometry) of the VGF8a-related RNA to the actin RNA as a function of the NGF concentration.

clones was then used to determine the level of induction of the corresponding mRNA sequence by Northern blot analysis with total RNA from control and NGF-treated PC12 cultures. One clone, VGF8a, which gave the strongest induction ratio, hybridized to an RNA of 2800 nucleotides (Fig. 1) and contained an Eco RI–Hind III insert of 2.2 kilobases. Primer extension studies with specific restriction fragments of the VGF8a insert indicated that the cDNA clone lacked approximately 650 nucleotides on the 5′ end, again suggesting that the full size cDNA clone would be approximately 2800 nucleotides. The same library was screened with a chicken β-actin cDNA probe (*9*) to obtain a rat β-actin cDNA clone for use as an internal reference in subsequent studies. Sequence analysis confirmed that the rat actin cDNA clone would encode the correct amino acid sequence.

The mRNA corresponding to the VGF8a cDNA clone, although not detectable in control cultures, was rapidly induced to the maximal level 5 hours after the addition of 2.5*S* NGF (250 ng/ml) to PC12 cultures (Fig. 1, lane 7). The higher concentration of NGF was chosen to obtain rapid binding to the NGF receptor. At the earliest time point investigated after induction (3 hours), the mRNA hybridizing with the VGF8a clone was expressed at levels comparable to that of actin (Fig. 1, lane 6). In addition, the VGF8a mRNA appeared to be tissue specific for it could not be detected in GH3 cells, a rat pituitary cell line (*10*) in HTC cells, a rat hepatoma cell line (*11*), or in FRTL5 cells, a rat thyroid cell line (*12*). However, all the RNA's from these cell lines were positive for actin mRNA (Fig. 1). The FRTL5 cell line is of interest since, unlike the GH3 and HTC lines, it has been shown to have NGF receptors (*13*). NGF treatment for 24 hours did not induce the VGF8a sequence in FRTL5 cells (Fig. 1, lane 2). Even though the actin band was weak in this particular RNA sample, overexposure did not reveal the induction of the VGF8a sequence in FRTL5 cells. In an initial survey of adult tissues, the VGF8a sequence could not be detected in rat liver or uterus RNA but was measurable in rat brain RNA.

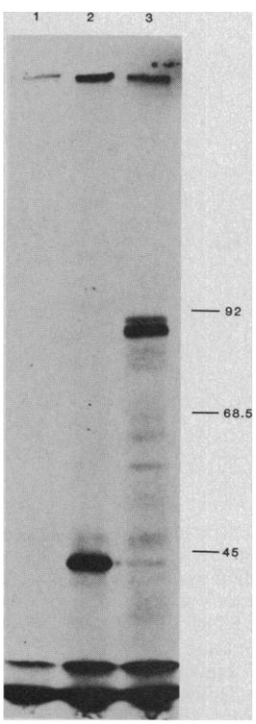

Fig. 3. In vitro translation of the mRNA hybrid selected by rat actin and VGF8a cDNA clones. VGF8a or actin plasmid DNA (20 μg) were linearized by digestion with Eco RI, denatured, and spotted on nitrocellulose paper as described (*22*). Oligo(dT)–selected RNA (25 μg) from PC12 cells that had been grown for 24 hours with NGF (100 ng/ml) were used for hybrid selection (*22*). The selected RNA was translated in vitro in a rabbit reticulocyte lysate system and the products examined by fluorography after sodium dodecyl sulfate–polyacrylamide gel electrophoresis (7.5 percent gel). (Lane 1) No RNA; (lane 2) RNA selected by the rat actin clone; (lane 3) RNA selected by the VGF8a clone. The molecular size markers are indicated in kilodaltons. The band just below the 92-kD marker in lane 3 is an endogenous translation product from the lysate.

The dose-response curve for the induction of the VGF8a sequence was determined by Northern blot analysis of total RNA extracted from PC12 cultures that had been grown in the presence of different concentrations of NGF for 6 hours. The blot was hybridized with the nick-translated VGF8a and actin cDNA clones and the ratio of the two signals was measured by scanning laser densitometry. The induction ratio was plotted as a function of NGF concentration (Fig. 2). A rough estimate of the NGF concentration necessary to give 50 percent maximum induction of the VGF8a sequence corresponded to 50 ng/ml, which is in good agreement with the reported value for the K_m of the receptor (*14*).

Hybrid selections were then made in an attempt to characterize the gene product encoded by the VGF8a-related mRNA. The in vitro translation products of the mRNA selected by the rat actin and VGF8a clones are shown (Fig. 3). The size of the polypeptide encoded by the VGF8a-selected mRNA was approximately 85,000 to 90,000 daltons, which is within the coding capacity of an mRNA of 2800 nucleotides.

The VGF8a cDNA clone described here corresponds to an mRNA whose level is increased at least 50-fold, as determined by dilution experiments, by 5 hours after the addition of NGF to PC12 cultures. Furthermore, the dose response for the induction is within the physiological range of NGF concentration necessary to bind to the receptor. We have not been able to detect VGF8a mRNA sequences in total RNA from a variety of nonneuronal rat cell lines, or in RNA from adult rat tissues, with the single exception of brain. Although more

work on the physiological role and tissue distribution of the VGF8a sequence is required, our results suggest that VGF8a is predominantly expressed in neuronal cells and tissues.

