Molecular Cloning and Expression of the cDNA for a Novel α₁-Adrenergic Receptor Subtype*

(Received for publication, September 7, 1989)

Debra A. Schwinn‡, Jon W. Lomasney§, Wulfing Lorenz∥, Pamela J. Szklut¶, Robert T. Fremeau, Jr.**, Teresa L. Yang-Feng‡‡, Marc G. Caron∥∥, Robert J. Lefkowitz∥∥∥, and Susanna Cotecchia¶

From the Departments of ‡Anesthesiology, §Pathology, ¶Medicine, ∥Cell Biology, ∥∥Biochemistry, **Neurobiology, and ∥∥∥Department of Human Genetics, Yale University School of Medicine, New Haven, Connecticut 06510

A novel α₁-adrenergic receptor subtype has been cloned from a bovine brain cDNA library. The deduced amino acid sequence is that of a 466-residue polypeptide. The structure is similar to that of the other adrenergic receptors as well as the larger family of G protein-coupled receptors that have a presumed seven-membrane-spanning domain topology. The greatest sequence identity of this receptor protein is with the previously cloned hamster α₁B-adrenergic receptor being ~72% within the presumed membrane-spanning domains. Localization on different human chromosomes provides evidence that the bovine cDNA is distinct from the hamster α₁B-adrenergic receptor. The bovine cDNA clone expressed in COS7 cells revealed 10-fold higher affinity for the α₁-adrenergic antagonists WB4101 and phentolamine and the agonist oxymetazoline as compared with the α₁B receptor, results similar to pharmacologic binding properties described for the α₁A receptor. Despite these similarities in pharmacological profiles, the bovine α₁-adrenergic receptor is sensitive to inhibition by the alkylating agent chloroethylclonidine unlike the α₁A-adrenergic receptor subtype. In addition, a lack of expression in tissues where the α₁A subtype exists suggests that this receptor may actually represent a novel α₁-adrenergic receptor subtype not previously appreciated by pharmacological criteria.

Epinephrine and norepinephrine mediate their effects via binding to adrenergic receptors (α₁, α₂, β₁, β₂) (1, 2). These receptors are encoded by different genes and are members of a much larger family of guanine nucleotide regulatory protein (G protein)-coupled receptors (3). Molecular cloning studies have revealed a growing heterogeneity of receptor subtypes. For example five different muscarinic cholinergic (4–8), three serotonergic (9–12), and two α₂-adrenergic receptor subtypes (13, 14) have been recently identified by molecular cloning. Heterogeneity of α₁-adrenergic receptors (α₁A and α₁B subtypes) has been suggested by several pharmacological studies (15–19) based on differential sensitivity of α₁-adrenergic receptor-mediated responses to a variety of agonists (e.g. oxymetazoline) and antagonists (e.g. WB4101 and phentolamine) as well as on different requirements of α₁-adrenergic receptor-induced responses for extracellular calcium (20).

Recently we reported the cloning of the cDNA which encodes the hamster α₁-adrenergic receptor purified from DDT7-MF₃ cells (21). The pharmacological properties of this α₁-adrenergic receptor resemble those described for the α₁B-receptor subtype. Here we present the cloning, sequencing, and expression of another α₁-adrenergic receptor subtype from bovine brain. This receptor shows the pharmacological properties proposed for the α₁A-adrenergic receptor subtype, but on the basis of a lack of expression in tissues where the α₁A subtype exists and its sensitivity to CEC (20), it may actually represent a novel α₁-adrenergic receptor subtype not previously appreciated by pharmacological criteria.

MATERIALS AND METHODS

Genomic and cDNA Library Screening—A human leukocyte genomic library in EMBL3 (10⁶ total recombinants, Clonetech, Palo Alto, CA) was screened with an oligonucleotide probe constructed from the hamster α₁-adrenergic receptor cDNA (nucleotide 1028–1094). Oligonucleotide probes were synthesized on an Applied Biosystems 380B DNA synthesizer, purified on a 15% (w/v) denaturing polyacrylamide gel, and labeled with [α-³²P]ATP at the 5'-hydroxyl group by T4 polynucleotide kinase. Duplicate filters were hybridized in 6 × SSC (1 × SSC = 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7), 0.5% polyvinylpyrrolidone, 0.2% Ficoll, 0.2% bovine serum albumin, 0.1% sodium pyrophosphate, 0.1% sodium dodecyl sulfate, 100 µg of sheared salmon sperm DNA/ml, 10⁶ cpm of ³²P-labeled probes/ml at 42 °C for 36 h. Filters were washed in 0.2 × SSC at 60 °C. A 0.32-kb PvuII/HindIII restriction fragment of the single genomic clone obtained was then used to screen a size-selected (20-4.4 kb) randomly primed bovine brain cDNA library in ZAP (500,000 total recombinants) generously provided by D.E.R. Bacon (Merck Sharp and Dohme) as described above. DNA fragments used as hybridization probes were labeled with [α-³²P]dATP by random priming. Standard recombinant DNA and microbiological procedures were used (22).

