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Expression Profile and Up-Regulation of Prax-1 mRNA by Antidepressant Treatment in the Rat Brain

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ABSTRACT

A protein associated with the peripheral-type benzodiazepine receptor (PRAX-1) has recently been cloned, but its regional distribution in the central nervous system and its function remain to be clarified. In situ hybridization was carried out to localize PRAX-1 mRNA in the rat brain and revealed a high expression of the transcript in limbic structures such as the CA1 region of the hippocampus, as well as the dentate gyrus, septum, amygdala, and the islands of Calleja. A dense hybridization signal was also observed in the nucleus accumbens, caudate nucleus, olfactory tubercle, pineal gland, and cerebellar cortex. PRAX-1 mRNA expression was largely neuronal; it colocalized with neuron-specific enolase but not glial fibrillary acidic protein. Long-term treatments (21 days) with the neuroleptic haloperidol increased PRAX-1 mRNA expression only in the dentate gyrus, whereas anxiolytic/anticonvulsant diazepam had no effect in any of the hippocampal region studied. Repeated electroconvulsive shock administration significantly enhanced PRAX1 expression in the CA1 subfield and dentate gyrus. Several classes of antidepressant treatment, including serotonin selective reuptake inhibitor (fluoxetine), mixed serotonin- and norepinephrine-uptake inhibitor (imipramine), and monoamine oxidase inhibitors (iproniazid and tranylcypromine), shared this effect. Furthermore, the selective nonpeptide NK2 receptor antagonist (*S*)-*N*-methyl-*N*-[4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide (SR48968), which shows an antidepressant profile in animal studies, also enhanced PRAX-1 mRNA expression. These results point to a potential role of PRAX-1 function in the central nervous system and suggest that the up-regulation of PRAX-1 mRNA represents a common action of chronic antidepressant treatment.

PRAX-1 has recently been isolated by the yeast two-hybrid system, using the peripheral-type benzodiazepine receptor (PBR) as bait (Galiegue et al., 1999). This 1857-amino acid protein is a single 220- to 250-kDa entity and is encoded by a 7.5-kilobase mRNA that is highly expressed in the central nervous system and at lower levels in some peripheral tissues. PRAX-1 was shown to interact with PBR in several cell lines, but this interaction has not yet been demonstrated in the central nervous system (CNS). Subcellular localization studies of PBR have shown that it is linked to mitochondria in the rat brain (Basile and Skolnick, 1986), as well as in the rat adrenal gland (Anholt et al., 1986) and many peripheral organs (Antkiewicz-Michaluk et al., 1988). More recently, the use of confocal microscopy further confirmed the abundant mitochondrial localization of PBR (Garnier et al., 1993). PRAX-1 protein was shown to be present in both mitochondrial and cytoplasmic compartments (Galiegue et al., 1999). A comparison of PRAX-1 and PBR expression profiles revealed that some tissues, like lung or testis, express PBR but are devoid of detectable PRAX-1 expression (Burgi et al., 1999). Furthermore, immunohistochemical analysis proved this protein to be highly expressed in the hippocampus, which demonstrates a very low level of PBR expression (Anholt et al., 1985). The function of PRAX1, particularly in the hippocampus, remains unknown.

The hippocampus is involved in many physiological and adaptive processes, including the long-term effects of stress and antidepressant drug efficacy (Duman et al., 1999; McEwen, 1999). Disturbed functioning of serotonin (5HT) and noradrenaline (NE) circuits may contribute to the cause of depression. Two main classes of antidepressants were discovered in the early 1950s: the tricyclic antidepressants and the monoamine oxidase inhibitors (reviewed by Nestler, 1998), both of which result in significant increases in synaptic con-

P.R. and C.R. contributed equally to this work.

ABBREVIATIONS: PRAX-1, peripheral benzodiazepine receptor-associated protein 1; CNS, central nervous system; 5HT, serotonin; NE, noradrenaline; CREB, cAMP response element-binding protein; BDNF, brain-derived neurotrophic factor; ECS, electroconvulsive shock; DG, dentate gyrus; SR48968, (*S*)-*N*-methyl-*N*-[4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl) butyl]benzamide; SR48965, (*R*)-*N*-methyl-*N*-[4-acetylamino-4-phenypiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide; RT-PCR, reverse transcription-polymerase chain reaction; NSE, neuron-specific enolase; PBS, phosphate-buffered saline; dig, digoxigenin; SSC, standard saline citrate buffer; GFAP, glial fibrillary acidic protein; TNT, Tris-HCl/NaCl/Tween 20; TSA, tyramide signal amplification; HRP, horseradish peroxidase; PBR, peripheral-type benzodiazepine receptor.

centration of the monoamines NE and 5HT. However, a lag time of several weeks is observed before the onset of the clinical efficacy of these antidepressants, suggesting that their mechanism of action requires neuronal adaptations. These adaptations may be caused by indirect regulation of other transduction systems or even changes at the molecular level by gene transcription. Indeed, chronic antidepressant administration was recently described to increase neurogenesis in adult rat hippocampus (Malberg et al., 2000). Recent developments in molecular neurobiology have identified many intracellular targets that are regulated by antidepressant treatment and have provided new tools to elucidate the mechanisms by which antidepressant produce their adaptive alterations in brain function.

