ATP, with multiple or multiphasic (*15*) kinetics (Michaelis constants, 3 and 67 n*M* and maximum velocities, 0.06 and 4.07 nmol per liter per hour, respectively).

We wondered whether 5′-nucleotidase could supply a substantial fraction of the P_i required for phytoplankton growth. In California coastal waters, a typical rate of P_i consumption was 1 to 2 nmol per liter per hour (Table 2). If we assume a total concentration of 5′-nucleotides of 10 nmol per liter and a hydrolysis rate of 10 percent per hour (Table 1), then the rate of P_i release was 1 nmol per liter per hour or 50 to 100 percent of the consumption rate. Although 5′-nucleotidase activity decreased in offshore samples (Table 1), P_i consumption also decreased (*10*); thus, 5′-nucleotidase remained a substantial source of regenerated P_i for primary productivity.

From 10 to 15 percent of the P_i resulting from 5′-nucleotidase activity did not mix with bulk-phase P_i but was taken up by the bacteria that produced it. The rate of uptake of this hydrolyzed P_i was 10 to 40 times greater than that of bulk-phase P_i (Table 2). At seawater concentrations of P_i (Table 2), this coupling between hydrolysis and uptake was loose, and the remaining 85 to 90 percent of the P_i hydrolyzed by 5′-nucleotidase was released into the environment. When P_i in seawater was depleted, however, coupling increased to 50 percent, and half the hydrolyzed P_i was taken up (Table 2).

Phosphorus controls primary productivity in many fresh and brackish waters (*3*). Picoplankton samples from fresh water (Lake Hodges, California) and brackish water (Northern Baltic Sea; salinity, 4 to 5 parts per thousand) hydrolyzed added ATP faster than marine picoplankton (*16*). Rates often exceeded 100 percent per hour, and initial competition data suggest 5′-nucleotidase to be responsible. From 10 to 50 μ*M* added P_i had little effect on hydrolysis but significantly inhibited uptake. Coupling between release and uptake of P_i was tight; often 100 percent of the released P_i was taken up.

Most of the ATP hydrolysis was due to the picoplankton fraction (probably bacteria), but most of the uptake of released P_i was in the microplankton fraction (probably phytoplankton). Apparently the phytoplankton rapidly took up the P_i released by the bacteria. 5′-Nucleotidase (unlike alkaline phosphatase) was not inhibited by P_i; it is therefore likely to be more widely distributed in aquatic ecosystems than alkaline phosphatase. Our results may necessitate a revision of the current concept of P_i regeneration in aquatic ecosystems so that the role of bacterial plankton may be incorporated.

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7. 5′-Nucleotidase activity in different aquatic environments was assayed by adding 10 p*M* to 13 n*M* [γ-^{32}P]ATP to water samples. Samples were incubated (0.3 to 6 hours) and filtered onto 0.2-μm Nuclepore filters to measure uptake of $^{32}P_i$ released from the substrate by 5′-nucleotidase (*A*). A portion of the filtrate was assayed for ^{32}P (*B*), and the remainder was acidified with 0.06*N* H_2SO_4, mixed with about 20 mg of activated charcoal, and filtered through a 0.45-μm Millipore filter. The ^{32}P in this second filtrate was the $^{32}P_i$ released from [γ-^{32}P]ATP by 5′-nucleotidase but not taken up by organisms (*C*). 5′-Nucleotidase activity was the sum of *A* and *C* divided by the total amount of label added (*A* + *B*). Blanks were boiled or zero-time samples. All measured enzyme activities and rates of uptake were the means of three replicate filtrations; values for the replicates almost always fell within 5 percent of the mean.
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Heterogeneity of 5*S* RNA in Fungal Ribosomes

Abstract. Neurospora crassa *has at least seven types of* 5S *RNA genes* (α, β, γ, ϵ, δ, ζ, *and* η) *with different coding regions. A high resolution gel electrophoresis system was developed to separate minor* 5S *RNA's from the major* 5S *RNA* (α). *A study of several* Neurospora crassa *strains, four other species in the genus* Neurospora, *members of two closely related genera, and three distantly related genera demonstrated that* 5S *RNA heterogeneity is common among fungi. In addition, different* 5S *RNA's are present in* Neurospora *ribosomes. The finding that fungal ribosomes are structurally heterogeneous suggests that ribosomes may be functionally heterogeneous as well.*

