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Cell-Specific Repressor or Enhancer Activities of Deaf-1 at a Serotonin 1A Receptor Gene Polymorphism

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The serotonin-1A (5-HT1A) receptor is the primary somatodendritic autoreceptor that inhibits the activity of serotonergic raphe neurons and is also expressed in nonserotonergic cortical and limbic neurons. Alterations in 5-HT1A receptor levels are implicated in mood disorders, and a functional C(-1019)G 5-HT1A promoter polymorphism has been associated with depression, suicide, and panic disorder. We examined the cell-specific activity of identified transcription factors, human nuclear deformed epidermal autoregulatory factor-1 (DEAF-1)-related (NUDR)/Deaf-1 and Hes5, at the 5-HT1A C(-1019) site. In serotonergic raphe RN46A cells, Deaf-1 and Hes5 repressed the 5-HT1A receptor gene at the C(-1019)-allele but not the G(-1019)-allele. However, in nonserotonergic cells that express 5-HT1A receptors (septal SN48, neuroblastoma SKN-SH, and neuroblastoma/glioma NG108–15 cells), Deaf-1 enhanced 5-HT1A promoter activity at the C(-1019)-allele but not the G-allele, whereas Hes5 repressed in all cell types. The enhancer activity of Deaf-1 was orientation independent and competed out Hes5 repression. To test whether Deaf-1 activity is intrinsic, the activity of a Gal4DBD (DNA binding domain)-Deaf-1 fusion protein at a heterologous Gal4 DNA element was examined. Gal4DBD-Deaf-1 repressed transcription in RN46A cells but enhanced transcription in SN48 cells, indicating that these opposite activities are intrinsic to Deaf-1. Repressor or enhancer activities of Deaf-1 or Gal4DBD-Deaf-1 were blocked by histone deacetylase inhibitor trichostatin A. Thus, the intrinsic activity of Deaf-1 at the 5-HT1A promoter is opposite in presynaptic versus postsynaptic neuronal cells and requires deacetylation. Cell-specific regulation by Deaf-1 could underlie region-specific alterations in 5-HT1A receptor expression in different mood disorders.

Key words: 5-HT1A receptor; transcription factor; epigenetic; raphe; polymorphism; anxiety; major depressive disorder

Introduction

The serotonin system originates from neurons of the raphe nuclei that are negatively regulated by presynaptic 5-HT1A autoreceptors (Penington et al., 1993; Bayliss et al., 1997). Postsynaptic 5-HT1A receptors are also strongly expressed in brain regions that regulate mood and emotion (Törk, 1990; Jacobs and Azmitia, 1992). Anxiety, depression, and suicide appear to result from reduced serotonergic activity and antidepressants such as 5-HT-selective reuptake inhibitors are thought to enhance serotonergic activity in part by desensitization of 5-HT1A autoreceptors. In contrast, in midbrain tissue from depressed suicides, elevated levels of presynaptic 5-HT1A receptors were observed (Stockmeier et al., 1998), an alteration that would reduce serotonergic activity.

We postulated that upregulation of 5-HT1A autoreceptors could be attributable to genetic alterations such as the 5-HT1A C(-1019)G functional promoter polymorphism. Several studies have associated the 5-HT1A G(-1019) allele with major depressive disorder, suicide, panic disorder or decreased response to antidepressants (Lemonde et al., 2003, 2004b; Strobel et al., 2003; Serretti et al., 2004). The 5-HT1A G(-1019) allele fails to bind identified repressors Deaf-1 and Hes5, leading to upregulation of autoreceptor expression. Consistent with a role of this site in 5-HT1A autoreceptor regulation, an increase in 5-HT1A autoreceptor expression in individuals with the G/G genotype has been observed (David et al., 2005; Parsey et al., 2006). Conversely, positron emission tomography imaging studies of depressed patients indicate decreased levels of postsynaptic 5-HT1A receptors in the cortex and hippocampus (Drevets et al., 1999; Sargent et al., 2000; Albert and Lemonde, 2004). Furthermore, 5-HT1A-null mice display increased anxiety-related behavior, which is rescued by early postnatal expression of the postsynaptic 5-HT1A receptor in the forebrain (Gross et al., 2002). We therefore addressed whether the C(-1019)G site might mediate differential regulation of the 5-HT1A Receptor in presynaptic versus postsynaptic cells.

