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Overview of the most prevalent hypothalamus-specific mRNAs, as identified by directional tag PCR subtraction

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ABSTRACT **We applied the directional tag PCR subtractive hybridization method to construct a rat hypothalamic cDNA library from which cerebellar and hippocampal sequences had been depleted, enriching 20–30-fold for sequences expressed selectively in the hypothalamus. We studied a sample of 94 clones selected for enrichment in the subtracted library. These clones corresponded to 43 distinct mRNA species, about half of which were novel. Thirty-eight of these 43 mRNAs (corresponding to 85 of the clones in the sample) exhibited enrichment in the hypothalamus; 23 were highly enriched. *In situ* hybridization studies revealed that one novel species was restricted to cells in a small bilaterally symmetric area of the paraventricular hypothalamus. Other novel mRNAs showed substantial enrichment in basal diencephalic structures, particularly the hypothalamus, without restriction to single hypothalamic nuclei. The data suggest that the hypothalamus utilizes at least two distinct strategies for employing its selectively expressed proteins. Secretory neuropeptides utilized for intercellular communication are produced by functionally discrete nuclei, while several other proteins are shared by structures that are unrelated in their physiological roles but may share biochemical systems.**

The hypothalamus is a phylogenetically ancient region of the mammalian brain. In contrast to laminar cortical structures such as the cerebellum and hippocampus whose final functions rely on innervation from the thalamus and brain stem, the hypothalamus is organized as a collection of distinct, autonomously active nuclei with discrete functions. Ablation and electrical stimulation studies and medical malfunctions have implicated several of these nuclei as central regulatory centers for major autonomic and endocrine homeostatic systems mediating processes such as reproduction, lactation, fluid balance, metabolism, and aspects of behaviors, such as circadian rhythmicity, basic emotions, feeding and drinking, mating activities, and responses to stress, as well as normal development of the immune system (1). Distinct hormones and releasing factors have been associated with some of these nuclei, but, at best, the organizations and molecular operations of these structures are only partially understood.

A substantial portion of a mammal's genetic endowment is dedicated to the function of its central nervous system, as evidenced by the substantial number of mRNAs selectively expressed in the brain (2). Many of these have been observed to be selectively associated with distinct neural subsets. We hypothesized that ensembles of mRNAs selectively associated with discrete hypothalamic nuclei encode proteins singularly associated with the unique functions of those nuclei. The hypothesis must be at least partially valid given existing knowledge on the expression of specific hypothalamic hormones and releasing factors, but the extent to which it can account for the function of this organ is not known. To address this hypothesis further and to illuminate additional molecules that contribute to the specialized functions of hypothalamic nuclei, we have embarked on a systematic analysis of the mRNAs whose expression is restricted to or enriched in the hypothalamus. Here we report our initial progress toward that goal and an overview of hypothalamus-specific gene expression in the rat.

MATERIALS AND METHODS

RNA Isolation and cDNA Library Construction. Young adult Sprague–Dawley rats of both genders were sacrificed under anesthesia by decapitation, and their brains were removed quickly. The hypothalamus, hippocampus, and cerebellum were immediately dissected on an ice-cold plate following the boundaries described by Glowinski and Iversen (3). The block of hypothalamic tissue was 2 mm deep and was taken using the optic chiasm as the rostral limit and the mammillary bodies as caudal reference. Cytoplasmic RNA was isolated rapidly from the dissected tissues (4) and enriched for poly(A)-containing species by oligo(dT)-cellulose chromatography (5). For the Northern blots, RNA was isolated (6) from frozen tissue purchased from Zivic–Miller. cDNA libraries were prepared as described (7), except that pBCSK$^+$ (Stratagene) was used for the subtracted library rather than pT7T3D because lower backgrounds have been found in the subsequent steps using the former vector (H. Usui, personal communication). The number of recombinants in the libraries were: pT7T3D hypothalamus, 8×10^6; cerebellum pGEM11Zf(−), 5×10^5; hippocampus pGEM11Zf(−), 1×10^6.

