triplet at amino acid position 237 and an amber codon at amino acid position 249; the NS gene of A/Berk/1/68 virus terminates NS1 with an ochre triplet (UAA) at position 220 followed by a UGA triplet 17 amino acids downstream (*2*). Thus, these two viruses may be conveniently used for the analysis of opal and ochre suppressor activity, respectively. Finally, the analysis system described here may not only be useful for measuring nonsense suppressor activity in cells transformed by genetic engineering techniques, but also for detecting suppressor activity in eukaryotic cells containing natural nonsense suppressor tRNA's (*7*).

JAMES F. YOUNG*
Department of Microbiology, Mount Sinai School of Medicine of City University of New York, New York 10029

MARIO CAPECCHI
Department of Biology, University of Utah, Salt Lake City 84112

FRANK A. LASKI
UTTAM L. RAJBHANDARY
PHILLIP A. SHARP
Center for Cancer Research and *Department of Biology, Massachusetts Institute of Technology, Cambridge 02139*

PETER PALESE
Department of Microbiology, Mount Sinai School of Medicine of City University of New York

References and Notes

1. F. Laski, R. Belagaje, U. L. RajBhandary, P. A. Sharp, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5813 (1982); R. M. Hudziak, F. Laski, U. L. RajBhandary, P. A. Sharp, M. Capecchi, *Cell* **31**, 137 (1982).
2. M. Krystal, D. Buonagurio, J. F. Young, P. Palese, *J. Virol.* **45**, 547 (1983); M. Baez, R. Taussig, J. J. Zazra, J. F. Young, P. Palese, A. Reisfeld, A. M. Skalka, *Nucleic Acids Res.* **8**, 5845 (1980); J. Parvin, J. F. Young, P. Palese, *Virology* **128**, 512 (1983).
3. W. P. Summers, W. C. Summers, F. Laski, U. RajBhandary, P. A. Sharp, *J. Virol.*, in press.
4. J. F. Young, U. Desselberger, P. Graves, P. Palese, A. Shatzman, M. Rosenberg, in *Origin of Pandemic Viruses*, W. G. Laver, Ed. (Elsevier, New York, in press).
5. L. Bossi, *J. Mol. Biol.* **164**, 73 (1983); C. T. Caskey and A. Campbell, in *Nonsense Mutations and tRNA Suppressors*, J. E. Celis and J. D. Smith, Eds. (Academic Press, London, 1979), pp. 81–96.
6. M. Capecchi, unpublished result.
7. A. I. Geller and A. Rich, *Nature (London)* **283**, 41 (1980); A. Diamond, B. Dudock, D. Hatfield, *Cell* **25**, 497 (1981); D. Hatfield, A. Diamond, B. Dudock, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6215 (1982).
8. J. V. Maizel, in *Methods in Virology*, K. Maramorosch and H. Koprowski, Eds. (Academic Press, New York, 1971), vol. 5, pp. 179–246.
9. J. F. Young and P. Palese, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 6547 (1979).
10. We thank M. Lin for technical assistance. Supported in part by NIH grants AI-11823 (P.P.), AI-18998 (P.P.), GM17151 (U.L.R.), GM21168 (M.C.), and PO1-CA26717 (P.A.S.); by American Cancer Society grant NP114 (U.L.R.); by NSF grant PAM823230 (P.A.S.); and by an I. T. Hirschl Career Research Award (P.P.) and a Sinsheimer Scholarship (J.F.Y.).
* Present address: Smith, Kline and French Laboratories, Philadelphia, Pa. 19101.

2 May 1983; revised 28 June 1983

Corticotropin Releasing Factor Decreases Postburst Hyperpolarizations and Excites Hippocampal Neurons

Abstract. *Corticotropin releasing factor in concentrations of 15 to 250 nanomoles per liter increased the spontaneous discharge frequency and decreased the size of hyperpolarizations after burst discharges in CA1 and CA3 pyramidal neurons of rat hippocampal slices. Concentrations greater than 250 nanomoles per liter also depolarized the cells. These excitatory actions of corticotropin releasing factor may involve a novel calcium-dependent process.*