In this study, the activity of Deaf-1 at the 5-HT1A C(-1019) site was compared in serotonergic raphe cells and nonserotonergic neuronal or glial cells that express similar levels of endogenous 5-HT1A receptors, including SN48 rat septal × mouse neuroblastoma cells, NG108–15 rat glioma × mouse neuroblastoma cells, and SKN-SH human neuroblastoma cells (Charest et al., 1993; Anborg et al., 2004; Fricker et al., 2005). Cell lines with intrinsic expression of 5-HT1A receptors were chosen to ensure the presence of relevant transcription factors necessary for 5-HT1A receptor expression. We show that Deaf-1 enhances 5-HT1A Promoter

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activity in the postsynaptic/glial cell models and that this enhancement is attenuated in the presence of the C(-1019)G polymorphism. In contrast, Hes5 represses 5-HT<sub>1A</sub> gene transcription in all cell types examined. The cell-specific activity of Deaf-1 in 5-HT<sub>1A</sub> gene regulation could account for region-specific decreases in postsynaptic 5-HT<sub>1A</sub> receptor levels reported in depressed patients.

Materials and Methods

**Reporter constructs.** The luciferase plasmids pG3P-RE-1 (repressor element 1), pcDNA1-REST/NRSF (RE-1-silencing transcription factor/neuron-restrictive factor), 5-HT<sub>1A</sub>(C) (-1128 to ATG), and 26bp-C(6)F, Gal4-DBD fusion constructs and X2G2P reporter constructs have been described previously (Lemonde et al., 2003; 2004a).

Briefly, the luciferase plasmid 5-HT<sub>1A</sub>(C) was obtained by insertion of a -1128 bp KpnI–BstNI fragment of the human 5-HT<sub>1A</sub> receptor gene into pG3-P Basic (Promega, Madison, WI) digested with KpnI and MluI. The 5-HT<sub>1A</sub>(G) construct was generated by unique site elimination mutagenesis (Amer sham Biosciences, Piscataway, NJ) of C(-1019)G in 5-HT<sub>1A</sub>(C). Appropriate oligonucleotides of the 26 bp element flanked with CC and GG 3′-overhangs were annealed, concatenated in six copies using T4 DNA ligase, blunted with Klenow, and subcloned into Smal-cut pGL3-promoter to generate 26bp-C(6)G and 26bp-C(6)F. Full-length Deaf-1 was obtained by PCR amplification using the human brain Marathon-Ready cDNA kit (BD Biosciences, Mississauga, Ontario, Canada). The PCR product was subcloned in pGEM-T Easy vector (Promega) before subcloning in the EcoRI site of pcDNA3 (Invitrogen, San Diego, CA). The 26bp-C(6)G was generated by ligation of a Nhel/Xhol blunted fragment, obtained from 26bp-C(6)F construct into pGL3B (Promega), digested by Smal. All plasmids were purified by CsCl equilibrium gradient centrifugation, quantified spectrophotometrically, and verified by dideoxynucleotide DNA sequencing.

**Cell culture and transfection.** Septal SN48, NG108–15, and SKN-SH neuroblastoma cells were grown in DMEM (Invitrogen BRL, Rockville, MD) supplemented with 10% v/v heat-inactivated fetal bovine serum at 37°C in 5% CO<sub>2</sub>. Binding studies of crude membranes from these cells using [H]<sup>–</sup>2-(di-n-propylamino)tetralin revealed (mean ± range; n = 2) 3.0 ± 0.5, 8.3 ± 1.6, and 2.7 ± 1.0 fmol/mg protein of 5-HT<sub>1A</sub> receptor sites, respectively. RN46A cells (2.4 ± 0.6 fmol/mg 5-HT<sub>1A</sub> sites) (Kushnaha and Albert, 2005) were grown on Primaria six-well plates (Falcon, Franklin Lakes, NJ) in Neurobasal medium (Invitrogen BRL) supplemented with 10% v/v heat-inactivated fetal bovine serum, 0.1% Pen-Strep, and 0.04% of 1-glutamine at 33°C in 5% CO<sub>2</sub> (Lemonde et al., 2003, 2004a). Media were changed 16–24 h before transfection. Except for the RN46A and SKN-SH cells, transfctions were done using calcium phosphate coprecipitation as described previously (Charest et al., 1993) with 20 μg of luciferase construct and 5 μg of pcMV<sub>β</sub>gal per 10 cm plate or with 10 μg of luciferase-expressing vector, indicated amounts of pcDNA expression constructs or empty vector and 0.5 μg of pcMV<sub>β</sub>gal to normalize transfection efficiency, maintaining the total amount of DNA transfected at 25 μg. Raphe RN46A cells and SKN-SH cells were transfected with 1:2 and 1:1.5 ratios, respectively, of plasmid: Lipofectamine2000 reagent (Invitrogen) using 10 μg/plate of luciferase plasmid and equal amount of protein-expression constructs or empty vector and 2 μg/plate pcMV<sub>β</sub>gal. In some experiments, 200 μm trichostatin A (TSA) or vehicle (0.75% ethanol) was applied to the culture medium at the time of transfection.

