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α_2-Adrenergic Receptors in Human Dorsal Root Ganglia

Predominance of α_{2b} and α_{2c} Subtype mRNAs

Rita R. S. Ongjoco, D.O.,* Charlene D. Richardson, Ph.D.,† Xiaowen L. Rudner, Ph.D.,‡
Mark Stafford-Smith, M.D.,§ Debra A. Schwinn, M.D.||

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Background: **Nonselective α_2-adrenergic receptor (α_2AR) agonists (*e.g.*, clonidine) mediate antinociception in part through α_2ARs in spinal cord dorsal horn; however, use of these agents for analgesia in humans is limited by unwanted sedation and hypotension. The authors previously demonstrated $\alpha_{2a} \approx \alpha_{2b}$ >>> α_{2c} mRNA in human spinal cord dorsal horn cell bodies. However, because 20% of dorsal horn α_2ARs derive from cell bodies that reside in the associated dorsal root ganglion (DRG), it is important to evaluate α_2AR expression in this tissue as well. Therefore, the authors evaluated the hypothesis that α_{2b} mRNA, α_{2c} mRNA, or both are present in human DRG.**

Methods: **Molecular approaches were used to determine α_2AR expression in 28 human DRGs because of low overall receptor mRNA expression and small sample size. After creation of synthetic competitor cDNA and establishment of amplification conditions with parallel efficiencies, competitive reverse transcription polymerase chain reaction was performed using RNA isolated from human DRG.**

Results: **Overall expression of α_2AR mRNA in DRG is low but reproducible at all spinal levels. α_{2b} and α_{2c}AR subtype mRNAs predominate ($\alpha_{2b} \approx \alpha_{2c}$), accounting for more than 95% of the total α_2AR mRNA in DRG at all human spinal nerve root levels.**

Conclusions: **Predominance of α_{2b} and α_{2c}AR mRNA in human DRG is distinct from α_2AR mRNA expression in cell bodies originating in human spinal cord dorsal horn, where α_{2a} and α_{2b} predominate with little or absent α_{2c} expression. These findings also highlight species heterogeneity in α_2AR expression in DRG. If confirmed at a protein level, these findings provide an additional step in unraveling mechanisms involved in complex neural pathways such as those for pain. (Key words: Anesthesiology; intensive care; nociception; spinal cord.)**

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MANAGEMENT of acute and chronic pain is important for clinicians in many subspecialties. Despite their potent analgesic effect, many available antinociceptive agents have unwanted properties, including respiratory depression, platelet dysfunction, and tolerance. Analgesia from α_2-adrenergic receptor (α_2AR) agonists has been shown in animals[1-4] and humans,[5-8] including patients who have become tolerant to opioids.[9-11] Because α_2AR agonists are relatively free of side-effects, they have been suggested as an attractive alternative to available analgesic agents; however, clinical use of α_2AR agonists re¹mains limited by sedation and hypotension. Furthermore, the exact role of α_2ARs in modulating human pain remains to be determined.

Three distinct human α_2AR subtypes (α_{2a}, α_{2b}, α_{2c}) have been described.#[12-14] Intracellular effects of α_2AR agonists include inhibition of adenylyl cyclase, inhibition of voltage-sensitive Ca^{2+} channels, and enhancement of

* Critical Care Fellow, Department of Pediatrics.

† Postdoctoral Fellow, Department of Anesthesiology.

‡ Postdoctoral Fellow, Department of Anesthesiology.

§ Assistant Professor, Department of Anesthesiology.

||Professor, Department of Anesthesiology, Pharmacology/Cancer Biology, and Surgery; Senior Fellow, Center for the Study of Aging and Human Development.

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Address reprint requests to Dr. Schwinn: Professor of Anesthesiology, Surgery, and Pharmacology/Cancer Biology, Box 3094, Department of Anesthesiology, Duke University Medical Center, Durham, North Carolina. Address electronic mail to: Schwi001@mc.duke.edu

The rat homolog of the human α_{2a}AR is called the α_{2d}AR; this is because a single amino acid change gives it slightly different pharmacology, even though it is the same gene product. For purposes of this manuscript (to avoid confusion), the term α_{2a}AR will be used for the human receptor and homologous gene products in other species.

