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Short Communication

α_1-Adrenoreceptor in human hippocampus: Binding and receptor subtype mRNA expression

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Abstract

α_1-Adrenoreceptors (AR), of which three subtypes exist (α_{1A}-, α_{1B}- and α_{1D}-AR) are G-protein-coupled receptors that mediate the actions of norepinephrine and epinephrine both peripherally and centrally. In the CNS, α_1-ARs are found in the hippocampus where animal studies have shown the ability of α_1-AR agents to modulate long-term potentiation and memory; however, the precise distribution of α_1-AR expression and its subtypes in the human brain is unknown making functional comparisons difficult. In the human hippocampus, ^{3}H-prazosin (α_1-AR antagonist) labels only the dentate gyrus (molecular, granule and polymorphic layers) and the stratum lucidum of the CA3 homogenously. Human α_{1A}-AR mRNA in the hippocampus is observed only in the dentate gyrus granule cell layer, while α_{1D}-AR mRNA expression is observed only in the pyramidal cell layers of CA1, CA2 and CA3, regions where ^{3}H-prazosin did not bind. α_{1B}-AR mRNA is not expressed at detectable levels in the human hippocampus. These results confirm a difference in hippocampal α_1-AR localization between rat and humans and further describe a difference in the localization of the α_{1A}- and α_{1D}-AR mRNA subtype between rats and humans.
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The α_1-adrenoreceptor (AR) is a G-protein-coupled receptor that mediates norepinephrine (NE) signaling via the phosphatidylinositol pathway [5,8]. α_1-ARs are localized postsynaptic to noradrenergic terminals where they modulate the release of other neurotransmitters [13,15]. Receptor autoradiography has shown a wide distribution of α_1-AR binding sites in the CNS of rodents [11,17,26,27]. Cloning of the α_1-AR has revealed three different subtypes which are classified as α_{1A} (formerly α_{1c})-, α_{1B}- and α_{1D}-AR (formerly α_{1a}/α_{1d}) [9,12,20,21,24,25]. Expression of each of the α_1-AR subtypes has been thoroughly characterized in rodents [6,14,19]. However, a comparison of α_1-AR expression between humans and rats has not been thoroughly investigated, even though earlier studies indicated differences in α_1-AR binding in the hippocampus between rats and humans [3,17,27]. In the hippocampus, α_1-ARs modulate the activity of many neurons and interneurons in all regions of the hippocampus [2,4,7], ultimately affecting long-term potentiation and memory [22], at least in rodents. Extrapolation of rodent α_1-AR function to humans requires knowledge of the distribution of α_1-AR subtypes in the human hippocampus. The purpose of this study was to

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examine by receptor autoradiography the binding distribution of the α_1-AR in human dorsal hippocampus and to determine by in situ hybridization the localization of each of the α_1-AR subtypes (α_{1A}-, α_{1B}- and α_{1D}-AR) mRNA.

Hippocampal tissue from eight control (4 men and 4 women) human subjects from the Alzheimer's Disease Research Center (ADRC) were used for these experiments. The ADRC has received approval for use of tissue from human subjects. The subjects were normal antemortem with no neurological or psychiatric illness and had no brain neuropathology at postmortem examination. The subjects prior to death did not use alcohol or drugs of abuse. Age (mean ± SD) for men was 70.3 ± 6.6 years (range 55–84) and 71.0 ± 11.1 years (range 38–85) for women; with postmortem delay of 8.3 ± 1.7 h for men and 7.9 ± 1.9 h for women. The fresh medial temporal 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 dorsal hippocampal sections were cut on cryostat onto Fisher Superfrost slides (Fisher Scientific, Houston, TX) and stored at −70 °C.

α_1-AR binding sites were measured using ^{3}H-prazosin (α_1-AR antagonist; Perkin Elmer, Boston, MA). Briefly, slides were thawed at room temperature for 10 min and then 400 μl/slide of incubation buffer (~0.2 nM ^{3}H-prazosin in 50 mM Tris buffer, 1 mM EDTA, pH 7.4) was placed over the tissue. Non-specific binding was defined in the presence of 10 μM phentolamine. Slides were incubated for 40 min at room temperature, washed twice for 2 min in ice-cold 50 mM Tris-buffer, pH 7.4, dipped in ice-cold distilled water to remove the salts and then rapidly dried under a stream of cool air. Slides were apposed to Biomax MR film (Eastman Kodak Co., Rochester, NY) for 8 weeks. For each human, 4 consecutive slides containing the hippocampus were run (each slide contained one section of hippocampal tissue), three slides for total binding and the fourth for non-specific binding. Specific binding was total binding minus non-specific binding in the same region. Specific binding for ^{3}H-prazosin constituted ~90% of total binding.