Preparation and Validation of Subtracted cDNA Probe and Library. Subtractive hybridization was performed in two cycles using the previously described procedure (7). Briefly, 1 μg of trace-labeled, tagged hypothalamus target cDNA prepared as described from the pT7T3D target library was annealed for 24 h at 68°C in 10 μl of hybridization buffer (7) with 20 μg of cerebellum cRNA (ratio 1:20). After hydroxyapatite chromatography, the single-stranded fraction corresponded to 10% of the input material, as judged by tracer quantitation. This was mixed with 20 μg of hippocampus cRNA (estimated ratio 1:200) for a second 24 h of hybridization, after which 30% of the input chromatographed at the single-strand position. Cumulatively, these steps removed more than 97% of the input tracer. An aliquot of the single-stranded material was used as template in a 30-cycle PCR (program: 94°C for 15 s, 60°C for 15 s, 72°C for 1 min) using primers corresponding to the tag sequences (7): 5′-AACTGGAAGAATTCGCGG-3′ and 5′-AGGCCAAGAATTCGGCACGA-3′. The amplification product was cleaved with *Not*I, then *Eco*RI, and inserted into pBCSK$^+$. A dot blot was prepared and screened with probes prepared from the target, subtracted target, and driver libraries as described previously (7) using serial dilutions of plasmid cDNA clones isolated previously in this laboratory. The target and subtracted target cDNA libraries were screened to determine the frequency of

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Abbreviation: POMC, proopiomelanocortin.

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oxytocin and VAT-1 (see below) cDNA clones using as probes clones isolated in the present study.

Other Methods. Grids of the subtracted library were prepared and probed as described (7). DNA sequence analysis, Northern blotting, and *in situ* hybridization were performed as described (7, 8).

For cDNA library Southern blotting, 2 μg of each library was digested with *Hae*III, separated by electrophoresis, transferred to nylon membranes, and hybridized to individual clones, as described (7).

RESULTS

To recognize mRNAs that are selectively expressed in the hypothalamus, we prepared poly(A)-enriched cytoplasmic RNA from carefully dissected rat hypothalami. We constructed a target cDNA library in vector pT7T3D. We also made driver libraries in pGEM11Zf(−) from analogously prepared cerebellum and hippocampus RNA samples. We applied the directional tag PCR subtractive hybridization method of Usui and colleagues (7) to produce tagged hypothalamic cDNAs from which cerebellar and hippocampal sequences were depleted in two consecutive steps, which removed more than 97% of the input target cDNA (see *Materials and Methods*). The tag sequences were used as PCR primer-binding sites to amplify the remaining material. An aliquot of the amplified product was cloned into pBCSK$^+$ to generate a subtracted hypothalamus library with 5×10^5 members, with inserts ranging from 400 to 1200 (average 700) nucleotide pairs, as judged by agarose gel electrophoresis of the released inserts (not shown).

Efficiency of the Subtraction. To validate the efficiency of the subtraction, we investigated the degree of depletion in the subtracted library of sequences known to be expressed panneurally and the enrichment of sequences known to be expressed specifically in the hypothalamus. Dot blots were prepared with dilutions of cDNA clones of the mRNAs encoding the following proteins: panneural neuron-specific enolase, ubiquitously expressed cyclophilin, hypothalamus-specific vasopressin, hypothalamus-enriched proopiomelanocortin (POMC), thalamus-specific protein kinase Cδ, and pituitary-specific growth hormone as well as the target vector itself. The blots were probed with cDNA inserts amplified by PCR from the unsubtracted target library, the subtracted target library, or a pool of the driver libraries (Fig. 1). The driver and unsubtracted library probes gave strong signals for cyclophilin and neuron-specific enolase and a weaker signal for POMC. Neither hippocampus nor cerebellum is known to express POMC. Although this finding could be explained if one of the drivers had suffered contamination with mRNA from another structure, for example brain stem, the studies below suggest that the signal with the driver libraries was probably due to background hybridization to sequences in the POMC clone. The unsubtracted target additionally gave a weak signal for vasopressin. The subtracted probe gave a very strong signal for vasopressin and POMC and otherwise only faint or undetectable signals. The increase in strength of the vasopressin signal was 20–30-fold. Thus, the subtraction protocol removed abundant, panneurally expressed sequences nearly quantitatively while enriching for hypothalamus-specific sequences. There was no apparent contamination with sequences from the anatomically adjacent structures, thalamus or pituitary. The effectiveness of the subtraction was quantitated further by measuring the frequencies of VAT-1 (see below) and oxytocin clones in the unsubtracted and subtracted libraries by colony hybridization with a probe corresponding to a mixture of clones of these two species. The frequency of positive clones in the unsubtracted target was 4/2775. After subtraction, the frequency increased to 33/1224. These frequencies indicate an approximately 19-fold increase in the specific activities of these known hypothalamus-enriched species, consistent with the estimates suggested by the data of Fig. 1.

Identification of Hypothalamus-Enriched Species. To identify species enriched by the subtraction, we picked 648 clones from the subtracted library into grid arrays and hybridized three replicate blots of grid images with probes prepared from the unsubtracted or subtracted target library or a pool of the driver libraries (not shown). Approximately 70% of the colonies gave significant signals with the subtracted target probe compared with 50% with the unsubtracted target probe. Only 10% of the colonies gave signals with the mixed-driver probe.