K^+ channel opening; these effects cause suppression of neurotransmitter release and neuronal firing at locations in brain and spinal cord associated with antinociception.[15-19] In addition, α_2AR activation has been shown to inhibit calcitonin gene-related peptide expression in cultured dorsal root ganglia (DRG) neurons[20]; this suggests a possible mechanism by which α_2ARs may decrease inflammation and vasodilation. During the past 5 yr, various studies designed to determine physiologic actions mediated by distinct α_2AR subtypes have been performed in cell culture and whole animals. Experiments using knockout mice reveal that specific α_2AR subtypes mediate murine sedation, centrally mediated hypotension, peripheral vasoconstriction, and temperature regulation.[21-24] Interestingly, α_2AR subtype expression and function appears to be species specific.[25-28] We previously demonstrated predominance of α_{2a} and α_{2b}AR mRNA in human spinal cord dorsal horn, with minimal α_{2c}AR expression in the lumbar region only.[25] However, because 20% of spinal cord dorsal horn α_2ARs are contributed by neurons with cell bodies located in DRG,[29] it is important to evaluate α_2AR subtype expression in human DRG as another possible site of action of α_2AR subtype-selective drugs. Overall, DRG α_2ARs are difficult to study because the small amounts of tissue result in extremely low levels of available protein and mRNA. We therefore used quantitative competitive reverse transcription polymerase chain reaction (RT-PCR) to test the hypothesis that heterologous α_2AR expression occurs in human DRG.

Materials and Methods

Human Tissues, Control Cells, and RNA Preparation

Human DRG was obtained with institutional approval from autopsy specimens (rapid and routine). DRG tissue samples were immediately frozen in liquid nitrogen and stored at −70°C until assays were performed. Representative 5-μ cryostat sections were evaluated histologically with hematoxylin and eosin staining to confirm that tissues collected were DRG and not thickened portions of nerve roots. Total cellular RNA was extracted from DRG tissues based on the guanidinium thiocyanate-phenol-chloroform method originally described by Chomczynski and Sacchi[30] using RNA STAT-60 (TEL-TEST "B" Inc., Friendswood, TX). As a positive control for final RT-PCR, RNA was also extracted from other human tissues (vena cava and aorta) known to express all three human α_2AR mRNA subtypes.[14,31]

Table 1. Human α_2AR Primers for Qualitative and Quantitative RT-PCR

α_2AR Primers	Nucleotide Sequence 5′→3′	Primer Position Relative to cDNA	No. of Bases Amplified
α_{2a} sense	AAACCTCTTCCTGGTGTCTC	204–223	
α_{2a} antisense	AGACGAGCTCTCCTCCAGGT	875–894	691
α_{2b} sense	CCTGGCCTCCAGCATCGGAT	519–538	
α_{2b} antisense	ATGACCACAGCCAGCACGAA	1111–1130	612
α_{2c} sense	GTGGTGATCGCCGTGCTGAC	214–233	
α_{2c} antisense	CGTTTTCGGTAGTCGGGGAC	767–786	573

α_2AR = α_2-adrenergic receptor; RT-PCR = reverse transcription polymerase chain reaction.

In addition to tissue controls, cells containing each αAR subtype (Chinese hamster ovary cells stably expressing individual human α_2AR subtypes and rat-1 fibroblasts expressing human α_1AR subtypes) were used as positive and negative controls, respectively, to test for specificity and sensitivity of α_2AR primers used in RT-PCR. Chinese hamster ovary cells were grown as monolayers in F-12 Nutrient mixture (Ham, Gibco/BRL, Gaithersburg, MD) supplemented with 10% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and G418 (500 μg/ml) in 5% CO_2 at 37°C. Rat-1 fibroblasts were grown similarly in Dulbecco's modified Eagle medium. RNA was extracted from these control cells using the protocol described previously, with final RNA pellets resuspended in ribonuclease-free water, and the RNA was quantitated using a spectrophotometer at 260 and 280 nm and stored at −70°C for later use.

Qualitative Reverse Transcription Polymerase Chain Reaction

To determine the presence or absence of specific α_2AR mRNAs in human DRG, qualitative RT-PCR was performed initially. Human α_2AR subtype-specific primers were synthesized at Duke University Medical Center. Sense and antisense primers (20 mer) were adapted from Eason and Liggett[31] with some modifications, as shown in table 1. Using GeneAmp RNA PCR Core Kit (Perkin Elmer, Foster City, CA), RT of 1 μg RNA was performed in a 20-μl reaction mixture containing a final concentration of 5 mM $MgCl_2$; 50 mM KCl; 10 mM Tris-HCl; 1 mM each of dGTP, dATP, dTTP, and dCTP; 20 U ribonuclease inhibitor; 50 U Muloney leukemia virus RT; and 2.5 μM random hexamers. As a negative control, reactions without RT were performed to ensure that contamination with genomic DNA did not occur. RT reactions were run for 60 min at 42°C, 10 min at 95°C, and 10 min at 4°C.