Endogenous hypothalamic factors capable of releasing adrenocorticotropic hormone (ACTH) and β-endorphin from the pituitary have been recognized for some time (*1*). However, only recently has a corticotropin releasing factor (CRF) been purified, sequenced, and synthesized (*2*). Synthetic CRF releases ACTH and β-endorphin from the pituitary and, when centrally administered, activates the sympathetic nervous system (*3*), suggesting that CRF may be a key hormone in mobilizing the organism under stress (*2*). This view is supported by the recent findings that centrally administered CRF causes hyperactivity and arousal in rats (*4*) and that CRF immunoreactivity is seen in cell bodies

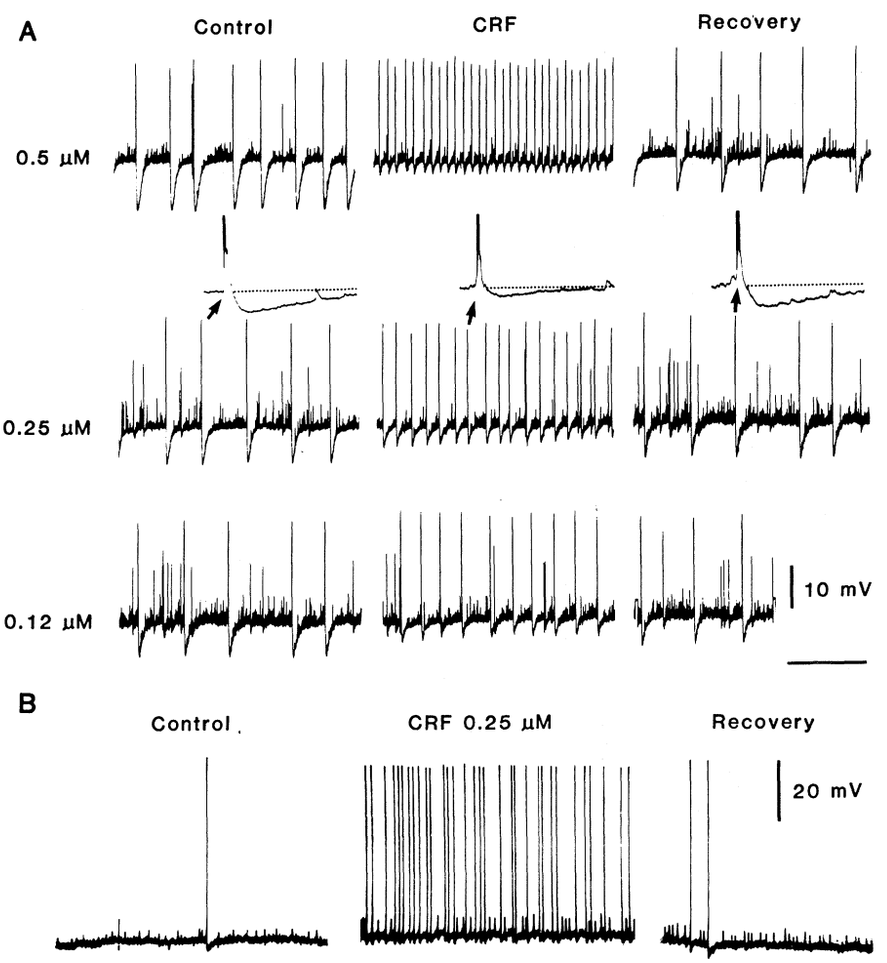

Fig. 1. Effects of CRF on spontaneous spikes and transmembrane properties of pyramidal neurons. (A) Dose-dependent increases in firing and reductions of postburst afterhyperpolarizations by CRF in a CA3 neuron. Spontaneous large spikes followed by afterhyperpolarizations are actually bursts of multiple spikes that appear as single attenuated spikes because of the slow speed and rise time of the chart recorder. Arrows indicate representative bursts displayed at faster speed (insets). Dotted lines represent the baseline potentials in each condition. The spike firing rate increases at all CRF concentrations. (B) Oscillographs from a CA1 neuron: 0.25 μ*M* CRF increases the spike discharge rate and slightly depolarizes (about 3 mV) this cell. There is an apparent increase in the rate and size of activity subthreshold for somatic spike generation (excitatory postsynaptic potentials or dendritic spikes). Horizontal calibration bars are 20 seconds in (A), 0.8 second in the insets, and 2 seconds in (B). All CRF records were obtained within 2 to 5 minutes of control records; recovery records were taken at 10 to 40 minutes after CRF washout.

and fibers of the rat and sheep limbic systems (*5, 6*). Reasoning that endogenous CRF might have a neuromessenger role in brain, we tested the effects of CRF on electrical properties of pyramidal neurons in the hippocampal slice preparation. We now report that nanomolar concentrations of CRF increase the spontaneous discharge of these neurons and decrease the afterhyperpolarizations that follow bursts or trains of action potentials; greater concentrations of CRF also depolarize these neurons (*7*).