Eukaryotic cytoplasmic ribosomes contain four RNA molecules. The three largest ribosomal RNA's are made by posttranscriptional processing of a single high molecular weight transcript. The smallest ribosomal RNA, 5*S* RNA, is transcribed by a different polymerase and coded separately (*1*). *Neurospora crassa* has approximately 100 5*S* RNA genes, widely dispersed over at least six of its seven chromosomes (*2*, *3*). Of the 21 distinct 5*S* genes that we have characterized, 13 encode α 5*S* RNA, which constitutes about 70 to 80 percent of *Neurospora* 5*S* RNA. Four of the genes encode β 5*S* RNA, which differs in sequence from α 5*S* RNA in 15 out of 120 nucleotides. We have also identified several other presumptive 5*S* RNA genes (γ, δ, ζ, and η) which, if functional, would have the capacity to produce RNA's different from α or β by 12 to 34 nucleotides. The sequences flanking 5*S* genes are extremely heterogeneous, suggesting that the various 5*S* genes may be differentially regulated. Sequencing studies of total 5*S* RNA have shown that both α and β 5*S* RNA genes are expressed in vegetative hyphae, perithecia, and aerial conidia and that at least two other *Neurospora* 5*S* RNA's are made (*2*). We have developed a high resolution polyacrylamide gel electrophoresis system for 5*S* RNA to detect expression of variant 5*S* genes which may be present in the genome as single copies.

The 5*S* RNA from *Aspergillus nidulans* has been separated into two bands in a 15 percent polyacrylamide gel containing 4*M* urea (*4*). We have found that, under certain conditions, *Neurospora* 5*S* RNA can also be resolved into two or

more bands on such a gel and that the resolution of *Neurospora* 5*S* RNA can be improved by performing high voltage electrophoresis with thin ("sequencing") polyacrylamide gels containing lower concentrations of urea (Fig. 1A). At room temperature, optimal separation of *Neurospora* 5*S* RNA was achieved by electrophoresis (30 to 35 V/cm; 20 to 40 hours) in a 0.3-mm-thick, 15 percent polyacrylamide gel containing 2*M* urea. The resolution was dependent on temperature, as would be expected if the separation were due to minor differences in shape or flexibility of partially denatured molecules.

To determine whether the multiple bands observed in the partially denaturing gels represented different 5*S* RNA's or different conformations of identical molecules, we analyzed the RNA between nucleotides 2 and ~90 from the five bands of the 40-cm gel (Fig. 1A) using an enzymatic RNA sequencing method (*5*). The 5′ end-labeled RNA's were partially digested with ribonucleases T_1, U_2, Phy M, or *Bacillus cereus*. These enzymes cleave RNA after guanine (G), adenine (A), uracil (U) and A, and U and cytosine (C) respectively. The RNA digests were then fractionated by size on sequencing gels and autoradiographed. The ribonuclease T_1 and ribonuclease U_2 digestion products in one 30-nucleotide region are shown (Fig. 1B).

