The genes encoding two functional human interleukin-8 (IL-8) receptors have been identified by molecular cloning techniques and they are members of the rhodopsin G-protein coupled receptor (GCR) superfamily. We report the molecular cloning of two rat GCR genes (rat CXCR1-like and rat CXCR2) whose conceptualized amino acid sequences are approximately 70% identical to the human IL-8 A and B receptor subtypes. The murine GRO-α-like peptide, macrophage inflammatory peptide-2 (MIP-2), elevates intracellular calcium levels in HEK293 cells expressing the rat CXCR2 receptor. Southern blot analysis of restriction-digested rodent and human genomic DNAs indicate that rat CXCR1-like and CXCR2 are: 1) each single copy gene in the rat genome; 2) most closely related to the human IL-8 receptor genes; and 3) orthologous to two previously identified murine genes. CXCR2 mRNA is detected in adult rat lung, spleen, and neutrophils. CXCR1-like mRNA can be detected in adult rat lung, native rat macrophages, and a rat alveolar macrophage cell line (NR8383). These data identify the rat orthologs of the human IL-8 receptors, and describe cellular and tissue targets of rat C-X-C chemokine peptides.

Chemoattractant cytokines (chemokines) are structurally related pro-inflammatory peptides (~70–90 amino acids in length) which elicit leukocyte migration and activation (reviewed extensively in Ref. 1). Chemokines are subclassified based on the position of four highly conserved cysteine residues. In the C-X-C chemokine family, the first two cysteines are separated by an amino acid while the C-C chemokines have these first two cysteines adjacent to one another. To date, 27 chemokines, 13 C-X-C, 13 C-C, and one C chemokine have been identified from humans (2). Most C-X-C chemokines (e.g. interleukin-8 (IL-8), 1 neutrophil activating peptide-2, and the GRO peptides) attract and activate neutrophils while C-C chemokines (e.g. monocyte chemoattractant protein-1, macrophage inflammatory peptide-1α and 1β, and RANTES) target monocytes/macrophages, eosinophils, basophils, and T-lymphocytes (1–4). Recently, a novel chemokine named lymphotactin has been identified by molecular cloning (3). This peptide differs from members of the other two subclasses in that it only has two conserved cysteine residues, corresponding to the cysteine residues in positions 2 and 4 in the C-X-C and C-C chemokine families. Lymphotactin represents the first of a potentially larger gene subfamily of “C” chemokine peptides.

Receptors for chemokine peptides have been identified by molecular cloning studies and they are members of the rhodopsin superfamily of G-protein coupled receptors (GCRs) (4). Structurally, these receptors are characterized as having seven transmembrane spanning domains. To date, the primary structures of eight functional human chemokine receptors have been determined. Two of these receptors bind, and are activated by, C-X-C chemokines (5, 6), while the remaining six receptors are activated by C-C chemokine peptides (7–14). C-C chemokine receptors are approximately 45–60% identical to each other, while the two functional C-X-C (IL-8) receptors are 78% identical to one another. Genes encoding chemokine receptors have also been identified in the mouse (15–22). Despite the apparent absence of a rodent ortholog of the human IL-8 peptide, a single murine ortholog of the human IL-8 receptors has been cloned (15–17). This receptor binds the rodent C-X-C chemokines, macrophage inflammatory peptide-2 (MIP-2), and KC/CINC (cytokine induced neutrophil chemoattractant) as well as human IL-8 and GRO-α. Targeted deletion of this murine gene has suggested the importance of this receptor in neutrophil chemotaxis, hematopoiesis, and myelopoiesis (23). Some studies suggest that this gene is the only murine IL-8 receptor-like gene (24), while others have identified, by Southern blot analysis, a second closely related gene in the mouse (17). Herein, we report the isolation and cloning of two novel rat C-X-C chemokine receptors. These receptor genes are members of the GCR superfamily, having high amino acid sequence identity to the human IL-8 receptors and two closely related genes in the mouse, one of which includes the murine IL-8RB receptor.