DNA Sequencing—Nucleotide sequence analysis of both DNA strands was done by the Sanger dideoxy chain termination method (23) with overlapping restriction fragments and by primer extension in pTZ18R (Pharmacia LKB Biotechnology Inc.) or ZAP pBluescript SK with T7 DNA polymerase (Sequenase, United States Biochemical Corp., Cleveland, OH).

Expression—In order to construct an expression vector for the newly cloned bovine cDNA, we used the expression vector pUC17 during...
Cloning and Expression of a Novel α1-Adrenergic Receptor Subtype

RESULTS

Screening of a human leukocyte genomic library with the labeled oligonucleotide probe constructed from the hamster α1β adrenergic receptor cDNA (nucleotides 1028–1094) identified one clone under high stringency conditions (0.2× SSC at 60 °C). This clone contained a 0.86-kb EcoRI/HindIII restriction fragment with an open reading frame between nucleotides 459 and 855. The open reading frame encoded a peptide where the first 43 residues were 67% identical with the hamster α1α-adrenergic receptor putative seventh transmembrane domain and the beginning of the carboxyl terminus (nucleotides 951–1077 of the hamster α1α-adrenergic receptor) (21). Therefore, the open reading frame diverged completely from the hamster α1α-adrenergic receptor sequence. In order to obtain a full-length clone, a size-selected (2.0–4.4 kb) bovine brain cDNA library was screened using as a probe a 0.32-kb PvuII/HindIII restriction fragment containing most of the open reading frame of the human genomic clone previously obtained. A single clone with a 3.1-kb insert was isolated under high stringency conditions (0.2× SSC at 60 °C). This bovine clone contained the same PvuII/HindIII restriction fragment as the human genomic clone. An initiator methionine at nucleotide 741 of the bovine cDNA clone started a 1388 bp open reading frame.

Fig. 1A shows the restriction map of the bovine cDNA clone. Fig. 1B shows the bovine cDNA nucleotide and deduced amino acid sequence (open reading frame together with a short stretch of 5′-untranslated sequence (96 bp) and the entire 3′-untranslated region (967 bp)). The open reading frame encodes a polypeptide of 466 amino acids with a calculated molecular mass of 51 kDa. Comparison of the deduced amino acid sequence of the bovine clone with that of previously cloned G protein–coupled receptors revealed striking amino acid identity in the putative transmembrane domains of the various adrenergic receptors but most strikingly with the hamster α1α-adrenergic receptor. The percentage identities in the putative transmembrane domains with each adrenergic receptor are the following: hamster α1β, 72.1%; human α1α-C4, 43.2%; human α1α-C10, 41.5%; human β1α, 43.2%; human β2α, 42.1% (13, 14, 21, 25, 34, 35). The level of amino acid identity within the putative transmembrane domains between the bovine cDNA clone and the hamster α1β-adrenergic receptor (72.1%) is similar to the level of amino acid identity within the transmembrane domains between the two α1α-adrenergic receptor subtypes (75%) or between the β1α- and β2α-adrenergic receptor subtypes (75%). Comparison of the entire bovine cDNA-deduced amino acid sequence and the hamster α1β-adrenergic receptor (Fig. 2, solid circles are identical amino acids) reveals that the NH2 terminus (27% amino acid identity), the COOH terminus (12% amino acid identity), and the third cytoplasmic loop (50% amino acid identity) represent the most divergent domains. These regions are also ones that differ the most in length and amino acid composition among other adrenergic receptors and G protein–coupled receptors. Since amino acid identity between hamster and human α1β-adrenergic receptors is 99%,4 this suggests that the bovine clone does not encode the bovine homolog of the hamster α1β-adrenergic receptor but rather a different α1α-adrenergic receptor subtype.