Many of the recently identified genes involved in depression were found to be up-regulated in the hippocampus of animals undergoing long-term treatment with antidepressants. Examples are regulatory elements such as CREB (Nibuya et al., 1996; Chen et al., 2001), immediate-early genes such as *NGFI* (Johansson et al., 1998), neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and its receptor trkB (Nibuya et al., 1995), or newly identified genes such as *ADRG34* (Yamada et al., 2000).

The aim of this study was to provide a detailed analysis of PRAX-1 mRNA distribution in the rat brain to try to identify its function. Our results demonstrated that, at least in the hippocampus, PRAX-1 up-regulation may be a defining characteristic common to the chronic administration of several different types of antidepressants but not other psychotropic drugs.

Materials and Methods

Animals and Treatments. Male Sprague Dawley rats (Charles River PharmServices Europe, Lyons, France) weighing 120 to 140 g were housed in groups of two to three and maintained on a constant dark/light cycle (lights on 7:00 AM–5:00 PM) with food and water available ad libitum. All procedures were approved by the Comité d'Experimentation Animal (Animal Care and Use Committee) at Sanofi-Synthelabo Recherche and were carried out in accordance with French legislation that implemented the European directive 86/609/EEC.

For long-term electroconvulsive treatment, rats ($n > 4$ per group) were given one electroconvulsive shock (ECS; 150 Hz, 0.1 s) per day each second day for 10 days or received sham treatment (handled identically to those that received ECS but without electrical stimulation). For short-term ECS treatment, the same shock was applied and rats were killed 4 h after the shock. For long-term drug treatments, rats ($n > 4$ per group) received fluoxetine (5 mg/kg) for 7, 14, or 21 days, or imipramine (15 mg/kg), tranylcypromine (10 mg/kg), iproniazid (10 mg/kg), haloperidol (2 mg/kg), diazepam (5 mg/kg), SR48968 (1 mg/kg), SR48965 (1 mg/kg), or saline (0.9% NaCl). Animals were decapitated 24 h after the last treatment; the brains were removed, frozen immediately on dry ice, and stored at −80°C. Sections (12 μm) were cut on a cryostat, mounted on microscope slides, and kept at −80°C until further study.

All the drugs were obtained from Sigma (St. Quentin-Fallavier, France) except SR48968 and SR48965, which were provided by Sanofi-Synthelabo Recherche (Montpellier, France).

Probe Synthesis. Riboprobes were isolated by RT-PCR (Access RT-PCR system; Promega France, Charbonnieres, France) using rat total RNA (RNAgents, Promega France). The rat *Eco*RI-*Hin*dIII cDNA fragment (539 base pairs) corresponding to nucleotides 4483 to 5021 of the human PRAX-1 cDNA (GenBank accession no. AF039571) was subcloned into the transcription vector pGEM 4Z (Promega France). The rat PBR riboprobe corresponding to a 580 base-pair fragment was subcloned in pGEM 4Z vector (GenBank accession no. NM_012515, fragment 53–632). For neuron-specific enolase (NSE; GenBank accession no. AF19973), a 650 base-pair fragment was amplified by RT-PCR using specific NSE primers (sense, 5′-CAA ACA GCG TTA CTT AGG-3′; antisense, 5′-GCT GGT CCC CAG TGA TGC ATC-3′. This RT-PCR product was subcloned in pCR II-TOPO vector (Invitrogen, Carlsbad, CA) and transformed according to the manufacturer's instructions. Clones bearing the riboprobes were isolated and sequenced.

Double Fluorescent in Situ Hybridization. Cryostat sections (12 μm) were heated for 15 min at 60°C and fixed for 1 h with 3.7% formaldehyde in phosphate-buffered saline (PBS), rinsed twice with PBS and dehydrated through a graded series of ethanol concentrations (50–70%). The riboprobes were labeled using T7 and sp6 polymerases (Promega France) using biotin- or dig-UTP (Roche Molecular Biochemicals, Mannheim, Germany). PRAX-1 (biotin-labeled) and NSE (dig-labeled) riboprobes (100 ng) were heat denatured and diluted in 100 μl of hybridization buffer (containing 50% formamide, 0.6 M NaCl, 10 mM Tris, 2 mM EDTA, 1× Denhardt's solution, 10% dextran sulfate, 250 μg/ml yeast tRNA) and added together to each slide. The sections were incubated overnight at 60°C. Slides were washed twice in 1× standard saline citrate (SSC) buffer with 50% formamide and 0.1% Tween 20 for 30 min, then rinsed twice in TNT buffer (0.1 M Tris-HCl, pH 7.4, 0.15 M NaCl, and 0.1% Tween 20) and treated with RNase (10 μg/ml) in 1× SSC buffer (1 h at 37°C). Endogenous peroxidase activity was quenched with 1% H_2O_2 in PBS. After that, the two probes were then detected sequentially. Slides were blocked (with the blocking agent provided in the TSA system kit; PerkinElmer Life Sciences, Boston, MA) and incubated with anti-digoxigenin horseradish peroxidase (HRP)-antibody conjugate (Roche Molecular Biochemicals) overnight at 4°C. Slides were washed in TNT and the conjugate was detected using the manufacturer's instructions (TSA-fluorescein system; PerkinElmer Life Sciences). Then slides were treated with 1% H_2O_2 in PBS to quench the residual HRP activity. The biotin PRAX-1 probe was detected with Streptavidin-HRP and the TSA-cyanin 3 system (PerkinElmer Life Sciences). For in situ hybridization coupled to glial fibrillary acidic protein (GFAP) detection, slides were hybridized with biotin–PRAX-1 probe and detected using a TSA-fluorescein system (PerkinElmer Life Sciences) as described above. Subsequently, immunofluorescence was carried out using anti-GFAP coupled to cy3 (1/1000; Sigma) for 1 h at 20°C in the same blocking reagent and rinsed with TNT.

