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RHS2, a POU domain-containing gene, and its expression in developing and adult rat

(DNA binding protein striatum/paraventricular nucleus/supraoptic nucleus/*in situ* hybridization)

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ABSTRACT **Gene expression within the central nervous system is regulated by complex interactions of DNA-binding proteins, among which are the POU domain-containing proteins, which are distantly related to homeobox proteins. These POU domain-containing proteins have been implicated in control of transcription and replication within the central nervous system. We used degenerate primers with the PCR to isolate another POU domain-containing cDNA, *RHS2*, from hypothalamic RNA. Isolation of a putative full-length cDNA was accomplished by using serial dilutions of a hypothalamic cDNA library grown on solid medium. This member of the class III POU family is expressed in rats from embryonic day 11.5 into adulthood, being especially prominent in the brain. We performed double-labeling hybridization histochemistry and determined that *RHS2* is coexpressed with a variety of neuropeptides in medium-sized neurons in the caudate putamen and with dynorphin in the paraventricular and supraoptic nuclei of the hypothalamus. Expression of *RHS2* in the caudate putamen was increased by elimination of its nigrostriatal dopaminergic innervation.**

Many distinct families and subclasses of DNA-binding transcription factors have been described in the brain (for review, see refs. 1 and 2). Among these families is the homeobox-related POU domain-containing proteins. These proteins, acting as monomers, or homodimers and heterodimers with other POU proteins or DNA-binding proteins, appear to regulate cell-specific gene expression, cell fates, and even replication (for review, see refs. 3 and 4). Furthermore, most of the identified POU proteins are expressed in various adult tissues, including brain, where they exhibit different patterns of expression and likely regulate gene expression, acting as positive or negative transcription factors. For example, SCIP appears to repress myelin-specific genes (5, 6), Pit-1 transactivates the growth hormone and prolactin genes in the anterior pituitary (7, 8), and Cf1a transactivates the dopamine decarboxylase gene in *Drosophila* neurons (9).

For several years, we have been interested in regulation of gene expression in the hypothalamus, especially in the paraventricular (PVN) and supraoptic (SON) nuclei. Recently, He *et al.* (10) identified several POU domain genes expressed in mammalian developing and adult brains, some of which are expressed in the hypothalamus. To identify specific transcription factors involved in regulation of genes expressed in these areas, we examined mRNA from the PVN and SON and cloned a POU domain-containing cDNA, *RHS2*. Subsequently, we isolated a putative full-length clone for *RHS2* from a rat hypothalamic cDNA library and we report here its sequence and expression patterns in developing and adult rat.*

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MATERIALS AND METHODS

Cloning Strategy. Total RNA was isolated from the PVN and SON of 11-g preweanling rats using 500-μm-diameter punches (11) and the method of Chomczynski and Sacchi (12). The RNA was primed with a poly(dT) primer and the first strand was synthesized with avian myeloblastosis virus reverse transcriptase (BRL). Two degenerate primers based on the sequences of the N terminus and C terminus of the Pit-1 POU domain [5′-TACGAAGCTTAT(A,C,T)AA(A,G)(C,T)TIGGITA(C,T)ACICA-3′ and 5′-TACGAAGCTT(C,T)TGIC(G,T)IC(G,T)(A,G)TT(A,G)CA(A,G)AACCA-3′] were then used to amplify DNA by PCR according to the manufacturer (Perkin–Elmer/Cetus) with 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 3 min. A 380-base-pair (bp) band was extracted and subcloned into pGEM3Z (Promega) and clones were sequenced by the chain-termination method (13) using a Sequenase kit (United States Biochemical). One clone, PVN19, displaying a high amino acid homology with the POU domain of other POU proteins, was isolated.

Hypothalamic mRNA was isolated from rats given 2% saline to drink for 7 days and a cDNA library with 2×10^7 independent recombinants was constructed according to the method of Okayama *et al.* (ref. 14; M. J. Brownstein and W.S.Y., unpublished results). Initially, the library was screened by a sequential subdivision method in LB/ampicillin cultures as described by Bonner *et al.* (15). However, we were not able to follow our clone further than the second subdivision, probably because of a growth disadvantage. To overcome this problem, we amplified our sublibraries on LB/ampicillin plates instead of in broth.