**Reporter assays.** Luciferase and β-galactosidase assays were performed as described previously (Ou et al., 2000; Lemonde et al., 2003). Activities were obtained from at least three independent experiments in which triplicate transfections were performed and corrected for transfection efficiency by calculating the ratio of luciferase/β-galactosidase activity and normalizing to vector-transfected extracts. Data are presented as mean ± SD. Statistical significance was evaluated using two-tailed unpaired t test and 95% confidence intervals with GraphPad Prism software (GraphPad, San Diego, CA) unless indicated otherwise.

**Nuclear and cytosolic fractionation.** Fractionation was adapted from methods previously (Lemonde et al., 2003; Subramanian and Chinnadurai, 2003; Zuccato et al., 2003). Cells (10<sup>7</sup>) were trypsinized and collected in 14 ml of HEPS-buffered balanced salts plus EDTA buffer (118 mM NaCl, 4.6 mM KCl, 10 mM d-glucose, 5 mM EDTA, 20 mM HEPS, pH 7.2), pelleted at 4°C for 4 min at 500 × g and resuspended in 1 ml of resuspension buffer (10 mM KCl, 10 mM Na-HEPS, pH 7.6, 1.5 mM MgCl<sub>2</sub>) and allowed to swell on ice for 10 min. The resulting cells were collected by spinning for 10 min at 4°C. The nuclear and cytosolic fractions were obtained by centrifugation at 10,000 × g for 2 min at 4°C and washed three times using 1 ml of wash buffer (50 mM NaCl, 20 mM Na-HEPS, pH 7.6, 25% glycerin, 0.2 mM EDTA, 1.5 mM MgCl<sub>2</sub>, 0.5 mM DTT, 0.5 μM spermidine, 0.15 μM spermine, 1 mM PMSF, 1 μM Protease Inhibitor Cocktail (Roche Diagnostics, Welwyn Garden City, UK). Nuclear pellets were collected by centrifugation at 10,000 × g for 2 min at 4°C and washed three times using 1 ml of wash buffer (50 mM NaCl, 20 mM Na-HEPS, pH 7.6, 25% glycerin, 0.2 mM EDTA, 1.5 mM MgCl<sub>2</sub>, 0.5 mM DTT, 0.5 μM spermidine, 0.15 μM spermine, 1 mM PMSF, 1 μM Protease Inhibitor Cocktail) and rotated for 30 min at 4°C. The nuclear proteins were collected from the supernatant after centrifugation for 15 min at 16,000 × g at 4°C to clear cell debris. Western blot analysis. Nuclear or cytosolic proteins (50 μg/reaction) were separated by SDS-PAGE on a 12% gel, transferred to nitrocellulose membrane, and blocked using TBS containing 5% dry milk and 0.02% Tween 20. Immunoactive proteins were detected with rabbit anti-Deaf-1 antibody (Lemonde et al., 2003) at a dilution of 1:5000 followed by a 1:2000 dilution of the secondary horseradish peroxidase-linked anti-rabbit antibody. Anti-histone 1 (nuclear marker) antibody (Upstate Biotechnology, Lake Placid, NY) was used at 1:1000 (Zuccato et al., 2003). Anti-c-Raf (cytosolic marker) antibody (BD Biosciences) was used at 1:3000 dilution (Brown et al., 2004).