Slides were mounted in Fluoromount (Southern Biotechnology Associates, Birmingham, AL) and analyzed using a confocal microscope (Leica, Wetzlar, Germany). Z assignments were kept constant for double labeling. Photomultiplier tube assignments and pinhole size were kept constant across different confocal sessions.

Colorimetric in Situ Hybridization. In situ hybridization was carried out as described above. Hybridized neurons were detected by exposing the sections to anti-digoxigenin antibodies conjugated to alkaline phosphatase (Roche Molecular Biochemicals) overnight at a dilution of 1:1000 and revealed using a mixture containing nitro blue tetrazolium (Roche Molecular Biochemicals) and 5-bromo-4-chloro-3-indoyl phosphate, toluidine salt (Roche Molecular Biochemicals).

In Situ UTP-^{35}S Hybridization. Analysis of PRAX-1 mRNA expression was performed by in situ hybridization as described previously (Hoefler et al., 1986). Briefly, coronal brain sections (12 μm) were pretreated successively in 4% paraformaldehyde for 20 min, 0.3% Triton X-100 for 15 min, and 0.25% acetic anhydride in triethanolamine (0.1 M, pH 8) for 10 min to reduce background in the autoradiographs and prehybridized in preheated (37°C) 50% formamide/2× SSC for 10 min. Sections were then hybridized with ^{35}S-labeled PRAX-1 riboprobes (2.5×10^6 cpm/section) for 15 h at 55°C in buffer containing 50% formamide, 0.6 M NaCl, 10 mM Tris, 2 mM EDTA, 1× Denhardt's solution, 10% dextran sulfate, 250 μg/ml yeast tRNA, and 50 μg/ml salmon sperm DNA. The sections were washed

in 2× SSC at 25°C and then treated with 20 μg/ml RNase A for 30 min in 0.5 M NaCl, 10 mM Tris, and 1 mM EDTA. The sections were then washed with 2× SSC for 10 min at 25°C, 1× SSC for 10 min at 25°C, 1× SSC for 10 min at 55°C, 0.5× SSC for 10 min at 55°C, 0.5× SSC for 10 min at 25°C. Sections were dehydrated in 30, 70, 95, and 100% alcohol and exposed to Kodak BioMax MR film (Eastman Kodak, Rochester, NY). The specificity of the hybridization was confirmed by demonstrating that ^{35}S-labeled sense PRAX-1 riboprobe did not yield any significant hybridization signal (data not shown).

Data Analysis. The levels of PRAX-1 mRNA were determined by densitometric quantification of autoradiograms by outlining the regions of interest of the hybridization sections: the dentate gyrus (DG) and the CA1 and CA3 regions of the hippocampus were quantified with an image analysis program (Autoradio ver. 4.03; Samba Technologies, Grenoble, France). For analysis, six to eight measurements from adjacent sections of the same animals were averaged.

Experiments containing three groups of rats (n = 4–5 per group) were subjected to analysis of variance, with a significance level of $p <$

