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NEURON SPECIFIC α-ADRENERGIC RECEPTOR EXPRESSION IN HUMAN CEREBELLUM: IMPLICATIONS FOR EMERGING CEREBELLAR ROLES IN NEUROLOGIC DISEASE

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Abstract

Recent data suggest novel functional roles for cerebellar involvement in a number of neurologic diseases. Function of cerebellar neurons is known to be modulated by norepinephrine and adrenergic receptors. The distribution of adrenergic receptor subtypes has been described in experimental animals, but corroboration of such studies in the human cerebellum, necessary for drug treatment, is still lacking. In the present work we studied cell-specific localizations of α_1 adrenergic receptor subtype mRNA (α_{1a}, α_{1b}, α_{1d}), and α_2 adrenergic receptor subtype mRNA (α_{2a}, α_{2b}, α_{2c}) by *in situ* hybridization on cryostat sections of human cerebellum (cortical layers and dentate nucleus). We observed unique neuron-specific α_1 adrenergic receptor and α_2 adrenergic receptor subtype distribution in human cerebellum. The cerebellar cortex expresses mRNA encoding all six α adrenergic receptor subtypes, whereas dentate nucleus neurons express all subtype mRNAs, except α_{2a} adrenergic receptor mRNA. All Purkinje cells label strongly for α_{2a} and α_{2b} adrenergic receptor mRNA. Additionally, Purkinje cells of the anterior lobe vermis (lobules I to V) and uvula/tonsil (lobules IX/HIX) express α_{1a} and α_{2c} subtypes, and Purkinje cells in the ansiform lobule (lobule HVII) and uvula/tonsil express α_{1b} and α_{2c} adrenergic receptor subtypes. Basket cells show a strong signal for α_{1a}, moderate signal for α_{2a} and light label for α_{2b} adrenergic receptor mRNA. In stellate cells, besides a strong label of α_{2a} adrenergic receptor mRNA in all and moderate label of α_{2b} message in select stellate cells, the inner stellate cells are also moderately positive for α_{1b} adrenergic receptor mRNA. Granule and Golgi cells express high levels of α_{2a} and α_{2b} adrenergic receptor mRNAs. These data contribute new information regarding specific location of adrenergic receptor subtypes in human cerebellar neurons. We discuss our observations in terms of possible modulatory roles of adrenergic receptor subtypes in cerebellar neurons responding to sensory and autonomic input signals, and review species differences in cerebellar adrenergic receptor expression.

Keywords

receptors; alpha-adrenergic; norepinephrine; *in situ* hybridization; cerebellum; locus coeruleus

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The cerebellar cortex receives three classes of afferents: mossy, climbing and multilayered fibers (Haines, 2002). The first two classes use glutamate to transmit sensorimotor, timing, error and error corrections signals, which are modified by GABAergic interneurons and fast chemical synapses (Ito, 1984). The third class uses catecholamines, including norepinephrine (NE), and slow volume transmission to modulate the response of the fast synapses (Abbot and Sotelo, 2000). NE axons from locus coeruleus (LC) and subcoeruleus innervate all layers of the cerebellar cortex (Moore and Card, 1984), transmitting phasic or tonic impulses (Aston-Jones et al., 1999) to activate specific adrenergic receptors (ARs).

ARs are G protein-coupled receptors with nine distinct AR subtypes (α_{1a}, α_{1b}, α_{1d}, α_{2a}, α_{2b}, α_{2c}, β_1, β_2 and β_3). ARs regulate neurotransmitter release in the CNS and play a vital role in various processes, including autonomic, somatosensory, motor, cognitive, nociceptive and endocrine functions, and the control of alertness, temperature, blood pressure and shivering (Kamibayashi and Maze, 2000). Distribution of AR subtypes in animal and human tissues has been described using both RNA (Northern, RNase protection, *in situ* hybridization) and protein (immunohistochemical, autoradiographic) approaches (Palacios et al., 1987; Jones and Palacios, 1991; Pascual et al., 1992; Nicholas et al., 1993; Aoki et al., 1994; Berkowitz et al., 1994; Pieribone et al., 1994; Price et al., 1994; Scheinin et al., 1994; Stafford Smith et al., 1995, 1999; Tavares et al., 1996; Wang et al., 1996; Day et al., 1997) that indicates some species differences making extrapolation from animal studies to human difficult. To our knowledge, no other reports on the distribution of αAR subtype mRNAs in human cerebellum are currently available.

NE involvement in signaling of cerebellar neurons has been documented (Moises et al., 1979; Yeh and Woodward, 1983; Basile and Dunwiddie, 1984; Bickford-Wimer et al., 1991; Woodward et al., 1991; Kondo and Marty, 1998), and abnormal levels of NE or αAR densities have been noted in cerebella of patients with olivocerebellar atrophy, dementia with Lewy bodies, Parkinson's and Alzheimer's diseases (Kish et al., 1984a,b; Shimohama et al., 1986; Meana et al., 1992; Grijalba et al., 1994; Russo-Neustadt and Cotman, 1997; Leverenz et al., 2001). Additionally, animal studies have shown that NE is essential for normal cerebellar development (Sievers and Klemm, 1982; Luthman et al., 1990; Podkletnova and Alho, 1998), and human studies indicate cerebellar involvement in neurodevelopmental disorders, such as autism, fetal alcohol, Joubert, Down's and fragile X syndromes, attention deficit hyperactivity disorder, and posterior fossa malformations (Holroyd et al., 1991; Reiss et al., 1991; Sowell et al., 1996; Courchesne, 1997; Berquin et al., 1998).

Given this involvement of NE and ARs in cerebellar function, development and disease, we investigated neuron specific expression of α_1 and α_2AR mRNAs in human cerebellum. We demonstrate distinct cell-specific distribution of α_1 and α_2AR mRNAs in cerebellar neurons, and show expression in some neurons where αAR subtypes were not previously known to exist. We discuss our findings in terms of species differences and functional activity of αAR subtypes in cerebellar neurons. These findings will enhance our understanding of noradrenergic actions in human cerebellum and should lead to emerging strategies of pharmacological interventions designed to treat neurological diseases of the cerebellum.

EXPERIMENTAL PROCEDURES

Human cerebellum

After institutional approval and family consent, human cerebellar tissue was obtained within 1–2 h postmortem from four patients (age range 74–86 years) without history of premorbid CNS disease who were enrolled in the Duke Rapid Autopsy Program. A neuropathologist grossly identified regions and areas of interest of the cerebellum at the time of tissue harvest; each cerebellar area was later confirmed by examination of tissue architecture using light

microscopy. Tissue blocks were chosen from the anterior lobe vermis (lobules I to V), the ansiform lobule (lobule HVII) and the uvula/tonsil (lobules IX/HIX) in order to represent tissue from anterior and posterior lobes, as well as spinocerebellum (vermis and paravermis) and pontocerebellum (lateral hemispheres). The tissue was placed immediately into ice cold 4% paraformaldehyde for 16 –24 h, then briefly rinsed in phosphate-buffered saline (PBS), and transferred to ice cold 20% sucrose (in PBS) until the tissue sank (to prevent freezing artifacts; at least 24–36 h). Thereafter, cerebellar samples were covered with embedding media (OCT©; Baxter Scientific; Deerfield, IL, USA) and slowly frozen in liquid nitrogen (3–5 min per tissue), and stored at −70 °C for future use.

αAR subtype selective cDNA probe constructs

Human α_1AR and α_2AR subtype cDNA constructs (α_{1a}, α_{1b}, α_{1d}, α_{2a}, α_{2b}, and α_{2c}) were used as templates for the generation of control (sense) and specific (antisense) RNA probes. Human α_1AR cDNA constructs consisted of the following: α_{1a}AR, 0.326 kb (kb) *Pvu*II/*Hind*III fragment in pGEM-4Z (Promega Corporation; Madison, WI, USA), nucleotides 958–1283 (GenBank #L31774); α_{1b}AR, 0.673kb *Xho*I/*Bam*HI fragment in pGEM-4Z (Promega), nucleotides 94–766 (GenBank #L31773); and α_{1d}AR, 0.377kb *Eco*RI/*Pst*I fragment, nucleotides 520–896 (GenBank #L31772). Human α_2AR cDNA constructs consisted of the following: α_{2a}AR, 0.324 kb *Bgl*II/*Acc*I fragment in pSP72 (Promega), nucleotides 318–642 (GenBank #M18415); α_{2b}AR 0.333kb *Pst*I/*Bam*HI fragment in pGEM-3Z (Promega), nucleotides 1395–1728 (Genbank #M34041); and α_{2c}AR, 0.348kb *Sac*I/*Acc*I fragment in pGEM-3Z (Promega), nucleotides 377–725 (Genbank #J03853). The control β-actin probe consisted of a 104-bp fragment of the human β-actin gene cloned into the *Acc*I/*Hind*II sites of the vector pGEM-4Z (Promega). Radiolabeled single strand sense and antisense RNA probes were made using linearized cDNA constructs, [^{35}S]-αUTP (DuPont, NEN, Boston, MA, USA), and either SP6 or T7 RNA polymerase, as previously described (Titus, 1992; Wilson et al., 1997). Control RNA probes were slightly larger than antisense probes due to increased amounts of polylinker transcribed with sense strand RNA.