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Table 2. Primers Used in Constructing Human α_2AR Subtype-Specific Competitor Templates

	Nucleotide Sequence 5′→3′	
Competitor α_2AR Primers	α_2AR Subtype Sequence	pGEM-7Zf(+) Sequence
α_{2a} sense	AAACCTCTTCCTGGTGTCTC	CTTACCGCTGTTGAGATCCA
α_{2a} antisense	AGACGAGCTCTCCTCCAGGT	GACTCTTGTTCCAAACTGGA
α_{2b} sense	CCTGGCCTCCAGCATCGGAT	CTTACCGCTGTTGAGATCCA
α_{2b} antisense	ATGACCACAGCCAGCACGAA	GACTCTTGTTCCAAACTGGA
α_{2c} sense	GTGGTGATCGCCCGTGCTGAC	CTTACCGCTGTTGAGATCCA
α_{2c} antisense	CGTTTTCGGTAGTCGGGGAC	GACTCTTGTTCCAAACTGGA

α_2AR = α_2-adrenergic receptor.

The resulting mixture was then amplified by PCR in a 100-μl reaction mixture containing a final concentration of 2 mM $MgCl_2$; 50 mM KCl; 10 mM Tris-HCl; 200 μM each of dGTP, dATP, dTTP, and dCTP; 2.5 U AmpliTaq DNA polymerase; 0.4 μM each of α_2AR sense and antisense primers; and 10% dimethyl sulfoxide. PCRs were performed using the Twin Block System Easy Cycler Series (ERICOMP, San Diego, CA). Optimal amplification conditions for human DRG were determined to be 3 min at 95°C; 35 cycles of 1 min denaturation at 95°C; 1 min annealing (57°C for α_{2a}, 65°C for α_{2b}, and 60°C for α_{2c}); and 1 min extension at 72°C. Final PCR products (10 μl) were separated by size electrophoretically through an agarose gel (1%) and visualized using ethidium bromide staining. Amplified products were confirmed to contain specific α_2AR target by DNA sequencing using the *fmol* DNA Sequencing System (Promega Corporation, Madison, WI).

Quantitative Competitive Reverse Transcription Polymerase Chain Reaction

Synthesis of Competitor Template. After the presence or absence of α_2AR subtypes in human DRG was established and general PCR conditions determined, quantitative competitive PCR was performed. For the heterologous competitor template, a sequence from the plasmid pGEM-7Zf(+) (Promega) was used. The use of a competitor rather than native DNA has the advantage of minimizing differences in GC content between subtypes, because it should be amplified with equal efficiency in each reaction. Using α_2AR sense and antisense primers to flank the pGEM-7Zf(+) sequence, competitor primers (40 mer) were constructed as shown in table 2. Using 9 ng pGEM-7Zf(+) in a final volume of 100 μl containing the PCR mixture (described previously) and 0.5 μl of each of the sense and antisense competitor primers, a 540-base pair (bp) product (20 bp on either end corresponding to the α_2AR subtype-specific primers) was amplified corresponding to pGEM-7Zf(+) nucleotides 2,021–2,520.[32] Conditions for amplification were 3 min at 95°C; 40 cycles of 30 s denaturation at 95°C and 30 s annealing at 58°C; and 1 min extension at 72°C. Final PCR products were separated by size on a 1% agarose gel and visualized using ethidium bromide staining.

Final construction of a human α_2AR subtype-specific plasmid was accomplished by ligating 1 μl of the PCR product (with deoxyadenosine 3′ overhangs produced by *Taq* polymerase) into 50 ng of the pCR 2.1 vector (which has 3′ deoxythymidine residues) in a final volume of 10 μl using the Original TA Cloning Kit (Invitrogen Corp., Carlsbad, CA); the product was subsequently transformed into INVαF′ cells. To analyze and confirm successful transformation, isolated plasmids were digested with *Eco*RI (which cleaves on either side of the competitor insert), and PCR was performed using the α_2AR subtype-specific 20-mer sense and antisense primers to amplify the competitor insert. Products from these reactions were visualized on a 1% agarose gel stained with ethidium bromide. These plasmids were used subsequently as templates to create competitor RNA transcripts.

To create competitor RNA transcripts, plasmids containing α_2AR competitor sequence were linearized with *Hind*III (New England Biolabs, Inc., Beverly, MA), treated with proteinase K (Boehringer Mannheim, Indianapolis, IN) at 37°C for 30 min, extracted with phenol-chloroform, and precipitated with isopropanol. Single-stranded RNA competitor transcripts were then synthesized using T7 polymerase and MEGAscript reagents (Ambion, Inc., Austin, TX) according to the manufacturer's directions. Molecules of competitor RNA were calculated (discussed later), and yeast transfer RNA was added to each competitor dilution at a final concentration of 10 ng/μl to stabilize the concentration and prevent degradation. Competitor dilutions were stored at −70°C until use.