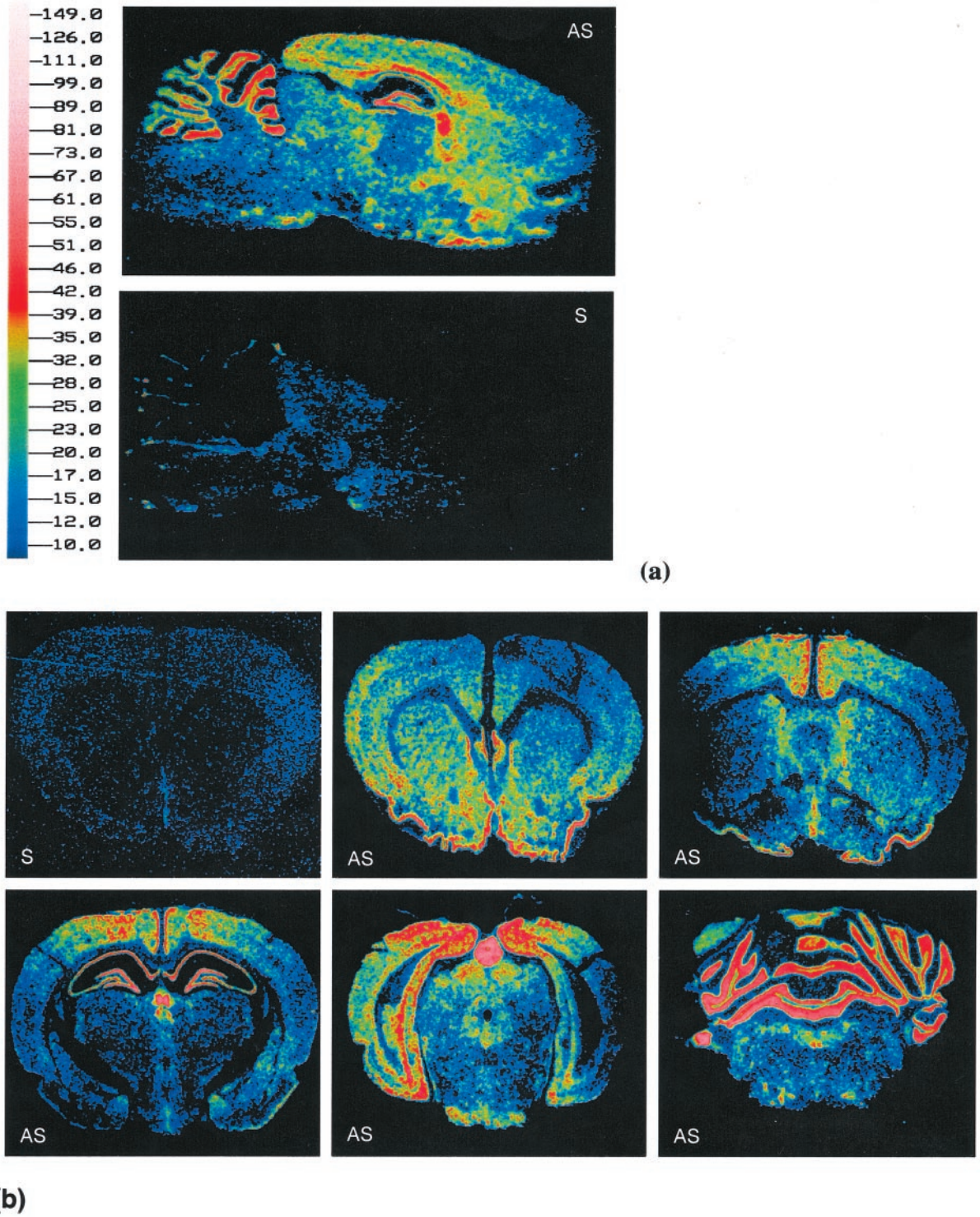

Fig. 1. Localization of PRAX-1 mRNA. Representative autoradiograms of the distribution of PRAX-1 mRNA depicted on sagittal (a) and coronal (b) sections of rat brain. The false color scale indicates the density of labeling. S, labeling achieved with sense probe; AS, specific PRAX-1 labeling.

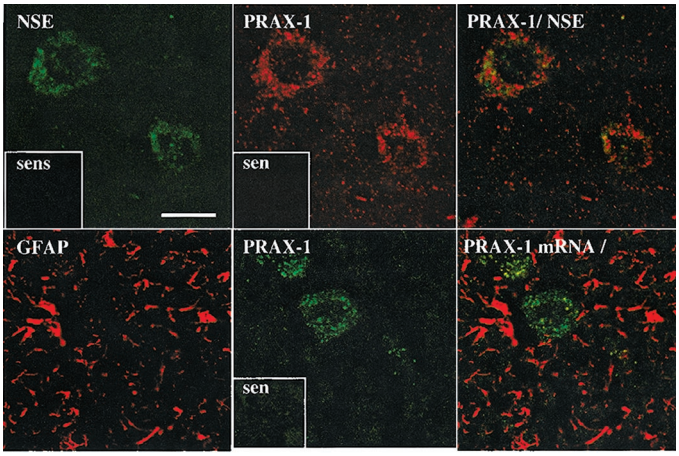

Fig. 2. PRAX-1 mRNA is expressed in neurons. Top row, colocalization of PRAX-1 mRNA and NSE mRNA in the rat cortex; NSE mRNA is labeled with fluorescein and PRAX -1 mRNA is labeled with Cy3. Bottom row, coupled immunofluorescence/in situ hybridization analysis; PRAX-1 mRNA is labeled with fluorescein and GFAP immunofluorescence is obtained using anti-GFAP Cy3-coupled antibody. Images are examined using confocal image analysis (magnification, 120×); insets correspond to labeling performed with the sense-probe. Scale bar, 10 μm.