Three potential sites for N-linked glycosylation are present in the NH2 terminus (asparagine residues 7, 13, and 22). Several threonines and serines are present in the second and third cytoplasmic loops of this CDNA clone, representing potential sites for protein kinase C phosphorylation. A con-
Cloning and Expression of a Novel \( \alpha_1 \)-Adrenergic Receptor Subtype

sensus sequence for protein kinase A phosphorylation (amino acid residues 211–215) is present in the bovine \( \alpha_1 \)-adrenergic receptor subtype cDNA in an analogous position to the conserved site seen in the hamster \( \alpha_1 \)-adrenergic receptor cDNA. Phosphorylation has been observed as a mechanism of regulation for the \( \alpha_{1B} \)-adrenergic receptor (37, 38). The presence of these consensus sequences suggests that this new \( \alpha_1 \)-adrenergic receptor may also be regulated by phosphorylation.

To further confirm that the bovine cDNA obtained represented a different gene product than the hamster \( \alpha_1 \)-adrenergic receptor, human somatic cell hybridization analysis was performed using as probes the 0.32-kb \( \text{PvuII} / \text{HindIII} \) fragment of the bovine cDNA and the 0.7-kb \( \text{XhoI} / \text{BanHI} \) fragment of the hamster \( \alpha_1 \)-adrenergic receptor. These studies showed that while the gene corresponding to the hamster \( \alpha_1 \)-adrenergic receptor is located on human chromosome 5, the gene corresponding to the bovine cDNA is located on human chromosome 8.3

To further assess the functional identity of the cDNA isolated, a 2.4-kb fragment representing the coding region and the entire 3'-untranslated region was inserted into the expression vector pBC12BI and used to transfect COS-7 cells. The COS-7 cells transfected with the vector containing the bovine cDNA were able to bind the \( \alpha_1 \)-adrenergic antagonist [\( \text{Iz51} \)] HEAT with high specific activity (15 pmol/mg of protein) and with an affinity (60–70 PM) similar to that of the cloned hamster \( \alpha_1 \)-adrenergic receptor. No binding activity was detected in untransfected COS-7 cells. Analysis of adrenergic agonist and antagonist competition curves showed the appropriate pharmacology for \( \alpha_1 \)-adrenergic receptor binding. To compare ligand binding characteristics between the newly cloned bovine \( \alpha_1 \)-adrenergic receptor and the hamster \( \alpha_{1B} \)-adrenergic receptor, competition curve analysis of agonists and antagonists for [\( \text{Iz51} \)] HEAT binding was performed on membranes prepared from COS-7 cells transfected with the cDNA for either the bovine \( \alpha_1 \)-adrenergic receptor subtype or the hamster \( \alpha_{1B} \)-adrenergic receptor subtype. Comparison of the \( K_i \) values for different compounds (Table I) reveals striking differences between these two \( \alpha_1 \)-adrenergic receptors.

Specifically, the bovine \( \alpha_1 \)-adrenergic receptor subtype has more than 10-fold higher affinity for the \( \gamma_2 \) selective antagonist prazosin shows the same affinity for both \( \alpha_1 \)-adrenergic receptor subtypes, while epinephrine and norepinephrine are slightly more potent at the hamster \( \alpha_1 \)-adrenergic receptor. Recently the existence of two \( \alpha_1 \)-adrenergic receptor subtypes (\( \alpha_1A \) and \( \alpha_1B \)) has been suggested on the basis of differences in the affinities of the antagonists WB4101 and phentolamine and the agonist oxymetazoline for the two putative receptor subtypes (15–20), with the \( \alpha_1A \)-adrenergic receptor having 10-fold higher affinity for WB4101 than the \( \alpha_1B \). The 10-fold higher affinities of WB4101, phenolamine, and oxymetazoline for the bovine versus the hamster \( \alpha_1 \)-adrenergic receptor subtypes are in close agreement with those described for the \( \alpha_1A \) and \( \alpha_1B \)-adrenergic receptor subtypes, respectively.