**Electrophoretic mobility shift assay.** Sense and antisense oligonucleotides of the 26 bp Deaf-1 element (C-allele) with CC/GG 3′-overhangs were hybridized and labeled with [α-<sup>32</sup>P]-dCTP using Klenow fragment DNA polymerase (Ou et al., 2000). Nuclear extract (5 μg/reaction) was preincubated with or without competitor DNA in a 20 μl reaction containing gel-shift DNA binding buffer (20 mM HEPS, 0.2 mM EDTA, 0.2 mM EGTA, 100 mM KCl, 5% glycerol, and 2 mM DTT, pH 7.9), 250 ng of herring sperm DNA, at room temperature for 20 min. Unlabeled double-stranded polyomavirus enhancer activator 3 (PEA3) sequences (5′GGGATCCAGGAAATGGA-3′) were used as nonspecific competitor and were electrophoresed on a nondenaturing 5% acrylamide/Tris-glycine gel at 4°C to resolve protein-DNA complexes. For SKN-SH extracts, the probe was trapped in poorly migrating nonspecific protein-DNA aggregate that was not competed by specific oligonucleotide.

**Results**

**Cell- and-allele-specific activity of Deaf-1 at the 5-HT<sub>1A</sub> promoter.** The transcriptional activity of Deaf-1 and Hes5 was compared in serotonergic (RN46A) (Fig. 1A) and nonserotonergic (SN48, NG108–15, and SKN-SH) (Fig. 1B) 5-HT<sub>1A</sub> receptor-positive neuronal cells cotransfected with C(-1019)G or G(-1019) human 5-HT<sub>1A</sub> promoter-luciferase reporter constructs [5-HT<sub>1A</sub>(C) or (G), respectively]. Cell lines with intrinsic expression of 5-HT<sub>1A</sub> receptors were chosen to ensure the presence of relevant transcription factors necessary for 5-HT<sub>1A</sub> receptor expression. As previously observed in RN46A raphe cells, both Deaf-1 and Hes5 repressed the activity of the 5-HT<sub>1A</sub>(C) construct by 50% but had no effect at the 5-HT<sub>1A</sub>(G) construct, but there was no significant difference in basal activity between the two alleles. In contrast, in SN48 and NG108–15, there was a 70% reduction in basal activity of 5-HT<sub>1A</sub>(G) compared with the 5-HT<sub>1A</sub>(C) construct, with a smaller but significant reduction in SKN-SH cells (Fig. 1B). Furthermore, cotransfection of Deaf-1 significantly increased the activity of 5-HT<sub>1A</sub>(C) construct but not the 5-HT<sub>1A</sub>(G) construct. Deaf-1 also enhanced 5-HT<sub>1A</sub> promoter activity in 5-HT<sub>1A</sub>—
expressing H19–7 rat hippocampal cells (data not shown). In contrast, Hes5 mediated a significant repression of the 5-HT1A(C) but not G-allele in SN48 and NG108–15 cells. Thus, in several models of postsynaptic or glial 5-HT1A gene expression, Deaf-1 enhanced 5-HT1A transcriptional activity at the C(-1019) site, whereas it repressed 5-HT1A transcription in serotonergic raphe cells.

Deaf-1 expression and localization

Because endogenous Deaf-1 could influence basal 5-HT1A transcription, Deaf-1 protein was detected in nuclear and cytosolic extracts by Western blot using a specific antibody, which recognized a single 60 kDa species (Lemonde et al., 2003) (Fig. 2A). Levels of Deaf-1 were highest in nuclear extracts from RN46A cells, with low or undetectable levels in SN48, SKN-SH, and NG108–15 nuclear fractions. In contrast, Deaf-1 was abundantly expressed in cytosolic extracts from these cell types. Markers specific for nuclear (H1-histone) or cytosolic (c-Raf protein) fractions indicated undetectable cross-contamination between fractions and similar protein loading among cell types. The DNA binding activity of Deaf-1 in nuclear fractions was determined (Fig. 2B). A specific Deaf-1/DNA complex with labeled human Deaf-1 element (C-allele) was observed that was completely competed with excess unlabeled Deaf-1 element (Sp) but weakly competed by unrelated PEA-3 site primers (NSp). Deaf-1 binding activity paralleled the nuclear protein content identified by Western blot (Fig. 2A), greatest in RN46A cells and weak but detectable in SN48, SKN-SH, and NG108–15 cells. Therefore, endogenous Deaf-1 from rat or human nuclei binds to the 5-HT1A site in accordance with its protein abundance. The abundance of nuclear Deaf-1 in RN46A cells suggests that Deaf-1 may repress endogenous 5-HT1A receptor expression.