Two primers, PVN19.3 and PVN19.4 (5′-CACGTACCACTTCTTTCTCC-3′ and 5′-CGACGTGGGGCTGGCATTGG-3′), targeted to either end of the PVN19 PCR fragment, were then used to screen the library. Pools from sequential subdivisions of the library (10^6, 2.5×10^5, 2.5×10^4, and 2.5×10^3 recombinants per pool) were plated on LB/ampicillin plates and grown overnight at 37°C. The bacteria were scraped off and resuspended in LB broth, and the plasmid DNA was isolated. PCRs (30 cycles of 94°C for 1 min, 60°C for 2 min, and 72°C for 1.5 min) were then performed with PVN19.3 and PVN19.4 primers on the plasmid DNA to follow pools containing the specific POU domain sequence. The initial PVN19 PCR fragment was then labeled with random primers (16) and [^{32}P]dCTP (NEN; 3000 Ci/mmol; 1 Ci = 37 GBq) and used as a probe to screen colonies of a positive pool containing 2.5×10^3 independent recombinants. One cDNA clone, *RHS2*, was isolated and both strands were sequenced as described above.

Abbreviations: PVN, paraventricular nucleus; SON, supraoptic nucleus.

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M84645).

Northern Analysis. Total RNA from rat brain and peripheral tissues was isolated (12), size-fractionated on a 1.2% agarose gel containing formaldehyde, transferred to a nylon membrane (Nytran; Schleicher & Schuell), and hybridized with a 515-bp *Nco* I restriction fragment (from the 5′ end of the cDNA upstream of the POU domain) labeled with random primers and [^{32}P]dCTP. After hybridization overnight at 55°C in hybridization buffer [50% formamide/5× Denhardt's solution/5× SSPE (1× SSPE = 0.15 M NaCl/10 mM phosphate, pH 7.4/1 mM EDTA)/0.1% SDS/tRNA (0.25 mg/ml)/single-stranded DNA (0.25 mg/ml)/0.1% pyrophosphate], the blot was washed with 0.1× SSPE/0.2% SDS at 55°C.

Hybridization Histochemistry. Adult male Sprague–Dawley rats (200–300 g, Taconic Farms) were sacrificed by decapitation. The brains were removed, frozen on dry ice, cut into 12-μm frontal sections, collected on twice gelatin-coated slides, and stored at −80°C until hybridized. Embryos from E9.5 (E0 = day of conception) onward were also removed, frozen, and cut as described above. To study the dopaminergic influence on the expression of *RHS2* in the basal ganglia, 6-hydroxydopamine (8 mg/ml in 0.02% ascorbic acid in saline) was injected (1 μl/min) into the right substantia nigra of 10 rats (17) that were sacrificed 21 days later, and coronal sections through the caudate putamen and the accumbens nucleus were cut. Sections adjacent to those examined for expression of *RHS2* were probed with an enkephalin probe [935-bp *Sac* I fragment (18)] as a control for the effectiveness of the lesions (19).

^{35}S-labeled antisense- and sense-strand RNA probes were prepared by *in vitro* transcription (according to Promega), using UTP[^{35}S] (NEN; >1000 Ci/mmol), SP6 or T7 RNA polymerases, and the 515-bp *Nco* I restriction fragment (cloned into pGEM3Z; Promega) as template. A ^{35}S-labeled RNA probe from the original PVN19 fragment was also used in some initial hybridization histochemistry studies, yielding identical results. Digoxigenin-labeled antisense RNA probes from rat enkaphalin (see above), substance P and neurokinin B (465-bp exon 7 and 690-bp cDNA, respectively; gifts of T. Bonner, National Institute of Mental Health), dynorphin [1.78-kilobase (kb) *Bgl* II/*Bam*HI fragment; gift of J. Douglass (20)], and oxytocin (476-bp *Sst* I fragment containing exon 1) cDNA templates were prepared (21) by using digoxigenin-UTP (Boehringer Mannheim).

Sections were hybridized as described (22, 23) with minor modifications. Briefly, sections were fixed with 4% formaldehyde in phosphate-buffered saline (PBS) for 5 min at room temperature, rinsed twice with PBS, and placed into 0.25% acetic anhydride in 0.1 M triethanolamine/PBS, pH 8, for 10 min at room temperature. After dehydration in 70%, 80%, 95%, and 100% ethanol and chloroform, the sections were hybridized overnight at 55°C with 10^6 dpm of ^{35}S-labeled probe in 50 μl of hybridization buffer [50% formamide/600 mM NaCl/80 mM Tris·HCl, pH 7.5/4 mM EDTA/0.1% sodium pyrophosphate/0.2% SDS/sodium heparin (0.2 mg/ml)/2% sodium polyacrylate/100 mM dithiothreitol]. After treatment with RNase A (20 mg/ml) for 30 min, sections were washed four times for 30 min in 0.2× standard saline citrate (SSC) at 60°C and dehydrated. Slides were then dipped in Kodak NTB2 or Ilford K5 emulsion, exposed 2–3 months, developed, and counterstained with thionin.