We cannot rule out a low level of expression of the VGF8a mRNA in untreated PC12 cells. Nevertheless, our data suggest a rapid activation of the VGF8a gene by NGF. Within 5 hours of the addition of NGF to PC12 cells, the VGF8a sequence was induced to maximal levels, comparable to those of actin mRNA, and this level of expression is only diminished two- to threefold in cultures treated with NGF for 2 weeks. On the basis of this amount of expression, the protein encoded by the VGF8a mRNA would be expected to represent a major product in NGF-treated cells. We have no explanation for the inability to detect such a protein in previous studies (*6, 7*). The polypeptide encoded by the VGF8a mRNA sequence may have an unusual isoelectric point, or the polypeptide may represent a precursor that is modified or processed such that the final products would not appear in the gel pattern.

Initial sequence analysis of the VGF8a clone does not indicate any significant sequence homology with sequences in the National Sequence Data Bank, and direct hybridization studies with oncogene probes have been negative. Hybridization of the VGF8a clone to Eco RI- and Hind III-digested genomic rat DNA indicates that this portion of the gene is in the single-copy range and is contained within fragments of less than 6 kilobases. It is conceivable that the VGF8a-related mRNA encodes one of the microtubule-associated proteins induced by NGF in PC12 cells, as described by Biocca *et al.* (*15*).

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Vasopressin-Stimulated Release of Atriopeptin: Endocrine Antagonists in Fluid Homeostasis

Abstract. *Administration of pharmacological doses of arginine-vasopressin, related peptides, and other pressor agents induced a profound release of atriopeptin immunoreactivity into the circulation. The stimulated release of atriopeptin apparently was related to increased arterial blood pressure. Neither the nonpressor vasopressin analog 1-deamino-D-Arg^8-vasopressin nor arginine-vasopressin in the presence of a specific pressor antagonist caused atriopeptin to be released into the circulation. Urine output was correlated with the level of atriopeptin released. Physiological levels of arginine-vasopressin suppress diuresis and produced vasoconstriction. Pharmacological levels of the hormone stimulated the cardiac endocrine system to release atriopeptin, which may cause diuresis and vasodilation to physiologically antagonize the effects of vasopressin.*

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Mammalian atrial myocytes contain peptides [atriopeptins (AP's)] that have potent natriuretic, diuretic, and vascular smooth muscle relaxant properties (*1*). Support for the hypothesis that AP's are involved in hormonal regulation of the electrolyte and extracellular fluid balance requires characterization both of the release of AP's from the heart and of their subsequent effects on target organs, including the kidneys and vasculature. Current efforts are focused on the direct demonstration of the endocrine nature of AP's. The release of low molecular weight AP's into the coronary venous circulation by isolated, perfused mammalian hearts (*2*) is facilitated by atrial stretching (*3*). Distention of the left atrium and expansion of intravascular volume in conscious dogs produce natriuresis and diuresis (*4*), possibly because of the release of AP's into the blood. Conversely, removal of the right atrial appendage decreases the natriuretic and diuretic response to acute hypervolemia in the rat (*5*). Muscarinic and adrenergic agonists as well as the intrinsic hormone arginine-vasopressin (AVP) have been reported to induce the release of AP's from isolated atria; however, appropriate tissue controls were lacking in this study (*6*). Recent radioimmunoassays indicate that rat plasma contains immunoreactive AP's (*7, 8*). Profound acute expansion of intravascular volume in rats results in the transient appearance in the circulation of an unidentified material that is immunologically related to AP's

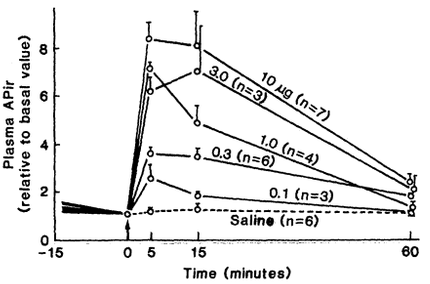

Fig. 1. Dose-dependent stimulation of AP release into the circulation by dAVP. Male Sprague-Dawley rats were anesthetized intraperitoneally (0.5 ml of 7 percent chloral hydrate per 100 g of body weight) and the jugular vein and carotid artery were cannulated (PE 50 tubing). After a 20-minute equilibration period, arterial blood samples were collected 15 minutes and immediately prior to, as well as 5, 15, and 60 minutes after, intravenous administration of various doses of dAVP. Blood samples were collected into one-tenth of a volume of 0.11*M* sodium citrate and centrifuged, and plasma was immediately frozen and stored at −70°C until being assayed for APir by radioimmunoassay (*8, 18*). The blood removed (0.3 ml) was replaced with an equivalent volume of saline. Values are means ± standard errors for *n* animals and are expressed relative to values at zero time. Mean basal plasma APir was 0.85 ± 0.07 ng/ml.