Rat hippocampal slices, prepared as described (*8*), were completely submerged and continuously superfused in warm, gassed, artificial cerebrospinal fluid (ACSF). After stable penetration (*8*) of a pyramidal neuron with a micropipette (100 to 200 megohms) containing potassium acetate, the perfusate was switched, without interruption of flow, from ACSF alone to ACSF containing CRF. Superfusion with CRF for 5 to 20 minutes was followed by washout with ACSF. All of the effects of CRF were reversible.

We tested CRF on 25 pyramidal cells (60 CRF trials). At concentrations of 60 n*M* or greater, CRF increased the frequency of action potential discharge in both CA1 and CA3 pyramidal neurons in a concentration-dependent manner (Fig. 1). At CRF concentrations of 15 to 250 n*M*, changes in membrane potential were not evident, but concentrations of 250 to 1000 n*M* depolarized these cells by 3 to 30 mV (Fig. 1). However, in all experiments, CRF at 15 to 500 n*M* decreased the afterhyperpolarizations that follow spontaneous bursts of spikes (CA3) or spike trains (CA1 and CA3) evoked by depolarizing current pulses injected through the recording pipette (Figs. 1 and 2). All CRF effects were accompanied by a small increase in input resistance, as assessed from current-voltage curves generated by intracellular current injection (data not shown). When CRF was deamidated at the carboxyl terminal, it was inactive in release studies (*2*) and had no effect on three pyramidal cells at 0.2 to 0.5 μ*M*.

Afterhyperpolarizations in hippocampal pyramidal cells are probably generated by a potassium conductance that depends on calcium influx during repetitive discharge (*9, 10*), a mechanism similar to that detailed for invertebrate neurons (*11*). Therefore, CRF may alter the afterhyperpolarization through reduction either of the inward calcium current or the outward potassium current. To evaluate these possibilities, we superfused 100 n*M* tetrodotoxin to block the sodium component of the spikes; current injection then elicited only slow humplike spikes, considered to be calcium spikes (*9, 10*). Afterhyperpolarizations occurred when calcium spikes were elicited, but not after subthreshold depolarizations. During superfusion with tetrodotoxin, CRF again decreased the afterhyperpolarization (Fig. 2), whereas the size of the calcium spikes was unaltered or increased. We interpret this finding as evidence for a CRF antagonism of the afterhyperpolarization by a mechanism secondary to calcium influx, perhaps by a direct antagonism of the potassium current or of some intermediate linkage between the two conductances.

In summary, we find that CRF increases the frequency of discharge, reduces the size of afterhyperpolarizations and, at greater concentrations, depolarizes CA1 and CA3 pyramidal neurons. The mechanism behind the increased discharge is unclear; remote effects such as transsynaptic activation could contribute but may not fully explain this effect (*10*). At high CRF concentrations the increased firing could arise from membrane depolarization. At low concentrations, depolarization was not detected, but remote dendritic depolarizations, undetected in the soma, could lead to increased firing by generation of dendritic spikes. Alternatively, antagonism by CRF of the hyperpolarizations that follow spontaneous bursts of spikes, perhaps in dendrites (*12*), may result in increased firing by a type of intrinsic disinhibition. A similar action also might account for the CRF-induced depolarizations: if pyramidal cells at resting membrane potentials show a finite calcium-activated potassium conductance, antagonism of this conductance could lead to depolarization. The small increase in input resistance seen during CRF is consistent with this speculation.