Deaf-1 enhancer/repressor activity at the C(-1019) Deaf-1 site

To determine whether the 26 bp Deaf-1 element is sufficient to confer cell-specific activity of Deaf-1, the activity of Deaf-1 at a reporter construct containing the Deaf-1 element (in six copies) placed upstream of the SV40 promoter [26bp-C(6)] was compared in RN46A and SN48 cells (Fig. 3). In RN46A cells, Deaf-1 almost completely inhibited transcriptional activity, whereas in SN48 cells, Deaf-1 mediated enhancer activity. Repressor or enhancer activity of Deaf-1 was observed in forward or reverse orientations of Deaf-1 sites, as expected for orientation-independent enhancer or repressor activity. In cells transfected with a control vector lacking Deaf-1 sites, Deaf-1 had no effect (data not shown). Therefore, the 26-bp Deaf-1 element is sufficient to confer cell-specific Deaf-1-mediated repressor or enhancer activity.

Regulation of the 5-HT1A promoter could involve interactions

Figure 1. Cell type-dependent activity of Deaf-1 at the 5-HT1A C(-1019) site. Constructs containing the C(-1019) or G(-1019) allele of the human 5-HT1A promoter (−1128 bp to ATG) fused to luciferase [5-HT1A(C) and 5-HT1A(G), respectively] were cotransfected with vector (pcDNA3), Deaf-1, or Hes5 expression plasmids as indicated in 5-HT1A-expressing neuronal cell lines: rat raphe RN46A (A), mouse septal SN-48, neuroblastoma-glioma NG108–15, or human SKN-SH neuroblastoma cells (B). Adjusted luciferase activity was corrected for transfection efficiency by calculating the ratio of luciferase activity/β-galactosidase activity and normalized to control (pcDNA3) transfections. Data are presented as mean ± SD of triplicate samples collected from at least three independent experiments as indicated. Statistical analysis compared with vector control (for Deaf-1 and Hes5) was determined by one-way ANOVA; significant differences between C and G alleles were evaluated by unpaired t test with two-tailed p values (**p ≤ 0.01; ***p ≤ 0.001).
between Deaf-1 and Hes5 at the 26 bp 5-HT1A palindrome. To address this, Deaf-1 and Hes5 were cotransfected in RN46A or SN48 cells, and transcriptional activity at the 26 bp palindrome was measured (Fig. 4). In RN46A cells, both Deaf-1 and Hes5 repressed transcriptional activity, and together Deaf-1 and Hes5 induced a similar repression (Fig. 4A). In SN48 cells, Deaf-1 enhanced activity of the 26bp-C(6) construct, whereas Hes5 repressed its activity (Fig. 4B). Interestingly, when cotransfected with Hes5, Deaf-1-mediated enhancement was the dominant effect. A similar pattern was observed using the 5-HT1A promoter-luciferase construct, with Deaf-1 enhancer activity dominant over Hes5 repression (Fig. 4C). Thus, the enhancer activity of Deaf-1 could result from a competition with endogenous repressors such as Hes5.

HDAC-dependent repressor/enhancer activity of Deaf-1

Because recruitment of histone deacetylase (HDAC) is implicated in gene repression (Ayer, 1999), we tested the effect of HDAC inhibitor TSA on Deaf-1-mediated repressor or enhancer activity at the 5-HT1A promoter (Fig. 5). We also tested the 5-HT1A RE-1 element placed upstream of the SV40 promoter in the pGL3P vector (pGL3P-RE1), the target of the prototypic REST/NRSF that mediates TSA-sensitive repression (Lemonde et al., 2004a). In RN46A cells, Deaf-1 reduced 5-HT1A promoter activity, and this action was attenuated by TSA treatment. Similarly, the silencer activity of REST at RE-1 was also partially reversed by TSA treatment in RN46A cells. In SN48 cells, Deaf-1-induced enhancer activity was also inhibited by TSA, indicating that HDAC activity is also required for Deaf-1-mediated enhancer activity. In SN48 cells, TSA treatment reversed REST-mediated repression to enhanced activity compared with vector (pGL3P), consistent with the finding that REST can recruit silencer or enhancer activities (Jepsen et al., 2000). Thus Deaf-1-mediated recruitment of HDAC can either repress or enhance 5-HT1A promoter activity, depending on the cellular context.