In situ hybridization

In situ hybridization in human tissues is often difficult due to lack of pristine tissue conditions resulting from inevitable delays in tissue harvesting. In order to produce the best signal-to-noise ratio, we used cerebellar tissue from one of the human brains to optimize the *in situ* hybridization methods previously used with rat tissue (Scheinin et al., 1994; Schambra et al., 1994). We reported in detail the protocol to accommodate to different conditions of human tissue processing, which included elimination of some procedural steps and decreases post fixation time and RNase levels (see Wilson et al., 1997). Therefore, *in situ* hybridization methods are presented only briefly below.

Cerebellum hybridization and photographic emulsion conditions

Ten micrometer horizontal frozen sections of human cerebellum were cut on a cryostat (Kryostat 1720 digital; Leitz, Wetzlar, Germany) using a −20 °C block, thaw mounted onto silylated microscope slides (CEL Associates, Houston, TX, USA), and stored at −70 °C with desiccant until hybridization. To start hybridization, slides were warmed to room temperature using a hair dryer at cool setting (15–30 min), then rinsed twice for 5 min in 2× saline-sodium citrate buffer (SSC; 1× SSC=0.15 M NaCl, 0.04 M Na citrate, pH 7.2). No permeabilization or prehybridization steps were performed. Hybridization buffer (0.02 M DTT, 1× Denhart's Solution [Sigma, St. Louis, MO, USA], 1 mg/ml salmon sperm DNA [Sigma] heated to 80 ° C before use, 50 μg/ml transfer RNA [Sigma], 2× SSC, 50% formamide, 9% dextran sulfate), and 5000–7000 cpm/μl linearized radiolabeled probe were applied to the slides. The slides were then incubated at 50 °C overnight in sealed plastic containers lined with Whatman filter

paper soaked with 50% formamide in 2× SSC buffer. To remove non-specific binding, slides were washed as follows: sequential immersion in 1 μl/ml β-mercaptoethanol solutions in 2× SSC (50 °C, brief dip) and 50% formamide in 2×SSC (50 °C, 10 min, then 20 min), followed by RNase treatment using 10 μg/ml RNase in 2× SSC (35 °C, 30 min). Subsequent washes at room temperature included the following: 2× SSC (5 min), 50% formamide in 2× SSC (50 °C, 5 min), and 2× SSC (10 min), followed by dehydration steps using 2 min immersions each in 0.3 M ammonium acetate solutions containing 50%, 70%, then 100% ethanol, followed by a brief wash in pure 100% ethanol. After air drying for 30–60 min, slides were dipped in warm (40 °C) autoradiography emulsion (Kodak NTB2; Rochester, NY, USA) in a darkroom illuminated with a Kodak safe-light #2, dried for several hours in the dark, then placed in light-sealed slide boxes with desiccant at 4 °C for 4 weeks. After warming for 90 min to room temperature, exposed slides were developed under safelight by sequential immersion in fresh D19 developer (Kodak) mixed 1:1 with distilled water (dH_2O; 15 °C, 4 min), followed by room temperature immersions in dH_2O (20 s), fixer (Kodak; 5 min), and dH_2O rinses (3×5 min). Slides were counterstained with hematoxylin and eosin, dehydrated in an ascending ethanol and xylene series, and cover slipped. Dry slides were examined and photographed under bright and dark field microscopy (high power: Leitz DMRB; low power: WILD M420).

Control experiments

In order to ensure that the detected signal represented specific probe hybridization, several positive and negative controls were performed. Negative controls included sense probe experiments and demonstration of loss of signal in known positive samples exposed to excess (50 μg/ml) RNase before hybridization. An antisense human βactin probe was used as a positive control for experimental conditions and general neuronal anatomy. Controls for α_1AR and α_2AR subtype specificity of antisense probes included simultaneously performed *in situ* experiments in lines of transfected cells stably expressing individual human α_1AR subtypes, inclusion of known positive and negative human non-cerebellar tissues, as well as RNase protection assays using mRNA derived from clonal α_1AR subtype cell lines with the same probes.

Detection and analysis

Analysis of general and cell-specific αAR mRNA expression was performed by examining silver granules present in cerebellar tissue sections and signaling neurons under darkfield conditions, and the observation confirmed and photographed using bright field microscopy. Semiquantitative image analysis was performed using the MCID system (Brock University, St. Catharines, Ontario, Canada). Silver grains resulting from *in situ* hybridization experiments with αAR mRNA probes were counted from multiple samples from each patient. For each representative cell type within the cerebellum, a minimum of five high power darkfield grain counts were performed; variability of αAR mRNA expression was ≤10–15% for the vast majority of cell types at all cerebellar layers. Although cerebellum samples were harvested within 2 h postmortem, some degradation of mRNA is theoretically possible prior to tissue collection; hence, in order to ensure uniformity in reporting of results, samples for each patient were analyzed separately, αAR subtype mRNA expression compared for each patient, and then averaged. The abundant signal of either α_{2a} or α_{2b}AR mRNA expression in Purkinje cells was arbitrarily defined as 100%. It should be noted that, despite of efforts to normalize data, signal assessment for *in situ* experiments is semi-quantitative, since some experimental artifacts cannot be ruled out (e.g. different probes may not hybridize exactly equally). Therefore, for final reporting we utilized a more general scale as follows: +++ denotes heavy signal (71–100%); ++ moderate signal (31–70%); + light signal (5–30%); +/0 represents signal just above background and 0 that signal is undetectable. When we found signal in select neurons only, we denoted that with brackets.

RESULTS

General

It is well known that the cerebellar cortex contains what can be called major cells, in terms of their numbers and extensive distribution throughout the cortex, and minor cells that are much fewer in number and/or found mainly in more geographically restricted areas of the cortex. Examples of the former are the granule, Golgi, basket, stellate and Purkinje cells; these cortical neurons are the target of this investigation. Examples of the latter are the cells of Lugaro and the unipolar brush cells. Due to their fewer numbers and more restricted distribution, these cells were excluded from the present study.

Pilot experiments to determine appropriate *in situ* hybridization conditions (hybridization time, temperature, and RNase concentration) were performed in *n*=1 patient samples. For final analysis tissue samples from three human cerebellar regions were compared from *n*=3 patients. These selected cerebellar regions are shown in Fig. 1A. The corresponding tissue sections are shown as follows: anterior lobe vermis (lobules I to V; Fig. 1B), ansiform lobule (lobule HVII; Fig. 1C), and uvula/tonsil (lobules IX/HIX; Fig. 1D).

Controls

Control experiments confirmed sensitive and specific hybridization of α_1AR and α_2AR subtype mRNA probes, with no evidence of cross-hybridization between αAR subtypes or binding of sense probes. For example, sense probes produced no hybridization signal, whereas antisense probes hybridized specifically to cerebellar tissue and control cells (clonal cell lines stably expressed an individual human αAR subtype) without cross-hybridization to other subtypes. The β-actin antisense probe served both as positive control and as a marker to delineate the cerebellar cortex and its neurons (Fig. 2A), as well as dentate nucleus and its neurons (Fig. 2B). Negative controls included hybridization with appropriate sense probes, i.e. the α_{1a}AR sense probe (Fig. 2C) and RNase treatment before hybridization (Fig. 2D).

General α_1 versus α_2AR mRNA expression in cerebellum

Both α_1AR and α_2AR mRNAs were present in the cerebellum in every patient, with α_2AR mRNA expression greater than α_1AR. Overall, the Purkinje cell layer contained the highest abundance of both α_1AR and α_2AR subtype mRNA in human cerebellum. Some subtype specificity was observed in the other two major layers of the cerebellar cortex, with signal intensity in the molecular layer> granule cell layer for α_1AR mRNA, and granule cell layer>molecular cell layer for α_2AR mRNA. In addition to general α_1AR versus α_2AR selectivity, unique αAR subtype specificity was evident, varying by cell layer and cell body location (Table 1).