EXPERIMENTAL PROCEDURES

Materials—DNA modifying enzymes were purchased from Promega (Madison, WI) with the exception of DNA polymerase (Pfu) which was purchased from Stratagene (La Jolla, CA). [α-32P]dCTP (3000 Ci/mmol) and [α-35S]ATP (1000 Ci/mmol) were purchased from DuPont/NEN (Wilmington, DE). [α-32P]UTP (3000 Ci/mmol) and [α-35S]ATP (1000 Ci/mmol) were from Amersham. The random primer labeling kit was from Promega (Madison, WI). Dulbecco’s modified essential medium, trypsin, penicillin/streptomycin, and TRIToR Reagent were from Life Technologies, Inc. Murine MIP-2 and KC/CINC were from R&D Systems (Minneapolis, MN). The rat genomic DNA library (Sau3AI partial, Lambda Dash II, Stratagene, La Jolla, CA) was a gift from Dr. T. J. Murphy (Emory University, Atlanta, GA). HEK293 cells were from the American Type Culture Collection (Rockville, MD). The rat alveolar...
Conserved regions of G-protein coupled receptors (and specifically chemogenomic DNA as the template) encoding novel rat GCRs. The amino acids were used as primers for the amplification of DNA fragments (using rat genomic DNA as the template) and subjected to DNA sequence analysis (Sanger dideoxy chain termination). The PCR products were subcloned into the Smal site of pGEM7Zf(+) (Promega) and subjected to DNA sequence analysis (Sanger dideoxy chain termination, Sequences kit, U. S. Biological Corp.).

Isolation of Rat Genomic Clones—Approximately 106 independent colonies of a StuII partially digested Wistar rat genomic DNA library (Lambda Dash II, Stratagene) were simultaneously screened with 32P-radiolabeled 0.6 (pCrec4) and 0.7 (pCrec8) kb PCR products. Positive signals were plaque purified and phage DNA purified by standard methods (15). Restriction-digested recombinant genomic DNA hybridizing to each of the pCrec4 and pCrec8 DNA were subcloned to pGEM7Zf(+) and subjected to partial DNA sequence analysis (Sanger dideoxy chain termination, Sequences kit).

Transfection of HEK293 Cells—The open reading frames (ORFs) of rat CXCR1-like and CXCR2 were amplified using the PCR and the respective genomic clones as the templates. To amplify the CXCR1-like ORF, two oligonucleotides were synthesized and used in the PCR (30 cycles of 94 °C for 1 min, 55 °C for 2 min, and 72 °C for 3 min). The oligonucleotides used were 5′-CAT GGA TCC AAC AAA CAA TG and 5′-AGA AGG GAA TCT AGA GGG GCC ACA GTT. The PCR product was restriction digested with BamHI and cloned subsequently to the same site of pcDNA3 (Invitrogen, San Diego, CA). The resultant expression plasmid construct was subject to DNA sequence analysis to verify the fidelity of the in vitro DNA polymerase used in the PCR. To amplify the rat CXCR2 ORF, two oligonucleotides were synthesized and used in the PCR (30 cycles of 94 °C for 1 min, 55 °C for 2 min, and 72 °C for 3 min). The oligonucleotides used were 5′-AGG ATT AAG TCT AGA TCA AAG ATG GGA and 5′-AGA AGG GAA TCT AGA GGG GCC ACA GTT. The PCR product was restriction digested with XbaI, cloned to the same site of pcDNA3 (Invitrogen), and subjected to DNA sequence analysis. The HindIII/BamHI restriction fragment of the pcDNA3 construct was then subcloned into the HindIII/BamHI sites of pcDNA3. HEK293 cells were grown in Dulbecco’s modified essential medium (Life Technologies, Inc.) according to the manufacturer’s recommended procedure. Approximately 72 h post-transfection, cells were replated in complete media containing the neomycin analog G418 (0.8 mg/ml). The cells resistant to G418 were isolated and propagated for further analysis. Cells expressing cRNA hybridizing to either rat CXCR1-like or CXCR2 DNA were characterized further.