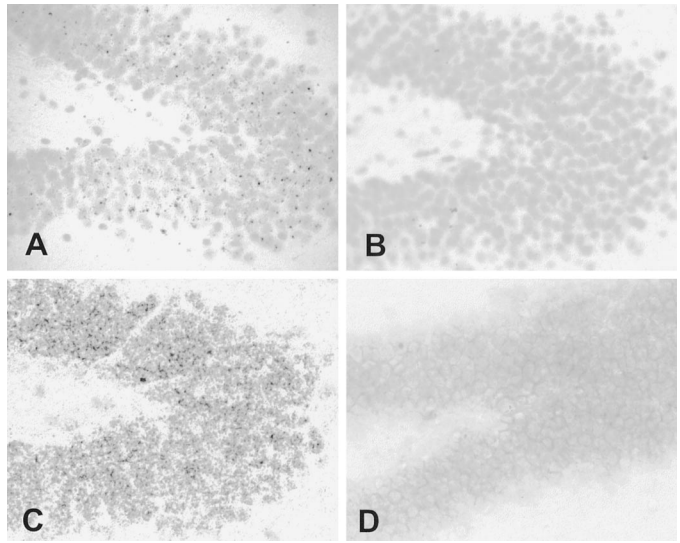

Fig. 3. Different pattern of expression of PBR mRNA and PRAX-1 mRNA in the DG region of the hippocampus. Detection was performed by in situ hybridization using dig-labeled probes. A, PBR mRNA; B, labeling achieved with the PBR sense-probe; C, PRAX-1 mRNA; D, labeling achieved with the PRAX-1 sense-probe.

0.05, whereas an experiment containing two groups (n = 4–5 per group) was subjected to Student's t test, with significance determined at the $p < 0.05$ level.

Results

The distribution of PRAX-1 mRNA in the rat brain was determined using in situ hybridization. The sagittal sections (Fig. 1A) consistently show significant levels of PRAX-1 mRNA throughout the CNS, with certain brain regions exhibiting a particularly high signal. Hybridization performed with the corresponding sense probe did not yield any labeling. In coronal sections of the rat brain (Fig. 1B), the highest levels of expression were seen in the piriform cortex, islands of Calleja, olfactory tubercles, hippocampus, habenular nuclei, pineal gland, and cerebellum. Moderate expression was seen in cingular and retrosplenial cortices, septum, accumbens nucleus, periaqueductal gray, and pontine nuclei.

In an attempt to define the cell type in which PRAX-1 mRNA is expressed, its cellular localization was studied using double fluorescent in situ hybridization on sections from the hippocampus (Fig. 2). The results show that the PRAX-1 transcript was colocalized with NSE, indicating that PRAX mRNA is expressed in the neuronal cells of the rat brain. On the contrary, colocalization studies using in situ hybridization coupled to the glial marker GFAP showed no detectable colabeling in the hippocampus (Fig. 2) or any other structure examined, indicating that PRAX-1 expression was largely if not exclusively neuronal. Furthermore, compared with PRAX-1 mRNA, PBR mRNA expression, a transcript known to be expressed in glial cells, yields a very distinct pattern of expression in sections of the hippocampus (Fig. 3) as well as in other brain areas studied (including thalamus, hypothalamus, cortex) suggesting that PRAX-1 and PBR are not overall colocalized in the same cells.

To gain insight into PRAX-1 function in the rat brain, we attempted to modulate PRAX-1 mRNA expression by an array of pharmacological agents or treatments.

First, chronic administration of several non-antidepressant drugs, including haloperidol (a typical neuroleptic) and diazepam (an anticonvulsant-anxiolytic), did not modify the expression of PRAX-1 mRNA in the CA1 and CA3 (Table 1). Only haloperidol yielded a modest up-regulation of this gene in the DG (19 ± 2%).

Next, the influence of ECS on the expression of PRAX-1 mRNA was examined. Four hours after acute ECS, no mod-

TABLE 1

Effects of various chronic treatments on PRAX-1 mRNA in the hippocampus

Rats were administered imipramine, iproniazid, tranylcypromine, haloperidol, diazepam, SR48968, or saline for 21 days and subjected to in situ hybridization analysis using ^{35}S-labeled PRAX-1 riboprobes. Quantification of PRAX-1 mRNA in the DG and CA1 and CA3 regions of the hippocampus was performed by optical densitometry. Values are expressed as percentage of saline and represent mean ± S.E.M., n = 4 to 5 rats per group.

	Dentate Gyrus Granule cell layer	CA1 Pyramidal cell layer	CA3 Pyramidal cell layer
		%	
Saline	100 ± 3	100 ± 9	100 ± 11
Fluoxetine	149 ± 12*	173 ± 25*	90 ± 7
Imipramine	155 ± 8*	214 ± 11*	93 ± 5
Iproniazid	119 ± 7	157 ± 18*	136 ± 12*
Tranylcypromine	118 ± 7*	186 ± 17*	83 ± 8
Haloperidol	119 ± 2*	101 ± 7	101 ± 6
Diazepam	103 ± 5	93 ± 9	83 ± 13
SR48968	161 ± 8*	150 ± 11*	103 ± 5

*, $p < 0.05$ versus saline-treated rats (Student's t test).