Double simultaneous hybridization histochemistry was performed with the ^{35}S-labeled antisense probe (for RHS2) and one of the digoxigenin-labeled antisense probes (for enkephalin, substance P, dynorphin, oxytocin, or neurokinin B) in the hybridization buffer. Slides were processed as described above. After being washed four times for 30 min in 0.2× SSC at 60°C, the sections were rinsed twice in buffer 1 (100 mM Tris·HCl/150 mM NaCl, pH 7.5) for 5 min, and then in buffer 1 containing 3% normal goat serum and 0.3% Triton X-100 for 30 min. After 5 hr of incubation with alkaline phosphatase-conjugated anti-digoxigenin antiserum (Boehringer Mannheim; 1:1000 in 3% normal goat serum/0.3% Triton X-100 in buffer 1), the sections were rinsed twice for 10 min in buffer 1 and then for 5 min in buffer 3 (100 mM Tris·HCl/100 mM NaCl/50 mM $MgCl_2$, pH 9.5). The sections were then incubated overnight at room temperature in the dark in buffer 3 containing nitroblue tetrazolium salt (0.34 mg/ml) and 5-bromo-4-chloro-3-indolyl phosphate toluidinium salt (0.18 mg/ml) (NBT/BCIP; Boehringer Mannheim). Sections were rinsed twice for 5 min in buffer 3 and then dipped briefly in