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Molecules of competitor per microliter were calculated from the following relation:

g/M of competitor = number of bp of competitor RNA transcript × 325 d/bp

g/μl competitor (calculated from abs_{260}) ÷ g/M competitor × 6.02 × 10^{23} (Avogadro number) molecules/M = molecules/μl competitor

Amplification Efficiency. To ensure that the amplification rate was identical for target and competitor species, amplification efficiency of each target α_2AR subtype and the corresponding α_2AR competitor was measured. Two to 5 ng α_2AR target plasmid and the corresponding competitor plasmid were coamplified in a final volume of 100 μl PCR reaction mixture using α_2AR sense and antisense primers (table 1). Amplification conditions were as described previously in the general RT-PCR section. For each α_2AR subtype, nine identical reactions were prepared and the appropriate sample removed every five cycles (0-40 cycles) and placed on ice. PCR products (5 μl) were separated on a 3% NuSieve GTG agarose gel (FMC BioProducts, Rockland, ME) and stained with ethidium bromide. By design, amplified target and competitor species could be distinguished on the basis of difference in size (competitor size is 540 bp; see table 1 for α_2AR subtype product size).

Final Competitive Reverse Transcription Polymerase Chain Reactions

For final competitive RT-PCRs, serial dilutions (10^8-10^2 molecules) of the competitor templates were added to a series of RT reaction tubes containing 0.5 μg of total cellular RNA from human DRG in a final 10-μl reaction mixture; final reagent concentrations were 5 mM $MgCl_2$; 50 mM KCl; 10 mM Tris-HCl; 1 mM each of dGTP, dATP, dTTP, and dCTP; 10 U ribonuclease inhibitor; 25 U Muloney leukemia virus RT; and 2.5 μM random hexamers. RT reactions were performed as described previously in the general RT-PCR section and subsequently amplified by PCR in a total volume of a 50-μl reaction mixture; final concentrations were 2 mM $MgCl_2$; 50 mM KCl; 10 mM Tris-HCl; 200 μM each of dGTP, dATP, dTTP, and dCTP; 1.25 U AmpliTaq DNA polymerase; 0.4 μM each of α_2AR sense and antisense primers, and 10% dimethyl sulfoxide). Amplification conditions also were identical to those described previously in the general section concerning RT-PCR. Final PCR products (5-10 μl) were separated by size on a 3% NuSieve GTG agarose gel and visualized using ethidium bromide staining. Unlike qualitative PCR, in which relative quantitation necessitates use of the linear portion of the amplification efficiency curve, in competitive PCR, the ratio of target to competitor remains constant throughout amplification; thus, valid quantitation continues into the plateau portion of amplification. Therefore, we used 35 cycles to optimize product visualization on the gel for capture by the digital camera.

Data Analysis

To determine amplification efficiency and competitive RT-PCR results, final gels were photographed using a DC-40 digital camera (Eastman Kodak Co., Rochester, NY). Band intensity was analyzed using Digital Science 1D Image Analysis software (Eastman Kodak Co.). Pixel data were exported and graphed using Microsoft Excel (Microsoft Corp., Seattle, WA). For the amplification efficiency curve, the logarithm of band intensity for the target and the competitor (*y*-axis) were graphed as a function of the number of cycles (*x*-axis). The slope of the linear portion of the curve (representing efficiency of amplification) was then compared for the two curves. For competitive RT-PCR, the logarithm of the ratio of the target and competitor products (*y*-axis) was graphed as a function of the logarithm of the initial amount of competitor molecules (*x*-axis) added to the reactions. The values obtained for band intensity were corrected for the difference in size between the target and the competitor (*i.e.*, the band intensity of the target was divided by the ratio of target size to competitor size). The amount of initial target molecules present was determined from the intersection of the curves (the *x*-intercept where target and competitor were equal [$\log_{10} 1 = 0$]; this $\log_{10}$ number, extrapolated from the curve, was then converted back to a real number to yield the number of molecules of target).

Statistical Analysis

Because DRG was obtained at variable intervals from death in each patient, potentially, more RNA degradation may have occurred in some patients compared with others. Therefore, to normalize for absolute amount of starting RNA, final molecules of α_2AR subtype mRNA from each patient were converted to percent of total α_2AR mRNA present before comparison with other patients. Because data were not distributed normally, the Wilcoxon signed rank test (a nonparametric version of the paired *t* test) was used to determine differences in percentages of α_2AR subtype expression in human DRG. These results were confirmed using a general estimating equation[33,34]; $P < 0.05$ was defined as statistically sig-

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nificant. Data are presented as the median [interquartile range] and reported to two significant figures.

Results

Human Dorsal Root Ganglia and Controls

Twenty-eight DRG samples were obtained from seven patients. Patient age ranged from 26-85 yr; all causes of death were unrelated to neurologic disease. Furthermore, no tissue was harvested from patients with indwelling epidural or spinal catheters or those who were administered long-term intravenous or intrathecal analgesic agents (*e.g.*, opioids). Time between death and DRG tissue harvest ranged from 2.5-20 h. No patient was administered medications known to activate or inhibit α_2ARs.

