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Changes In Adrenoreceptors In The Prefrontal Cortex Of Subjects With Dementia: Evidence Of Compensatory Changes

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Abstract

In Alzheimer's disease (AD) there is a significant loss of locus coeruleus (LC) noradrenergic neurons. However, recent work has shown the surviving noradrenergic neurons to display many compensatory changes, including axonal sprouting to the hippocampus. The prefrontal cortex (PFC) is a forebrain region that is affected in dementia, and receives innervation from the LC noradrenergic neurons. Reduced PFC function can reduce cognition and disrupt behavior. Because the PFC is an important area in AD, we determined if noradrenergic innervation from the LC noradrenergic neurons is maintained and if adrenoreceptors are altered postsynaptically. Presynaptic PFC α_2-adrenoreceptor (AR) binding site density, as determined by ^{3}H-RX821002, suggests that axons from surviving noradrenergic neurons in the LC are sprouting to the PFC of subjects with dementia. Changes in postsynaptic α_1-AR in the PFC of subjects with dementia indicate normal to elevated levels of binding sites. Expression of α_1-AR subtypes (α_{1A}- and α_{1D}-AR) and α_{2C}-AR subtype mRNA in the PFC of subjects with dementia is similar to what was observed in the hippocampus with one exception, the expression of α_{1A}-AR mRNA. The expression of the α_{1A}-AR mRNA subtype is significantly reduced in specific layers of the PFC in subjects with dementia. The loss of α_{1A}-, α_{1D}- and α_{2C}-AR mRNA subtype expression in the PFC may be attributed to neuronal loss observed in dementia. These changes in postsynaptic AR would suggest a reduced function of the PFC. Consequence of this reduced function of the PFC in dementia is still unknown but it may affect memory and behavior.

Keywords

norepinephrine; RX 821002; prazosin; α_1-adrenoreceptor; α_2-adrenoreceptor; sprouting; Alzheimer's disease

Alzheimer's disease (AD), a neurodegenerative disorder, is characterized by cognitive impairment and the loss of neurons in the cortex and hippocampus. An area of the cortex that has shown the greatest amount of neuronal loss in AD is the prefrontal cortex (PFC) (Braak and Braak, 1991; Masliah et al., 1993; Salat et al., 2001). A change in PFC neuronal

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function can affect learning and memory (Brozoski et al., 1979; Carli et al., 1983; Arnsten, 1993) as well as behavior (Birnbaum et al., 2004; Arnsten and Li, 2005). The role of the PFC in behavior may be important in the expression of disruptive agitation that is commonly observed in AD.

Noradrenergic neurons in the locus coeruleus (LC) send projections to the PFC where they can modulate neuronal activity. There is a significant loss of noradrenergic neurons in the LC in AD (Mann et al., 1980; Tomlinson et al., 1981; Bondareff et al., 1982; Marcyniuk et al., 1986; Chan-Palay and Asan, 1989; German et al., 1992). However, the surviving neurons in the LC appear to be compensating for the neuronal loss (Adolfsson et al., 1979; Cross et al., 1981; Mann et al., 1981; Perry et al., 1981; Tomlinson et al., 1981; Gottfries et al., 1983; Raskind et al., 1984; Palmer et al., 1987; Reinikainen et al., 1988; Tohgi et al., 1992; Elrod et al., 1997; Russo-Neustadt et al., 1998; Hoogendijk et al., 1999; Szot et al., 2000). Recently, our laboratory showed that the remaining noradrenergic neurons in the LC of AD and a related dementing disorder, dementia with Lewy bodies (DLB), showed three different compensatory changes: (1) an increase in tyrosine hydroxylase mRNA expression in the remaining neurons; (2) sprouting of dendrites into the peri-LC dendritic zone, as determined by α_2-adrenoreceptor (AR) and norepinephrine transporter binding sites; and (3) sprouting of axonal projections into the hippocampus as determined by α_2-ARs (Szot et al., 2006). In AD and DLB subjects, the number of hippocampal postsynaptic α_1-ARs was normal to elevated (Szot et al., 2006). Expression of α_{1A}- and α_{2A}-AR mRNA in the hippocampus of AD and DLB subjects was not altered, but expression of α_{1D}- and α_{2C}-AR mRNA was significantly reduced in the hippocampus of AD and DLB subjects (Szot et al., 2006). The focus of this work is to examine noradrenergic innervation to the PFC and postsynaptic AR status in the PFC, a region substantially affected in AD (Braak and Braak, 1991; Masliah et al., 1993; Salat et al., 2001) and important in cognition and behavior (Brozoski et al., 1979; Carli et al., 1983; Arnsten, 1993, Birnbaum et al., 2004; Arnsten and Li, 2005). To determine if noradrenergic axons are sprouting to the PFC in AD and DLB subjects, α_2-AR binding sites were measured. To determine if postsynaptic ARs are affected in the PFC, α_1-AR binding sites were measured as well as *in situ* mRNA expression of the different α_1- and α_2-AR subtypes.