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    ile ile thr ser arg gly ser ser ser thr met ala thr ala ala ser asn pro tyr      9
 AC ATT ATA ACT AGT AGA GGA TCC TCA TCG ACC ATG GCC ACA GCT GCC TCG AAT CCC TAC     59
ser ile leu ser ser ser ser leu val his ala asp ser ala gly met gln gln gly ser     29
AGC ATT CTC AGT TCC AGC TCC CTC GTC CAT GCG GAC TCT GCG GGC ATG CAG CAG GGA AGT    119
pro phe arg asn pro gln lys leu leu gln ser asp tyr leu gln gly val pro ser asn     49
CCT TTC CGC AAT CCT CAG AAA CTT CTC CAA AGT GAC TAC TTG CAG GGA GTT CCC AGC AAT    179
gly his pro leu gly his his trp val thr ser leu ser asp gly gly pro trp ser ser     69
GGG CAT CCC CTC GGG CAT CAC TGG GTG ACC AGT CTG AGC GAC GGG GGC CCG TGG TCC TCC    239
thr leu ala thr ser pro leu asp gln gln asp val lys pro gly arg glu asp leu gln     89
ACA TTG GCC ACC AGC CCC CTG GAC CAG CAG GAC GTG AAG CCG GGA CGT GAA GAT CTG CAA    299
leu gly ala ile ile his his arg ser pro his val ala his his ser pro his thr asn    109
CTG GGA GCA ATC ATC CAT CAC CGC TCG CCT CAC GTA GCC CAC CAC TCG CCC CAC ACT AAC    359
his pro asn ala trp gly ala ser pro ala pro asn ser ser ile thr ser ser gly gln    129
CAT CCG AAC GCC TGG GGA GCG AGC CCG GCT CCA AAC TCG TCC ATC ACG TCC AGC GGC CAA    419
pro leu asn val tyr ser gln pro gly phe thr val ser gly met leu glu his gly gly    149
CCT CTC AAT GTG TAC TCG CAG CCA GGC TTC ACG GTG AGT GGT ATG CTA GAG CAC GGG GGA    479
leu thr pro pro pro ala ala ala ser thr gln ser leu his pro val leu arg glu pro    169
CTC ACT CCA CCA CCA GCT GCT GCC TCC ACA CAG AGC CTG CAT CCA GTG CTC CGG GAG CCT    539
pro asp his gly glu leu gly ser his his cys gln asp his ser asp glu glu thr pro    189
CCA GAC CAT GGT GAG CTG GGC TCG CAC CAC TGC CAG GAC CAC TCG GAT GAA GAG ACT CCA    599
thr ser asp glu leu glu gln phe ala lys gln phe lys gln arg arg ile lys leu gly    209
ACC TCT GAT GAG TTG GAA CAG TTC GCC AAA CAA TTC AAA CAA AGA AGA ATC AAG TTG GGT    659
phe thr gln ala asp val gly leu ala leu gly thr leu tyr gly asn val phe ser gln    229
TTC ACG CAA GCC GAC GTG GGG CTG GCA TTG GGC ACA CTG TAT GGC AAC GTG TTC TCG CAG    719
thr thr ile cys arg phe glu ala leu gln leu ser phe lys asn met cys lys leu lys    249
ACT ACC ATC TGC AGG TTC GAG GCC TTG CAA CTG AGC TTC AAG AAC ATG TGC AAG CTG AAA    779
pro leu leu asn lys trp leu glu glu ala asp•ser ser thr gly ser pro thr ser ile    269
CCA CTG CTA AAT AAG TGG CTG GAG GAG GCT GAT TCA TCC ACA GGA AGC CCG ACC AGC ATT    839
asp lys ile ala ala gln•gly arg lys arg lys lys arg thr ser ile glu val ser val    289
GAC AAG ATC GCC GCT CAG GGC CGT AAA CGC AAG AAG CGA ACC TCC ATT GAG GTG AGT GTC    899
lys gly val leu glu thr his phe leu lys cys pro lys pro ala ala gln glu ile ser    309
AAG GGC GTA CTG GAA ACG CAT TTC CTC AAG TGT CCC AAG CCT GCA GCA CAG GAG ATC TCC    959
ser leu ala asp ser leu gln leu glu lys glu val val arg val trp phe cys asn arg    329
TCG CTG GCA GAC AGT CTC CAG TTG GAG AAA GAA GTG GTA CGT GTC TGG TTC TGT AAT AGA   1019
arg gln lys glu lys arg met thr pro pro gly asp gln gln pro his glu val tyr ser    349
AGA CAA AAA GAA AAA AGA ATG ACT CCG CCA GGG GAT CAG CAG CCA CAC GAG GTT TAT TCG   1079
his thr val lys thr asp ala ser cys his asp leu OPA                               361
CAC ACG GTG AAA ACA GAT GCG TCC TGC CAC GAT CTC TGA  CTGGAGGAAGCAAGGAGGTGGCCCGC   1144
TGCACTGGGAGCAGTGCTTGATACAATTCTCTCTTTACCTT ...                                   1185
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FIG. 1. Nucleotide and amino acid sequences for the coding region of RHS2. The presumptive initial methionine is in boldface type, the POU domain is underlined, and a potential glycosylated Asn-121 is italicized. Solid circles separate the POU-specific, linker, and POU homeodomain portions of the POU domain.

distilled water and 70% ethanol. The dried sections were dipped in Ilford K5 emulsion, exposed 2.5 months, and developed.

Quantitation of *RHS2* Expression. To examine *RHS2* expression in rats with unilateral 6-hydroxydopamine injections into the substantia nigra, quantitative hybridization histochemistry was performed with a Fujix Bas2000 Image System (Fujifilm I & I, Stamford, CT). The intensity of labeling was measured in the caudate putamen and in the accumbens nucleus of the ipsilateral striatum and was compared to the contralateral side after subtraction of the background signal (n = 10). Values were expressed in arbitrary units of radioactivity. Statistical analyses were performed with a paired Student's t test.

RESULTS

The *RHS2* cDNA. This cDNA encodes a protein belonging to class III of the POU protein family (10). The *RHS2* insert is ≈3.8 kb long and contains an open reading frame of 1083 bp encoding 361 amino acids, beginning with the first methionine (Fig. 1), and predicting a molecular mass of ≈43 kDa. A potential N-linked glycosylation site is present at Asn-121. Northern analysis showed RHS2 transcripts of ≈3.8 kb in the rat brain but none was detected in skeletal muscle, liver, spleen, small intestine, thymus, kidney, testis, or lung (data not shown).