Measurement of Intracellular Calcium Levels—HEK293 cells (5 × 105) which stably express rat CXCR1-like or CXCR2 were dissociated from the culture dish and diluted subsequently into 10 ml of Dulbecco’s modified essential medium (serum-and antibiotic-free). The suspension was made in 5 μl of FURA-2 AM and was incubated for 30 min in the dark at 37 °C. The cells were then washed twice with Kreb’s buffer and subsequently resuspended in 2 ml of Krebs buffer. The cell suspension was transferred to cuvettes and placed in a SLM 8090 model spectrofluorimeter (SLM-AMINCO, Urbana, IL) driven by an IBM PC. FURA-2 fluorescence was monitored at 510 nm, with automatic slushing of the excitation wavelength at 340 and 380 nm. Ratios at these two excitation wavelengths were automatically calculated.

Southern Analysis of Rodent and Human Genomic DNA—Human genomic DNA (from K562 cells), rat genomic DNA (from normal adult Wistar rat liver), and murine genomic DNA (adult SV129 mouse liver) were isolated by standard procedures (25). Genomic DNAs (15 μg) were restriction endonuclease-digested and were subsequently electrophoresed through 0.8% agarose and subjected to Southern blot analysis. The transferred DNAs were hybridized to either the 32P-labeled 1.0-kbp EcoRI fragment of the rat CXCR1-like gene or the 0.7-kbp PCR product (pCrec8) encoding part of rat CXCR2, using the method of Church and Gilbert (26). Final wash temperatures are indicated in the legend to Fig. 3.

RNA Isolation, Northern Analysis, and RNase Protection Assay—Total RNA was isolated using TRIZol Reagent (Life Technologies, Inc./BRL) according to the manufacturer’s recommended procedure. Total RNA (10 μg/lane) was electrophoresed through denaturing 1.2% agarose and subjected to Northern analysis using the method of Church and Gilbert (26). A 1.0-kbp EcoRI fragment of the rat CXCR1-like gene was 32P-radiolabeled by the random primer method (1 × 106 dpm/μg) and used as the hybridization probe. The final wash temperature was 65 °C. Exposure to Kodak X-AR film was for 3 days at −80 °C with an intensifying screen. The RNase protection assay was performed as described previously (27, 28). The antisense riboprobe for rat CXCR1-like was generated from an in vitro transcription reaction using a plasmid construct containing an EcoRI/AccI fragment of the rat CXCR1-like gene. The antisense probe for rat CXCR2 was made in a similar manner using a HindIII/BamHI restriction fragment of rat CXCR2.