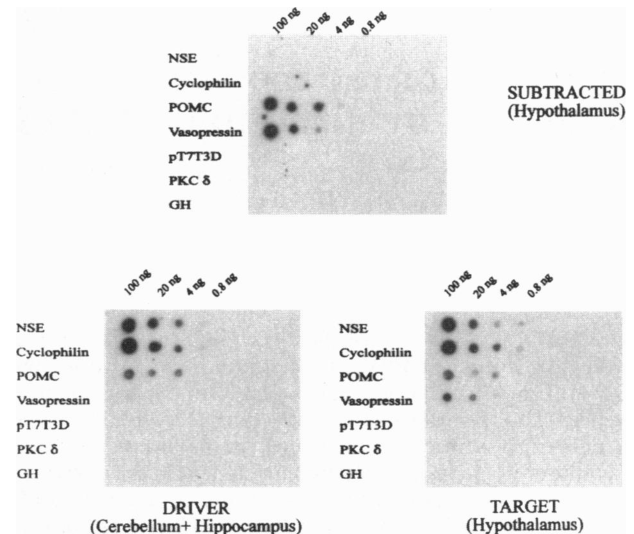

FIG. 1. Subtraction enriched for sequences selectively expressed in hypothalamus. Replicate dot blots on which the indicated masses of plasmid DNA for clones of neuron-specific enolase (NSE), cyclophilin, POMC, vasopressin, the vector pT7T3D, protein kinase Cδ (PKCδ), and growth hormone (GH) were manually spotted and hybridized with cDNA probes made from cRNA transcribed from the target or subtracted libraries, or an equal mixture of the cerebellum and hippocampus driver libraries. Comparison of the signal intensities for the vasopressin dilution series dots at several levels of autoradiographic exposure suggested a 20–30-fold increase in the specific activity of vasopressin cDNA.

Plasmid DNA was prepared individually from 100 of the colonies that gave strong signals with target-derived probes but no signal with the mixed-driver probe. Partial sequences of the inserts were determined for 94 of these, using a sequencing primer that annealed to the vector region adjacent to the 3′ ends of the inserts. The remaining six clones were not pursued further because clear sequences were not obtained. More than 90% of the 3′ sequences appeared to be derived from bona fide 3′ ends of mRNAs as they contained recognizable poly(A) addition consensus hexads (9) 12–22 nucleotides upstream from the poly(A) tracts used in their directional cloning. The sequences were searched by BLAST analysis (10) against the GenBank database. For those that appeared to be novel, the sequence at the 5′ end of the insert was also determined and compared with the database.

A compilation of those data is presented in Table 1, and database accession numbers are given for those prototypes for which a match was found. The 94 clones from the subtracted library for which data were obtained corresponded to 43 distinct mRNA species. Twenty-nine of these were encountered only once in the set of 94 clones, while 14 species were seen between 2 and 13 times. Among the 43 distinct species were 21 that were unambiguously matched to known mRNA species and 22 that were novel species. Among the novel species were six that appear to correspond (greater than 80% nucleotide sequences identity across an extensive span) to rat homologues of so-called "expressed sequence tags," mRNAs of as yet unknown function compiled in the databases. Two species exhibited similarities in both their partial nucleotide sequences and putative encoded amino acid sequences that suggest them to represent members of

Table 1. Cumulative data from 100 clones.

Clone*	BLAST Homology†	Accession no.‡	No.§	Pattern¶
2 +	Oxytocin	M25649	13	A/A
6 +	VAT1-like	T05306	11	B/B
1 +	CART	U10071	7	C
35 +	Novel		6	A/A
15 +	Novel		4	B/C
25 +	POMC	J00759	4	A
12 +	Novel (E)	R75926	3	B/B
16 +	Vasopressin	M25646	3	A
18 +	Glutathione perox	U13705	3	B
29 +	Novel CaM kinase		3	B/C
3 +	Novel		2	B/C
10 +	Novel		2	B/B
51 +	Ubiquitin carrier	M91679	2	C
62 +	Novel		2	C
5 −	Calbindin	U08290		B
14 +	Melanin-conc hormone	M62641		C
17 +	Asp aminotrans	M18467		D
19 −	Novel (E)	R74893		D
20 +	Novel			A/B
21 −	Novel (E)	T32756		A/D
22 −	Novel			D
33 +	Novel (E)	R67552		A/A
34 −	Cl^-/HCO_3^- exchanger	J05167		C
37 +	Novel			B/D
39 −	Novel			C
45 +	Novel			C
46 +	Fibromodulin	X82152		C
47	Perox enolhydratase	U08976		C
48 +	Galanin	J03624		B
52 −	5-HT_2 receptor	L31546		B
53 +	MHC orf	M32010		E
55 +	HNF dimer cofactor	M83740		C
56 +	Carbonyl reductase	X84349		C
57 +	Tyrosine hydroxylase	M10244		A
63 +	Novel			D
67 +	Novel			B/B
73 +	Novel			C
74 +	Novel (E)	T93996		C
75 +	Lamin C2	D14850		A
86 +	Novel			C
92 −	Novel (E)	R49544		C
98 +	Novel			B/D
99 −	Neuronal kinesin	U06698		B/D