Experimental Procedures

Subjects

All postmortem tissue was obtained from the University of Washington Alzheimer's Disease Research Center, where permission for use of tissue in scientific experiments was obtained. AD is characterized by the insidious onset and gradual progression of impaired memory, language, and executive function. Psychosis, agitation, and other behavioral disturbances characteristically appear late in the disease course. DLB, which accounts for ~20% of patients with late-life dementia, presents early in its course with psychotic symptoms such as visual hallucinations and with fluctuating cognition and pronounced attentional deficits and often with bradykinesia and increased muscle tone (McKeith et al., 1996; Ballard et al., 1999; Barber et al., 2001). The subjects used in this study were the same subjects used in a previous study measuring changes in the noradrenergic nervous system in the LC and hippocampus and were described in detail in the previous publication (Szot et al., 2006). Briefly, PFC was studied in the following 17 nondemented age-comparable control subjects, with an age range of 38-90 years (mean ± SEM, 71.4 ± 3.5 years), seven males and 10 females with an average postmortem delay (PMD) of 8.5 ± 0.9 h; 15 AD subjects with an age range of 37-94 years (mean ± SEM, 68.6 ± 4.4 years), six males and nine females with an average PMD of 7.4 ± 0.9 h; and 22DLB subjects with an age range of 63-98 years (mean ± SEM, 79.5 ± 1.6 years), 16 males and six females with an average PMD of 8.0 ± 0.7 h. AD subjects met the National Institute on Aging Reagan criteria for AD (Braak stage IV/C

or higher with no vascular dementia, frontotemporal dementia, or Lewy body pathology) (McKhann et al., 1984). DLB subjects met the same neuropathological diagnostic criteria for AD plus had the presence of Lewy body pathology in the brainstem and limbic regions, confirmed by α-synuclein immunohistochemistry.

Tissue

PFC tissue was accessed at autopsy using a protocol that provided a portion of the PFC in snap-frozen blocks. The region of the cortex where the PFC was obtained was consistent among all subjects. The size of the block was based on the dimensions of a standard slide that was used in the experiments. The fresh tissue block for each individual was dissected into 1-cm-thick coronal blocks, snap frozen in liquid nitrogen-cooled isopentane, and stored at −70°C. Serial coronal sections (20 μm) were cut on a cryostat, thaw mounted onto FisherSuper frost slides, and stored at −70°C for each individual.

^{3}H-Prazosin and ^{3}H-RX821002 binding sites

α_1-AR binding sites were measured in the PFC with ^{3}H-prazosin (PerkinElmer, Boston, MA) as previously described (Szot et al., 2005, 2006). Briefly, for each subject four consecutive slides, each containing a section of the PFC was run: three slides for total binding and the fourth for nonspecific binding. Nonspecific binding was defined in the presence of 10 μm phentolamine. Slides and ^{3}H standards were apposed to Biomax MR film (Eastman Kodak, Rochester, NY) for 8 weeks. Films were developed and analyzed as described previously (Szot et al., 1997). Density measurements (microcuries per gram) were determined using MicroComputer Imaging Device system (MCID) (Imaging Research, St. Catherines, Ontario, Canada) in as many distinguishable layers as possible in the PFC (see Zilles, 2004 for extensive details on architecture of the human cortex), and the values are expressed as a mean (microcuries per gram) ± SEM for each subject group. Specific binding was obtained by taking the total average value minus nonspecific value in the same region. Specific binding for ^{3}H-prazosin constituted ~90% of total binding. Data were analyzed in layers I/II, III/IV and V/VI with ANOVA, followed by a *post hoc* Fisher's test; statistical significance was taken at $p<0.05$.

α_2-AR binding sites were measured in the PFC with ^{3}H-RX821002 (PerkinElmer) according to Happe et al., (2004). For each subject, four consecutive slides, each containing a section of the PFC, were run: three slides for total binding and the fourth for nonspecific binding. Nonspecific binding was defined in the presence of 10 μm rauwolscine. Slides and ^{3}H standards were apposed to Biomax MR film (Eastman Kodak, Rochester, NY) for 8 weeks. Films were developed and analyzed as described previously (Szot et al., 1997). Density measurements (microcuries per gram) were determined using MCID (Imaging Research) in as many distinguishable layers as possible in the PFC, and the values are expressed as a mean (microcuries per gram) ± SEM for each subject group. Specific binding was obtained by taking the total average value minus nonspecific value in the same region. Specific binding for ^{3}H-RX821002 constituted ~90% of total binding. Data were analyzed layers I/II, III, IV, V and VI as described above.