The different bands on the 2*M* urea gel (Fig. 1A, lane 4) represented different RNA species (Fig. 1B). The sequence of the RNA from the most prominent band (c) matched the α 5*S* gene sequence and RNA from the second strongest band (e) matched the β gene sequence (*2*). The RNA in band d was a mixture of at least two 5*S* RNA's, one of which matched the γ gene sequence except for a single nucleotide difference at position 17, while the other RNA matched the β sequence in the region examined. The G residue at position 8 is characteristic of γ 5*S* RNA (*2*). The sequence of RNA from band a matched the α gene sequence with the exception of an A to G substitution at position 23. Band b appeared to be a mixture of two α-like 5*S* RNA's. The only difference detected between the RNA in band b and the α sequence was an A to G transition at position 25. Minor variants of both α and β *Neurospora* 5*S* genes (designated α′ and β′ genes) have been found (*2*, *6*). Genes corresponding to the α′ 5*S* RNA's of bands a and b have not yet been isolated. The sequence differences for both of these α′ 5*S* RNA's are in a region of four consecutive A residues that are not base-paired in the proposed 5*S* RNA secondary structure (Fig. 2). Since we did not completely sequence these RNA's, it is possible that they have additional differences from the standard α sequence. However, we have found that 5*S* RNA transcribed in vitro from an α′ 5*S* gene variant with a single nucleotide difference relative to the standard α sequence (T instead of C at position 69) could be separated cleanly from α 5*S* RNA on a 2*M* urea gel (*7*). Thus, a single nucleotide substitution is sufficient to cause large mobility differences in this high resolution polyacrylamide gel electrophoresis system. We can routinely resolve *Neurospora* 5*S* RNA into 8 to 12 bands on a 90-cm 2*M* urea gel (Fig. 1A, lane 5). Partial sequence analysis of RNA from each of these bands indicated that most, and perhaps all of the bands represent distinct 5*S* RNA's (*8*). Thus, many different 5*S* RNA's are made in *Neurospora*. Nevertheless, we have not yet detected RNA corresponding to *Neurospora* δ, ζ, or η 5*S* genes (*2*, *3*).

It was possible that the variant 5*S* RNA's would not be incorporated into ribosomes. In eukaryotes, a substantial amount of 5*S* RNA is found in the nucleus, not associated with ribosomes (*9*). However, more than one type of 5*S* RNA can be used in ribosomes of *Xenopus* (*10*) and *Escherichia coli* (*11*). We analyzed the 5*S* RNA from *Neurospora* ribosomes to determine whether they contain heterogeneous 5*S* RNA, and if so, whether the various 5*S* species are present in the same proportions as found in total 5*S* RNA. The pattern of ribosomal 5*S* RNA bands (Fig. 3, lane 1) resembled the pattern of total 5*S* RNA bands (Fig. 3, lane 2), indicating that *Neurospora* ribosomes contain a variety of 5*S* RNA's and thus are structurally heterogeneous. Nevertheless, there were minor differences. For example, one of the bands (asterisk, Fig. 3) was much