*Number of prototype clone in set of 100 followed by indication (+/−) as to whether 3′ sequence contained poly(A) addition hexad (no 3′ sequence for clone 47).
†Short name of matching species or novel for no match: (E) indicates EST match.
‡GenBank database reference.
§Number of representatives in set of 100.
¶Hybridization pattern in cDNA library Southern assay/Northern blot assay. Code: A, target only; B, target highly enriched; C, hypothalamus and hippocampus; D, not highly enriched; E, too faint to categorize.

protein families: a protein related to the VAT-1 secretory vesicle protein (clone 6) and a new calmodulin-dependent protein kinase (clone 29). Their complete sequences will be reported separately.

Validation of the Subtraction. The cDNA insert from at least one representative of each of the 43 mRNA species was used as a probe in a Southern blot with lanes corresponding to the hypothalamus, hippocampus, and cerebellum target and driver cDNA libraries, each cleaved with the restriction endonuclease *Hae*III. Assuming that the cDNA libraries are representative of the mRNAs expressed in their corresponding tissues, this assay serves as a low cost, high throughput surrogate for more expensive and time consuming Northern blot analyses. The hybridization results of the clones in this so-called "cDNA library Southern blot" assay were classified in one of five patterns (Table 1): hybridization to bands detected exclusively in the hypothalamus library (A), to bands highly enriched in hypothalamus but still detectable in hippocampus and/or cerebellum lanes (B), to bands in hypothalamus and hippocampus but not in cerebellum (C), to bands in all three tissues (D), or too faint to categorize (E). Examples of classes A–D are shown in Fig. 2. Twenty-three of the 43 distinct mRNA species were exclusive to or highly enriched in the hypothalamus library, and an additional 15 species were undetectable in the cerebellum library, indicating the effectiveness of our protocols for identifying species selectively present in the target library. It may be significant that the patterns classified as D corresponded to clones that were isolated only once; similarly, none of the species lacking a poly(A) addition signal turned up more than once. The existence in the collection of species present in hippocampus, but not cerebellum, libraries presumably is explained by their enrichment during the first subtraction step with cerebellum driver to an extent that did not allow their complete depletion in the second step with hippocampus driver. POMC gave an A pattern in this assay, demonstrating that the driver libraries were not significantly contaminated with POMC-expressing structures. Thus the low POMC signal observed with the driver probes in Fig. 1 is mostly likely accounted for by vector cross-hybridiation.

mRNA Distributions in CNS and Peripheral Tissues. Northern blots were performed for 15 of the species that showed hypothalamus-enriched or -specific distributions (group A or B) in the cDNA Southern blot assay. The blots (Fig. 3) included RNA samples from six grossly dissected regions of rat brain in

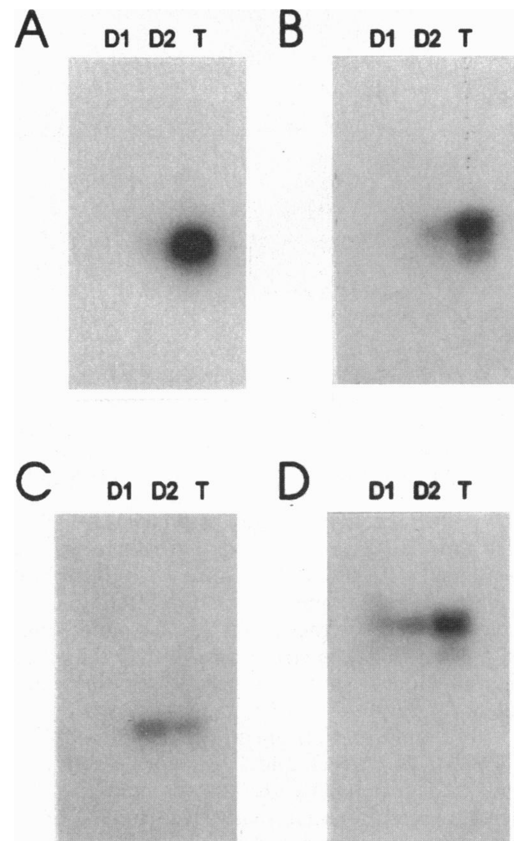

FIG. 2. cDNA library Southern blotting with clones representative of the four distribution classes. The electrophoretic lanes contain the cerebellum first driver library (D1), the hippocampus second driver library (D2), and the hypothalamus target library (T) cleaved with *Hae*III and hybridized with the inserts from clone 35 (*A*), clone 10 (*B*), clone 86 (*C*), and clone 19 (*D*).