Another approach used to pharmacologically discriminate different \( \alpha_1 \)-adrenergic receptor subtypes has been the irreversible inactivation of \( \alpha_1 \)-adrenergic receptor binding by the alkylating derivative of clonidine, CEC (16, 17, 19). Stopp and others (17, 20) have shown that treatment with CEC inactivates 50–60% of the \( \alpha_1 \)-adrenergic receptors in rat cerebral cortex. Since the population of receptors left after CEC

Fig. 1. Restriction map (A) and nucleotide sequence and deduced amino acid sequence (B) of the bovine cDNA clone (5'-untranslated region). 590 bp, open reading frame of 1308 bp. 3'-Untranslated region of 967 bp. 5'-Untranslated region of 103 bp. Underlined amino acids indicate potential N-linked glycosylation sites.
Cloning and Expression of a Novel \(\alpha_1\)-Adrenergic Receptor Subtype

**EXTRACELLULAR**

![Diagram of seven-transmembrane-spanning model of the new bovine \(\alpha_1\)-adrenergic receptor subtype showing amino acid identity with the hamster \(\alpha_{1B}\)-adrenergic receptor subtype. Solid circles indicate amino acids common to the corresponding position in the hamster \(\alpha_{1B}\)-adrenergic receptor (21). Transmembrane domains are defined based on hydropathy analysis (36). Potential N-linked glycosylation sites are shown as crosses.](image)

**INTRACELLULAR**

**FIG. 2.** Seven-transmembrane-spanning model of the new bovine \(\alpha_1\)-adrenergic receptor subtype showing amino acid identity with the hamster \(\alpha_{1B}\)-adrenergic receptor subtype. Solid circles indicate amino acids common to the corresponding position in the hamster \(\alpha_{1B}\)-adrenergic receptor (21). Transmembrane domains are defined based on hydropathy analysis (36). Potential N-linked glycosylation sites are shown as crosses. Inactivation shows only high affinity for WB4101, the \(\alpha_{1A}\)-adrenergic receptor subtype has been suggested to be insensitive to CEC. To assess the effect of CEC on the bovine and hamster \(\alpha_1\)-adrenergic receptors, transfected COS cell membranes were treated with 100 \(\mu\)M CEC for 20 min at 37 °C, and \(\alpha_1\)-receptor ligand binding was measured with a saturating concentration of \([\text{I}^{125}\text{I}]\)HEAT. CEC treatment inactivated 95 ± 1 and 68 ± 3% (mean ± S.E., \(n = 3, p < 0.02\)) of the \(\alpha_1\) binding in COS cells individually expressing the hamster \(\alpha_{1B}\)- and bovine \(\alpha_1\)-adrenergic receptor, respectively. In rat cortex membranes, the same treatment with CEC inactivated 72 ± 7.5% of \(\alpha_1\)-adrenergic receptors, in agreement with the values previously reported (17, 19, 20). These results indicate that the bovine \(\alpha_{1A}\)-adrenergic receptor subtype is less sensitive to CEC inactivation than the hamster \(\alpha_{1B}\)-adrenergic receptor subtype but more sensitive than has been suggested for the \(\alpha_{1A}\)-adrenergic receptor subtype (16, 17, 19).

In order to explore the tissue expression of the bovine \(\alpha_1\)-adrenergic receptor and compare it with that of the hamster \(\alpha_{1B}\) subtype, we performed Northern blot analysis on poly(A)+-selected mRNA of various rat and bovine tissues as well as utilized the DNA PCR with cDNA prepared from RNA of various bovine tissues. Northern blot analysis of rat mRNA has confirmed that the hamster \(\alpha_1\)-adrenergic receptor previously cloned corresponds to the subtype described as the \(\alpha_{1B}\)-receptor as indicated by strong hybridization to rat liver and cerebral cortex mRNA (15, 17, 19). However, no signal was observed with the bovine \(\alpha_1\) subtype in any of the rat tissues (cerebral cortex, pituitary, hippocampus, brainstem, liver, heart, lung, kidney, spleen, aorta, adipose tissue, skeletal muscle, vas deferens) or bovine tissues (cerebral cortex, liver, heart, kidney, lung, adrenal) examined by Northern blot analysis or by PCR (data not shown). In situ hybridization of human dentate gyrus using antisense RNA probes derived from the human homolog of the bovine \(\alpha_{1A}\)-adrenergic receptor subtype revealed a restricted pattern of expression only in the granular cell layer (Fig. 4). These data suggest that the expression of this receptor subtype is either very low or restricted to specific tissues or cellular populations.