Control Reactions and Initial Qualitative Reverse Transcription Polymerase Chain Reactions

Specificity of α_2AR PCR primers was confirmed by lack of amplified products in RT-PCRs using RNA isolated from stable cell lines that expressed either of the other two α_2AR subtypes or all three α_1AR subtypes (data not shown) and expression profile in human vena cava and aorta, which matched that previously described by our laboratory.[14] To ensure contaminating genomic DNA

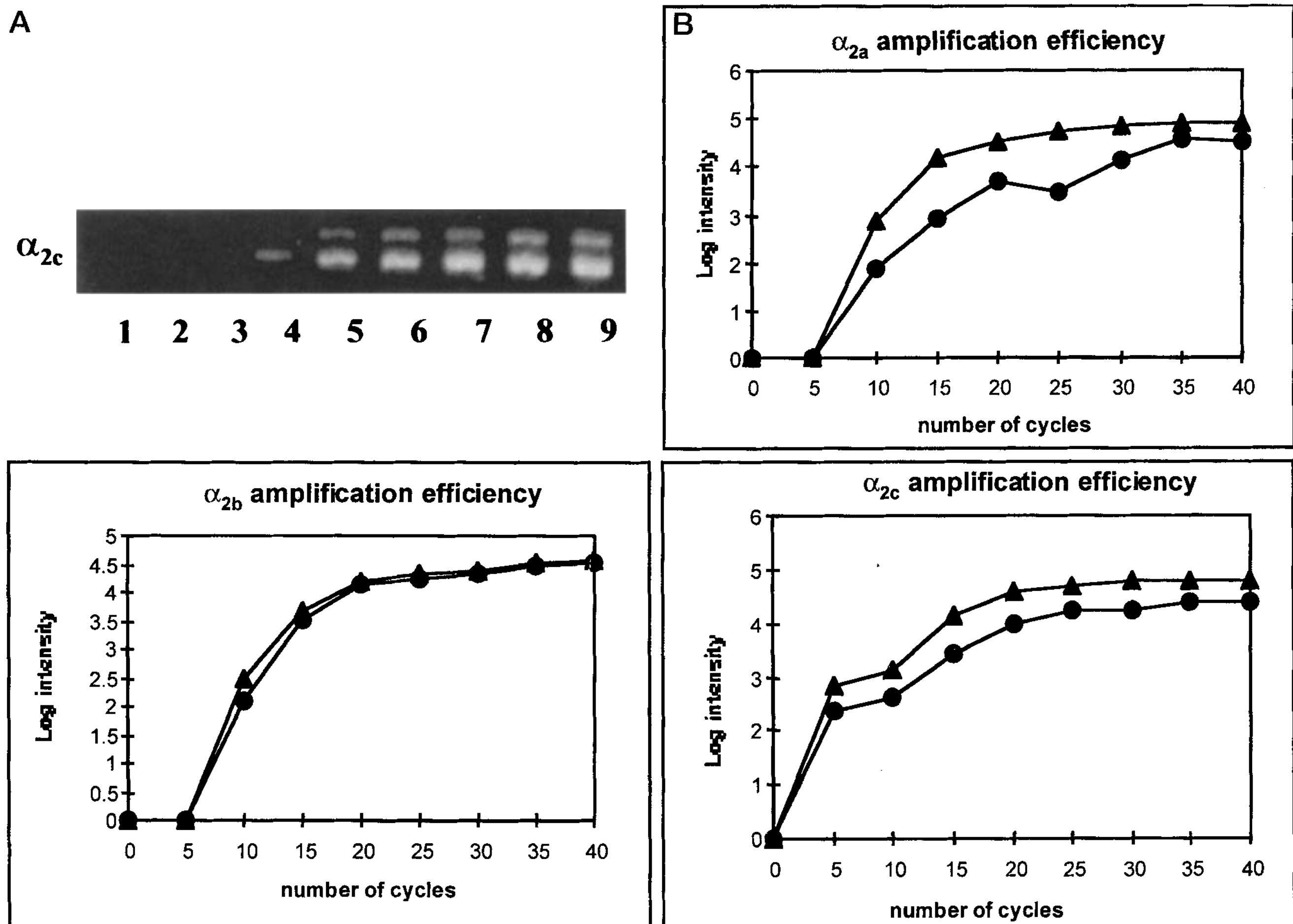

Fig. 1. Amplification efficiencies for target α_2-adrenergic receptor (α_2AR) subtype and corresponding competitor. (*A*) Representative agarose gel analysis of α_{2c}AR subtype (upper band, α_{2c} = 573 bp) and its competitor (lower band, α_{2c} competitor = 540 bp). Polymerase chain reactions were removed from the cycler every five cycles from 0 to 40 cycles; lane 1 represents zero cycles, lane 2 represents five cycles, and so forth (see text). (*B*) Log_{10} of the band intensity of the products (*y*-axis) was graphed as a function of the number of cycles (*x*-axis). Slopes for each set of target α_2AR subtype (●) and corresponding competitor (▲) were similar, indicating that the products were amplified with equal efficiency; α_2AR slope = 0.123, α_{2a} competitor slope = 0.130; α_{2b}AR slope = 0.122, α_{2b} competitor slope = 0.122; α_{2c}AR slope = 0.092, α_{2c} competitor slope = 0.097.