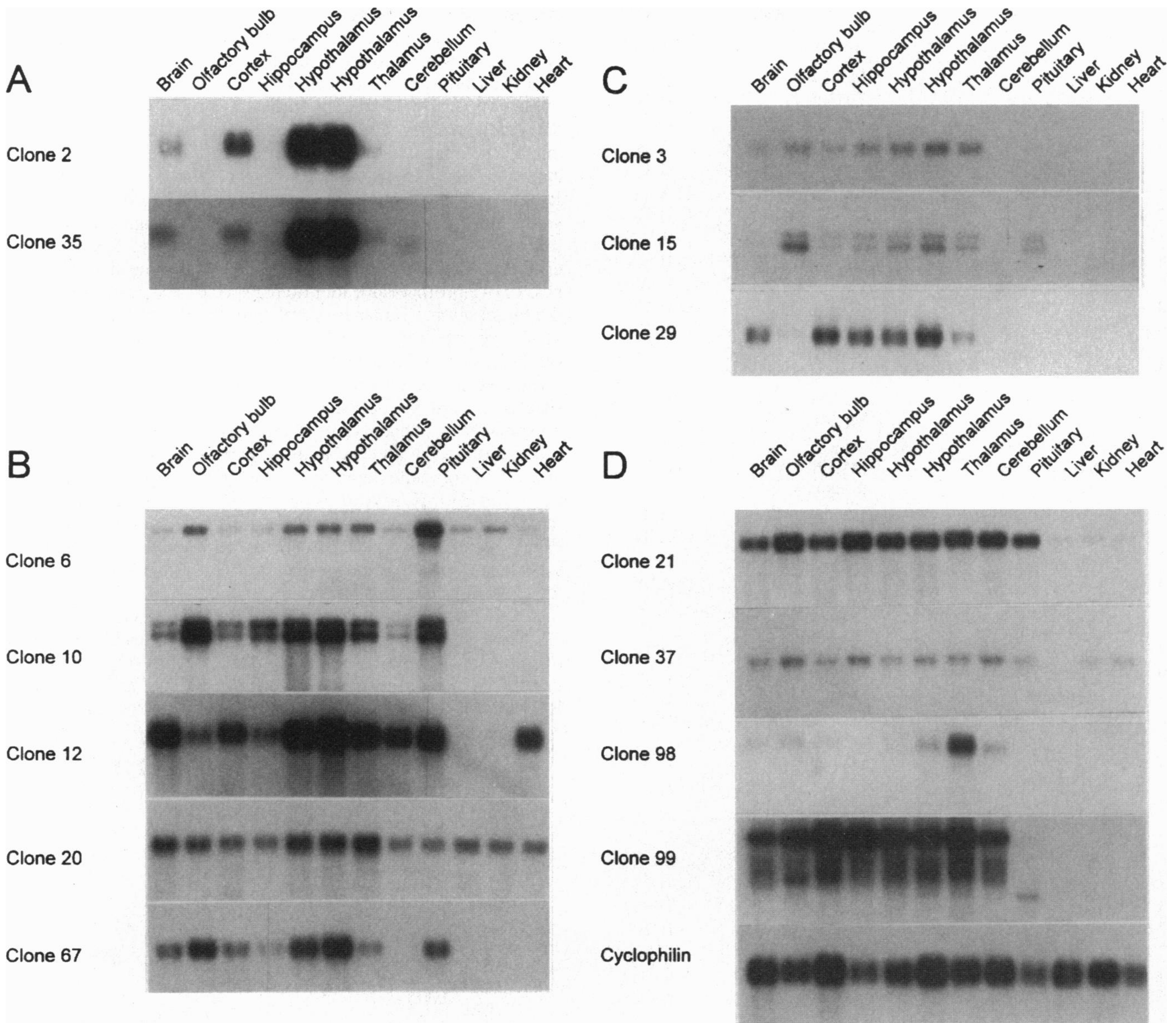

FIG. 3. Distribution of hypothalamic mRNAs. Northern blots with poly(A)$^+$ RNA isolated from extracts of whole brain, olfactory bulb, cerebral cortex, hippocampus, hypothalamus, thalamus, cerebellum, pituitary, liver, kidney, and heart were probed with cDNA inserts from the indicated clones. A cyclophilin probe was included in the series as a control for comparable blot loading and RNA integrity. The two hypothalamus samples represent inadvertent mixtures of approximately equal parts of hypothalamus and striatum. The expression patterns are grouped into four classes (A, B, C, and D). Only the regions of the blots containing the hybridized signal are shown.