Intrinsic repressor/enhancer activity of Deaf-1

Deaf-1-mediated repressor/enhancer activity could result from competition with other factors (e.g., Hes5) or represent an intrinsic property of Deaf-1. To test the latter possibility, a completely heterologous hybrid system was used: full-length Deaf-1 was fused to the Gal4 DNA binding domain (DBD) and its activity tested at a reporter construct containing Gal4 elements (Fig. 5A). Full-length Deaf-1 was used to preserve protein–protein interactions. Compared with the vector (Gal4DBD), Gal4DBD-Deaf-1 repressed the SV40 promoter in RN46A cells (Fig. 6), indicating that Deaf-1 confers repression when recruited to a heterologous element and promoter. This effect was entirely blocked by TSA,
indicating that Deaf-1 repression is dependent on HDAC activation, whereas LexA-mediated repression was not reversed by TSA, as observed previously (Lemonde et al., 2004a). When transfected in SN48 cells, Gal4-Deaf-1 induced a small but significant enhancement of transcription that was inhibited to below basal activity by TSA, whereas LexA induced HDAC-independent repression (Fig. 6C). The effect of TSA indicates a primary role for HDAC in Deaf-1 enhancer activity and reveals a possible HDAC-independent Deaf-1 repressor activity. Thus Deaf-1-induced enhancer activity in SN48 cells is HDAC dependent and is conferred by Deaf-1 itself and is not attributable to displacement of the binding of another repressor to the 26 bp palindrome (such as Hes5).
Figure 6. Cell-specific and TSA-sensitive repressor and enhancer activities are conferred by Deaf-1 at a heterologous DNA element. A, A model for the action of effectors Gal4-DBD (Gal4 vector), Gal4DBD-Deaf-1 fusion protein, or LexA (positive control) at the X2G2P reporter construct containing two LexA and Gal4 sites upstream of SV40 promoter-luciferase. These constructs were cotransfected in 5-HT1A-expressing rat RN46A (A) or myeloid, nervy, and Deaf-1 (B) domain (Gross and McGinnis, 1996). Deaf-1 also contains both nuclear import (Jensik et al., 2004) and export sites (Huggenvik et al., 1998) to regulate trafficking. In this study, we show that Deaf-1 binds to the C(-1019) site to repress 5-HT1A receptor gene transcription in a presynaptic serotonergic cell line (RN46A), whereas Deaf-1 enhanced transcription in postsynaptic neuronal or glial cells (SN48, NG108–15, SKN-SH), as shown in the model (Fig. 1) (supplemental material, available at www.jneurosci.org). The finding that the G(-1019) allele blocked both enhancer and repressor actions of Deaf-1 argues for a direct effect of Deaf-1 at the 5-HT1A promoter.

Discussion

Deaf-1, a cell-specific repressor or enhancer

In this study, we show that Deaf-1 binds to the C(-1019) site to repress 5-HT1A receptor gene transcription in a presynaptic serotonergic cell line (RN46A), whereas Deaf-1 enhanced transcription in postsynaptic neuronal or glial cells (SN48, NG108–15, SKN-SH), as shown in the model (Fig. 1) (supplemental material, available at www.jneurosci.org). The finding that the G(-1019) allele blocked both enhancer and repressor actions of Deaf-1 argues for a direct effect of Deaf-1 at the 5-HT1A promoter. Hes5-mediated repression in all cell lines, which was attenuated in postsynaptic models by the presence of Deaf-1-mediated enhancer activity. This suggests that Deaf-1 and Hes5 compete for binding to the 26 bp palindrome, but that Deaf-1 may have higher affinity for binding than Hes5. However, Deaf-1-induced enhancement did not result from competition with an endogenous stronger repressor of the 26 bp 5-HT1A palindrome element, because cell-specific Deaf-1 enhancer activity was present in the heterologous GAL4-DBD system. The possibility that differences in activator or repressor activity result from competition with binding to endogenous promoters is unlikely, because the number of genomic sites for Deaf-1 is at least 100-fold lower than that of transfected reporter construct, based on 10% transfection efficiency. We postulate that Deaf-1 recruits cell-specific protein complexes to the DNA to either repress or enhance transcription.