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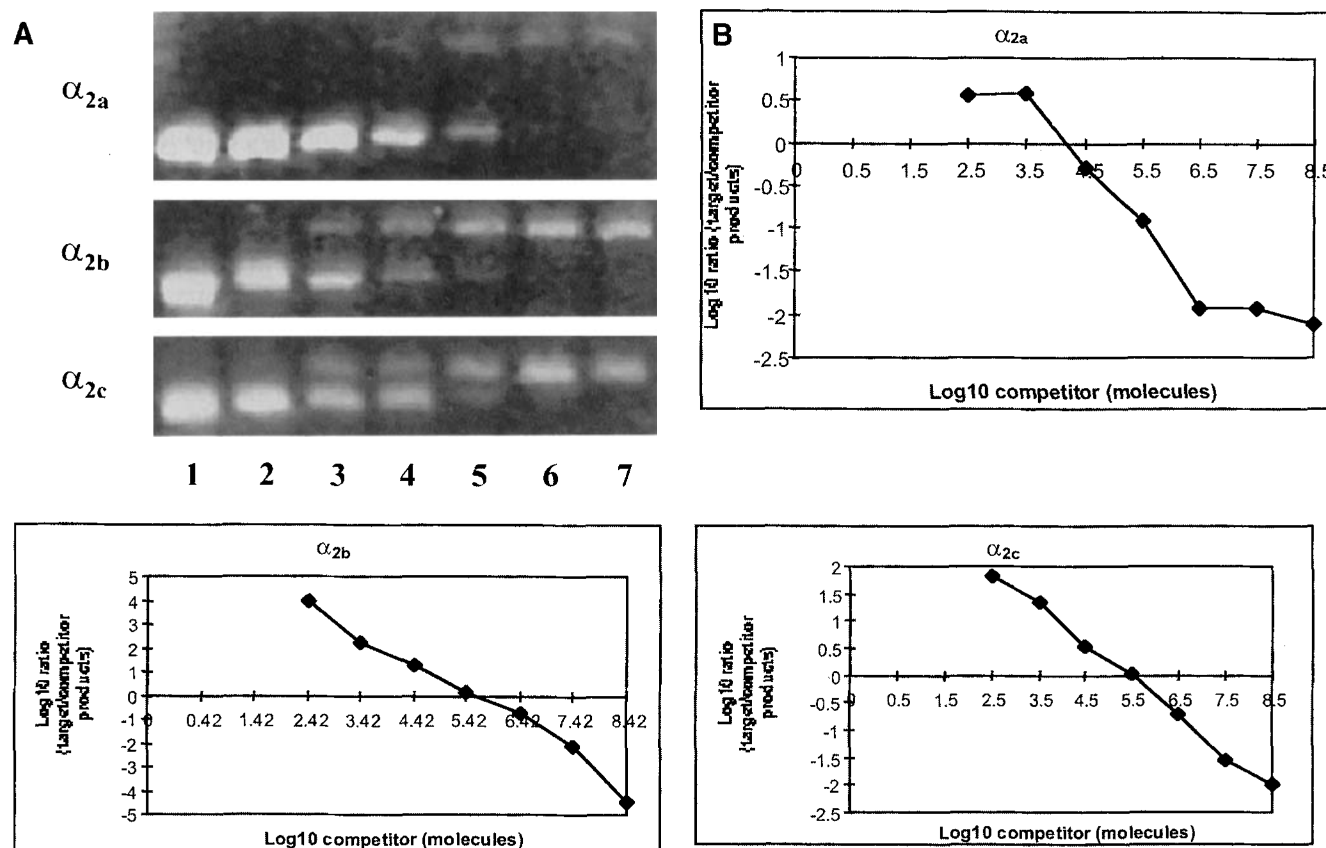

Fig. 2. α_2-Adrenergic receptor (α_2AR) subtype mRNA in human dorsal root ganglion. (*A*) Representative agarose gel analysis of α_2AR subtype (upper band) and corresponding competitor (lower band); α_{2a}AR = 691 bp, α_{2b}AR = 612 bp, α_{2c}AR = 573 bp, and all three competitors = 540 bp. Lanes 1–7 correspond to serial dilutions of competitor present in the reaction starting from 10^8 to 10^2 molecules (see Materials and Methods). Each competitive reverse transcription polymerase chain reaction contains 0.5 μg total cellular RNA from dorsal root ganglion (cervical level). (*B*) After correcting for the difference in size between target and competitor (*i.e.*, target band intensity divided by the ratio of target size to competitor size), $\log_{10}$ ratio of target and competitor products (*y*-axis) was graphed as a function of the $\log_{10}$ of the initial amount of competitor molecules (*x*-axis) present in the reactions. The amount of initial target molecules present (the *x*-intercept for y = 0) was determined for each α_2AR subtype in each set of reactions.

was not present, reactions were performed without RT; these reactions yielded no product. Initial qualitative α_2AR RT-PCRs showed that all three α_2AR subtype mRNAs are capable of being amplified from human DRG; therefore, quantitative competitive RT-PCRs were performed for all three α_2AR subtypes.