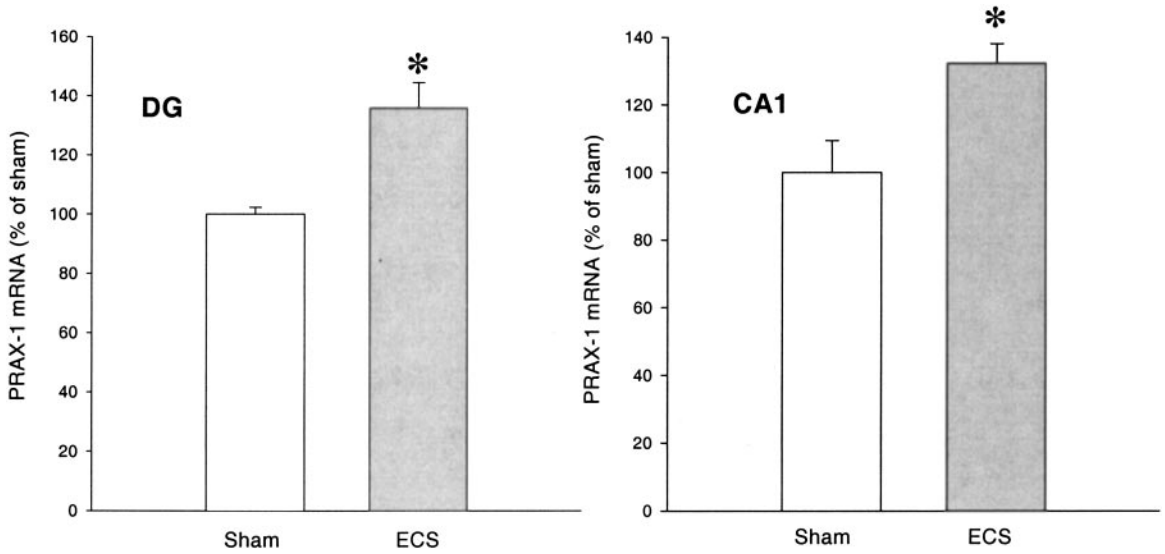

Fig. 4. Regulation of PRAX-1 mRNA in DG and CA1 regions of the hippocampus by chronic ECS treatment. PRAX-1 mRNA was quantified by optical densitometry after in situ hybridization using ^{35}S-labeled PRAX-1 riboprobes. Values are expressed as percentage of sham and represent mean ± S.E.M., $n = 4$ per group. *, $p < 0.05$ versus sham treatment (Student's t test).

ification of PRAX-1 mRNA expression in rat hippocampus was observed (data not shown). In contrast, repeated administration of ECS (five sessions over ten days) increased levels of PRAX-1 mRNA in the dentate gyrus (36 ± 9%) and CA1 region of hippocampus (32 ± 6%) (Fig. 4).

Long-term (21 days) administration of fluoxetine (a selective serotonin-reuptake inhibitor) increased the expression of PRAX-1 mRNA in both DG (49 ± 12%) and CA1 region (73 ± 25%) of the hippocampus (Fig. 5), whereas a 7- (not shown) or 14-day treatment was without effect in both regions. Furthermore, no modifications were observed in other structures examined, such as the hypothalamus, the frontal cortex, and the habenular nucleus (data not shown).

Long-term administration (21 days) of several different types of antidepressant drugs increased levels of PRAX-1 mRNA in the CA1 and DG (Table 1). The antidepressant drugs tested included imipramine, a nonselective 5-HT and NE reuptake inhibitor, and tranylcypromine and iproniazid, two monoamine oxidase inhibitors. Only chronic treatment with iproniazid affected PRAX-1 mRNA in the CA3 region of the hippocampus (Table 1).

Finally, we studied SR48968, a selective neurokinin NK2 receptor antagonist active in neurochemical and behavioral models sensitive to known antidepressants. As illustrated in Fig. 6, a 21-day treatment with SR48968 (1 mg/kg) induced an increase of PRAX-1 mRNA expression in both the DG (61 ± 8%) and the CA1 regions (50 ± 11%) of the hippocampus. In contrast, SR48965, the optical antipode of SR48968, which is devoid of affinity for NK2 receptors, was unable to modulate PRAX-1 mRNA expression.