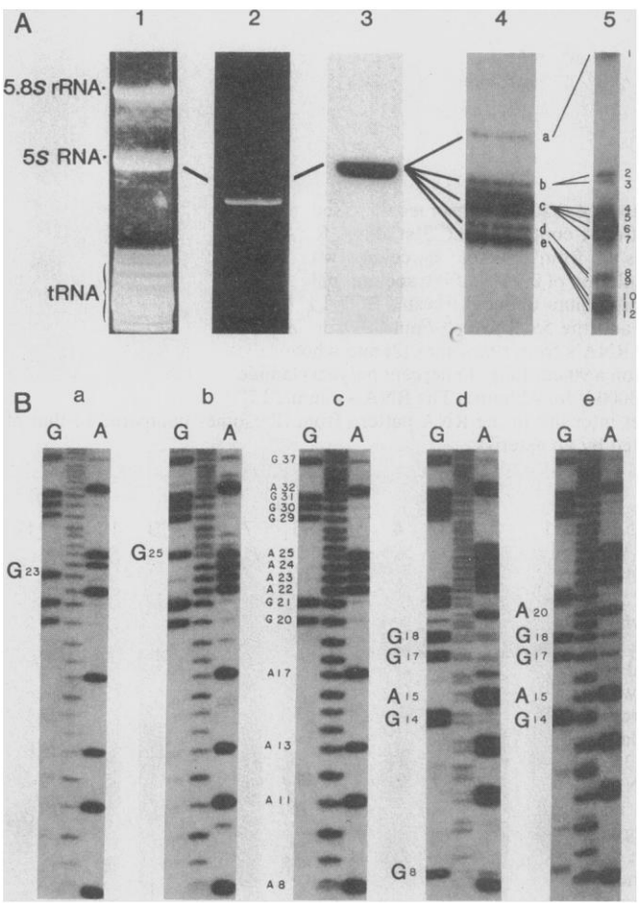

Fig. 1. Fractionation and analysis of *Neurospora* 5*S* RNA (A). Total soluble RNA, extracted from *Neurospora crassa* 74-0R8-1a (*2*), was resolved into 5.8*S* ribosomal RNA, 5*S* RNA and transfer RNA by electrophoresis through a 10 percent polyacrylamide (acrylamide:bisacryl) amide, 29:1) gel containing 7*M* urea (lane 1). The RNA's were visualized by staining with ethidium bromide and the 5*S* RNA was eluted and fractionated on a nondenaturing 10 percent polyacrylamide gel (lane 2). RNA was then 5′ end-labeled with T_4 polynucleotide kinase and [γ-^{32}P]ATP and separated from degradation products on an 8 percent polyacrylamide gel containing 7*M* urea and 40 cm in length (lane 3). Pure 5*S* RNA was finally obtained by fractionation on a 0.3-mm-thick, 40-cm (lane 4) or 90-cm (lane 5) 15 percent polyacrylamide (29:1) gel containing 2*M* urea, prepared in 50 m*M* tris-borate (*p*H 8.3), 1 m*M* EDTA. Electrophoresis of RNA's was at 21°C (gel temperature about 28°C) at approximately 1100 V for 24 hours (40-cm gels), or 3000 V for 42 hours (90-cm gels). The separated RNA's were eluted and sequenced by size-fractionation of end-labeled oligonucleotides generated by partial digestion with base-specific ribonucleases (*5*). (B) Autoradiograms showing the position of G and A residues in a 30-nucleotide-long region of the 5*S* RNA's from the five bands (a to e) observed in lane 4 of Fig. 1A. The reference ladders between the A and G lanes were generated by hydrolysis for 10 minutes at 90°C in formamide containing 0.4 m*M* $MgCl_2$ (*21*). G and A nucleotide designations for α 5*S* RNA are indicated in panel c and G and A differences from α are indicated in the other panels.

less prominent in the ribosomal 5S RNA than in total 5S RNA. It contained an RNA species that was initiated two nucleotides upstream of the usual initiation site (*8*). Possibly this RNA is rejected during ribosome assembly. Anomalous initiation of synthesis at position −2 has been observed in vitro (*12*).

To determine if 5S RNA heterogeneity was peculiar to one strain of *Neurospora crassa*, we examined 5S RNA from three other strains of *N. crassa*, four other species of *Neurospora* (*N. discreta, N. intermedia, N. tetrasperma*, and *N. sitophila*), and members of the related genera *Sordaria* (*S. brevicollis*) and *Gelasinospora* (*G. tetrasperma*). In addition we analyzed 5S RNA from the more distantly related ascomycetes, *Schizosaccharomyces pombe, Aspergillus nidulans, Cochliobolus heterostrophus*, and *Saccharomyces cerevisiae*. In every case, evidence of 5S RNA heterogeneity was observed (Fig. 4). The pattern of 5S RNA's from the *N. crassa* strains, the other *Neurospora* species, and *G. tetrasperma* were similar or identical. The α RNA was invariably the most abundant species and β RNA was second in abundance. Variations among minor bands were seen, however. For example, *G. tetrasperma* had a β-like 5S RNA not seen in the other strains (*8*). The *S. brevicollis* had the same α 5S RNA as *G. tetrasperma* and the *Neurospora* species, but lacked a β 5S RNA. Nevertheless, sequence analysis of total 5S RNA from *Sordaria* showed that this organism does have at least one minor 5S RNA (*8*). The 5S RNA from the maize pathogen, *C. heterostrophus*, produced the most complex pattern of prominent bands. *Aspergillus* had two major bands plus two minor bands, which agrees with the results of previous investigators (*4*). The *S. pombe* 5S RNA was resolved into three major bands and several minor bands. The *S. cerevisiae* 5S RNA could be separated into one major and one or more minor bands, consistent with results showing the existence of a minor species of 5S RNA in this yeast (*13*). Heterogeneity in 5S RNA has been reported for a wide range of organisms including bacteria (*11*), fungi (*14*), echinoderms (*15*), arthropods (*16*), amphibia (*10, 17*), birds, and mammals (*9*). However, only in *E. coli, Xenopus*, and *Neurospora* is it known that heterogeneous 5S RNA exists in ribosomes.