RESULTS

Isolation of Rat Genomic DNA Containing Sequences Encoding Novel G-Protein Coupled Receptors—Degenerate oligonucleotide primers were designed based on the highly conserved regions (TM2, TM3, and TM7) of the known human chemokine receptors (huIL-8RA, huIL-8RB, huCCCR1, and huCCCR2b) and the orphan rat chemokine receptor-like gene, RBS11 (5–9, 29). DNA fragments encoding novel members of the GCR superfamilies were generated by PCR using these oligonucleotides and rat genomic DNA as the template. Rat genomic DNA was used because the protein coding sequences of most GCRs in the rhodopsin family identified to date are contained within a single exon. DNA sequence analysis of several PCR products identified DNA fragments encoding novel members of the GCR superfamily. Two of these (pCrec4 and pCrec8) were used subsequently to screen a rat genomic DNA library under high stringency conditions. Six bacteriophages were isolated hybridizing to pCrec4, while a single bacteriophage clone hybridizing to pCrec8 was identified. DNA sequence analysis of each of these clones revealed ORFs encoding novel G-protein coupled receptors. Hereafter, we designate the ORF containing the pCrec4 sequence as rat CXCR1-like and the ORF containing the pCrec8 sequence as rat CXCR2. A search of the GenBank revealed that rat CXCR1-like was most similar to human receptors for IL-8 (huIL-8RA and huIL-8RB). Similarly, rat CXCR2 was also related to these human C-X-C chemokine receptors. The sequence of rat CXCR2 is essentially identical to an unpublished sequence found in a GenBank release (accession number X77797). Fig. 1 depicts a multiple alignment of these two rat receptors (rat CXCR1-like [ratCXCR1] and rat CXCR2) with the two functional human IL-8 receptors (huIL-8RA and huIL-8RB) and the murine IL-8 receptor (murIL-8R). The rat CXCR1-like gene encodes a protein of 349 amino acids with a calculated molecular mass of 39,944 Da, while rat CXCR2 is a protein of 359 amino acids and a calculated molecular mass of 40,532 Da. Both receptors contain structural features characteristic of chemokine receptors, including a preponderance of acidic amino acid residues in their respective N termini and putative N-linked glycosylation sites (N-X-S/T). The C termini of each of these rat receptors are serine-rich, suggesting possible receptor regulation by phosphorylation. A comparison of amino acid identities of the rodent and human receptors is shown in Table I. Rat CXCR1-like and CXCR2 proteins share 65% amino acid sequence identity. Rat CXCR1-like protein has 71 and 67% amino acid sequence identity to huIL-8RA and huIL-8RB, respectively, while rat CXCR2 has 70 and 72% sequence identity to these human receptor subtypes, respectively. The 86% amino acid identity between rat CXCR2 and murine IL-8R receptors is similar to other orthologous pairs in the GCR superfamilies.

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Signal Transduction Properties of Rat CXCR2—MIP-2 and KC/CINC are the GRO-like peptides in rodents (31, 32) and hence are likely ligands for the rat CXCR1-like and CXCR2 gene products. HEK293 cells which stably expressed rat CXCR2 responded to MIP-2 (Fig. 2), illustrated by an elevation in intracellular calcium ([Ca^{2+}]_{i}). The effect of MIP-2 on intracellular calcium levels was dose-dependent. The EC\textsubscript{50} value for this effect is approximately 20 nM. In contrast, HEK293 cells stably transfected with DNA encoding the rat CXCR1-like protein did not show a detectable change in [Ca^{2+}]_{i} in the presence of either MIP-2 or KC/CINC.

Genomic Analysis—Southern blot analysis of restriction-digested rat genomic DNA probed with radiolabeled rat CXCR1-like and CXCR2 cDNAs revealed single hybridizing genomic fragments (Fig. 3A). The sizes of these restriction fragments were identical to the hybridizing restriction-digested fragments from the CXCR2 cDNA clones. These data suggest that the rat genome contains single copy genes encoding the CXCR1-like and CXCR2 receptor proteins. Hybridization of restriction-digested rat genomic DNA with either rat CXCR1-like or CXCR2 radiolabeled DNA under conditions of reduced stringency demonstrated identical hybridization patterns, consistent with the presence of single copy genes encoding these receptors.
strated that these two genes cross-hybridize with one another (Fig. 3B). Additional, albeit fainter bands were observed in each hybridization, indicating the possibility of other closely related genes.

In order to determine the human genes most closely related to rat CXCR1-like and CXCR2 genes, radiolabeled rat CXCR1-like and CXCR2 DNAs were used to probe restriction-digested human genomic DNA under conditions of reduced stringency. Fig. 3C demonstrates that both CXCR1-like and CXCR2 DNAs hybridize to 1.8-, 3.3-, and 5.0-kbp *Pst*I human genomic DNA fragments. These *Pst*I fragments are identical in size to the *Pst*I restriction endonuclease fragments corresponding to the genes for the huIL-8RA (1.8 kbp), huIL-8RB (3.3 kbp), and huIL-8RB pseudogene (5 kbp), previously identified by Ahuja et al. (33). These data suggest that two rat genes, CXCR1-like and CXCR2, are orthologous to the human IL-8 receptor (huIL-8RA) and murIL-8 receptor. Rat CXCR1-like DNA, however, showed preferential hybridization to the 3.8-kbp *Pst*I and 6.0-kbp BanHI restriction fragments. These data suggest that the rat CXCR1-like gene is orthologous to the second murine gene suggested by Bozic et al. (17).