The CRF effects seen in the hippocampus appear to involve an unusual pattern of actions that could represent a new interneuronal signal in the central nervous system. Similar direct excitatory actions on cells in the hypothalamus or pituitary could provide a physiological basis for the reported release of neuropeptides such as ACTH and β-endorphin by CRF (*2*). Although early immunohistochemical studies showed CRF immu-

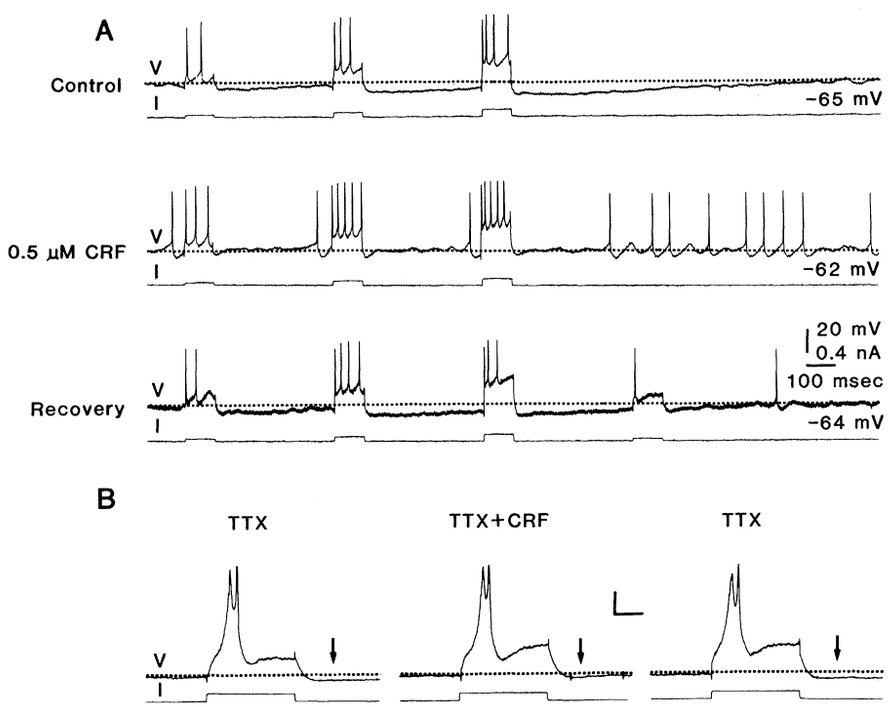

Fig. 2. Effect of CRF on current-evoked bursts of action potentials and associated afterhyperpolarizations. (A) In a CA1 neuron, 0.5 μ*M* CRF reduces the afterhyperpolarizations that follow trains of spikes generated by passing depolarizing current pulses of three different intensities through an active bridge circuit and the recording electrode. There is complete loss of late components of the afterhyperpolarizations during CRF, in spite of the greater number of spikes generated by equivalent current strengths. CRF also increases spontaneous firing and depolarizes the cell (indicated by the values of membrane potentials at the end of each trace). (B) Effect of CRF in the presence of tetrodotoxin (*TTX*; 100 n*M*): CA1 neuron. Tetrodotoxin alone abolishes the early fast action potentials, leaving a slower, calcium spike. CRF (400 n*M*) added to the tetrodotoxin solution then nearly abolishes the afterhyperpolarization (arrows), but did not significantly alter the Ca^{2+} spike. Recovery is shown on the right. *V*, voltage trace; *I*, current trace. Current pulse, 0.2 nA; calibration bars, 10 mV and 0.2 second.

noreactivity in cell bodies and fibers primarily in the hypothalamus (*5*), recent studies reveal scattered CRF-positive cells in rat and sheep hippocampus (*5, 6*). Radioimmunoassay also reveals CRF in the hippocampus (*13*). Furthermore, the subependymal location of CRF-positive fibers near the third ventricle (*5*) might release sufficient CRF into the cerebrospinal fluid to activate hippocampal neurons. Indeed, central injection of as little as 10 ng of CRF activates the hippocampal electroencephalogram (*14*). If endogenous CRF is involved in activation of brain neurons for response to stress (*2*), the novel pattern of neuronal excitation seen in this study could provide the cellular basis.

Note added in proof: A study in our laboratory indicates that CRF also has excitatory actions on locus ceruleus neurons in the rat in vivo (*15*).