Oligonucleotides

α_{2C}-, α_{1A}- and α_{1D}-AR mRNA expression were measured in the PFC as previously described (Szot et al., 2006). α_{2A}-AR mRNA expression was not measured in the PFC because preliminary work showed α_{2A}-AR mRNA levels to be undetectable (data not shown). Tissue preparation and labeling of α_{2C}-, α_{1A}- and α_{1D}-AR oligonucleotide probes was performed as described previously for oligonucleotide labeling (Szot et al., 1997). Three consecutive slides in the PFC were used for each receptor subtype.

The α_{2C}-AR probe consisted of a single oligonucleotide probe to the following nucleotides of the published human sequence (Lomasney et al., 1990): 875-925. The α_{2C}-AR probe contained 0.16×10^6 cpm/50 μl for the PFC. Slides were apposed to film (Eastman Kodak) for 17 h at room temperature. The α_{1A}-AR probe consisted of three separate oligonucleotide probes to the following nucleotides of the published human sequence (Schwinn et al., 1990; Hirasawa et al., 1993): 1-45, 1102-1156, and 1435-1483. The α_{1A}-AR probe contained 1.4×10^6 cpm/50 μl for the PFC. Slides were apposed to film (Eastman Kodak) for 4 d at room temperature. The α_{1D}-AR probe consisted of three separate oligonucleotide probes to the following nucleotides of the published human sequence (Weinberg et al., 1994; Schwinn et al., 1995): 587-635, 990-1038, and 1668-1716. The α_{1A}-AR probe contained 0.4×10^6 cpm/50 μl for the PFC. Slides were apposed to film (Eastman Kodak) for 7 d at room temperature.

Film was developed and analyzed as described previously (Szot et al., 1997). α_{2C}-,α_{1A}- and α_{1D}-AR mRNA expression in the PFC was measured as optical density (OD) using MCID as previously described (Szot et al., 2006). α_{2C}-AR mRNA expression was analyzed in layers II, III/IV and V/VI. α_{1A}- and α_{1D}-AR mRNA expression was analyzed in layers I/II, III/IV and V/VI. The data are expressed as the average OD ± SEM for each group. Statistical analysis was performed as described above.

Results

α_2-AR binding sites are reduced in dementia subjects but not to the degree of neuronal loss in LC

In control subjects, α_2-AR binding sites have the greatest density in layer I/II, moderate levels in layers III and IV, and the lowest density in layers V and VI (Fig. 1 A). α_2-AR binding sites are not statistically different between control, AD and DLB subjects in any layer of the PFC (Fig. 1), but there is a tendency for a reduction in binding sites in specific layers of the PFC in AD and DLB subjects. Since there are few differences between AD and DLB subjects as to cognitive loss, behavioral changes and expression of adrenergic receptors in the LC and hippocampus (this manuscript and Szot et al., 2006), combining data from AD and DLB subjects as a dementia group demonstrated a significant reduction in α_2-AR binding sites in layers I/II ($p<0.05$) and III ($p<0.05$) from control subjects. No differences were observed in α_2-AR binding sites between dementia subjects and controls in layers IV, V and VI. Because the α_{2A}-AR subtype is not expressed in the PFC (data not shown) and ^{3}H-RX821002 binds predominately to the α_{2A}-AR (Ordway et al., 1993; Sastre and Garcia-Sevilla, 1994), the binding sites in the PFC represent presynaptic α_2-AR binding sites from the LC. Therefore, there is a reduction in the presynaptic α_2-AR binding sites in specific layers of the PFC in dementia, though the reduction in binding sites (~18%) isn't near the reduction in noradrenergic cell bodies in the LC (~50-80%) (Szot et al., 2006). This data suggests that there is sprouting from the surviving LC noradrenergic neurons in the LC to the PFC, similar to what was observed in the hippocampus (Szot et al., 2006).

Postsynaptic α_{2C}-AR mRNA is reduced only in layer II of the PFC in dementia subjects

In control subjects, α_{2C}-AR mRNA expression is greatest in layer II of the PFC with lower expression in layers III/IV and V/VI (Fig. 2 A). Quantitation of α_{2C}-AR mRNA expression in AD and DLB subjects demonstrated a significant reduction only in layer II of the PFC, the layer with the greatest amount of expression in control subjects (Fig. 2). The reduction in α_{2C}-AR mRNA expression in layer II of the PFC was comparable between AD and DLB subjects.