Neuron-specific expression of α_1 and α_2AR mRNA

All *Purkinje cells* showed strong α_{2a}AR (Fig. 3A; Table 1) and α_{2b}AR mRNA expression (Fig. 3B; Table 1). Purkinje cells of the anterior lobe vermis and uvula/tonsil expressed moderate levels of α_{2c}AR mRNA (Fig. 3C; Table 1). In contrast, expression of the three α_1AR subtypes was variable and specific to select Purkinje cells in cerebellar regions (Table 1). Thus, Purkinje cells of the anterior lobe vermis and uvula/tonsil expressed moderate levels of α_{1a}AR (Table 1), whereas Purkinje cells of the ansiform lobule and uvula/tonsil showed a moderate signal for α_{1b}AR mRNA (Table 1), and a strong label for α_{1d}AR mRNA (Fig. 3D; Table 1). *Granule cells* labeled intensely for α_{2a}AR (Fig. 4A; Table 1) and α_{2b}AR mRNA (Table 1), but not for the remaining AR subtypes (Fig. 4B shows α_{2c}AR; Table 1). *Golgi cells* also expressed high levels of α_{2a} (Fig. 4C; Table 1) and α_{2b}AR mRNA (Table 1), but none of the other subtypes. *Basket cells* showed a strong α_{1a}AR mRNA signal (Fig. 4D; Table 1), moderate levels of

α_{2a}AR mRNA and a weaker signal for α_{2b}AR mRNA (Table 1). *Inner stellate cells* showed high levels of α_{2a}AR (Table 1) and moderate levels of α_{1b}AR mRNA in the inner half of the molecular layer (Fig. 4E; Table 1). *Outer stellate cells* expressed strong signal for α_{2a}AR mRNA (Fig. 4F; Table 1) and moderate signal for α_{2b}AR mRNA in select cells (Table 1). *Dentate nucleus neurons* expressed moderate levels of α_{1a}AR mRNA (Fig. 5A;Table 1), high levels of α_{1d}AR (Fig. 5B; Table 1) and α_{2b}AR mRNA (Fig. 5C; Table 1), light levels of α_{1b}AR mRNA (Table 1), and low or background signal of α_{2c}AR mRNA (Fig. 5D;Table 1), while α_{2a}AR mRNA was absent.

DISCUSSION

Principal findings

This study provides unique information on subtype-specific mRNA expression of six α-ARs in human cerebellum, taken from anterior lobe vermis (lobules I–V), ansiform lobule (lobule HVII), and uvula/tonsil (lobules IX/HIX). AR subtype mRNAs are differentially expressed in cerebellar neurons (Table 1). The α_{2a}AR and α_{2b}AR mRNAs are present in all neurons of the cerebellar cortex, but α_{2a}AR mRNA is not found in dentate nucleus neurons. Message of the $\alpha_{1a,}$ $\alpha_{1b,}$ α_{1d} and α_{2c}AR subtypes also is observed in region-specific Purkinje cells. In addition, α_{1a}AR mRNA is expressed specifically by basket cells and dentate nucleus neurons, α_{1b}AR by inner stellate cells, and α_{1d}AR by dentate nucleus neurons.

Species-specific differences

Species-specific differences in distribution of α_1 and α_2AR subtypes have been described for CNS and peripheral tissues (Palacios et al., 1987; Pazos et al., 1988; Jones and Palacios, 1991; Schwinn et al., 1991; Go et al., 1993; Pascual et al., 1992; Van Liefde et al., 1993; Zilles et al., 1993; Aoki et al., 1994; Berkowitz et al., 1994; Price et al., 1994). The present *in situ* hybridization results contribute to our knowledge of interspecies differences in expression of αARs in cerebellum, and add important new information on their localization in human. In addition, while earlier animal studies mostly described αAR subtypes in different cerebellar layers (Tables 2–4), our study identified cell-type specific expression of αAR transcripts. Finally, we optimized our *in situ* hybridization conditions previously described in rat (Scheinin et al., 1994; Schambra et al., 1994), to control for variations in postmortem human tissue and to allow detection of rare or fragile mRNAs (Wilson et al., 1997).

Species-specific differences of α_2ARs in cerebellum

A comparison of our α_2AR subtype expression data in human cerebellum (Table 1) with the expression, immunocytochemical and autoradiographic data in cerebella of experimental animals (Tables 2– 4) confirms ubiquitous localization of the α_{2a}AR subtype. However, localization of α_{2b}AR and α_{2c}ARs in human differs from that in animal cerebella. Our observations of moderate and widespread expression of α_{2b}AR mRNA in human cerebellum are reflected in some previous rodent studies (Tavares et al., 1996;Wang et al., 1996, 2002;Winzer-Serhan and Leslie, 1997a), but not in others (Table 2). In contrast, while we observed α_{2c} expression only in select human Purkinje cells, strong expression and immunocytochemical signals of this subtype were reported for the three cerebellar layers and cerebellar nuclei of rat (Nicholas et al., 1993;Rosin et al., 1996). These discrepancies might theoretically be due to differences in probe preparation, i.e. our using probes against sequences encoding subtype-specific intracellular loops of the subtype genes versus others using full length clones which cross-hybridize unless more stringent conditions are used (Wang et al., 2002), or may be due to technical difficulties, such as sensitivity of α_{2b}AR mRNA to degradation after poor fixation (Winzer-Serhan and Leslie, 1997a). We observed similar technical problems in preliminary experiments, but optimized fixation time, RNase concentration and buffer stringency to overcome these issues (Wilson et al., 1997). More likely,

true species difference may exist between primate and rodent α_{2b} and α_{2c}AR expression patterns. In support of this interpretation, Go et al. (1993) observed α_{2b}AR protein in Purkinje cells of monkey but not rat; further divergence of the promoter regions between rodent and human α_{2b}AR was recently reported (Cayla et al., 2004). Thus, in parallel with the expansion of both cerebrum and cerebellum in primates and the addition of fine motor control, cognitive and language functions (Petersen et al., 1989;Leiner et al., 1991,1993;Desmond and Fiez, 1998;Thach 1998;Wickelgren, 1998;Schmahmann, 1997;Ito, 2000) to the control of posture, movement and reflexes (Ito, 1984;Jueptner and Weiller, 1998), the α_{2b}AR may have gained functional importance and replaced the α_{2c}AR.

Species-specific differences of α_1ARs in cerebellum

Expression of α_1AR mRNAs in human cerebellum was more limited than that of the α_2AR mRNAs and specific to certain cerebellar neurons (Table 1). Because the animal data are still scant (Tables 2–4), only partial comparisons are possible. Overall, our finding of relatively low abundance of α_1ARs in human cerebellum agrees with the animal data. However, our most striking findings, expression of α_{1a}AR in basket cells, α_{1b}AR in inner stellate cells and α_{1d}AR in dentate nucleus neurons, have not been reported previously. These three subtypes were also present in select Purkinje cells, as was noted in rat (Day et al., 1997), and our finding of moderate levels of α_{1a}AR mRNA in dentate neurons confirms an earlier report in rat (Domyancic and Morilak, 1997). Using α_1AR ligands, species differences were noted in one study, such that the α_1AR was observed in mouse, rat and cat, but not guinea-pig cerebella (Palacios et al., 1987). Also, the α_{1b}AR subtype may be more abundant in rodent than human cerebellar cortex (Wilson and Minneman, 1989;Yang et al., 1998;Acosta-Martinez et al., 1999).

Effects of NE on cerebellar function

NE axons from LC and subcoeruleus innervate all cells of the cerebellar cortex and cerebellar nuclei (Bloom et al., 1971; Landis et al., 1975; Kimoto et al., 1981; Moore and Card, 1984; Powers et al., 1989; Nelson et al., 1997). NE is released by volume transmission in a paracrine fashion from varicosities of thin axons and diffuses over more or less extended distances to activate specific AR subtypes in non-synaptic junctions (Abbot and Sotelo, 2000). The synchronized release of NE by LC neurons throughout the neuraxis can be either tonic (steady, low to high levels of NE, depending on state of vigilance), or phasic (additional bursts of high levels of NE during stimulus-evoked activity; Aston-Jones et al., 1991, 1999). LC stimulation and iontophoretical applications of NE to rat cerebellar slices or cells increase spontaneous Purkinje cell firing at low concentrations (0.5–10 μM), increase the frequency of spontaneous firing of stellate cells at > 16 μM, resulting in depression of Purkinje cell spontaneous activity, and cause consistent depression of Purkinje cell spontaneous activity at higher concentrations (25–100 μM; Basile and Dunwiddie, 1984; Mori-Okamoto and Tatsuno, 1988; Kondo and Marty, 1998). Furthermore, 10 mM NE has been reported to potentiate the inhibitory activity of stellate and basket cells (Llano and Gerschenfeld, 1993; Saitow and Konishi, 2000), while application of 100 mM NE for 30 min causes both a short-term enhancement of GABAergic inhibition and prolonged inhibition lasting more than 2 h. This is suggestive of the long-term depression involved in motor learning that occurs after simultaneous stimulation of climbing fibers (CF) and parallel fibers (PF) (Mitoma and Konishi, 1999; Gao et al., 2003). Thus, NE enhances stimulus-evoked signals, i.e. increases the “signal-to-noise ratio” of firing for both glutamatergic excitatory and GABAergic inhibitory synapses of cerebellar interneurons (Freedman et al., 1977; Moises et al., 1979, 1990; Mori-Okamoto and Tatsuno, 1988; Woodward et al., 1991). In contrast, depletion of cerebellar NE impairs motor performance (Watson and McElligott, 1984). Activation of specific ARs has been reported for some NE concentrations: α_{2a}AR for > 16 μM NE, αAR below that range, α_2AR at 10 mM and α_1AR at 0.5 M (Yeh and Woodward, 1983; Basile and Dunwiddie, 1984; Mori-Okamoto and Tatsuno,

1988; Kondo and Marty, 1998). Thus, modulation of cerebellar neuron activity appears to be regulated by specific AR subtypes that selectively respond to certain levels or exposure times of NE.