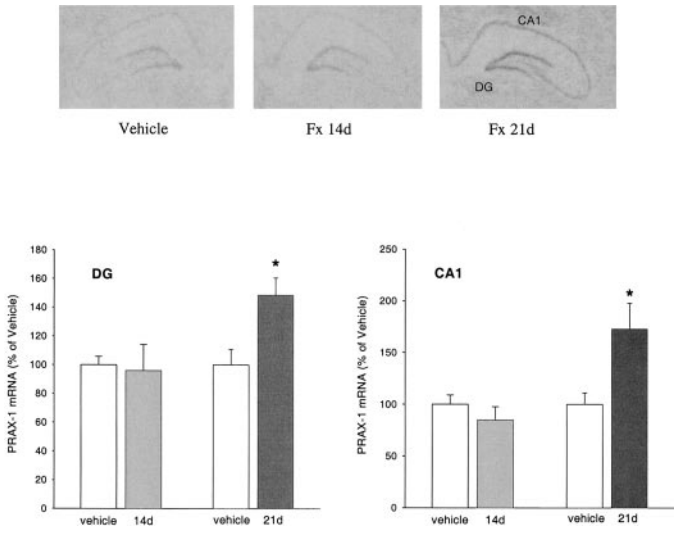

Fig. 5. Long-term fluoxetine (Fx) administration increases levels of PRAX-1 mRNA in hippocampus after a 21-day (21d) treatment but not a 14-day (14d) treatment. Representative autoradiograms for each of the conditions are shown. The DG and CA1 regions of the hippocampus are indicated. PRAX-1 mRNA was quantified by optical densitometry after in situ hybridization using ^{35}S-labeled PRAX-1 riboprobes. Values are expressed as percentage of saline and represent mean ± S.E.M., $n = 4$ per group. *, $p < 0.05$ versus saline-treated rats (Student's t test).

Discussion

In the yeast two-hybrid system as well as in several cell lines, PRAX-1 has been shown to interact with the PBR (Galiegue et al., 1999). Although PRAX-1 mRNA was known to be expressed in the CNS, its detailed distribution and function remained unknown. The present study partially replies to these questions by reporting on the regional and cellular localization of PRAX-1 mRNA and the pharmacological modulation of its expression.

PRAX-1 mRNA showed a distinctive distribution in the rat brain, being particularly enriched in certain regions of the limbic system, including the nucleus accumbens, septum, and hippocampus, as well as on the cerebellum and pineal gland. Thus, even though PRAX-1 originally was identified as a binding partner for PBR, its brain distribution is clearly distinct from that of PBR (Richards and Möhler, 1984). This observation indicates that the main function of PRAX-1 in the CNS may not be associated with the PBR.

The cellular localization of PBR has been studied extensively and is mainly confined to glia (Richards and Möhler, 1984; Kuhlmann and Guilarte, 2000). To study the cellular

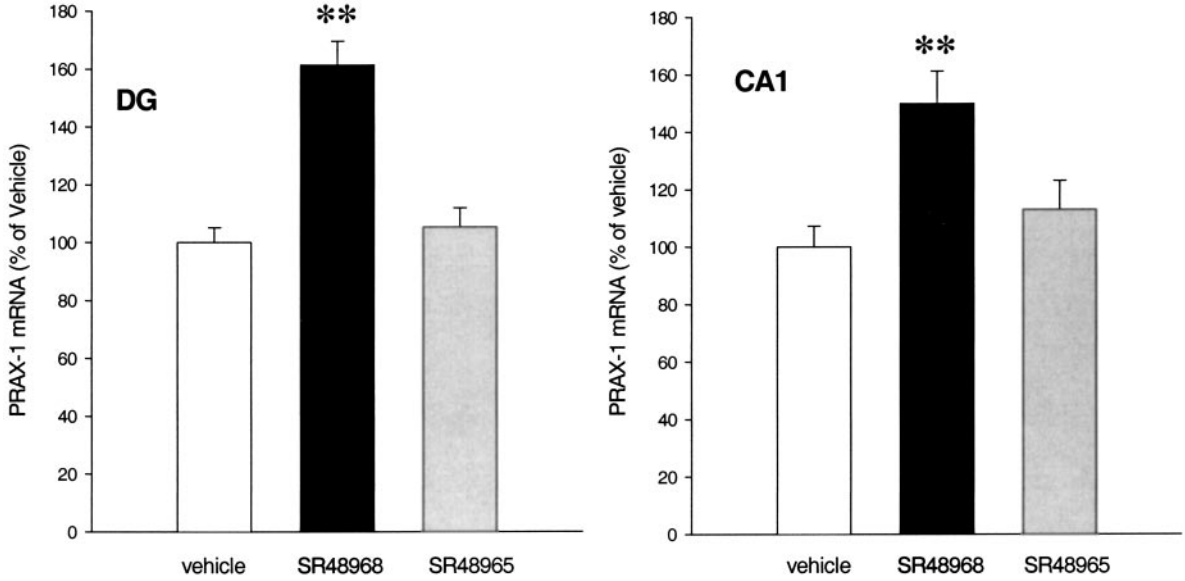

Fig. 6. PRAX-1 mRNA increases after long-term SR48968 treatment and but not after treatment with its inactive enantiomer SR48965. SR48968 and SR48965 were administrated for 21 days. PRAX-1 mRNA was quantified by optical densitometry after in situ hybridization using ^{35}S-labeled PRAX-1 riboprobes. Values are expressed as percentage of saline and represent mean ± S.E.M., $n = 4$ per group. **, $p < 0.01$; *, $p < 0.05$ versus compared with control (analysis of variance).

distribution of PRAX-1 mRNA, we concentrated our efforts on the hippocampus, because this region is rich in PRAX-1 mRNA expression and poor in PBR and, as part of the limbic system, is intimately involved in CNS pathologies. PRAX-1 transcript colocalized with NSE but not with GFAP, suggesting that PRAX-1 expression is largely, if not exclusively, neuronal. In the hippocampus, the study of PBR expression, known to be largely glial, yields a different pattern of expression compared with PRAX-1 mRNA, thus confirming that, at least in this brain region, the function of PRAX-1 is independent of PBR.