Although the different 5S RNA's may be functionally equivalent, it seems just as likely that ribosomes with different RNA's are functionally different. There is a wide range of initiation rates for different messenger RNA's, and structural changes of a messenger RNA, such as in folding or capping of the 5′ end, may affect its efficiency of translation or its stability (*18, 19*). Translation can be controlled by the availability of charged tRNA or by phosphorylation of initiation factors (*20*). If ribosomes containing different 5S RNA's have different translational properties, this would be another means of regulating gene expression, and possibly protecting translation from fluctuations in the environment.

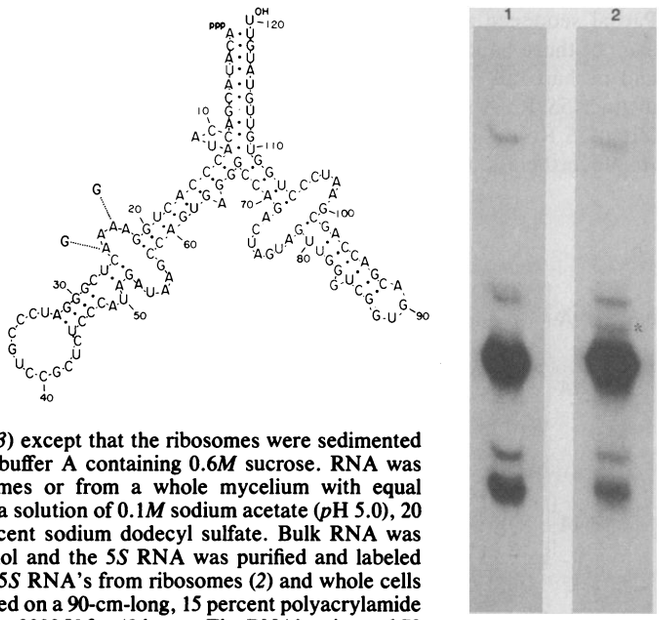

Fig. 2 (left). Predicted secondary structure of *Neurospora* α 5S RNA (*2*) showing position of differences in two α′ 5S RNA's. Fig. 3 (right). Heterogeneity of 5S RNA from *Neurospora* ribosomes. *N. crassa* 74-0R8-1a was grown from conidia at 30°C for 15 hours in Vogel's medium (*22*) containing 2 percent sucrose. Crude ribosomes were isolated by differential centrifugation as described (*23*) except that the ribosomes were sedimented (300,000*g*, 3 hours) in buffer A containing 0.6*M* sucrose. RNA was extracted from ribosomes or from a whole mycelium with equal volumes of phenol and a solution of 0.1*M* sodium acetate (*p*H 5.0), 20 m*M* EDTA, and 1 percent sodium dodecyl sulfate. Bulk RNA was precipitated with ethanol and the 5S RNA was purified and labeled (legend to Fig. 1). The 5S RNA's from ribosomes (*2*) and whole cells (*3*) were then fractionated on a 90-cm-long, 15 percent polyacrylamide gel containing 2*M* urea at 3000 V for 42 hours. The RNA's migrated 70 to 80 cm. A band of lesser intensity in the RNA pattern from ribosomes compared to that of whole cell RNA is indicated by an asterisk.