addition to pituitary, liver, kidney, and heart. For the clones of species that had been isolated two or more times, the correspondence with the cDNA library Southern blot assay was excellent. Thus, clones 2 (oxytocin) and 35 (novel), which gave A patterns in the cDNA Southern blot study, each detected a band that was strong in the hypothalamus lanes but only very faint or undetectable in the other lanes. The faint signals were possibly due to low expression in those tissues or to contamination during tissue dissection. Clones 6 (VAT1-like), 10 (novel), and 12 (novel), which had given B patterns, each detected bands that were considerably more intense in the hypothalamus than hippocampus or cerebellum lanes, although each was detected in the pituitary lane (6 strongly) and in the samples from some other structures. Clones 3 (novel), 15 (novel), and 29 (novel calmodulin-dependent protein kinase), although classified originally as B patterns, are more properly considered as C patterns, as their expression profiles in this assay are not enriched in hypothalamus per se, but rather are low in the cerebellum.

The clones encountered only once behaved, as a group, less well. Clones 21 (novel), 37 (novel), 98 (novel), and 99 (kinesin) failed to show substantial enrichment in hypothalamus over hippocampus or cerebellum (although 98 was thalamus-enriched). However, clone 33 (novel) detected an RNA species more prevalent in hypothalamus and thalamus than cortex, pons, or olfactory bulb and was undetectable in hippocampus, cerebellum, or peripheral tissues (not shown); thus, technically speaking, clone 33 maintained its A pattern classification. Clone 20 (novel) detected an RNA species with ubiquitous expression but enrichment in hypothalamus and thalamus, thus it is more properly classified as B pattern. Clone 67 (novel) detected a species enriched in hypothalamus and olfactory bulb that was detectable in other brain regions and pituitary but was not detectable in cerebellum.

In situ hybridization on coronal sections of brain from adult male rats was performed using the inserts from clones representing all four classes (A–D): 6, 10, 20, 21, 29, and 35 (Fig. 4). For all clones, the hybridization pattern was consistent with the