Tissue preparation and labeling of the α_{1A}-, α_{1B}- and α_{1D}-AR oligonucleotide probes was performed as previously described for oligonucleotide labeling [23]. For each human subject 3 consecutive slides (each slide containing one section of dorsal hippocampal tissue unilateral) were labeled with either the α_{1A}-, α_{1B}- or α_{1D}-AR probes. The α_{1A}-AR probe consisted of three separate oligonucleotide probes to the following nucleotides of the published human sequence [9,20]: (a) 1–45, (b) 1102–1156 and (c) 1435–1483. The α_{1B}-AR oligonucleotide probe consisted of three separate oligonucleotide probes to the following nucleotides of the human α_{1B}-AR sequence (PubMed NM_000679.2): (a) 247–297, (b) 334–384 and (c) 910–960. The α_{1D}-AR oligonucleotide probe consisted of three separate oligonucleotide probes to the following nucleotides of the human α_{1D}-AR sequence [21,25]: (a) 587–635, (b) 990–1038 and (c) 1668–1716.

The oligonucleotide probes were 3′-end-labeled with [^{33}P]-dATP (Perkin Elmer, Boston, MA) using terminal deoxyribonucleotidyl transferase (Invitrogen, Carlsbad, CA) and then purified with MicroSpin G-25 columns (Amersham Biosciences, Piscataway, NJ). The α_{1A}-AR probe contained 1.4×10^6 cpm/50 μl, the α_{1B}-AR probe contained

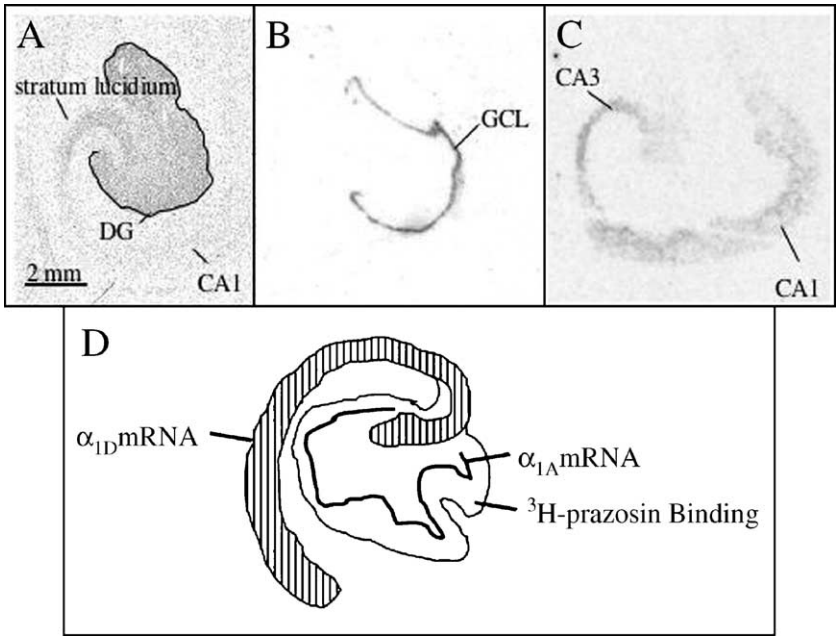

Fig. 1. α_1-AR sites in human hippocampus. (A) ^{3}H-Prazosin binding pattern (dark line indicates the location of the hippocampal fissure), (B) α_{1A}- and (C) α_{1D}-AR mRNA expression in adjacent sections of the same human hippocampus. (D) Cartoon composite of ^{3}H-prazosin binding in relation to where α_{1A} (dark line)- and α_{1D}-AR mRNA (hatched area) is expressed in the same subject. GCL; granule cell layer of the dentate gyrus, CA1 and CA3; pyramidal cell layer of the hippocampus.