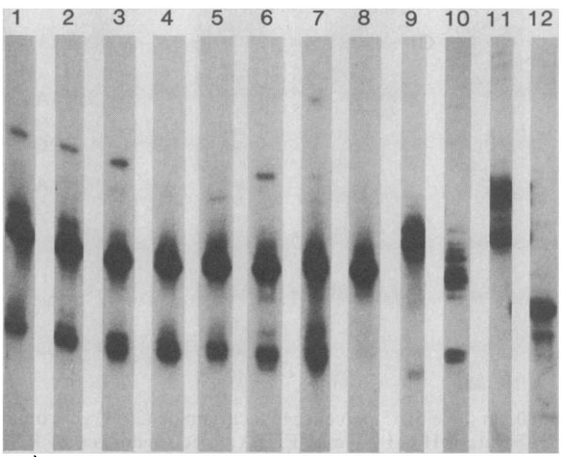

Fig. 4. Heterogeneity of 5S RNA among fungi. RNA was extracted directly from whole cells of all fungi except *Sordaria* and *Cochliobolus*, which were homogenized in the extraction solution (legend to Fig. 1). The 5S RNA's were resolved on a 90-cm 15 percent polyacrylamide gel containing 2*M* urea and subjected to 3300 V for 40 hours. The 5S RNA's from *Saccharomyces* and *Schizosaccharomyces* migrated faster than those from the other strains and were therefore loaded on the gel 12 hours later than the rest of the samples. (Lane 1) *Neurospora crassa* 74-0R8-1a (FGSC 988); (lane 2) *N. crassa* Beadle and Tatum 25a (FGSC 353); (lane 3) *N. tetrasperma* Honduras UFC-200 A/a (FGSC 850); (lane 4) *N. sitophila* Panama UP203 (FGSC 1134); (lane 5) *N. discreta* Kirbyville-6A (FGSC 3228); (lane 6) *N. intermedia* type tester strain P17a (FGSC 1767); (lane 7) *Gelasinospora tetrasperma* Dowding Standard type (FGSC 966); (lane 8) *Sordaria brevicollis* wild-type a (FGSC 1904); (lane 9) *Aspergillus nidulans* (derived from Glasgow wild-type); (lane 10) *Cochliobolus heterostrophus* c2; (lane 11) *Schizosaccharomyces pombe* N0738 h–; (lane 12) *Saccharomyces cerevisiae* (derived from S288C).

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Interaction of Calcitonin and Calcitonin Gene–Related Peptide at Receptor Sites in Target Tissues

Abstract. *Discrete receptor sites for calcitonin (CT) and calcitonin gene–related peptide (CGRP) were found in the nervous system and in peripheral tissues. Each peptide was capable of cross-reacting with the specific receptor of the other. In contrast to CT receptors, CGRP receptors were not linked to adenylate cyclase. However, CGRP could stimulate adenylate cyclase in CT target tissues apparently by interacting with CT receptors. The relative abilities of CGRP and mammalian CT to inhibit CT binding suggest that CGRP could serve as an endogenous ligand for CT receptors in the central nervous system.*

Distinct messenger RNA's encode the hormone calcitonin (CT) in the C cells of the rat thyroid, and a novel peptide, calcitonin gene–related peptide (CGRP) within the rat nervous system (*1*). Based on complementary DNA (cDNA) sequence analysis, CGRP was predicted to be a peptide (37 amino acids in length) with a 2,7-disulfide bridge and a phenylalanine amide at the carboxyl terminus (*1*). RNA blotting techniques and immunochemical studies have revealed the production of CGRP messenger RNA (mRNA) and CGRP in multiple locations within the nervous system (*1*). Calcitonin is a peptide (32 amino acids in length) with a 1,7-disulfide bridge and a proline amide at the carboxyl terminus. Although immunochemical studies have suggested that this peptide occurs within the nervous system (*2*), CT mRNA has not been revealed in neural tissue by hybridization with specific calcitonin cDNA probes (*1*). Despite the similar size of calcitonin and CGRP, and the presence in both peptides of a disulfide ring within the amino terminal region and an amide group at the carboxyl terminus, little sequence homology exists; furthermore, specific antisera to the CGRP sequence fail to recognize synthetic calcitonin (*1*). Specific binding sites have been demonstrated for CT in kidney, bone, other peripheral tissues (*3*), and the central nervous system (*4*); binding of CT to its receptor is associated with stimulation of adenylate cyclase. We now report on the distribution of receptor sites for CGRP, relationship of these receptors to the adenylate cyclase system, and the interaction of CGRP and CT at their respective sites in target tissues.