Postsynaptic α_1-AR binding sites tend to be elevated in layer I/II of PFC in subjects with dementia despite a reduction in α_{1A}-AR mRNA

The α_1-AR is solely a postsynaptic AR. ^{3}H-Prazosin labels all layers of the PFC with the greatest density in layer I/II, and less expression in layers III/IV and V/VI (Fig. 3 A). Quantitation of ^{3}H-prazosin binding sites in AD and DLB subjects are not statistically different from control subjects in any layer of the PFC, though there is a tendency for an increase in binding sites in layer I/II (Fig. 3). Combining α_1-AR binding site values of AD and DLB subjects results in a significant increase in α_1-AR binding sites in layer I/II as compared to control ($p<0.05$). These data indicate that the postsynaptic α_1-AR is normal to elevated in subjects with dementia, similar to what has been observed in the hippocampus (Szot et al., 2006).

Similar to the α_2-AR, the α_1-AR is composed of several subtypes, the α_{1A}-, α_{1B}- and α_{1D}-AR mRNA. ^{3} H-Prazosin binds mainly to the α_{1A}- and α_{1B}-AR subtype; however, only the α_{1A}-(Fig 4. A-C) and α_{1D}-AR (Fig 5. A-C) subtypes are expressed at detectable levels in the PFC. In control subjects, α_{1A}-AR mRNA is expressed in all layers of the PFC with greatest amount of expression observed in layer V/VI, with lower levels in the other layers (Fig. 4 A). Quantitation of α_{1A}-AR mRNA in AD and DLB subjects demonstrated a significant reduction in expression in layers I/II and V/VI (Fig. 4). The reduction in α_{1A}-AR mRNA in the PFC was comparable between AD and DLB subjects.

In control subjects, α_{1D}-AR mRNA expression is mainly observed in layer V/VI, with the other layers having very low expression (Fig 5. A). Quantitation of α_{1D}-AR mRNA in the PFC of AD and DLB subjects demonstrated a significant reduction of expression in layer V/VI (Fig 5). The reduction in α_{1D}-AR mRNA in the PFC was comparable between AD and DLB subjects.

Discussion

In subjects with dementia, the surviving noradrenergic LC neurons are sprouting to the PFC

In subjects with dementia (AD and DLB subjects), the loss of noradrenergic neurons in the LC does not necessarily mean a loss of noradrenergic innervation to forebrain regions such as the PFC. Axonal sprouting of the surviving noradrenergic LC neurons to the PFC is occurring in subjects with dementia, though the sprouting does not result in normal noradrenergic innervation. In subjects with dementia, there is a reduction in presynaptic innervation to the PFC, but the reduction in presynaptic α_2-AR binding is not as extensive as the loss of noradrenergic neurons in these subjects (Szot et al., 2006), indicating the presence of axonal sprouting. The data presented here support the lack of change in α_2-AR binding in the PFC of AD subjects with other radiolabeled compounds (Shimohama et al., 1986; Leverenz et al., 2001; Matthews et al., 2002). α_2-AR binding sites in the PFC represent presynaptic noradrenergic terminals because α_{2A}-AR mRNA, the subtype ^{3}H-RX821002 binds to (Ordway et al., 1993; Sastre and Garcia-Sevilla, 1994), is not expressed to detectable levels in the PFC. Although it would have been ideal to measure norepinephrine transporter (NET) binding sites in the PFC to confirm the data generated with ^{3}H-RX821002, NET binding sites are not expressed at detectable levels in the PFC of humans (Szot, unpublished observation) or non-human primates (Smith et al., 2006).

The sprouting of noradrenergic innervation observed in the PFC of subjects with dementia is also observed in the hippocampus (Szot et al., 2006). However, it is unclear if in dementia noradrenergic sprouting is observed in all forebrain regions that receive noradrenergic innervation. Sprouting of noradrenergic neurons in the LC following neuronal loss has been observed in rats after the administration of the noradrenergic neurotoxin N-(2-chloroethyl)-