RHS2 mRNA Distribution During Development. RHS2 mRNA was detected in the rat from E11.5 onward. At this stage, RHS2 transcripts were prominent throughout the developing neuraxis, from the ventricular germinal zone to the marginal layer. At E13.5, RHS2 transcripts were conspicuous throughout the neuraxis and otic vesicles. By E15.5 (Fig. 2), RHS2 transcripts were largely confined to the central nervous system and otic vesicles but were also present in the outer sheath cells of the whisker roots. The same patterns held through E19.5 (Fig. 2), with the basal telencephalon (caudate putamen) being especially prominent. In the cere-

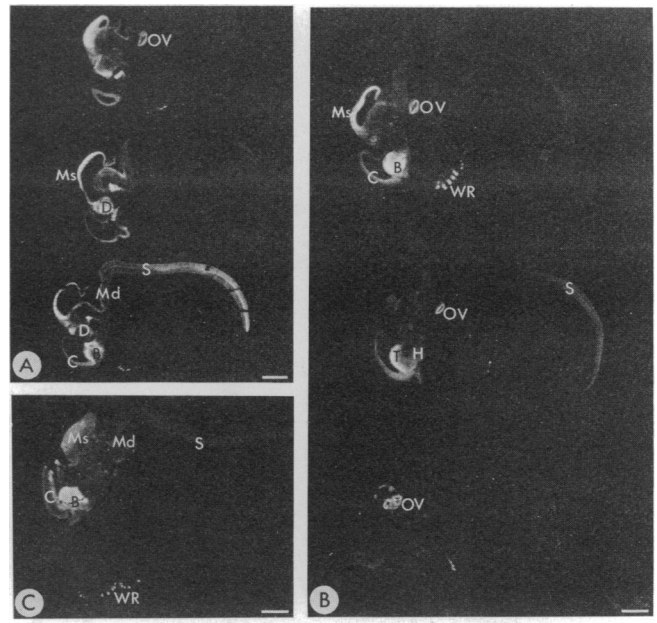

FIG. 2. Distribution of RHS2 mRNA in three sagittal sections from E15.5 (*A*), E17.5 (*B*), and E19.5 (*C*) rat embryos. Hybridization histochemistry was performed with a ^{35}S-labeled RNA probe from the original PVN19 PCR fragment. The top and bottom sections flank more laterally the center midline sections in *A* and *B*. RHS2 mRNA was present at high levels in the central nervous system, especially the cortex (C), basal telencephalon (B), diencephalon (D), mesencephalon (Ms), medulla (Md), spinal cord (S), thalamus (T), and hypothalamus (H), as well as in the otic vesicle (OV) and whisker roots (WR). Exposures were for 2 weeks. (Bars = 2 mm.)