**DISCUSSION**

We have cloned a cDNA encoding a novel \(\alpha_1\)-adrenergic receptor subtype from bovine brain. Sequence homology with the previously cloned hamster \(\alpha_{1B}\)-adrenergic receptor (72.1% identity in the putative transmembrane domains) suggests the bovine cDNA is an \(\alpha_{1A}\)-adrenergic receptor. High affinity for the \(\alpha_1\)-adrenergic antagonist \([\text{I}^{125}\text{I}]\)HEAT and overall rank order of potencies of agonists and antagonists confirms that the cDNA encodes an \(\alpha_{1A}\)-adrenergic receptor. However, human chromosome analysis provides evidence that the bovine \(\alpha_1\)-adrenergic receptor (localized to chromosome 8) is distinct from the hamster \(\alpha_{1B}\)-adrenergic receptor (localized to chromosome 5). In addition, the homology between the bovine \(\alpha_1\)-adrenergic receptor and the hamster \(\alpha_{1B}\)-adrenergic receptor...
Cloning and Expression of a Novel $\alpha_1$-Adrenergic Receptor Subtype

The $\alpha_1$-adrenergic receptor subtype in the putative transmembrane domains of the receptor is similar to that between $\alpha_2$-adrenergic subtypes (75%) and $\beta$-adrenergic receptor subtypes (75%), suggesting that these receptors represent distinct $\alpha_1$-adrenergic receptor subtypes. Ligand binding studies confirm that the bovine $\alpha_1$-adrenergic receptor subtype and the hamster $\alpha_{1B}$-adrenergic receptor subtype have clear pharmacological differences. Specifically, the $\alpha$-antagonists WB4101, phentolamine, corynanthinine, and indoramin, as well as agonists oxymetazoline and methoxamine, have 10-fold higher affinity for the bovine $\alpha_1$-adrenergic receptor subtype compared with the hamster $\alpha_{1B}$-adrenergic receptor subtype. These data are in close agreement with ligand binding properties of the $\alpha_1A$- and $\alpha_{1B}$-adrenergic receptors described in the literature, indicating that the bovine cDNA encodes a receptor with pharmacologic properties similar to the $\alpha_{1A}$-adrenergic receptor subtype.

In contrast to the clear agreement between ligand binding properties reported for the $\alpha_{1A}$-adrenergic receptor in the literature and those described for the bovine $\alpha_1$-adrenergic receptor subtype, CEC inactivation studies show some differences. $\alpha_{1B}$- and $\alpha_{1A}$-adrenergic receptors have been described as being sensitive or insensitive to the alkylating agent CEC, respectively (17, 19, 20). This classification originated from experiments in various tissues where CEC inactivation correlated with a loss of low affinity sites for WB4101 (corresponding to $\alpha_{1B}$-adrenergic receptors), leaving only high affinity sites (corresponding to $\alpha_{1A}$-adrenergic receptors). Our data with transfected COS cell membranes treated with 100 $\mu$M CEC for 20 min at 37 °C show that the previously cloned $\alpha_{1A}$-adrenergic receptor subtype is in fact totally (95%) inactivated by CEC while the bovine $\alpha_1$-adrenergic receptor subtype is only partially (68%) inactivated. It is very difficult to conclude based on ligand binding studies with membranes derived from tissue homogenates containing mixtures of $\alpha_1$-receptor subtypes whether the $\alpha_{1A}$-receptor is completely insensitive to CEC or whether it is merely less sensitive than the $\alpha_{1B}$. Although it seems clear that the $\alpha_1$-receptors remaining after CEC treatment in such preparations have ligand binding properties identical to those of the cloned bovine $\alpha_1$-adrenergic receptor (e.g. high affinity for WB4101), it is also possible that some unspecified proportion of the $\alpha_{1A}$-receptor in the tissue homogenate was inactivated. Moreover, it is possible that another subtype of $\alpha_1$-adrenergic receptor exists with

### Table I

**Competition by agonists and antagonists for the binding of $[^{35}S]$HEAT to membranes prepared from COS-7 cells transfected with the pBC12BI expression vector containing the new bovine $\alpha_1$ or the hamster $\alpha_{1B}$-adrenergic receptor cDNA**

Each concentration for competition curves was done in triplicate; all isomers are (−) unless otherwise stated. Ratios (hamster $\alpha_{1B}$/bovine $\alpha_1$) were rounded to the nearest digit. Results shown are the means of two experiments which agreed within 10%.