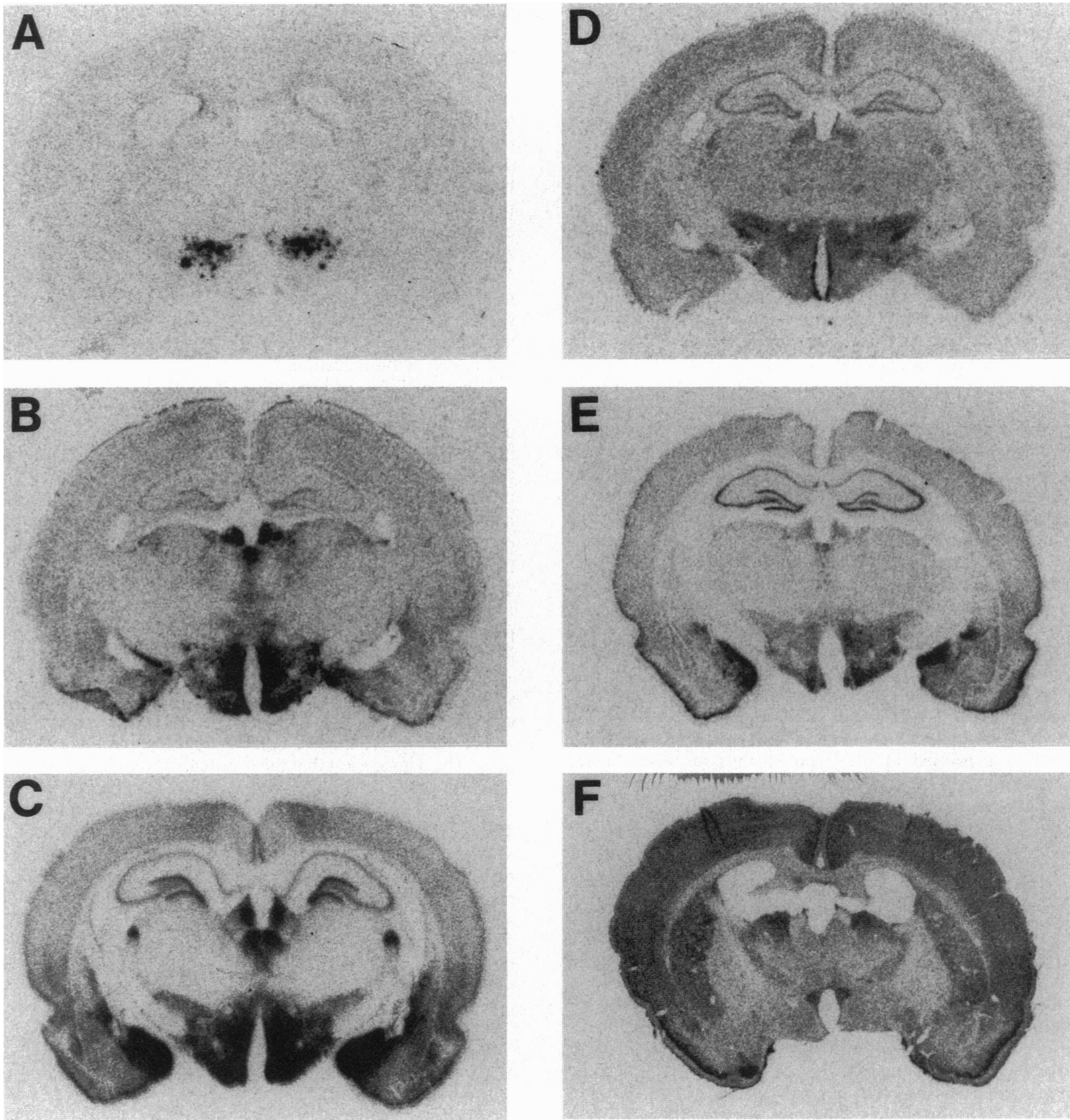

FIG. 4. Expression patterns analyzed by *in situ* hybridization. Coronal sections of rat brain were hybridized with the inserts of clone 35 (*A*), clone 6 (*B*), clone 10 (*C*), clone 20 (*D*), clone 29 (*E*), and clone 21 (*F*).

Northern blot data. In the A class, the clone 35 mRNA displayed a striking pattern of bilaterally symmetric expression restricted to a few cells in the paraventricular hypothalamic area (Fig. 4*A*) and ependymal cells surrounding the brain ventricles. We detected no clone 35 signals outside of the hypothalamus.

Clones 6, 10 and 20, belonging to class B, displayed somewhat more complex distributions. Clone 6 (Fig. 4B) gave strong signals in the periventricular hypothalamic nucleus, anterior hypothalamic area, preoptic, and arcuate nuclei. Very strong hybridization could also be seen in the centromedial thalamic nucleus and medial habenula. Clone 10 (Fig. 4*C*) displayed almost the same pattern, but additional strong signals could be seen in the laterodorsal thalamic nucleus and dentate gyrus, with weak signals in the hippocampal CA fields and the entire neocortex. Interestingly this mRNA showed a marked enrichment in basal diencephalic structures that included nuclei not only of the hypothalamus but also of the amygdaloid complex. Clone 20 (Fig. 4*D*) exhibited low levels of expression in several areas of the brain but displayed especially strong signals in the ventral hypothalamus, most notably in the anterior hypothalamic and periventricular nuclei.

Clone 29 (class C), which encodes a novel calmodulin kinase-like protein, was also very strongly expressed in the anterior hypothalamic area and arcuate nucleus, as well as in the pyramidal cell layer of all hippocampal fields and in the medial and central nuclei of the amygdala (Fig. 4*E*). Clone 21 represents a class D cDNA, whose distribution includes hypothalamic as well as extrahypothalamic structures. In particular, the clone 21 mRNA was found in cortex, amygdala, hippocampus, caudate, and several thalamic (centrodorsal and reticular nuclei) and hypothalamic

nuclei (Fig. 4*F*). Within the hypothalamus, clone 21 mRNA was especially abundant in the paraventricular hypothalamic nucleus.

DISCUSSION

Our hypothesis was that ensembles of mRNAs would be selectively expressed within discrete hypothalamic nuclei and that these mRNAs would encode proteins singularly associated with the unique functions of those nuclei. To approach this hypothesis experimentally, we employed directional tag PCR subtractive hybridization to enrich a cDNA library for clones of mRNA species selectively expressed in the hypothalamus. Candidate clones identified by their hybridization to a subtracted hypothalamus probe were validated in three stages. First, a high throughput cDNA library Southern blot was used to demonstrate that the candidate corresponded to a species enriched in the subtracted library. Second, candidate clones positive in the first assay were used as probes for Northern blots with RNA from several brain regions and peripheral tissues. Finally, candidate clones that were still positive were subjected to *in situ* hybridization analysis to detect the hypothalamic regions that express the corresponding mRNAs.