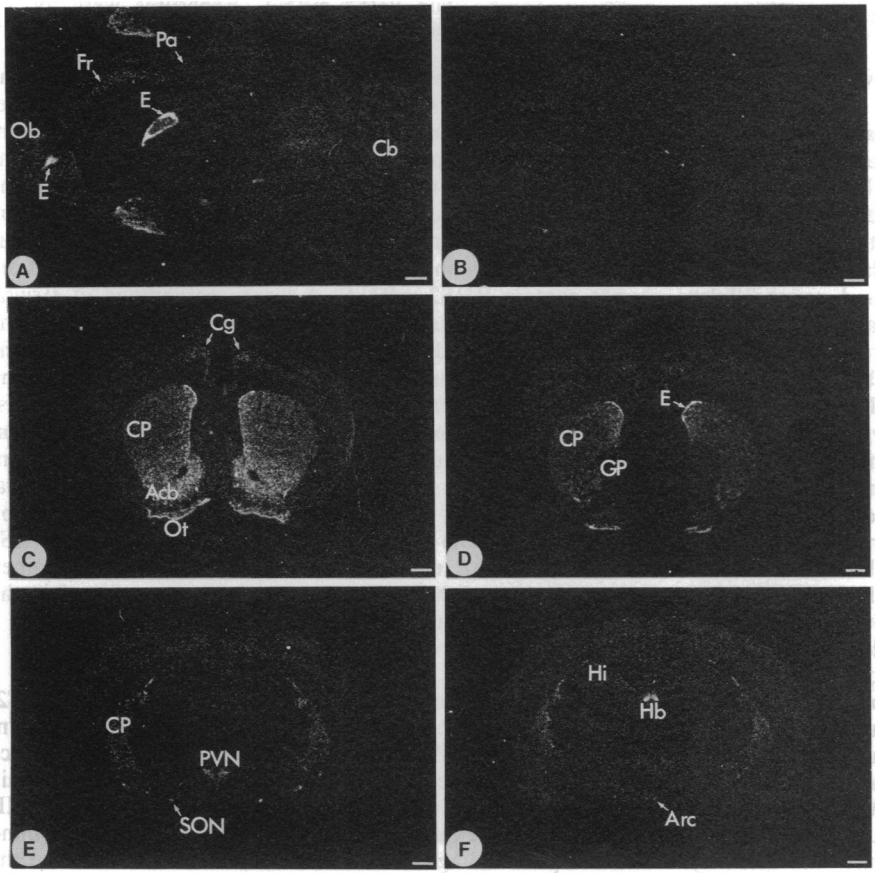

FIG. 3. Distribution of RHS2 mRNA in sagittal (*A* and *B*) and coronal (*C–F*) sections from adult rat brain. Hybridization histochemistry was performed with a ^{35}S-labeled RNA probe from the 515-bp *Nco* I restriction fragment. Antisense probes were used in *A* and *C–F*; sense probe was used in *B*. RHS2 mRNA was especially prominent in the ventricular ependyma (E), olfactory bulb (Ob), caudate putamen (CP), accumbens nucleus (Acb), olfactory tubercle (Ot; cell-dense layer and islands of Calleja), PVN and SON, habenula (Hb), and cingulate cortex (Cg). Lower levels of RHS2 mRNA were also detected in the frontal (Fr) and parietal (Pa) cortices, hippocampus (Hi), cerebellum (Cb), and arcuate nucleus (Arc). GP, globus pallidus. The sense probe (*B*) displayed only background levels of signal in a section adjacent to *A*. Exposures were for 2 months. (Bars = 1 mm.)