N-ethyl-2-bromobenzylamine (DSP-4), a selective noradrenergic neurotoxin which results in widespread degeneration of noradrenergic axon terminals in rodents. Shortly after the loss of terminals, a reduction in noradrenergic cell bodies is observed in the LC (Fritschy and Grzanna, 1991). However, with time the remaining noradrenergic neurons in the LC demonstrate sprouting (Fritschy et al., 1990; Fritschy and Grzanna, 1992), and this sprouting may account for the normal levels of α_2-AR binding sites in the cortex and hippocampus seen in DSP-4 treated rats (Dooley et al., 1983; Hume et al., 1992; Heal et al., 1993; Wolfman et al., 1994). The similarity in noradrenergic sprouting following DSP-4 treatment in rats and the sprouting of noradrenergic neurons in AD and DLB subjects suggests the terminals of noradrenergic neurons in dementia may be destroyed first and then the cell bodies die later. This supports the hypothesis that the deposition of amyloid plaques in the forebrain may have degenerative properties in areas like the cortex and hippocampus. However, transgenic animal models of AD where plaques develop in the cortex and hippocampus fail to produce neuronal loss (Hock and Lamb, 2001; German and Eisch, 2004). Recent work in rats (injected with amyloid β) and amyloid precursor protein 23 (APP23) transgenic mice suggests that a loss of noradrenergic function, as observed with DSP-4 treatment, enhances plaque deposition, neuronal and memory loss (Heneka et al., 2002, 2006). This suggests that the loss of noradrenergic terminals in the cortex and hippocampus following DSP-4 treatment may result in the deposition of plaques and not that plaques causes the loss of terminals. If it is correct that a loss of noradrenergic function contributes to plaque deposition, then it still needs to be determined why noradrenergic neurons are destroyed in AD.

Another possible explanation for the preservation of α_2-AR binding sites in the PFC is the presence of AR on astrocytes. α_1-, α_2- and β-AR binding sites have been observed in cultured astrocytes and these sites appear to be functional (McCarthy et al., 1995; Muyderman et al., 1998; Kilik et al., 1999; Kotter and Klein, 1999; Hosli and Hosli, 2000). The problem with these studies is that the work was done in cultures of just astrocytes. Electron microscope studies show the amount of β-AR and α_{2A}-AR in astrocytes in the rat hippocampus to be significantly less than on neurons (Milner et al., 1998, 2000), and α_{1B}- and α_{2C}-AR are not even detected in astrocytes (Enkvist et al., 1996; Papay et al., 2004). Even though ARs are found on astrocytes, the level of these binding sites appears to be much lower than that of neurons; therefore, AR binding sites in astrocytes probable do not contribute to the α_2-AR (or α_1-AR) binding sites measured in the PFC of postmortem human subjects.

Postsynaptic α_{2C}-AR mRNA is maintained in some layers of the PFC in subjects with dementia

Postsynaptic α_{2C}-AR mRNA expression in the PFC of subjects with dementia is significantly reduced only in layer II of the PFC. Layers III/IV and V/VI, which have moderate levels of α_{2C}-AR mRNA expression, remains unchanged in subjects with dementia. Expression of α_{2C}-AR mRNA in the PFC is more preserved than in the hippocampus of subjects with dementia (Szot et al., 2006). This is important because the postsynaptic α_2-AR in the PFC has been shown to increase cognitive performance with α_2-AR agonists (Arnsten, 1993, 2003; Franowicz and Arnsten, 1999, 2002; Birnbaum et al., 2000; Franowicz et al., 2002). Preservation of postsynaptic α_2-AR receptors in the PFC of AD subjects theoretically could be used to enhance cognitive function. The reduction in α_{2C}-AR mRNA expression in the PFC in layer II may be attributed to the neuronal loss in this portion of the cortex in dementia (Braak and Braak, 1991; Masliah et al., 1993; Salat et al., 2001), because α_{1A}-AR mRNA, which is also expressed in this layer, is also significantly reduced.

Postsynaptic α_1-AR binding sites are elevated in subjects with dementia even though α_{1A}- and α_{1D}-AR mRNA expression is reduced

In subjects with dementia, α_1-AR binding sites are normal to elevated in the PFC, supporting previously published work (Shimohama et al., 1986). These data also support the prior observation in the hippocampus of subjects with dementia where α_1-AR binding sites were unchanged in the hilus, but statistically elevated in the molecular level of the dentate gyrus (Szot et al., 2006). These data indicate that this major postsynaptic AR is not altered in subjects with dementia despite the loss of neurons in this region in dementia (Braak and Braak, 1991; Masliah et al., 1993; Salat et al., 2001). The α_1-AR binding sites are maintained despite the reduction in α_{1A}- and α_{1D}-AR mRNA, suggesting the remaining expressing neurons in the PFC of subjects with dementia are sprouting.