Quantitative Competitive Reverse Transcription Polymerase Chain Reaction to Determine α_2AR Subtype Expression in Human Dorsal Root Ganglia

Quantitative competitive RT-PCR was used to determine relative α_2AR subtype mRNA expression in human DRG. Amplification efficiency was equal for each α_2AR target and its corresponding competitor (fig. 1). A representative competitive PCR (agarose gel and quantitation) for all three α_2AR subtypes in the same DRG is shown in figure 2. Summative results from quantitative competitive RT-PCR for all patients revealed the presence of all three α_2AR subtypes in human DRG (table 3). Although total overall expression of α_2AR is low, it is reproducible and present at all levels of human spinal cord. Although evaluation of table 3 suggests that α_2AR subtype mRNA expression may vary between levels, limitations in sample size (28 DRG samples from four distinct levels of spinal cord) provide only enough statistical power to compare α_2AR subtype mRNA expression overall in human DRG (results pooled from all levels of human spinal cord). Overall, α_{2b} and α_{2c}AR mRNA predominate in human DRG, accounting for more than 95% of α_2AR mRNA in DRG at all spinal cord levels ($P <$

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Table 3. α_2AR mRNA Expression in Human DRG at Various Spinal Cord Levels

Spinal Cord Level	α_{2a} (%)	α_{2b} (%)	α_{2c} (%)	α_2 mRNA Molecules (All Subtypes) per μg Total RNA
Cervical	0.61 (0.01–3.7)	66 (57–86)	34 (14–39)	8.4×10^5 (0.64–13×10^5)
Thoracic	2.6 (1.3–3.3)	49 (35–60)	48 (38–62)	8.2×10^5 (3.3–13×10^5)
Lumbar	2.0 (0.19–3.2)	41 (29–58)	56 (40–67)	3.3×10^5 (2.9–13×10^5)
Sacral	2.0 (1.8–3.5)	54 (29–58)	45 (40–67)	7.1×10^5 (2.9–9.6×10^5)

Data are reported as percent total α_2AR mRNA present in human DRG at the appropriate spinal cord level or as absolute α_2 mRNA molecules (all subtypes) per μg total RNA. Because data are not distributed normally, the median value for each α_2AR mRNA subtype is shown, with the interquartile range given in parenthesis.

n = 7 patients.

α_2AR = α_2-adrenergic receptor; DRG = dorsal root ganglion.

0.001). Although there is a trend toward overall $\alpha_{2b} > \alpha_{2c}$ mRNA expression, it does not reach statistical significance; this is not surprising, because evaluation of table 3 reveals $\alpha_{2b} > \alpha_{2c}$ only at cervical and sacral spinal cord levels. These results contrast with our previously reported α_2AR mRNA expression in human spinal cord, where $\alpha_{2a} \approx \alpha_{2b}$ mRNA predominates at all spinal cord levels, with α_{2c}AR mRNA present only in very small quantities in the lumbar region.[25]

Discussion

This study is the first to describe α_2AR subtype mRNA heterogeneity in human DRG. Overall expression of α_2AR mRNA in DRG is low but reproducible. α_{2b} and α_{2c}AR subtypes predominate ($\alpha_{2b} \approx \alpha_{2c}$), accounting for 95% of the total α_2AR mRNA in DRG at all levels of human spinal cord. Although ribonuclease protection assays are generally considered the gold standard for quantitating mRNA levels, ribonuclease protection is not as sensitive as RT-PCR in analyzing low levels of RNA. A potential drawback to RT-PCR is the issue of reproducibility for quantitation. In the current study, we addressed this issue by strictly implementing competitive RT-PCR conditions; similarity in the percentage of α_2AR subtype in RNA among patients gives us confidence in our results. Our results in human DRG α_2AR subtype expression contrast with mRNA expression in both human spinal cord and rat DRG; therefore, it is important to evaluate each of these tissues in more detail.