Properties of α_2AR subtypes

Although the α_2AR subtypes are similar in structure, binding properties and coupling to G proteins, differences have been observed in subcellular localization, second messenger coupling, desensitization and trafficking properties in various transfection systems (Jones et al., 1991; Pepperl and Regan, 1993; Wozniak and Limbird, 1998; Olli-Lähdesmäki et al., 1999). For instance, when α_{2a} and α_{2b}ARs are co-expressed in a rat pheochromocytoma (PC12) cell line (Olli-Lähdesmäki et al., 1999) or mouse embryonic spinal cord neurons in culture (Wozniak and Limbird, 1998), they both appear dispersed over the plasma membrane and concentrated at the tips of neurites. However, when α_{2b}AR is expressed alone, it is evenly distributed in the plasma membrane of the cell body (Olli-Lähdesmäki et al., 1999). If these findings also hold true for cerebellar neurons, and future studies reveal comparable receptor locations in human cerebellar neurons, then the α_{2a} and α_{2b}ARs co-expressed in the present study in human cortical neurons could act both as pre- and postsynaptic receptors on axons and dendrites. In contrast, the α_{2b}AR expressed without the α_{2a}AR in human dentate nucleus neurons may instead reflect its function as a postsynaptic receptor, located on cell bodies and dendrites. Different from the α_{2a} and α_{2b}ARs, the α_{2c}AR subtype is thought to be located mainly intracellularly (Wozniak and Limbird, 1998; Olli-Lähdesmäki et al., 1999; Hurt et al., 2000).

In assessing α_2AR subtype-specific desensitization in PC12 cells, Olli-Lähdesmäki et al. (1999) found that the α_{2a}AR was rapidly internalized (with some receptors remaining at the tips of the neurites), but the α_{2b}AR was more extensively internalized after agonist-induced activation (NE; 10 μM, 30min). No change was observed for α_{2c}AR. In human choriocarcinoma (JEG-3) cells, the α_{2a}AR responded to nanomolar concentrations of agonist with inhibition of reporter gene activity, while micromolar concentrations reversed the inhibition and caused potentiation (Pepperl and Regan, 1993). In contrast, the α_{2b}AR responded only with stimulation of activity to either concentration, and the α_{2c}AR only with inhibition. Also, signaling and trafficking of α_2AR subtypes is regulated in many ways by specific kinases, arrestins and spinophilin. For instance, arrestin-3 (β-arrestin-2) promotes internalization of α_{2b} and α_{2c}AR subtypes, but not α_{2a}ARs, and arrestin-2 (β-arrestin-1) selectively promotes internalization of α_{2b}ARs only (DeGraff et al., 2002; Wang et al., 2004). Further distinguishing features of α_2AR subtypes are their response to stimulation frequencies and their affinity to agonist. Thus, Hein et al. (1999) observed in neurons from AR subtype knock-out mice that the α_{2a}AR inhibits neurotransmitter release with higher speed and at higher action potential frequencies than the α_{2c}AR, but that NE had a higher affinity for α_{2c}ARs than α_{2a}ARs, and the latter were deactivated faster after agonist stimulation (Bünemann et al., 2001).

If agonist response of cerebellar α_2ARs is similar, it could be reasoned that the α_{2a}AR inhibits neurotransmission during tonic and low levels of stimulation from the LC. However, when external signals produce LC phasic stimulation, both the α_{2a} and α_{2b}ARs may activate cerebellar neurons and potentiate their firing at inhibitory and excitatory synapses, respectively. Due to the limited expression of the α_{2c}AR subtype, we speculate that this AR is involved in slow inhibition of transmitter release from dentate nucleus neurons and select Purkinje cells in humans, possibly similar to what was observed in mouse brain, where α_{2a}AR inhibited dopamine release at higher speed than α_{2c}AR, and α_{2c}AR signaling was attenuated after prolonged agonist exposure (Bücheler et al., 2002).

Potential roles of α_2ARs in cerebellar function

To clarify the role of NE and its receptors, it is important to know where specific AR subtypes are located. For instance, are they located on the pre- and/or postsynaptic membrane, on cell bodies, dendrites or axons, proximal or distal to classic synapses, and preferentially associated with excitatory or inhibitory synapses? Several studies of other brain regions, using combined light and electron microscopy with dual immunocytochemical labels for the α_{2a}AR and tyrosine hydroxylase (catecholamine synthesizing enzyme) or dopamine beta-hydroxylase have shown that α_{2a}ARs can be located presynaptically on catecholaminergic and non-catecholaminergic axons and terminals, postsynaptically near catecholaminergic terminals on dendritic shafts and spines of various non-adrenergic neurons, and on some glial processes (Aoki et al., 1998; Milner et al., 1998; Glass et al., 2001). Similar to these findings, our observation of abundant α_{2a} and α_{2b}AR message in non-catecholaminergic cells may represent not only postsynaptic heteroreceptors located on dendrites, but also presynaptic receptors located near GABAergic and/or glutamatergic axon terminals.

It is interesting that we observed a similar abundance of both α_{2a} and α_{2b}AR subtype mRNAs in neurons of the cerebellar cortex, but no α_{2a}AR mRNA in dentate nucleus neurons. Both cortical and dentate nucleus neurons receive glutamatergic excitatory afferents that originate in pontine and spinal cord nuclei and terminate as mossy fibers (MF), as well as feed-back and timing information through CF from the inferior olives (Blumenfeld, 2002; Haines, 2002). These inputs are much more numerous in primates than in rodents, no doubt reflecting the expansion of the cerebrum and cerebellum during evolution (Leiner et al., 1991, 1993). We speculate that the α_{2b}AR subtype is associated with glutamatergic synapses related to this expansion and therefore more abundant in primates than in rodents. The absence of α_{2a}AR mRNA in dentate nucleus neurons may on the other hand point to a role of this subtype in states of vigilance (Aston-Jones et al., 1991, 1999). We suggest that cerebellar cortical neurons need to be able to respond to changes in vigilance, i.e. reducing their activity during sleep or increasing activity during stages of high alertness. This response may be regulated, depending on the levels of NE transmitted from the LC, by αARs. In contrast, the lack of the α_{2a} and α_{2a}AR subtype in dentate nucleus neurons may allow independence of these neurons from states of vigilance.

We observed that both granule and Golgi cells abundantly express α_{2a}AR and α_{2b}AR subtypes. Granule cells, the principal excitatory neurons of the cerebellar cortex, receive excitatory MF inputs which they propagate to synapses of their ascending axon segment with Purkinje cell dendrites and, after bifurcation of their axons and formation of PF, to additional Purkinje, Golgi, basket and stellate cell dendrites (Eccles et al., 1967; Gundappa-Sulur et al., 1999; Cohen and Yarom, 2000; Haines, 2002). Golgi cells, inhibitory interneurons, are located in the outer part of the granule cell layer and extend their dendrites throughout all layers of the cerebellar cortex. These dendrites are oriented in all planes to receive excitatory MF input at their bases in the granule cell layer, and from PF and CF collaterals at their apices in the molecular layer. They are inhibited by recurrent axon collaterals of Purkinje cells. Golgi cell axons arborize profusely in the granule cell layer to terminate on granule cell dendrites in cerebellar glomeruli, where they exert GABAergic feedback inhibition (Eccles et al., 1967; Haines, 2002). Granule and Golgi cells are reciprocally innervated and fire synchronously and rhythmically after MF stimulation, but the firing level can vary according to strength of incoming MF signal (Maex and Schutter, 1998). It is, therefore, possible that the α_{2a}AR subtype modulates activity of these cells according to states of vigilance, while the α_{2b}AR subtype provides modulation appropriate to the strength of glutamatergic input.

Properties of α_1AR subtypes

Several non-neuronal transfection systems have been used to define differences between α_1AR subtypes with similar results, but confirmation in neuronal systems is lacking. In these transfection studies, α_{1a}ARs are localized on the cell surface and intracellularly, α_{1b}ARs mainly on the cell surface, and α_{1d}ARs predominantly intracellularly. Both α_{1a} and α_{1b}ARs undergo rapid agonist-mediated phosphorylation, desensitization and internalization, apparently via common GRK2/β-arrestin pathways, but faster and at lower agonist concentrations for α_{1b}ARs than α_{1a}ARs (Fonseca et al., 1995; Yang et al., 1999; McCune et al., 2000; Chalothorn et al., 2002; Price et al., 2002; Morris et al., 2004). The α_{1d}AR subtype also undergoes agonist-mediated phosphorylation and desensitization; however, internalization appears to be primarily constitutive and may result from direct activation of protein kinase C, or cross-talk with other receptors endogenously expressed in the same cell (McCune et al., 2000; García-Sáinz et al., 2001).