Stimulation of postsynaptic PFC α_2-AR binding sites enhance cognitive function, while stimulation of PFC postsynaptic α_1-AR binding sites can impair cognition and disrupt behavior (Birnbaum et al., 2004; Arnsten and Li, 2005). An enhanced stimulation of the PFC α_1-AR can be observed under stressful conditions when an excess of norepinephrine is released, otherwise released norepinephrine will preferentially stimulate the α_2-AR (Arnsten and Li, 2005). In dementia, PFC function may be shifted towards impaired cognition and disruptive behavior due to the normal to elevated levels of the postsynaptic α_1-AR with the loss of postsynaptic α_2-AR; this enhanced α_1-AR would most likely be observed under stressful conditions. In dementia, a common behavior problem is increased agitation, and agitation is more frequently observed under stressful conditions. Interestingly, preliminary data from the Alzheimer's Disease Research Center at the University of Washington (Murray Raskind; personnel communication) has found blocking the α_1-AR with prazosin reduces disruptive agitation in subjects with dementia.

Conclusion

In AD and DLB subjects there is a significant loss of noradrenergic neurons in the LC (Mann et al., 1980; Tomlinson et al., 1981; Bondareff et al., 1982; Marcyniuk et al., 1986; Chan-Palay and Asan, 1989; German et al., 1992; Szot et al., 2006); however, in the hippocampus (Szot et al., 2006) and PFC, there is evidence of sprouting of the survivng noradrenergic neurons in the LC subjects with dementia. Sprouting of the surviving noradrenergic neurons in the LC of subjects with dementia has been suggested by a variety of other studies (Adolfsson et al., 1979; Cross et al., 1981; Mann et al., 1981; Perry et al., 1981; Tomlinson et al., 1981; Gottfries et al., 1983; Raskind et al., 1984; Palmer et al., 1987; Reinikainen et al., 1988; Tohgi et al., 1992; Elrod et al., 1997; Russo-Neustadt et al., 1998; Hoogendijk et al., 1999; Szot et al., 2000). The sprouting observed in postmortem dementia subjects is consistent with the sprouting of noradrenergic neurons in rodents after DSP-4 induced neuronal loss (Fritschy et al., 1990; Fritschy and Grzanna, 1992). What remains unknown at the present time is if these new terminals, due to sprouting, are replacing the old ones that have died or forming new connections in areas that have never received noradrenergic innervation before. A great deal of work is required to determine how noradrenergic neuronal loss and the subsequent sprouting contributes to pathophysiology and symptomatic expression of AD.

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Abbreviations

AD	Alzheimer's disease
Alpha	α
AR	adrenoreceptors
DLB	dementia with Lewy bodies
LC	locus coeruleus
PFC	prefrontal cortex
NE	norepinephrine

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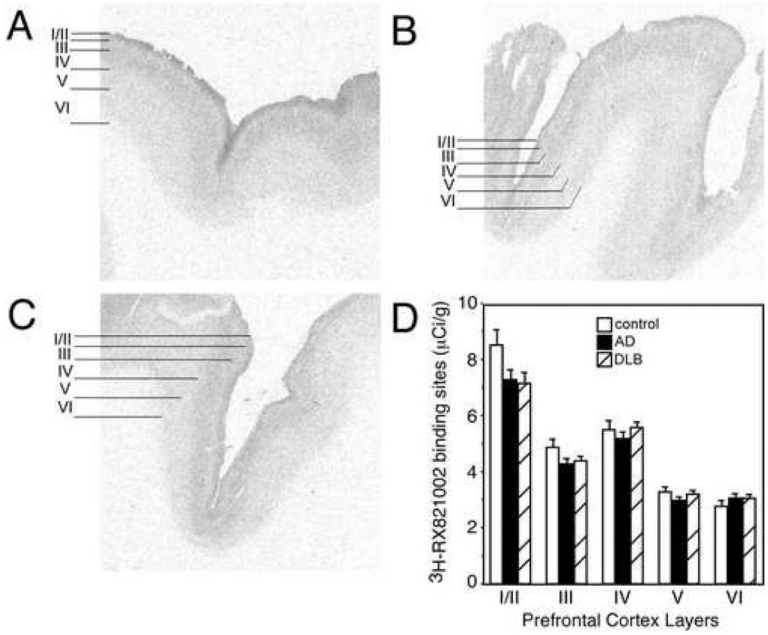

Figure 1. α-AR binding sites subjects with dementia tend to be reduced

^{3}H-RX 821002 labeling of α_2-AR binding sites in the PFC of (A) control (n=14), (B) AD (n=15), and (C) DLB (n=19). The greatest amount of ^{3}H-RX 821002 labeling is observed in layers I/II, with moderate levels observed in layers III and IV, and lowest levels of expression in layers V and VI. Definitions of PFC layers for data analysis are shown to the left of the autoradiograms for control, AD and DLB subjects. (D) Quantification of α_2-AR binding sites in the different layers of the PFC in control, AD and DLB subjects. Data are represented as mean ± SEM.