Although α_2AR agonists have been used clinically to treat pain, their exact site or sites of action remain to be determined. Potential sites of action include brain, spinal cord, DRG, and sensory neurons. In knockout mice, the α_{2a}AR has been linked to nociception,[21,35] although some α_{2b} or α_{2c}ARs may also be present.[35] These later findings are bolstered by the presence of predominantly α_{2a} and α_{2c}ARs mRNA and protein in rat spinal cord.[26,27,36,37] In rat spinal cord, α_{2a}AR protein is colocalized with afferent substance P–containing neurons, whereas α_{2c}ARs are present on spinal interneurons.[38] Although animal models classically have been used to evaluate nociceptive pathways, species differences in spinal cord adrenergic receptor expression may limit extrapolation of results to humans. α-Adrenergic receptor subtypes in human spinal cord have been evaluated by several investigators, including us.[25,28,39] Human spinal cord α_2AR distribution is limited to gray matter, ventral more than dorsal, with the following general regional abundance: sacral > cervical > thoracic = lumbar.[25] Cell bodies containing α_2AR mRNA are located in intermediolateral (thoracic and lumbar) and intermediate (sacral) cell columns (lamina VII), the dorsal nucleus of Clarke (thoracic and lumbar), and at all levels in sensory dorsal horn laminae I, II, III, IV, and V and motor ventral horn lamina IX.[25] In terms of α_2AR subtype mRNA distribution, α_{2a} and α_{2b}AR mRNAs predominate at all sites in human spinal cord, with α_{2c}AR mRNA present at only a few locations restricted to the lumbar spinal cord[25]; α_{2a}ARs predominate *via* ligand binding; however, because these studies used spinal cord homogenate membranes, nothing can be stated regarding regional localization of α_2AR subtype protein.[28]

To understand the role of α_2ARs in human DRG, it is important to remember that the portion of α_2AR subtype mRNAs in DRG cells translated into protein are then transported to the spinal cord dorsal horn. These receptors constitute the population of presynaptic α_2ARs on primary dorsal horn afferent neurons, representing approximately 20% of all dorsal horn α_2ARs.[29] Although activation of presynaptic α_2ARs, including those originating in DRG, has been associated with neuronal inhibition,[18] activation of DRG neurons by norepinephrine has also been reported to cause both depolarization and

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hyperpolarization.[40-42] Norepinephrine-mediated suppression of afferent dorsal horn signaling is a known mechanism for spinal cord inhibition of nociceptive input to the brain.[43,44] Although the presynaptic location of DRG-generated α_2ARs in spinal cord is clear, spinal cord dorsal horn-generated α_2ARs may be presynaptic or postsynaptic, with postsynaptic locations on spinal cord dorsal horn interneurons or neurons directed to more rostral locations. The significance of differences in presynaptic *versus* postsynaptic α_2AR subtype distribution between DRG and spinal cord dorsal horn for the processing of spinal cord pathways is not understood.

In terms of receptors involved in pain, rat models have been used to determine specific α_2AR subtypes in DRG in the absence or presence of neuropathic pain. Cho *et al.*[45] described $\alpha_{2c} >> \alpha_{2a} >> \alpha_{2b}$AR subtype mRNA DRG expression in control rats, compared with increased α_{2a} and decreased α_{2c} mRNA expression in a model of neuropathic pain. These findings are supported by Nicholas *et al.*,[27] who also described α_{2a} and α_{2c} (and absence of α_{2b}) mRNA in rat DRG. In contrast, Gold *et al.*[46] reported all three subtypes present in rat DRG, α_{2b} and α_{2c}AR mRNAs using *in situ* PCR and α_{2a} protein using a specific polyclonal antibody. In another interesting study, Graham *et al.*[47] demonstrated that the α_2AR subtype involved in rat pain is species dependent, with $\alpha_{2b} >> \alpha_{2a}$ARs involved in mediating hot-plate responses in Harlan rats but neither subtype involved in Sasco rats; in contrast, α_{2a}AR and α_{2b}AR both are important in nociceptive responses in the tail-flick test. In terms of human DRG, data from the current study shows that α_{2b} and α_{2c}AR subtype mRNAs predominate ($\alpha_{2b} \approx \alpha_{2c}$) in human DRG at all spinal cord levels. Compared with rat DRG, these findings suggest significant species and subtype heterogeneity in DRG α_2AR subtype expression. Development of highly sensitive and specific α_2AR subtype antibodies and ligands are necessary to further clarify the relation between α_2AR mRNA and protein in human spinal cord dorsal horn and DRG; such information may contribute to understanding nociception at the DRG-spinal cord level. Furthermore, such studies also may contribute to our understanding of α_2ARs in modulating other neural pathways.

We demonstrate the presence and heterogeneity of α_2AR subtype mRNA in human DRG ($\alpha_{2b} \approx \alpha_{2c}$). In addition, α_2AR subtype mRNA in DRG contrasts with that found in human and rat spinal cord dorsal horn and rat DRG. If confirmed at a protein level, these findings provide an additional step in unraveling mechanisms involved in complex neural pathways such as pain.

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