J. B. ALDENHOFF*
D. L. GRUOL, J. RIVIER
W. VALE, G. R. SIGGINS†
A. V. Davis Center for Behavioral Neurobiology and *Peptide Biology Laboratory, Salk Institute, San Diego, California 92138*

References and Notes

1. A. Witter and D. deWied, in *Handbook of the Hypothalamus*, P. Morgane and J. Panksepp, Eds. (Dekker, New York, 1980), p. 307; W. Vale, C. Rivier, M. Brown, in *ibid.*, p. 165.
2. W. Vale, J. Spiess, C. Rivier, J. Rivier, *Science* **213**, 1394 (1981); J. Spiess, J. Rivier, C. Rivier, W. Vale, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6517 (1981).
3. M. Brown, L. A. Fishers, J. Rivier, J. Spiess, C. Rivier, W. Vale, *Life Sci.* **30**, 207 (1982); M. Brown *et al.*, *Regul. Peptides*, in press.
4. R. E. Sutton, G. F. Koob, M. Le Moal, J. Rivier, W. Vale, *Nature (London)* **297**, 331 (1982).
5. F. E. Bloom, E. L. F. Battenberg, J. Rivier, W. Vale, *Regul. Peptides* **4**, 43 (1982); G. Bugnon, D. Fellman, A. Guoget, J. Cardot, *C. R. Acad. Sci.* **294**, 599 (1982); F. E. Bloom, E. Battenberg, J. Morrison, in preparation.
6. L. Swanson, P. E. Sawchenko, J. Rivier, W. Vale, *Neuroendocrinology* **36**, 165 (1983). In this study, larger amounts of colchicine were used than in earlier studies.
7. These data have been published in preliminary form [J. B. Aldenhoff, D. L. Gruol, G. R. Siggins, *Soc. Neurosci. Abstr.* **8**, 983 (1982)].
8. G. R. Siggins and P. Schubert, *Neurosci. Lett.* **23**, 55 (1981); Q. Pittman and G. R. Siggins, *Brain Res.* **221**, 402 (1981). After being cut on a McIlwain chopper, slices were incubated for 1 hour, with the top surface exposed to warm, moist atmosphere consisting of 95 percent O_2 and 5 percent CO_2; the slices were then submerged and superfused at 2 to 4 ml/min with ACSF gassed with 95 percent O_2 and 5 percent CO_2 (*p*H 7.4) and maintained at constant temperature (32° to 35°C). The composition of the ACSF, in millimoles per liter, was NaCl, 124; KCl, 5; $MgSO_4$, 2; KH_2PO_4, 1.3; $NaHCO_3$, 26; $CaCl_2$, 2; and glucose, 10. The superfusion rate allowed the CRF in the chamber to reach 90 percent of the reservoir concentration within 2.5 minutes (Q. Pittman and G. Siggins, unpublished data). Cells accepted for analysis had stable membrane potentials of 55 mV or greater; recording durations were 1 to 5 hours.
9. J. R. Hotson and D. A. Prince, *J. Neurophysiol.* **43**, 409 (1980); P. A. Schwartzkroin and C. E. Stafstrom, *Science* **210**, 1125 (1980); R. K. S. Wong and D. A. Prince, *J. Neurophysiol.* **45**, 86 (1981). Our experiments also support the Ca^{2+}-dependent K^+ mechanism for the afterhyperpolarizations: (i) afterhyperpolarizations and Ca^{2+} spikes were blocked by 10 m*M* Mg^{2+}; (ii) the afterhyperpolarizations were associated with decreased input resistance; (iii) the afterhyperpolarization was decreased by membrane hyperpolarization; and (iv) the afterhyperpolarization was not sensitive to chloride injection.
10. With superfusion of ACSF with 20 m*M* $MgCl_2$ to block calcium channels and synaptic transmission, a slight CRF-induced increase of discharge frequency was still observed, although the increase was much smaller than in normal ACSF. ACSF with 11.5 m*M* Mg^{2+} also somewhat reduced the depolarizations evoked by large CRF concentrations but did not block them. The posttrain afterhyperpolarizations and the "calcium spikes" seen after tetrodotoxin were antagonized by both Mg^{2+} concentrations. In CA1 neurons, CRF in normal ACSF often increased the frequency of subthreshold activity (Fig. 1), composed either of attenuated dendritic spikes or synaptic potentials. In tetrodotoxin, synaptic activation and spontaneous discharge were both blocked; under these conditions high CRF concentrations only occasionally depolarized pyramidal neurons.
11. G. Hofmeier and H. D. Lux, *Pfluegers Arch.* **391**, 242 (1981).
12. R. K. S. Wong, D. A. Prince, A. I. Basbaum, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 986 (1979).
13. A. J. Fischman and R. L. Moldow, *Peptides* **1**, 149 (1982).
14. C. L. Ehlers, S. J. Henriksen, F. E. Bloom, J. Rivier, W. J. Vale, *Soc. Neurosci. Abstr.* **8**, 1013 (1982).
15. R. Valentino, S. Foote, G. Aston-Jones, *Brain Res.* **270**, 363 (1983).
16. Supported by PHS grants AM 26741 and AA 03504 and by grants from the Alexander-von-Humboldt Foundation (to J.B.A. and G.R.S.) and the F. M. Kirby Foundation, Inc. (to J.B.A.). We thank F. E. Bloom and L. Koda for criticism, C. Franklin for technical help, and N. Callahan for manuscript preparation.
* Present address: BKH Kaufbeuren, Kemnaterstrasse 16, D-895 Kaufbeuren, West Germany.
† Address reprint requests to G.R.S.