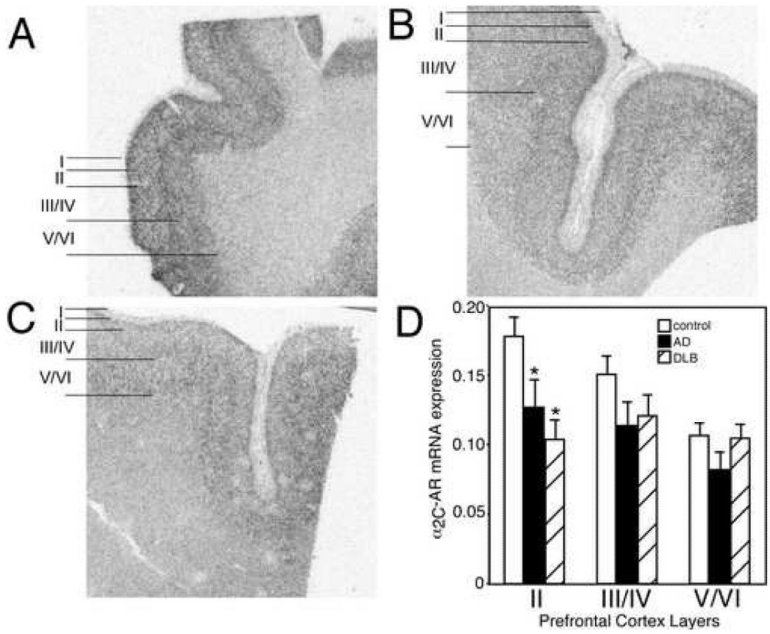

Figure 2. α_{2C}-AR mRNA expression is reduced in the PFC of AD and DLB subjects

α_{2C}-AR mRNA expression in the PFC of (A) control (n=15), (B) AD (n=15), and (C) DLB (n=19). The greatest amount of α_{2C}-AR mRNA expression in control subjects is observed in layer II, with less in layer III/IV and lowest expression in layer V/VI. Definition of PFC layers for data analysis are shown to the left of the autoradiograms for control, AD and DLB subjects. (D) Quantification of α_{2C}-AR mRNA expression in the different layers of the PFC in control, AD and DLB subjects. * Significant difference ($p<0.05$) compared with control subjects. Data are represented as mean ± SEM.

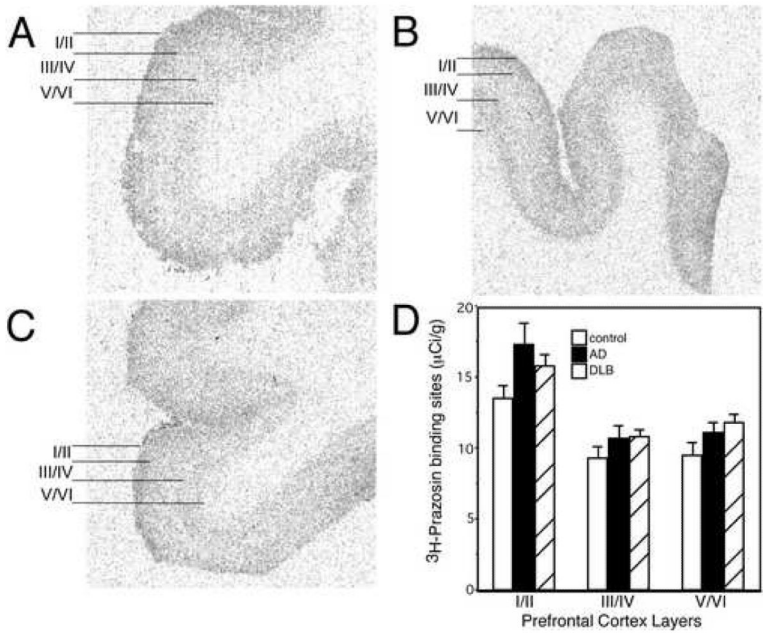

Figure 3. α_1-AR binding sites tend to be elevated in subjects with dementia

^{3}H- prazosin labeling of α_1-AR binding sites in the PFC of (A) control (n=17), (B) AD (n=15), and (C) DLB (n=22). The greatest amount of ^{3}H-prazosin labeling in control subjects is observed in layers I/II, with moderate levels observed in layers III/IV and V/VI. Layers for data analysis are shown to the left of the autoradiograms for control, AD and DLB subjects. (D) Quantification of α_1-AR binding sites in the different layers of the PFC in control, AD and DLB subjects. Data are represented as mean ± SEM.