Typically, subtractive hybridization protocols utilize a single target–driver dichotomy for enrichment of target-specific species. In our present implementation, we utilized a two-step subtraction, first depleting hypothalamus sequences with a cerebellum driver and then with a hippocampus driver. We included the second subtraction because, in some of our previous studies using this methodology, we had been successful in finding clones of species enriched in a target compared with a single driver tissue, only to find considerable expression in other brain regions. The present protocol was designed to provide a more stringent selection for clones of mRNAs with high selectivity for the target. The data compiled in Table 1 suggest that this strategy was effective: 53 of the 94 clones studied were shown to correspond to mRNAs expressed in the hypothalamus at much higher concentrations than in either the hippocampus or cerebellum. An additional 32 of the clones were enriched in both hypothalamus and hippocampus over cerebellum, indicating that the first subtraction was more efficient, probably because the target concentration was higher in the hybridization reaction; thus a greater portion of the common species were driven into hybrids. Cumulatively, 85 of the 94 candidates were found to be enriched in the target hypothalamus compared with the cerebellum, a quite acceptable success rate. It is noteworthy that in eight cases, the cDNA library Southern blot assay suggested a higher degree of hypothalamus enrichment than was later observed by Northern blotting, presumably due to artifactual enrichment in the target libraries compared with the driver libraries. In a few cases, this can be explained by artifactual cloning of an internal or intronic cDNA fragment. Other cases may be explained by difficulties in achieving proportional representation of low prevalence mRNAs in cDNA libraries.

The subtraction steps provided an approximately 30-fold enrichment. In the secondary screen, approximately 60% of the clones were positive with the subtracted probe but not the target probe. Of the 94 clones selected from this screen, 53 were clones of mRNAs selectively expressed in hypothalamus. These 53 clones correspond to approximately 1% of the clones examined in this pilot study, and represented 16 distinct mRNA species, suggesting that a complete characterization of hypothalamus mRNAs might reveal 100–200 species that were specific to or highly enriched in the hypothalamus. Of the 16 mRNA species detected here, nine corresponded to already known proteins, among them oxytocin, vasopressin, and POMC, three neuropeptides known to be highly enriched in the hypothalamus. However, seven mRNA species were novel. Among mRNA species not detected in the 94-clone sample were those encoding the releasing factors, which are less abundant than most of the species detected here. Thus, a complete survey would require a much larger number of clones.

How do the data collected here fit with the starting hypothesis? Oxytocin and vasopressin mRNAs are predominantly associated with discrete hypothalamic nuclei, as was previously known. The *in situ* hybridization images indicate that several additional mRNAs, including several novel species, are enriched in the hypothalamus. Among the novel species, only clone 35 meets the hypothesis in its strictest sense; the mRNA appears to be restricted to a single nucleus in the paraventricular area of the hypothalamus. Preliminary nucleotide sequence data suggest that the clone 35 mRNA encodes a novel small secretory protein that contains sites for proteolytic maturation, possibly the hallmarks of a new hypothalamic peptide hormone precursor. More detailed anatomical studies are required to assign the cells that express the mRNA precisely to known hypothalamic structures. However, it seems likely that this protein will be found to be exclusively involved in a particular physiological system, probably involved in cell-to-cell communication.

Other mRNAs corresponding to novel clones exhibit enrichment in basal diencephalic structures, especially the hypothalamus, but within the hypothalamus none is restricted to a single nucleus. These species presumably encode proteins whose functions are not dedicated to single physiological systems. Nevertheless, their roles seem to have selective utility within the central nervous system. Previous studies looking at mRNAs enriched in the caudate revealed several involved in signal transduction pathways (7). That is not the finding for the hypothalamus-enriched species encountered thus far.

The study described here provides an overview of those most prevalent mRNA species whose expression is enriched in the hypothalamus, including several novel species. A much larger sample will clearly be required to identify all mRNAs with selective hypothalamic expression. Nevertheless, some comments can be made as to the extent to which selective gene expression within the hypothalamus is related to specific physiological functions. The data suggest that the hypothalamus utilizes at least two different strategies for employing selectively expressed proteins. Some specific mRNAs are discretely correlated to distinct nuclei. Thus far, all of these mRNAs encode secretory signaling proteins. We have also recognized a class of mRNAs expressed prominently in hypothalamus and amygdala. These do not appear to be restricted to functionally discrete regions, but their comparable anatomical restrictions suggest that they might participate in a series of biochemical processes that are selectively distributed to these regions, which are developmentally related. Thus these regions may share molecular properties that are not apparent at the anatomical level.

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