Potential roles of α_1AR subtypes in cerebellar function

Localization of α_1AR messages is more limited than that of the α_2AR transcripts and lacking in Golgi and granule cells. Only dentate nucleus neurons express all three α_1AR subtypes with abundant α_{1d}AR, moderate α_{1a}AR and low α_{1b}AR levels. This co-expression may be functionally significant, because α_{1a} and α_{1b}ARs can form heterodimers which may alter their protein expression and trafficking (Stanasila et al., 2003; Uberti et al., 2003). These dentate neurons send cerebellar output to nuclei in the thalamus and red nucleus, are excited by MF and CF collaterals, and are either inhibited by Purkinje cells, or released from this inhibition when the Purkinje cells themselves are inhibited by basket and stellate cells (Haines, 2002). It is interesting that these dentate neurons express abundant signal for both α_{2b}AR and α_{1d}AR transcripts. As discussed above, we suggest that the α_{2b}AR is related to the incoming glutamatergic signals from MF or CF, and speculate that α_{1a} and α_{1b}ARs may play a role in the timing and receipt of the inhibitory signals from Purkinje cells, while α_{1d}ARs are constitutively active in association with other endogenous receptors to modulate the activity of dentate nucleus neurons.

It is well known that the cerebellum influences movement by synaptic mechanisms and circuits that utilize spatial and temporal timing. The limited or expansive domain of the dendritic arbor, the number of synaptic delays within a given circuit, and the constant flow of inputs from various body parts all combine to produce muscle synergy. The results of the present study, showing a differential preference for certain AR subtypes on functionally different cerebellar cell populations, suggest that timing most likely also has a molecular component. Basket cells express abundant α_{1a}AR mRNA and inner stellate cells moderate levels of α_{1b}AR mRNA. Basket cells, located in the inner molecular layer near Purkinje cell bodies, receive excitatory PF (axosomatic) and CF input. Basket cell dendrites arborize in the inner 2/3 of the molecular layer, and their axons extend along the Purkinje cell layer, where axon collaterals wrap around numerous Purkinje cell bodies to form the characteristic baskets. PF excitation of basket cell dendrites produces “off beam” GABAergic inhibition of Purkinje cells lateral to those excited by the same signal, which can be sustained up to 1 min and emphasizes the zone of Purkinje cell activity (Eccles et al., 1967; Palay and Chan-Palay, 1974; Haines, 2002). Inner stellate cells are located near basket cells in the inner molecular layer and some of their axons participate in the formation of baskets around Purkinje cell soma. In contrast, the short axons of outer stellate cells contact nearby Purkinje cell dendrites. Excitation of stellate cells though PF produces GABAergic inhibition of Purkinje cells, which narrows the spatial extent of Purkinje cell excitation in a “patchy” manner (Eccles et al., 1967; Cohen and Yarom, 1998, 2000; Blumenfeld, 2002; Haines, 2002). If the α_{1a}AR in basket cells and the α_{1b}AR in inner stellate cells function similarly as in non-neuronal transfected cells (Chalothorn et al., 2002), then the ability of the α_{1b}AR subtype to react faster and to lower levels of NE may be important

in the selective inhibition by stellate cells of specific populations of Purkinje cells. This inhibition might be followed by basket cell inhibition, modulated by α_{1a}ARs at a slightly slower speed, but requiring a more intense NE signal, resulting in a focused incoming excitatory signal from PF to select groups of Purkinje cells.

Purkinje cells express six αAR subtypes

While we observed an abundance of both α_{2a}AR and α_{2b}AR mRNA in all Purkinje cells, the remaining four AR subtypes are located in Purkinje cells of specific cerebellar lobules as follows: α_{1a}AR and α_{2c}AR in the anterior lobe vermis (lobules I to V); α_{1b}AR and α_{1d}AR in the ansiform lobule (lobule HVII); and α_{2c}AR, α_{1a}AR, α_{1b}AR and α_{1d}AR messages in the uvula/tonsil (lobule IX/HIX). This uneven location of α_1 and α_2AR subtype mRNAs may be related to the organization of Purkinje cells into sagittal microzones or functional compartments (Oberdick et al., 1998). Purkinje cells, their cell bodies located in a sheet between the granular and molecular layers, receive excitatory input from granule cell PF and inferior olive CF in their dendritic trees in the molecular layer. As the only output neurons of the cerebellar cortex, they inhibit cerebellar and vestibular nuclei. Their output, in part, is modulated by feed-forward inhibition from granule and stellate cells, as well as from Purkinje cells (Eccles et al., 1967; Gundappa-Sulur et al., 1999; Bower, 2002; Haines, 2002).

The abundance of α_{2a}AR and α_{2b}AR mRNA in all Purkinje cells may again be related to a role of these receptors in modulating Purkinje cell activity in response to states of vigilance, controlling signal-to-noise ratios and supporting glutamatergic synapses, as discussed above. In contrast, it is interesting that we observed α_{1b}AR and α_{1d}AR messages only in the two posterior lobe regions, the ansiform lobule and uvula/tonsil. The posterior lobe is phylogenetically the youngest part of the cerebellum and greatly expanded in primates (Leiner et al., 1991; Schmahmann, 1991). It is also interesting that in the two regions which are part of the spinocerebellum, the anterior lobe vermis and the uvula/tonsil, Purkinje cells are positive for α_{1a}AR and α_{2c}AR mRNA, while Purkinje cells of the ansiform lobule, part of the cerebrocerebellum, are not. The spinocerebellum receives spinal, trigeminal and vestibular inputs and is involved in motor execution, whereas the cerebrocerebellum receives corticopontine inputs and functions in motor planning. It is possible, therefore, that α_{1a}AR and α_{2c}AR subtypes play a role in motor execution. In support of this idea, in rat, α_{2c}AR transcripts have been found prominently in basal ganglia, nuclei important in motor function (Scheinin et al., 1994; Winzer-Serhan et al., 1997c).

CONCLUSIONS

Recent neuroimaging, neuroanatomical and lesion-behavioral studies have expanded our understanding of cerebellar function and diseases. No longer is the cerebellum thought to only control posture, voluntary movement and certain reflexes (Ito, 1984; Thach et al., 1992; Jueptner and Weiller, 1998), but also to control aspects of behavior, visceral responses, and cognitive processing that include language and forms of learning (Petersen et al., 1989; Haines et al., 1984, 1990; Leiner et al., 1991; Desmond and Fiez, 1998; Thach, 1998; Wickelgren, 1998; Schmahmann, 1991, 1997; Ito, 2000). Localized or global, pre- or postnatal cerebellar damage often is reflected in a variety of cerebellar dysfunctions which include ataxia, autism, Joubert syndrome and cerebellar cognitive affective syndrome (Holroyd et al., 1991; Schmahmann, 1991; Kemper and Bauman, 1993; Bastian et al., 1996; Courchesne, 1997; Schmahmann and Sherman, 1998). Also in some diseases, such as Alzheimer's and Parkinson's disease, olivocerebellar atrophy and dementia with Lewy bodies, alterations of NE projections to the cerebellum and ARs have been documented (Kish et al., 1984a,b; Shimohama et al., 1986; Meana et al., 1992; Grijalba et al., 1994; Russo-Neustadt and Cotman, 1997; Leverenz et al., 2001. It is, therefore, important to fully know the location and understand the function

of NE modulation by AR subtypes in the cerebellum and to be cognizant of possible species differences. Thus, the present study demonstrates the heterogeneous distribution of α_1 and α_2AR subtype mRNAs in human cerebellum, reports previously unknown expression of subtypes in certain cerebellar neurons and points out important species differences. This information should promote our understanding of the modulatory roles in cerebellar signaling exerted by the autonomic nervous and reticular activating systems through NE and αARs, cerebellar function in normal and disease states, and be suggestive of possible novel pharmacologic therapies.