Sequence analysis of the rat genomic CXCR2 clone revealed divergence of the nucleotide sequence 20 bases upstream of the ATG initiator codon (Fig. 4A) when compared to the cDNA sequence reported in the GenBank release (accession number X77797). The AG dinucleotide sequence, typically present at the 3' end of an intron, is found at this site of divergence between the genomic and cDNA clones. The length and content of the 5' noncoding sequence in the exon containing the CXCR2 ORF is identical to the murine IL-8 receptor genomic DNA sequence (34) in the analogous region. In addition, these rodent genomic sequences are similar in both length and sequence to 5' exon sequences of the human IL-8RB orf-containing exon (35). Two complimentary and overlapping oligonucleotides were designed and synthesized based on sequences in this upstream rat CXCR2 exon. The radiolabeled oligonucleotides were used to probe for the presence of this putative exon sequence in the single isolated genomic clone. We did not detect
any hybridization to this genomic clone. Based on restriction map analysis of our rat CXCR2 genomic clone, this putative exon sequence resides greater than 3 kbp away from the exon containing the CXCR2 protein coding sequences.

We also obtained a cDNA sequence of the 5'-untranslated region of the rat CXCR1-like gene using the technique of 5'-rapid amplification of cDNA ends. Similar to rat CXCR2, the DNA sequences of the rat CXCR1-like genomic and cDNA (5'-rapid amplification of cDNA ends) clones diverged just upstream of the translation initiator ATG. The CXCR1-like genomic and cDNA sequences diverged at position –13. Two complimentary and overlapping oligonucleotides were synthesized, radiolabeled, and hybridized to restriction endonuclease-digested bacteriophage clones containing rat CXCR1-like genomic DNA. This 5'-exon sequence was found within the identical 5-kbp HindIII/BamHI restriction fragment that contains the ORF. Fig. 4B shows the sequence of the rat CXCR1-like genomic clone(s) and the relationship of the 5'-exon sequence defined by the 5'-rapid amplification of cDNA ends clone. The intron has the expected splice donor and acceptor boundary sequences and is estimated to be approximately 1.4 kbp in size.

**Tissue Specific Expression of Rat CXCR1-like and CXCR2 Genes**—The tissue and cell distribution of rat CXCR1-like and CXCR2 mRNAs were determined by RNase protection assay and Northern blot analysis. Analysis of total RNA from solid rat organs and leukocytes probed with a pCree8 (CXCR2) riboprobe reveals the lung, spleen, and neutrophil as sites of expression of the rat CXCR2 gene (Fig. 5). Total RNA isolated from adult rat lung and NR8383 cells probed with DNA encoding rat CXCR1-like DNA showed hybridization to 2.6- and 1.3-kilobase RNA species (Fig. 6A). The hybridizing RNAs are large enough to encode the rat CXCR1-like gene product. The appearance of two hybridizing RNA bands is not unusual for GCRs and may indicate different starts of transcription of the rat CXCR1-like gene, alternative splicing of exons containing non-coding sequences, and/or use of more than one 3'-polyadenylation site(s). Total RNA from adult rat organs and leukocytes probed with 32P-antisense CXCR1-like riboprobe also identified CXCR1-like gene expression in the lung and native macrophage (Fig. 6B). In situ hybridization analysis of adult rat lung using a 35S-antisense rat CXCR1-like riboprobe was performed. Fig. 7 shows a representative section of rat lung hybridized with antisense riboprobe derived from the rat CXCR1-like gene. This receptor is expressed in a limited number of cells within the lung.