Male Sprague-Dawley rats (150 to 200 g), were decapitated and the brains, cervical and thoracic spinal cords, pituitary glands, adrenal glands, and kidneys were removed. The brains were frozen on dry ice, and discrete regions were macroscopically dissected. Pituitary glands, adrenal glands, and fractions of brain tissue were homogenized and prepared for analysis as described (*4*). Portions of each tissue containing 1 to 5 mg of protein were immediately frozen in dry ice and stored at −70°C. Purified renal membranes were prepared from dissected kidneys by differential centrifugation and ultracentrifugation (*5*), and skeletal tissue was prepared from the calvariae of 20-day-old Sprague-Dawley rat fetuses (*3*).

The salmon (s) CT and rat (r) CGRP (*6*) were each labeled with [^{125}I]NaI by the chloramine-T method (*7*) to a specific activity of 500 Ci/mmol, and purified (*4*). For binding studies, ^{125}I-labeled peptide (70×10^3 count/min) was incubated with or without unlabeled peptide in 0.5 ml of a buffer solution of 0.05*M* tris-HCl (*p*H7.5) and bovine serum albumin (20 g/liter) containing 200 μg of tissue protein (*8*). At the end of the incubation period, triplicate portions (100 μl) were each layered onto 200 μl of chilled assay buffer in a microcentrifuge tube kept at 4°C. Bound ^{125}I-labeled peptide was obtained after centrifugation (*4*), and the radioactivity was counted in a Packard Tricarb γ-spectrometer (Packard Corporation) at an efficiency of 50 percent.

For measurement of adenylate cyclase activity, the incubation mixture contained, in a 100-μl volume, 25 m*M* tris-HCl (*p*H 7.4), 0.1 m*M* adenosine triphosphate (ATP), [γ-^{32}P]ATP (0.8 to 2×10^6 count/min), 9 m*M* theophylline, 2 m*M* $MgCl_2$, bovine serum albumin (1 mg/ml),

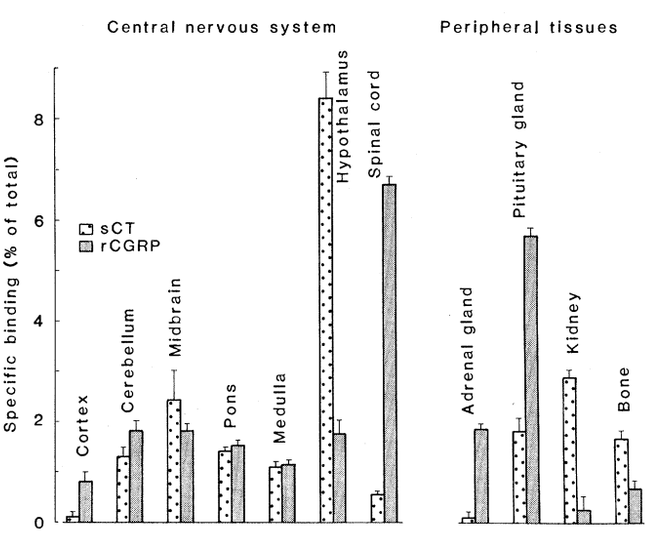

Fig. 1. Distribution of CT receptors and of CGRP receptors in the nervous system and in peripheral tissues of the rat. For assessment of CT receptors ^{125}I-labeled sCT was incubated with individual tissues in the absence or presence of excess unlabeled sCT (10^{-5}*M*). Specific binding represents the numerical difference between binding of the radioactive tracer in the absence of unlabeled sCT and binding not inhibited by excess sCT. Results are expressed as a percentage of added ^{125}I-labeled sCT specifically bound. For assessment of CGRP receptors similar studies were performed with ^{125}I-labeled rCGRP and excess unlabeled rCGRP. Each bar represents the mean ± standard error of triplicate determinations.