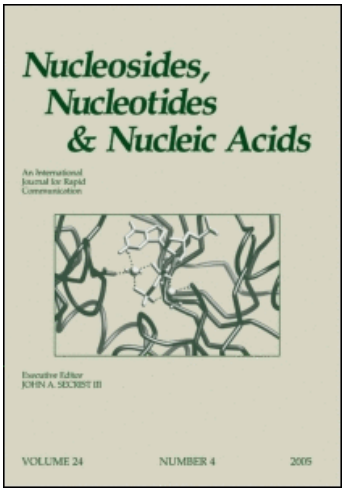

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Detection of Neuropeptide mRNA in Rat Brain by*In Situ*Hybridization with Radiolabeled Synthetic Oligonucleotide Probes

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DETECTION OF NEUROPEPTIDE mRNA IN RAT BRAIN BY IN SITU HYBRIDIZATION WITH RADIOLABELED SYNTHETIC OLIGONUCLEOTIDE PROBES

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SUMMARY: Radiolabeled synthetic oligonucleotide probes were used for detection of somatostatin and vasopressin mRNA in rat brain by in situ hybridization.

Recent advances in DNA synthesis, including fully automated synthesis, have made synthetic oligonucleotides more generally available. Thus, with the determination of the nucleic acid sequences for rat somatostatin (SS) and vasopressin (VP) mRNA, in situ localization of these mRNAs in the central nervous system (CNS) with specific oligonucleotide probes has become feasible. A 46-nucleotide probe complementary to the 3' coding end of SS mRNA was constructed by enzymatic ligation of two shorter sequences; one was 5' end-labeled with ^{32}P using T4 polynucleotide kinase and γ-^{32}P-ATP. A 48-nucleotide probe, complementary to part of the unique glycoprotein region of VP mRNA, was similarly constructed. The single-stranded ^{32}P-labeled probes ($\sim 10^8$ cpm/μg) were isolated by polyacrylamide gel electrophoresis under denaturing conditions.

For in situ hybridization, tissue blocks containing the hypothalamus from formalin-fixed, cryoprotected rat brains were serially sectioned in a transverse plane, from the level of the preoptic area to the level of the paraventricular nucleus. Every other section was slide mounted, deproteinated, and delipidated for hybridization with the radiolabeled probes. Alternate sections were processed for peptide immunoreactivity to anti-somatostatin or anti-vasopressin with the avidin-biotin method.

The results demonstrate that the periventricular portions of the preoptic and paraventricular hypothalamus are particularly rich in both SS-immunoreactive neurons and SS mRNA, while VP-immunoreactive neurons and VP mRNA are concentrated within the supraoptic and suprachiasmatic nuclei and the magnocellular portion of the paraventricular nucleus. More importantly, the results demonstrate the feasiblity of rapidly identifying specific neuropeptide mRNAs in discrete brain regions in situ by autoradiography. The regulation of gene transcription and neuropeptide expression in the CNS can now be studied at the molecular level.

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