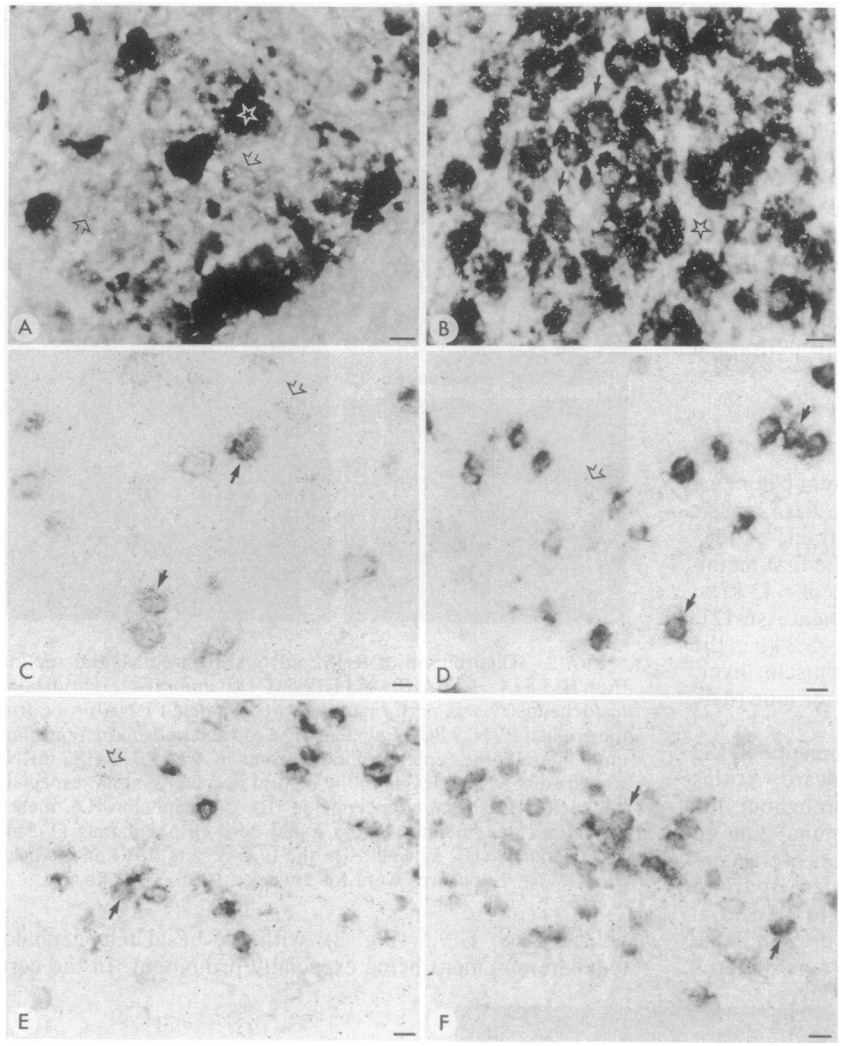

FIG. 4. Double simultaneous hybridization histochemistry for RHS2 mRNA using a ^{35}S-labeled RNA probe from the 515-bp *Nco* I fragment and for various neuropeptide mRNAs: oxytocin (*A*), dynorphin (*B* and *E*), substance P (*C*), enkephalin (*D*), and neurokinin B (*F*), using digoxigenin-labeled RNA probes. The digoxigenin-labeled cells have black staining and the radioactive cells display silver grains. (*A* and *B*) PVN and SON, respectively; (*C–E*) Caudate putamen; (*F*) olfactory tubercle. In *A*, RHS2 mRNA (open arrows) is not present in oxytocin mRNA-containing neurons (star). In *B*, RHS2 mRNA is present in neurons containing dynorphin mRNA (solid arrows). In the caudate putamen, subsets of enkephalin (*C*), substance P (*D*), and dynorphin (*E*) mRNA-containing neurons also contain RHS2 mRNA (solid arrows). Open arrows show neurons that contain only RHS2 mRNA. In *F*, most, if not all, neurokinin B neurons in the cell-dense clusters (except islands of Calleja) of the olfactory tubercle contain RHS2 mRNA. Exposures were for 2 months. (Bars = 10 μm.)

bral cortex, expression was present in the ventricular proliferative zone and cortical plate.

RHS2 mRNA Distribution in Adult Rat Brain. RHS2 mRNA was found prominently in the glomerular and internal granular layers of the olfactory bulb as well as the anterior olfactory nucleus, olfactory tubercle (cell dense layer, islands of Calleja, and cell dense clusters), cingulate cortex, neocortex (especially frontal and parietal), caudate putamen, accumbens nucleus, PVN (magnocellular and parvocellular neurons), SON, arcuate nucleus, medial habenula, and ventricular ependyma of the olfactory and lateral ventricles (Fig. 3). Within the striatum, the intensity of labeling was higher ventrally (accumbens nucleus) than dorsally (caudate putamen). RHS2 was also expressed in the amygdala, pyramidal, and granule cells of the hippocampal formation, parasubiculum, substantia nigra (pars compacta), and cerebellar Purkinje and granular cells. A sense ^{35}S-labeled probe gave only background signals on adjacent sections in all these areas.

To learn more about cells in the hypothalamus, caudate putamen, and accumbens nucleus in which the RHS2 POU protein was expressed, we developed a double-labeling procedure using ^{35}S- and digoxigenin-labeled RNA probes to identify neuropeptide transcripts in neurons expressing the *RHS2* gene (Fig. 4). Double-labeling experiments showed that RHS2 mRNA was not detected in neurons of the PVN or SON containing oxytocin mRNA. In contrast, RHS2 mRNA was detected in magnocellular neurons of the PVN and SON that contained dynorphin mRNA. In the striatum, RHS2 mRNA was detected mainly in medium-sized neurons, with a higher level of expression ventrally and medially (along the lateral ventricle and in the accumbens nucleus) as compared to dorsally (in the caudate putamen). Double-labeling experiments showed that RHS2 mRNA was present in subsets of enkephalin, substance P, and dynorphin neurons that represent the main populations of striatal neurons. At the level of exposure we used, there was no selective localization of RHS2 mRNA in these populations as a significant proportion of enkaphalin, substance P, and dynorphin neurons did not contain RHS2 mRNA. A larger percentage of striatal neurokinin B neurons contained RHS2 transcripts than the other neuropeptidergic neurons and most, if not all, of the neurokinin B neurons in the cell-dense clusters (non-island cells) of the olfactory tubercle contained RHS2 transcripts.

Effects of Dopamine Depletion on *RHS2* Gene Expression. As expected, after nigral 6-hydroxydopamine lesions, enkephalin mRNA levels were increased in the ipsilateral caudate putamen and accumbens nucleus, respectively, by 108% ($P < 0.001$) and 43% ($P < 0.01$). The amount of RHS2 mRNA was also increased in the ipsilateral caudate putamen by 30% ($P < 0.001$), whereas no change was found in the accumbens nucleus.