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Abbreviations

AR
adrenergic receptor

CF
climbing fibers

dH_2O
distilled water

LC
locus coeruleus

MF
mossy fibers

NE
norepinephrine

PBS
phosphate-buffered saline

PF
parallel fibers

SSC
saline-sodium phosphate

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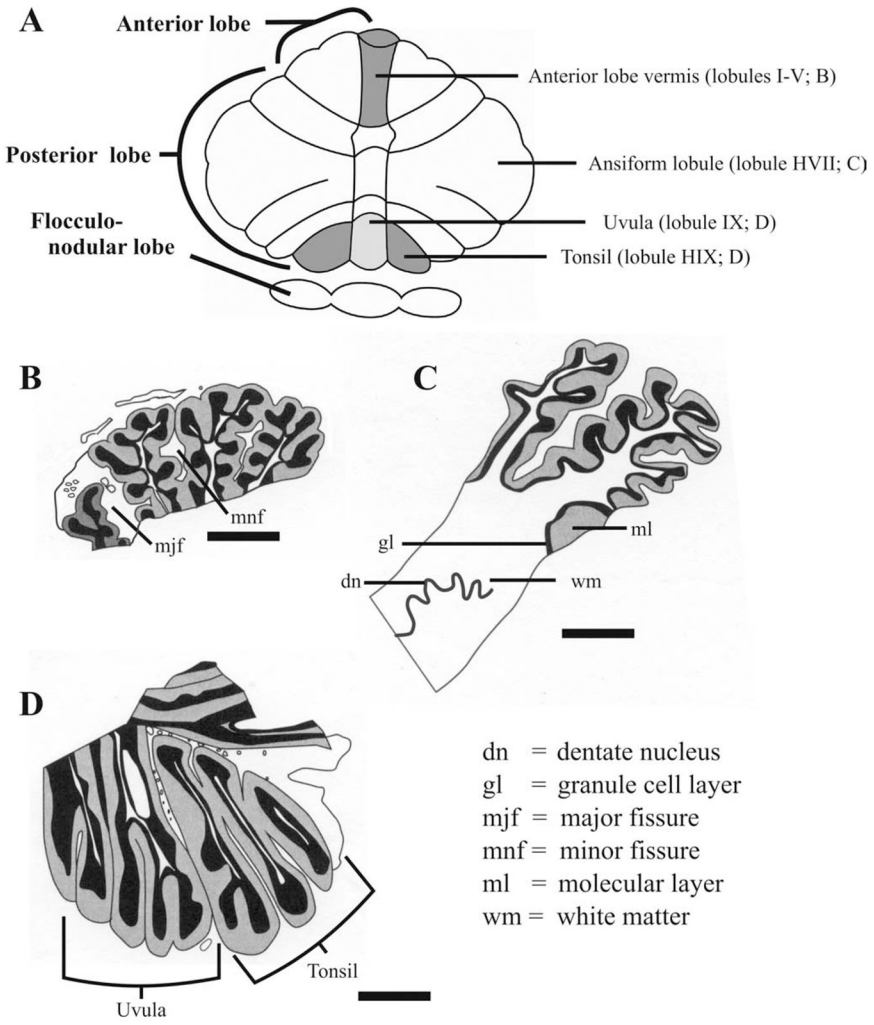

Fig. 1.

(A) Drawing of unfolded view of human cerebellar cortex indicating the regions analyzed in the current study. (B–D) Drawings of regions analyzed in this study; scale bar=0.5 cm: (B) Anterior lobe vermis (lobules I–V); (C) Ansiform lobule (lobule H VII); D: Uvula/tonsil (lobules IX/HIX).

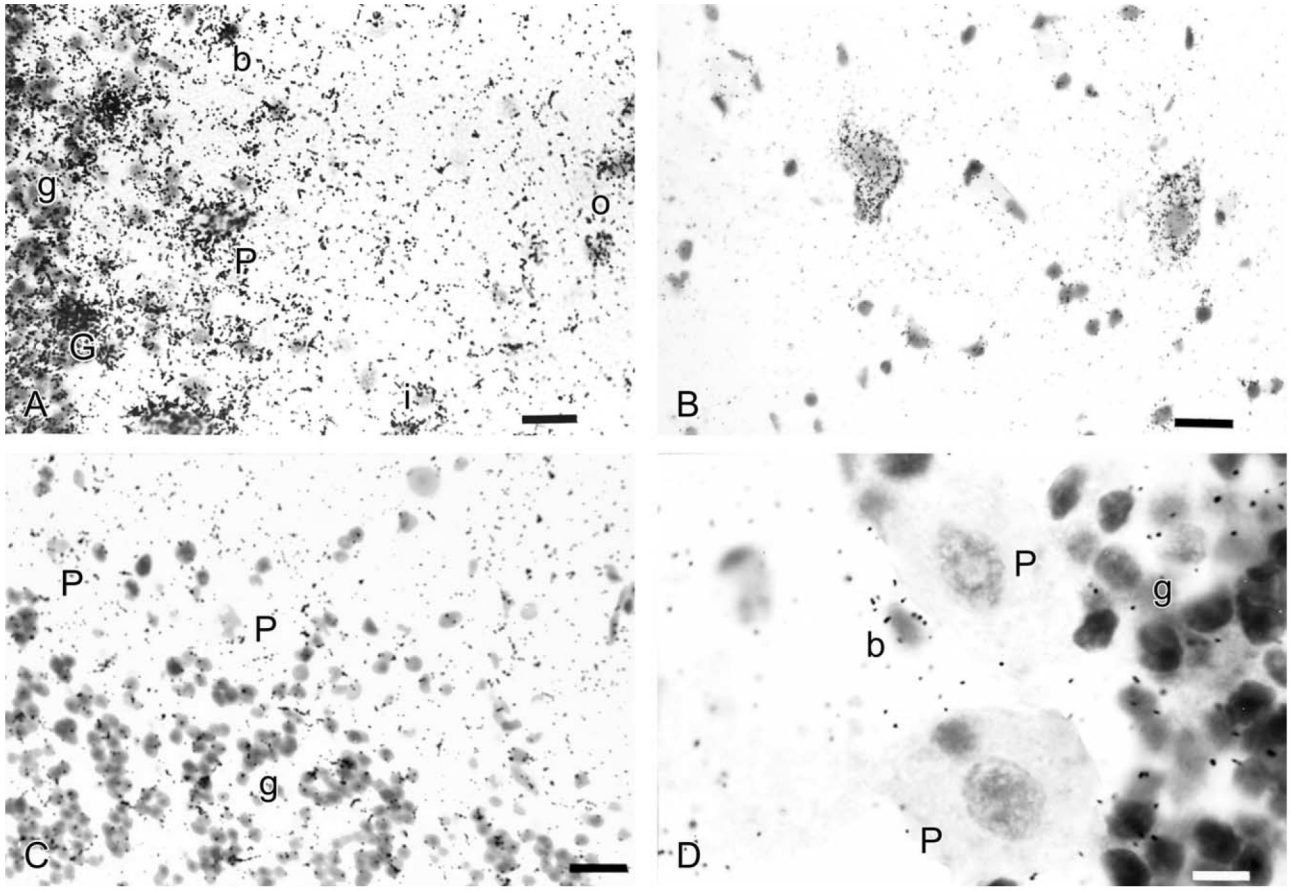

Fig. 2.

Control experiments were performed in human cerebellar tissue to ensure reported signal represents specific αAR mRNA signal: (A) Positive control: β-actin antisense, showing (left to right) granule cells (g), Golgi cell (G), Purkinje cell (P), basket cell (b), inner stellate cell (i), and outer stellate cell (o). (B) Positive control: β-actin antisense, two dentate nucleus neurons; ansiform lobule. (C) Negative control: α_{1a}AR sense probe. (D) Negative control: RNase treatment before hybridization. Note (left to right) unlabeled basket cells (b), Purkinje cells (P) and granule cells (g); bright field. Magnification: A–C: 40×, scale bar=125 μm; D: 100×, scale bar=10 μm.