Multiple trans-acting functions of Deaf-1

Deaf-1 has been shown previously to have either repressor or enhancer function depending on the gene analyzed. NUDR is the human homolog of Deaf-1, a DNA-binding protein that was first shown to bind the autoregulatory element of the deformed gene in Drosophila melanogaster (Gross and McGinnis, 1996). In these studies, Deaf-1 synergistically activated deformed protein autoregulation of the 120 bp deformed response element, thus displaying enhancer activity. Human Deaf-1 trans-activated preproenkephalin promoter activity by 30-fold, but this effect appeared to be independent of DNA binding to the promoter (Huggenvik et al., 1998). However, in monkey kidney CV-1 cells, Deaf-1 repressed the hnRNP A2/B1 and Deaf-1 promoters via sites located downstream from transcriptional initiation (Michelson et al., 1999). In contrast to these studies in different genes, depending on the cell type Deaf-1 repressed or enhanced gene transcription at the same C(-1019) site of the 5-HT1A receptor gene. This Deaf-1 site conferred cell-specific regulation at a heterologous promoter, implying that Deaf-1 recruits cell-specific regulatory factors to repress or enhance transcription.

Different functional domains within Deaf-1 could mediate its enhancer or repressor activities. The structure of Deaf-1 includes the conserved DNA-binding SAND domain (Sp100, AIRE-1, NucP41/75, Deaf-1) and a C-terminal MYND (myeloid, nervy, and Deaf-1) domain (Gross and McGinnis, 1996). Deaf-1 also contains two protein-interaction domains for its interaction with both LIM-only (LMO4) and nuclear LIM-binding protein (NLI/CLIM) transcriptional regulators, which mediate embryonic pattern formation and cell fate (Sum et al., 2002). Deaf-1-null mice display phenotypic abnormalities, including exencephaly and transformation of cervical segments that resemble those of LMO4-null mice, further suggesting functional overlap between Deaf-1 and LMO4 (Hahn et al., 2004; Tse et al., 2004; Lee et al., 2005). LMO4 has been shown to recruit various NLI proteins that lead to transcriptional activation or repression (Bach et al., 1997; Jurata and Gill, 1997). Therefore, recruitment by Deaf-1 of different protein complexes could direct enhancement or repression of gene transcription.

Deaf-1 also contains both nuclear import (Jensik et al., 2004) and export sites (Huggenvik et al., 1998) to regulate trafficking. The finding that nuclear Deaf-1 content is greatest in RN46A cells (Fig. 2A) suggests that Deaf-1 may be stabilized in the nucleus (perhaps in heterochromatin) to exert its repressor activity. We observed little effect of the C(-1019)G mutation on basal activity of 5-HT1A reporter constructs in RN46A cells (Fig. 1A), where endogenous Deaf-1 may be bound to the endogenous 5-HT1A gene and inaccessible to the reporter construct. Yet, there was a clear effect of the C(-1019)G change on basal activity in postsynaptic models by the presence of Deaf-1-mediated enhancer activity. This suggests that Deaf-1 and Hes5 compete for binding to the 26 bp palindrome, but that Deaf-1 may have higher affinity for binding than Hes5. However, Deaf-1-induced enhancement did not result from competition with an endogenous stronger repressor of the 26 bp 5-HT1A palindrome element, because cell-specific Deaf-1 enhancer activity was present in the heterologous GAL4-DBD system. The possibility that differences in activator or repressor activity result from competition with binding to endogenous promoters is unlikely, because the number of genomic sites for Deaf-1 is at least 100-fold lower than that of transfected reporter construct, based on 10% transfection efficiency. We postulate that Deaf-1 recruits cell-specific protein complexes to the DNA to either repress or enhance transcription.
aptic cells where Deaf-1 is mostly cytosolic and may be free to translocate to the reporter construct. At reporter constructs, transfected Deaf-1 displayed greater repressor activity than enhancer activity (Figs. 3, 4), because the background activity of endogenous Deaf-1 to repress is lower than basal enhancer activity (Fig. 1, compare A and B). Therefore, the preferential localization of Deaf-1 in the nucleus of RN46A cells appears to correlate with its greater activity as a repressor.