DISCUSSION

The newly discovered POU domain-containing cDNA, *RHS2*, based on sequence similarities within the POU domain, encodes a class III POU transcription factor (10). The sequence homologies at the amino acid level within the POU domain range from 92% to 95% with the other mammalian class III POU proteins expressed in the brain. The predicted 361 amino acid open reading frame of the RHS2 clone is based on the

assignment of the initial methionine by consensus (24). Remarkably, our sequence exhibits 100% amino acid identity with the mouse homolog, Brain-4 (25). XLPOU2 appears to be the *Xenopus* homolog, having 89.7% identity over the published sequence (26). Interestingly, RHS2, unlike other class III POU proteins with available sequence information [e.g., SCIP (27) and Cf1-a (9)], has no extensive homopolymeric stretches or over-representations of particular amino acids.

Despite the sequence similarities with *Brain-1*, *Brain-2*, *SCIP*, and *RHS1* [another putative class III POU domain-containing cDNA isolated in our laboratory (ref. 28; GenBank accession no. M84644)], and despite regions of overlap in expression, *RHS2* exhibits unique expression patterns in developing rat and adult rat brain. For example, *RHS2* is prominently expressed in the striatum, unlike *Brain-1* and *Brain-2*, and in the PVN, SON, and ventricular ependyma, unlike *SCIP* (*Tst-1*) (10). These unique patterns of expression, as well as different transcript sizes on Northern analyses reported by others, show that our probe is unlikely to be cross-hybridizing with these members of the POU family.

The developmental patterns of expression of *RHS2* in the neuraxis suggest that *RHS2* may be involved in development of neuronal phenotypes. For example, in the E19.5 cerebral cortex (Fig. 2), expression is found in areas of proliferating neuroblasts (ventricular proliferative zone) as well in areas of neuronal differentiation (cortical plate). Similar cell specification of peripheral tissues like the otic vesicles and the vibrissae may also be regulated by *RHS2*. Such roles have already been suggested for other POU proteins expressed in adult and developing brain (10). In many areas, such as the cortex, the degree of expression wanes postnatally. There may be a reduction in the number of cells expressing *RHS2*, a reduction in the level of expression per cell, or a dilution by nonexpressing cells. Detailed qualitative and quantitative studies should allow us to choose among these possibilities.

The selective expression of *RHS2* in the nonoxytocin, dynorphin (i.e., vasopressin) magnocellular neurons of the PVN and SON suggests a possible role for RHS2 in regulation of expression of vasopressin, dynorphin, or coexpressed genes in the adult. In contrast, *RHS2* is not restricted to a single peptidergic population (e.g., enkephalin, substance P, neurokinin B, or dynorphin neurons) in the caudate putamen or accumbens nucleus. These observations suggest that actions of *RHS2* alone or in concert with other transcription factors (DNA-binding or POU proteins) may define additional neuronal subsets in the striatum, as has been suggested (3). It is also possible that these subsets could receive and/or dispatch signals in a unified fashion. To learn whether *RHS2* expression can be regulated in the adult, we investigated whether *RHS2* expression is under dopaminergic control of the nigrostriatal pathway. As expected (19), 6-hydroxydopamine lesions elevated enkephalin expression ipsilaterally in the caudate putamen and accumbens nucleus. *RHS2* expression in adjacent sections was increased in the caudate putamen, suggesting a role for *RHS2* in regulation of gene expression mediated by dopamine. Since lesions of the substantia nigra mainly affect dopaminergic projections to the caudate putamen and the increase in enkephalin expression was lower in the accumbens nucleus, we cannot exclude the fact that a more extensive dopamine depletion of the accumbens nucleus would elevate *RHS2* expression there. Further experiments with lesions that include the ventral tegmental area, which projects mainly to the accumbens nucleus, should answer this question. It is possible that selective manipulation of the dopaminergic innervation of the striatum with selective agents will identify responsive subsets of neurons that express *RHS2*—e.g., those that express a particular dopamine receptor subtype (17, 29). In any case, our results suggest that *RHS2* is involved, directly or indirectly, in striatal gene regulation by dopamine.

Our anatomical, especially double-labeling, and physiological data suggest potential genes that may be regulated by RHS2. If RHS2 indeed proves to be a DNA-binding protein, it will be important to further examine those genes with which it is coexpressed for the presence and functionality of the cognate cis-acting element.

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