**DISCUSSION**

In this study, we report the isolation and cloning of two genes encoding rat GCRs. These two genes (rat CXCR1-like and rat CXCR2) are members of a subfamily of chemokine GCRs. These conclusions are based upon the high amino acid identity of rat CXCR1-like and rat CXCR2 gene products to the known human IL-8 receptor proteins (huIL-8RA and huIL-8RB), and the ability of the GRO-like peptide, MIP-2, to elevate intracellular calcium levels in rat CXCR2 expressing cells. Each of these genes are expressed in cells and tissues of the rat known to be responsive to chemokine peptides (e.g., lungs, spleen, neutrophils, and macrophages).

The rat CXCR1-like and CXCR2 receptor proteins are approximately 70% identical to the two functional human IL-8 receptors. This level of identity in the GCR superfamily suggests that the products of these genes should bind to similar ligands; the two functional IL-8 receptors are approximately 78% identical to each other (5, 6). Rat CXCR2 and CXCR1-like proteins are also 86 and 65% identical to a murine IL-8 receptor ortholog. The higher identity of the murine IL-8R to the rat CXCR2 suggests that these two genes are orthologous pairs. Rat CXCR1-like and CXCR2 proteins contain a number of highly conserved amino acids, including a preponderance of negatively charged amino acids in the N termini, a characteristic of chemokine receptors whose primary structures are known. In addition, each of these receptors has consensus sites for N-linked glycosylation, although this feature is shared by most of the members of the rhodopsin family of GCRs.

Unlike humans, rodents do not express an IL-8 peptide homologue. However, they do contain two distinct genes encoding GRO-like peptides, namely MIP-2 and KC/CINC (31, 32). HEK293 cells expressing rat CXCR2 responded (increased intracellular calcium levels) to application of MIP-2. We were unable to detect increased intracellular calcium in rat CXCR1-like transfected HEK293 cells stimulated with either MIP-2 or KC/CINC. It is possible that the rat CXCR1-like receptor in transfected HEK293 cells utilizes a different signal transduction pathway, is expressed only at a very low level, or that we have not yet identified the rat C-X-C chemokine peptide that is the endogenous ligand for the rat CXCR1-like protein.

Southern blot analysis of restriction endonuclease-digested human genomic DNA also indicated the high sequence similarity of rat CXCR1-like and CXCR2 to the human IL-8 receptors (huIL-8RA and huIL-8RB). The human genome contains three closely related IL-8 receptor genes, _i.e._ two functional receptors (A and B subtypes) and one pseudo gene. Both rat CXCR1-like and CXCR2 hybridized to the three _PstI_ fragments corresponding to these three human IL-8 receptor genes that were previously identified by Ahuja and Murphy (33). Most notably, there were not any additional, more strongly hybridizing, fragments identified in this analysis. This indicates that the three human IL-8 receptor genes are most similar to the rat CXCR1-like and CXCR2 genes and that there are not any additional, more similar genes in the human genome. Southern blot analysis of murine genomic DNA, using the human IL-8 B receptor, also suggests the presence of two IL-8 receptor-like genes in the rodent genome.
mouse (17). To date, only a single gene encoding a murine IL-8 receptor-like protein has been isolated (15–17). However, hybridization analysis of mouse genomic DNA suggests (Fig. 3D) that this second closely related gene in the mouse genome is orthologous to the rat CXCR1-like gene. Since the rat CXCR1-like amino acid sequence identity to the human IL-8 A receptor subtype is only slightly higher than to the B subtype, it is not clear if the rat CXCR1-like gene and this second related murine gene are orthologous to the human IL-8 A receptor. One can only speculate on the evolutionary relationship of the IL-8 receptor genes. It is possible that a single IL-8 receptor existed prior to the species divergence of ancestral rodent and human species. This ancestral IL-8 receptor gene could likely have been more closely related to the IL-8 B receptor subtype. Subsequent to rodent and human speciation, separate gene duplication events would have given rise to the human IL-8 A receptor (and pseudo) genes as well as the rodent CXCR1-like gene(s). Alternatively, the rat CXCR1-like gene is orthologous to the human IL-8 A receptor, and subsequent to rodent and human speciation the DNA sequences which regulate cell or tissue specific expression (neutrophil versus macrophage) of these genes diverged.