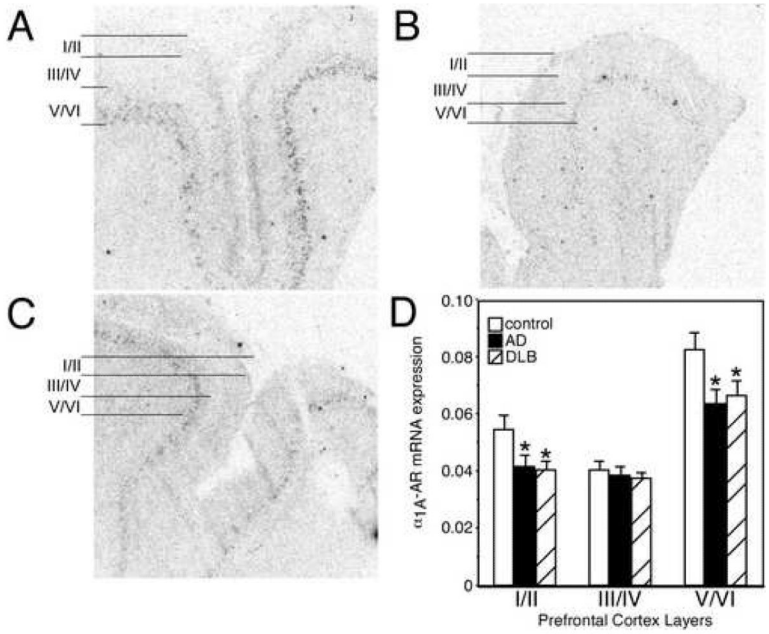

Figure 4. α_{1A}-AR mRNA expression is reduced in the PFC of AD and DLB subjects

α_{1A}-AR mRNA expression in the PFC of (A) control (n=16), (B) AD (n=15), and (C) DLB (n=21). The greatest amount of α_{2A}-AR mRNA expression in control subjects is observed in layer V/VI with moderate levels in layer I/II and lowest expression in layer III/IV. Layers for data analysis are shown to the left of the autoradiograms for control, AD and DLB subjects. (D) Quantification of α_{1A}-AR mRNA expression in the different layers of the PFC in control, AD and DLB subjects. * Significant difference ($p<0.05$) compared with control subjects. Data are represented as mean ± SEM.

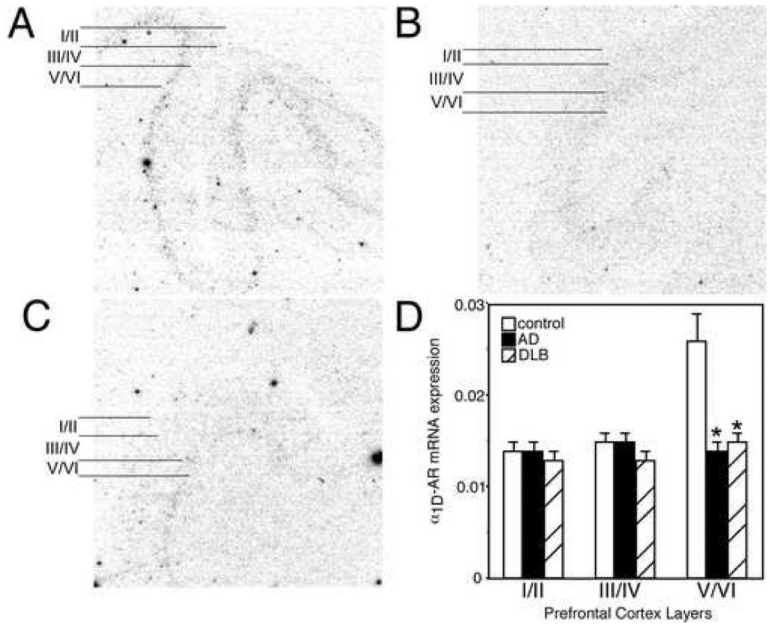

Figure 5. α_{1D}-AR mRNA expression is reduced in the PFC of AD and DLB subjects

α_{1D}-AR mRNA expression in the PFC of (A) control (n=17), (B) AD (n=15), and (C) DLB (n=21). The greatest amount of α_{2A}-AR mRNA expression in control subjects is observed in layer V/VI with lowest level of expression in layer I/II and III/IV. Layers for data analysis are shown to the left of the autoradiograms for control, AD and DLB subjects. (D) Quantification of α_{1D}-AR mRNA expression in the different layers of the PFC in control, AD and DLB subjects. * Significant difference ($p<0.05$) compared with control subjects. Data are represented as mean ± SEM.