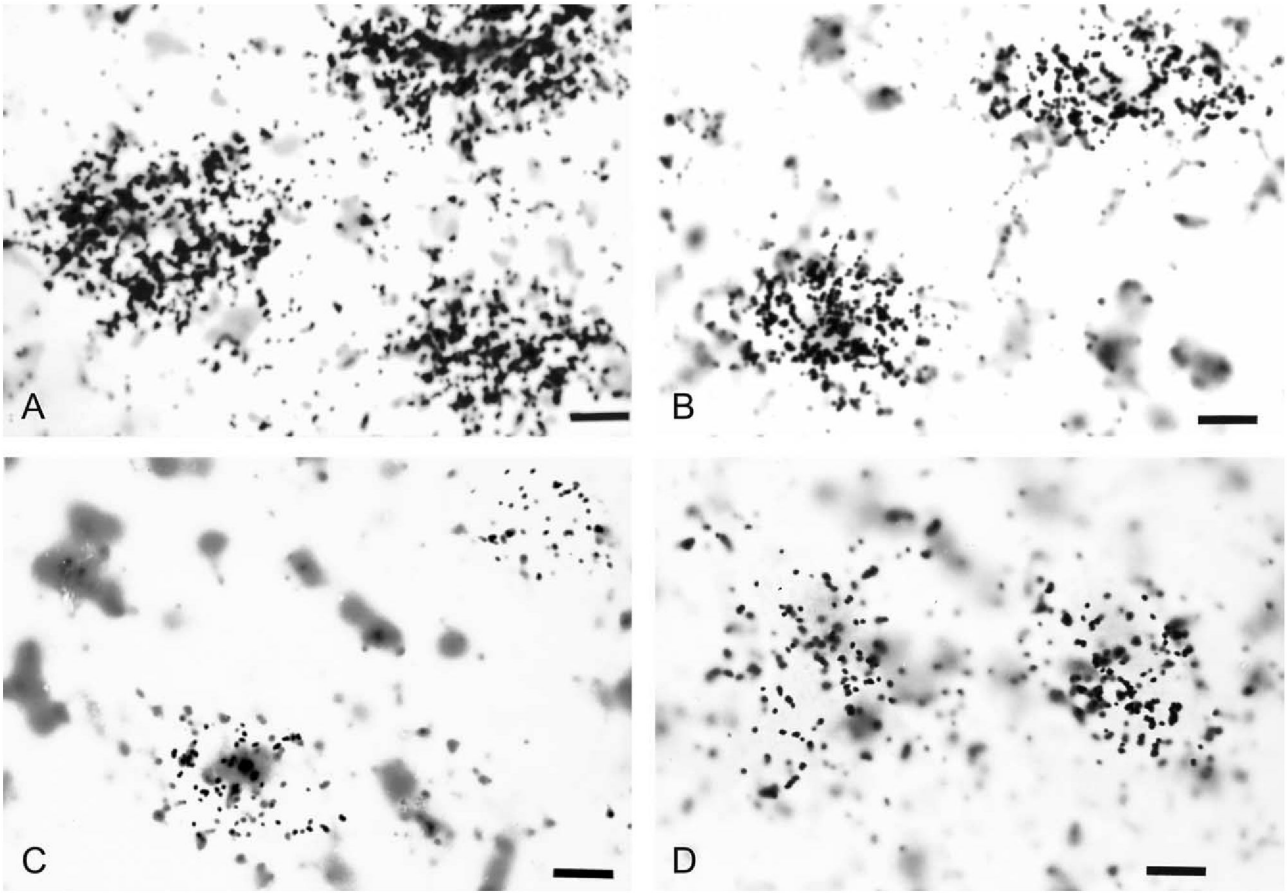

Fig. 3.

Representative expression of αAR subtypes in Purkinje cells. (A) Strong expression of α_{2a}AR mRNA in three Purkinje cells. (B) Similar high expression levels of α_{2b}AR mRNA in two Purkinje cells. (C) Significantly lower abundance of α_{2c}AR mRNA in two Purkinje cells. (D) Moderate expression of α_{1d}AR mRNA in two select Purkinje cells. Magnification: 100×, scale bar=10 μm.

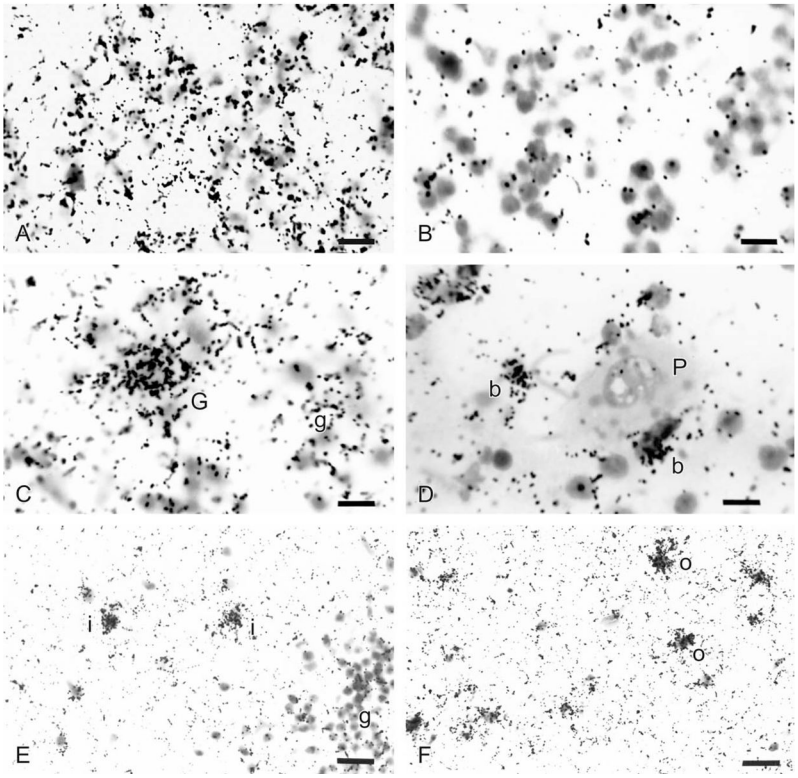

Fig. 4.

Cell specific expression of α_1AR and α_2AR mRNAs in human cerebellar interneurons. (A) α_{2a}AR mRNA is abundant in granule cells. (B) Granule cells do not express α_{2c}AR mRNA above background label (grains may represent pooling artifacts). (C) Strong expression of α_{2a}AR mRNA in a Golgi cell (G) and granule cells (g). (D) Basket cells (b), close to a Purkinje cell (P), express abundant α_{1a}AR mRNA. (E) Inner stellate cells (i) express moderate levels of α_{1b}AR mRNA in the inner half of the molecular layer. (F) Outer stellate cells (o) abundantly express α_{2a}AR mRNA. Magnification: A–D: 100×; E, F: 40×, scale bar=125 μm, scale bar=10 μm.

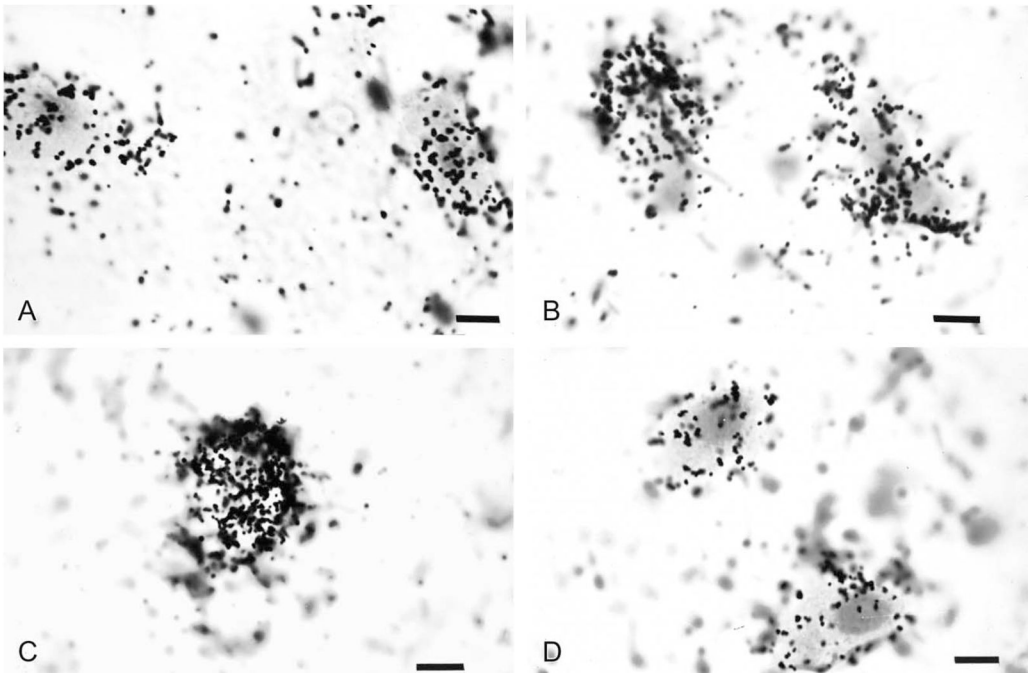

Fig. 5.

Dentate nucleus neurons of human ansiform lobule. (A) Moderate expression of α_{1a}AR mRNA. (B) Abundant α_{1d}AR mRNA expression in two dentate nucleus neurons. (C) High abundance of α_{2b}AR mRNA in a dentate nucleus neuron. (D) α_{2c}AR mRNA expression in dentate nucleus neurons is just above background. Magnification: 100×, scale bar=10 μm.

Table 1

Subtype-specific cellular distribution of α_1 and α_2AR mRNA in human cerebellum

Location	α_{1a}	α_{1b}	α_{1d}	α_{2a}	α_{2b}	α_{2c}
Molecular layer						
Outer stellate cells	0	(+)	0	+++	(++)	0
Inner stellate cells	0	++	0	+++	(++)	0
Basket cells	+++	0	0	++	+	0
Purkinje cell layer						
Purkinje cells	(++)[1,3]	(++)[2,3]	(+++)[2,3]	+++	+++	(++)[1,3]
Granule cell layer						
Golgi cells	0	0	0	+++	+++	0
Granule cells	0	0	0	+++	+++	0
Cerebellar nuclei						
Dentate nucleus	++	+	+++	0	+++	+/0

Symbols represent relative levels of mRNA signal: +++ denotes heavy signal (71–100%); ++ moderate signal (31–70%); + light signal (5–30%); +/0 signal just above background; 0 signal undetectable. Brackets denote select cells, and specifically: 1=anterior lobe vermis, 2=ansiform lobule, 3=uvula/tonsil.