A limited analysis of the gene structure of these two rat genes also indicates that rat CXCR2, murine IL-8, and human IL-8 B receptor subtypes are orthologous genes. The nucleotide sequences in the genomic region just upstream of the translation initiation ATG codon of rat CXCR2 and the murine IL-8 receptor (34) are identical to each other (over 42 nucleotides). These regions of the rodent receptor genes are also highly similar to the analogous region of the human IL-8 B receptor gene (35, 36) evidence in further support of the designation of each of these receptors as orthologous genes.

Both rat CXCR1-like and CXCR2 genes are expressed in tissues and cells known to be responsive to or sites of synthesis of chemokine peptides (1). Rat CXCR2 mRNA was detected in rat lung, spleen, and neutrophils. CXCR1-like gene expression is also evident in rat lung, but is both notably present in rat macrophage and absent in either resting or stimulated (IL-1 and lipopolysaccharide) rat neutrophils. In general, C-X-C chemokine peptides have chemotactic activity toward monocytes. An interferon-γ inducible protein, named IP-10, lacks activity toward neutrophil yet is active on C-C chemokine-responsive cells (e.g. monocytes and T-lymphocytes) (37). In addition, expression of IP-10 has been detected in alveolar macrophages (38, 39). Despite the similar tissue and target cell specificity of rat CXCR1-like and IP-10, we were unable to detect rat CXCR1-like transfected cell responsiveness to application of IP-10. We have demonstrated limited cellular expression of the rat CXCR1-like gene in the adult lung by the technique of in

**Fig. 6.** Rat CXCR1-like RNA tissue distribution in rat solid organs and cells. A, Northern analysis of rat CXCR1-like mRNA expression in rat lung and NR8383 cells. Ten μg of total rat RNA from Sprague-Dawley rat lung and NR8383 cells was subjected to Northern analysis (see “Experimental Procedures”). The blots were exposed to Kodak X-AR film with an intensifying screen at −80 °C for 3 days. The positions of migration of 18 S and 28 S ribosomal RNAs are indicated. B, RNase protection assay of rat CXCR1-like mRNA in rat tissues and cells. Neutrophils were either untreated or treated for 4 h with IL-1 (5 ng/ml) alone or in combination with lipopolysaccharide (1 μg/ml). Two μg of total RNA from rat tissues or cells were subjected to RNase protection assay (see “Experimental Procedures”). The tissue and cell RNAs were simultaneously hybridized to 32P-labeled 280-base pair rat CXCR1-like and 114-base pair glyceraldehyde-3-phosphate dehydrogenase (GAPDH) riboprobes. The dried gel was exposed to x-ray film at −80 °C for 10 h.

**Fig. 7.** In situ hybridization analysis of rat CXCR1-like gene expression in adult rat lung. The reaction of: A, “antisense” rat CXCR1-like gene riboprobe; and B, “sense” CXCR1-like gene riboprobe to sections of adult rat lung. The section was counterstained with hematoxylin and eosin. Arrows in A indicate areas of dense silver grains.
situ hybridization. At this point, we have not definitively identified the specific lung cell type expressing the CXCR1-like mRNA. However, we can detect CXCR1-like mRNA in NR8383 cells, a rat alveolar macrophage cell line. Therefore, it is likely that rat alveolar macrophages express the rat CXCR1-like gene in vivo.

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