**HDAC dependence of Deaf-1 function**

Both repressor and enhancer activities of Deaf-1 were blocked by inhibition of HDAC using the HDAC-specific (class I and II) inhibitor TSA (Yoshida et al., 1990), implicating HDAC in these activities of Deaf-1. HDACs regulate the acetylation of histone and nonhistone proteins, as well as the recruitment of transcriptional regulatory protein complexes to chromatin (Yang, 2004). In addition to their roles in transcriptional repression, deacetylases can also positively regulate gene expression by deacetylation of transcription factors (Yang and Gregoire, 2005). For example, recruitment of HDAC by STAT3 (signal transducer and activator of transcription 5) proteins leads to deacetylation of C/EBPβ (CCAAT/enhancer-binding protein β), resulting in transcriptional activation of the Id-1 repressor gene (Xu et al., 2003). Therefore, TSA-mediated inhibition of HDAC activity can block repression as well as enhancer activity.

Deaf-1 provides an unusual example of a transcription factor that, after directly binding to the same DNA regulatory element, can recruit HDAC-dependent mechanisms to either repress or enhance gene expression. Transcription factors, such as nuclear receptors or myocyte enhancer factor-2, can function as repressor or enhancer but repressor activity involves recruitment of HDAC, whereas enhancer activity requires recruitment of histone acetylase activity and the absence of HDAC (Glass and Rosenfeld, 2000; McKinsey et al., 2001). Alternately, enhancer activity can be mediated through indirect protein–protein interactions. For example, REST can both repress corticotrophin releasing hormone gene expression by binding to its RE-1 element and enhance its transcription via a mechanism independent of RE-1 in NG108–15 cells (Seth and Majzoub, 2001). Generally, REST represses most target genes by association with corepressors including Sin3A, nuclear receptor corepressor (NCoR), or CoREST. However, the corepressor NCoR can mediate HDAC-dependent gene activation (Jepsen et al., 2000), a mechanism that could account for the HDAC-dependent activation of the 5-HT₁A gene in postsynaptic cells by Deaf-1.

**Implications for depression**

Identifying mechanisms that control 5-HT₁A receptor expression has strong clinical relevance, because the 5-HT₁A receptor is a major direct or indirect target of antidepressant drugs (Pineyro and Blier, 1999; Blier and Ward, 2003; Albert and Lemond, 2004). An increase in 5-HT₁A autoreceptor expression has been associated with major depression (Stockmeier et al., 1998). In normal subjects and especially unmedicated depressed subjects, in vivo imaging studies reveal increased 5-HT₁A autoreceptors in the raphe area in G/G versus non-G/G genotype, consistent with de-repression of Deaf-1 at the 5-HT₁A G-allele (David et al., 2005; Parsey et al., 2006). Postsynaptically, however, subjects with major depressive or panic disorder have a significant decrease in 5-HT₁A mRNA (Lopez-Figueroa et al., 2004) and decreased 5-HT₁A receptor levels in hippocampus and cortex (Sargent et al., 2000; Bhagwagar et al., 2004; Neumeister et al., 2004). Furthermore, after chronic antidepressant treatment, 5-HT₁A autoreceptors are preferentially desensitized, whereas postsynaptic 5-HT₁A receptors are not (Pineyro and Blier, 1999). The cell-specific function of Deaf-1 could underlie differential regulation of pre- and postsynaptic 5-HT₁A receptor expression in vivo (Fig. 1) (supplemental material, available at www.jneurosci.org). The G/G genotype could confer de-repression of the 5-HT₁A gene in the raphe but reduced Deaf-1 enhancer activity in postsynaptic neurons. The extent of Deaf-1 enhancer activity varied depending on the cell type, with strongest activity in septal SN48 cells. By analogy, the contribution of Deaf-1 to 5-HT₁A receptor expression in various brain regions and the influence of the C(-1019)G genotype will differ depending on the neuronal cell type.

In summary, we identified a novel HDAC-dependent regulatory mechanism by which Deaf-1 mediates cell-specific enhancer or repressor activity to regulate the 5-HT₁A receptor gene. These divergent regulatory mechanisms by Deaf-1 may confer differential regulation of the 5-HT₁A receptor in presynaptic versus postsynaptic brain regions in vivo.

**References**