15 March 1983

Neuropeptide Y Distribution in the Rat Brain

Abstract. *A massive neuronal system was detected by immunocytochemistry and radioimmunoassay with antibodies to neuropeptide Y, the recently isolated peptide of the pancreatic polypeptide family. Immunoreactive cell bodies and fibers were most prevalent in cortical, limbic, and hypothalamic regions. Neuropeptide Y was extracted in concentrations higher than those of any other peptide hitherto discovered in the mammalian brain. Column chromatography of brain extracts and double immunostaining experiments indicate that neuropeptide Y is the endogenous brain peptide responsible for immunostaining of pancreatic polypeptide–like immunoreactivity in the mammalian brain.*

It has been thought for some time that a peptide chemically related to the pancreatic hormone pancreatic polypeptide (PP) is present in the mammalian central nervous system (CNS). Immunocytochemical studies with antisera to avian PP (APP) (*1–6*) and bovine PP (BPP) (*7*) have suggested a new and widespread PP-like immunoreactive neuronal system. However, no significant APP- or BPP-like immunoreactivity has been found on radioimmunoassay, so the true nature of the PP-like immunoreactivity has remained unknown. Recently, a peptide consisting of 36 amino acid residues, neuropeptide Y (NPY), was isolated from extracts of porcine brain (*8*) by a novel method for detecting peptides having a COOH-terminal α-amide (*9*), a characteristic feature of many biologically active peptides (*10*). It shares major sequence homologies with the putative gut hormone peptide YY (PYY) (*11–14*) and with PP (*14, 15*). NPY is, therefore, considered to be the latest member of the emerging PP family (*14*).

Table 1. Distribution of NPY-like immunoreactivity in the rat brain. Values (means ± standard errors; $N = 6$) are based on porcine NPY cross-reactivity.

Region	Immunoreactivity (picomoles per gram of wet tissue)
Olfactory bulb	82.2 ± 4.2
Frontal cortex	178.5 ± 23.7
Parietal cortex	183.6 ± 30.2
Occipital cortex	188.7 ± 31.5
Preoptic hypothalamus	729.8 ± 164.0
Periventricular hypothalamus	980.0 ± 162.0
Caudate	312.5 ± 68.6
Putamen	516.0 ± 104.3
Globus pallidus	311.2 ± 47.6
Nucleus accumbens	891.4 ± 80.9
Septum	762.2 ± 142.7
Amygdala	714.7 ± 56.2
Thalamus	103.4 ± 20.9
Hippocampus	205.2 ± 31.6
Periaqueductal gray region	894.4 ± 169.9
Cerebellum	27.0 ± 5.2
Pons	45.8 ± 16.0

By raising antibodies to NPY (*16*) that did not show significant cross-reactivity with PYY or APP (*17*), we have detected by immunocytochemistry (*18*) and radioimmunoassay (*19*) a widespread NPY-immunoreactive system in the rat brain that strongly resembles the reported distribution of PP-like immunoreactivity. Indeed, double immunostaining by an elution technique (*20*) confirmed that NPY and an APP-like immunoreactive material are located in the same cells. Analysis of brain extracts by reverse-phase high-performance liquid chromatography (HPLC) (*21*) showed NPY-like immunoreactivity to elute in a position similar to that of pure NPY. NPY may, therefore, be the true peptide of the PP family in the rat brain.

A salient feature of the distribution of