Table 2

Subtype-specific distribution of α_1 and α_2AR mRNA in cerebella of experimental animals

Location	α_{1a}	α_{1b}	α_{1d}	α_{2a}	α_{2b}	α_{2c}	Species	References
Molecular layer								
				0	0	0	Rat	Scheinin et al., 1994
				0	0	0	Mouse	Wang et al., 1996
				+++	++		Rat	Tavares et al., 1996
				0	0	0	Rat	Winzer-Serhan and Leslie, 1997a; Winzer-Serhan et al., 1997b,c
		0	(+)				Rat	Day et al., 1997
Purkinje cell layer								
Purkinje cells				0	0	+++	Rat	Nicholas et al., 1993
		+					Rat	Pieribone et al., 1994
				+++	++		Rat	Tavares et al., 1996
				0	++	0	Mouse	Wang et al., 1996
	++	+	+				Rat	Day et al., 1997
				(+)	++	+	Rat	Winzer-Serhan and Leslie, 1997a; Winzer-Serhan et al., 1997b,c
					++		Mouse*	Wang et al., 2002
Granule cell layer								
Golgi cells				++	0	+	Rat	Scheinin et al., 1994
				+++	0		Mouse	Wang et al., 1996
Granule cells		+	0	+		+	Rat	McCune et al., 1993
				++	0	+++	Rat	Nicholas et al., 1993
		+					Rat	Pieribone et al., 1994
				+++	++		Rat	Tavares et al., 1996
				+	+/0	++	Rat	Winzer-Serhan and Leslie, 1997a; Winzer-Serhan et al., 1997b,c
						0	Mouse*	Wang et al., 2002
Cerebellar nuclei								
General				+++	0	0	Rat	Nicholas et al., 1993
				+	0	+	Mouse	Wang et al., 1996
	0						Rat	Day et al., 1997
						+	Mouse*	Wang et al., 2002
Dentate n.	++						Rat	Domyancic and Morilak, 1997

Symbols represent relative levels of mRNA signal from individual studies as follows: +++ denotes heavy signal; ++ moderate signal; + light signal; +/0 signal just above background; 0 signal undetectable;

* denotes promoter reporter transgenic model. Brackets denote select cells within a cerebellar layer.

Table 3

Subtype-specific distribution of α_1 and α_2ARs in cerebella of experimental animals demonstrated by immunocytochemistry

Location	α_{1a}	α_{1b}	α_{1d}	α_{2a}	α_{2b}	α_{2c}	Species	References
Molecular layer								
				+/0			Monkey	Aoki et al., 1994
						+++	Rat	Rosin et al., 1996
				(++)			Rat	Talley et al., 1996
Purkinje cell layer								
Purkinje cells					0		Rat	Go et al., 1993
					++		Monkey	Go et al., 1993
				+			Rat	Aoki et al., 1994
				++			Monkey	Aoki et al., 1994
						0	Rat	Rosin et al., 1996
				++			Rat	Talley et al., 1996
		++					Rat	Acosta-Martinez et al., 1999
Granule cell layer								
Granule cells				+++			Rat	Aoki et al., 1994
				+++			Monkey	Aoki et al., 1994
						+++	Rat	Rosin et al., 1996
				+++			Rat	Talley et al., 1996
Cerebellar nuclei								
Dentate						+++	Rat	Rosin et al., 1996
Interposed						+++	Rat	Rosin et al., 1996
		++					Rat	Acosta-Martinez et al., 1999
Medial		+++					Rat	Acosta-Martinez et al., 1999
Glia								
Oligodendrocytes				++			Rat	Talley et al., 1996

Symbols represent relative levels of mRNA signal from individual studies as follows: +++ denotes heavy signal; ++ moderate signal; + light signal; +/0 signal just above background; 0 signal undetectable. Brackets denote select cells within a cerebellar layer.

Table 4

Subtype-specific distribution of α_1 and α_2ARs in cerebella of experimental animals demonstrated by autoradiography and ligand binding

Location	α_1	α_{1a}	α_{1b}	α_{1d}	α_2	α_{2a}	α_{2b}	α_{2c}	Species	References
Cerebellar cortex										
	++[a]				+[2]				Human	Shimohama et al., 1986
	+/−[c]								Rat, Cat, Mouse	Palacios et al., 1987
	0[c]								Guinea-pig	Palacios et al., 1987
					++,[510]				Human	Meana et al., 1989
	++[j]	0	++[j−1]	0					Rat	Wilson et al., 1989
					+[9]				Chick	Fernandez-Lopez et al., 1990
					+/++,[34]				Human	De Vos et al., 1991
	+,[ab]								Rat	Jones and Palacios, 1991
					+++[3]				Human	Jones and Palacios, 1991
					++[12]	++[12]			Human	De Vos et al., 1992
					+/−[9]				Rat	Pascual et al., 1992
					++,[27]	++,[27]			Human	Ordway et al., 1993
					+++,[3610]				Human	De Vos et al., 1994
					++,[6811]	++,[6811]	0	0	Human	Sastre et al., 1994
					++,[611]	++,[611]	0	0	Human	Grijalba et al., 1996
	+++[a]	0[d−i]	++[d−i]	0[d−i]					Mouse	Yang et al., 1998
	++[a]				+[3]				Mouse	Strazielle et al., 1999
Molecular layer										
	++[b]								Rat	Jones et al., 1985
					+,[34]				Rat	Boyajian et al., 1987
					(+)[3]				Rat	Brüning et al., 1987
					++[9]				Human	Pascual et al., 1992
					+[4]		+[8]	+[8]	Rat	Wamsley et al., 1992
					+/0[13]	+/0[1]			Rat	Alburges et al., 1993
					++[3*]				Mouse	Strazielle et al., 1999
					[+/−][4]			[+/−][4]	Rat	Winzer-Serhan et al., 1997c
					+/−,[46]			+/−,[46]	Mouse	Holmberg et al., 2003
					+/−[6]				Rat	Happe et al., 2004
Purkinje cell layer										
					++,[34]				Rat	Boyajian et al., 1987
					++[9]				Human	Pascual et al., 1992
Granule cell layer										
					+[4]				Rat	Unnerstall et al., 1984
	+[b]								Rat	Jones et al., 1985
					+,[34]				Rat	Boyajian et al., 1987
					+[3]				Rat	Brüning et al., 1987
					+[4]				Monkey	Jones and Palacios, 1991
					++[4]		+[8]	+[8]	Rat	Wamsley et al., 1992
					+++[9]				Human	Pascual et al., 1992
					+,[913]				Rat	Pascual et al., 1992
					++[13]	+[1]			Rat	Alburges et al., 1993
					[++/−][4]			[++/−][4]	Rat	Winzer-Serhan et al., 1997c
					++,[46]			+/−,[46]	Mouse	Holmberg et al., 2003
					+[6]				Rat	Happe et al., 2004

Location	α_1	α_{1a}	α_{1b}	α_{1d}	α_2	α_{2a}	α_{2b}	α_{2c}	Species	References
Cerebellar nuclei										
General	+[a]				+[3] +[6] +[9]				Mouse	Strazielle et al., 1999
									Rat	Happe et al., 2004
Dentate	+[b]								Rat	Jones et al., 1985
									Human	Pascual et al., 1992
Interposed	+[b]								Rat	Jones et al., 1985

Ligands used include the following: α_1AR:

[a] = [^{3}H]prazosin,

[b] = [^{125}I]HEAT,

[c] = [^{125}I]BE2254,

[d] =BMY7378,

[e] =methoxamine,

[f] = (+)-niguldipine,

[g] =noradrenaline,

[h] =SB 216469,

[i] =tamsulosin,

[j] = [^{125}I]IBE,

[k] =WB4101,

[l] =chloroethylclonidine. α_2AR:

[1] = [^{3}H]para-aminoclonidine ([^{3}H]PAC),

[2] = [^{3}H]yohimbine,

[3] = [^{3}H]idazoxan,

[4] = [^{3}H]rauwolscine,

[5] = [^{3}H]UK 14304,

[6] = [^{3}H]RX821002,

[7] =oxymetazoline,

[8]=ARC-239,

[9]= [^{3}H]bromoxidine ([^{3}H]UK-1434),

[10]= [^{3}H]clonidine,

[11]=BRL-44408,

[12]=prazosin[^{3}H]RX821002,

[13]= [^{125}I]para-iodoclonidine ([^{125}I]PIC). Curved brackets denote label only in lobules 9 and 10. Square brackets denote presence of α_{2C}AR in neonatal rat (P7–P17) but absence in adult rats.

*Inner 1/3 of molecular layer.