The localization of PRAX-1 mRNA in limbic regions, implicated in higher brain function, suggests that PRAX-1 might play a role in pathophysiology of the CNS. This hypothesis is also supported by its expression in the pineal gland. The pineal hormone melatonin regulates the circadian rhythm of many functions, and alteration of this secretory pathway has been found in various psychiatric disorders, including seasonal affective disorder, bipolar disorder, and unipolar depression (Pacchierotti et al., 2001).

Thus, to elucidate the functional role of PRAX-1, we studied its potential regulation by therapeutic treatment for the major psychiatric disorders, anxiety (diazepam), schizophrenia (haloperidol), and depression, concentrating on the hippocampus. For depression, we initially chose a repeated ECS treatment generally recognized as one of the most efficacious approaches for this disorder. No effect on PRAX-1 mRNA expression was observed after long-term administration of haloperidol and diazepam, with the exception of a slight increase in the DG for haloperidol.

However, long-term ECS, but not short-term ECS, increases the expression of PRAX-1 mRNA in the rat hippocampus. To examine whether PRAX-1 is implicated in the antidepressant effects of ECS, we extended our studies to the effect of other antidepressant treatments on PRAX-1 mRNA levels.

Levels of PRAX-1 mRNA were increased after administration of several classes of antidepressants with different modes of action, including 5HT and NE transporter blockers as well as monoamine oxidase inhibitors. Up-regulation of PRAX-1 mRNA was observed after long-term, but not short-term, administration of antidepressants, consistent with the time course for the therapeutic action of these drugs. As described previously for CREB, BDNF, and TrkB mRNA (Nibuya et al., 1996), this effect was more pronounced in the CA1 subfield than in the DG region. No differences were observed in the CA3 region of the hippocampus, except after treatment with the monoamine oxidase inhibitor iproniazid. This discrepancy is in contrast with the results obtained with other genes (i.e., *CREB*, *TrkB,* and *BDNF*) regulated by chronic antidepressant treatment in the CA3 region (Nibuya et al., 1996), suggesting decreased responsiveness of PRAX-1 in our experimental conditions. Furthermore, no modification was observed after chronic antidepressant treatment in other structures examined, supporting the specificity of this up-regulation.

Finally, these results suggest that an increase in PRAX-1 mRNA in the hippocampal formation may be common to various therapeutic approaches in antidepressant treatment, possibly in terms of long-term neuronal regulation and synaptic plasticity.

Preclinical studies on a novel approach to the treatment of depression suggested that neurokinin receptor antagonists could be of potential interest (Kramer et al., 1998; Rupniak and Kramer, 1999; Saria, 1999). Indeed, the NK2 receptor antagonist, SR48968 (Edmonds-Alt et al., 1992; Steinberg et al., 2001), exhibits many characteristics of antidepressant activity, such as up-regulation of CREB mRNA, reduced activity in the forced swimming test, or decrease in the amount of maternal separation-induced vocalization in guinea pig pups (Steinberg et al., 2001). Most interestingly, SR48968 also elicited the same effects on PRAX-1 mRNA in the CA1 and DG regions of the hippocampus.

Taken together, our results demonstrate that ECS, classic

antidepressants, and new potential antidepressants have the ability to up-regulate PRAX-1 mRNA and suggest that PRAX-1 could be involved in neuronal adaptations, intracellular messengers, and the gene regulation mechanism of antidepressant treatment. One possible pathway for this plasticity could implicate the cAMP cascade as seen with the regulation of CREB and BDNF, which contains a CRE element in its promoter. The promoter region of the *PRAX-1* gene has not yet been characterized, but its regulation might also be dependent on the cAMP pathway. In fact, the time course of PRAX-1 mRNA regulation during antidepressant treatment is in accordance with that of CREB, which is up-regulated only after a 21-day treatment (Nibuya et al., 1995).

Although the present studies thus point to a role for PRAX-1 in the treatment of depression, its cellular function remains unknown but might be related to that of RIM-BP1, a rat homolog of PRAX-1 (Wang et al., 2000). This protein has 69% identity in its nucleic acid sequence with human PRAX-1 and was shown, by RNA blotting experiments, to be expressed specifically in the brain. RIM-PB seems to be a binding protein for RIM1, a putative effector protein for Rab3, a synaptic G protein. Wang et al. (2000) suggest that the structure of RIM-BP is consistent with a role in the presynaptic cytomatrix next to the active zone as a scaffolding molecule and as a cytoskeletal organizer. Examining the functional relationship between RIM1 and PRAX-1 or RIM-BP could lead to a better understanding of these interactions. However, additional studies must be conducted before an intracellular function for PRAX-1 can be proposed.

In conclusion, we demonstrate that in the CNS, the majority of PRAX-1, unexpectedly, is not associated with PBR but shows a neuronal localization and is expressed in limbic regions of the brain. Although the role of PRAX-1 remains unknown, it is one of the common functional proteins up-regulated after chronic antidepressant treatment, including ECS and the NK2 receptor antagonist SR48968, and might thus represent a new target for the relief of depression.

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