<table>
<thead>
<tr>
<th></th>
<th>Bovine $\alpha_1$</th>
<th>Hamster $\alpha_{1B}$</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Agonist</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxymetazoline</td>
<td>27</td>
<td>290</td>
<td>11</td>
</tr>
<tr>
<td>$p$-Aminoclonidine</td>
<td>590</td>
<td>680</td>
<td>1</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>6,650</td>
<td>4,800</td>
<td>0.7</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>15,000</td>
<td>18,800</td>
<td>1</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>16,500</td>
<td>9,600</td>
<td>0.6</td>
</tr>
<tr>
<td>(+)-Epinephrine</td>
<td>43,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methoxamine</td>
<td>75,000</td>
<td>1,200,000</td>
<td>16</td>
</tr>
<tr>
<td>Dopamine</td>
<td>230,000</td>
<td>400,000</td>
<td>2</td>
</tr>
<tr>
<td>Serotonin</td>
<td>230,000</td>
<td>539,000</td>
<td>1</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>1,100,000</td>
<td>1,400,000</td>
<td>1</td>
</tr>
<tr>
<td><strong>Antagonist</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prazosin</td>
<td>0.27</td>
<td>0.26</td>
<td>1</td>
</tr>
<tr>
<td>WB4101</td>
<td>0.55</td>
<td>8.5</td>
<td>16</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>4.8</td>
<td>165</td>
<td>32</td>
</tr>
<tr>
<td>Indoramin</td>
<td>6</td>
<td>84</td>
<td>14</td>
</tr>
<tr>
<td>Corynanthinine</td>
<td>78</td>
<td>640</td>
<td>8</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>390</td>
<td>1,300</td>
<td>4</td>
</tr>
<tr>
<td>Rauwolscine</td>
<td>1,400</td>
<td>3,200</td>
<td>2</td>
</tr>
<tr>
<td>Idazoxan</td>
<td>1,500</td>
<td>1,100</td>
<td>0.7</td>
</tr>
</tbody>
</table>

**FIG. 3.** WB4101 (A) and oxymetazoline (B) competition for the binding of $[^{35}S]$HEAT to membranes prepared from COS-7 cells transfected with the expression vector pBC12BI containing the cDNA for the new bovine $\alpha_1$ (circles) or the hamster $\alpha_{1B}$-adrenergic (squares) receptor subtype. $[^{35}S]$HEAT was 90 pm, and 100% of $[^{35}S]$HEAT bound was 10 pm. Results are representative of two experiments done in triplicate which agreed within 10%. The $K_i$ estimates for these and other agonists and antagonists are listed in Table I.
Cloning and Expression of a Novel α₁-Adrenergic Receptor Subtype

Fig. 4. *In situ* localization of the bovine α₁-adrenergic receptor subtype mRNA in the human hippocampus. A, x-ray autoradiographs of coronal section of the human dentate gyrus hybridized with the ³⁵S-labeled antisense strand probe specific for the human homolog of the bovine α₁-adrenergic receptor. Specific labeling occurs over the granular cell layer. B, x-ray autoradiographs of serial sections hybridized with the ³⁵S-labeled sense strand control probe resulted in only background labeling. C, emulsion autoradiographs (Kodak NTB2, hematoxylin and eosin) of the section shown in panel A magnified × 250. Specific hybridization to the granular cell layer (dense band of cells running through picture) is seen; pyramidal cells were not labeled. D, emulsion autoradiographs of the section shown in panel B magnified × 250 result in background labeling only.

similar pharmacological properties which may be completely insensitive to CEC inactivation.

In agreement with this hypothesis is the fact that while the pharmacological properties of the bovine α₁-adrenergic receptor subtype suggest that it might represent the α₁A subtype, we have been unable to establish this identification by observing mRNA species in tissues where the α₁A-adrenergic receptor subtype has been previously described such as rat vas deferens and hippocampus. These observations strongly suggest that the bovine α₁-adrenergic receptor represents a novel α₁-adrenergic receptor subtype and that expression of this receptor is quite specialized in either tissue distribution or developmental stage. In fact, even if at present we have not identified the bovine or rat tissues where the bovine α₁AR subtype is expressed, *in situ* hybridization of human dentate gyrus slices reveals the presence of the human homolog of the bovine α₁AR specifically in the granular cell layer. Recently the human and rat genes of a fifth muscarinic receptor have been cloned, but the expression of this receptor could not be detected in several rat tissues (8). These observations indicate that receptor heterogeneity might be more complex than predicted by pharmacological studies, and the isolation of different receptor subtypes by molecular cloning provides the most direct tool for the attribution of distinct receptor subtypes to specific tissues. They also indicate the sensitivity and power of techniques such as PCR and *in situ* hybridization studies in determining the localization of new receptor subtypes. Since we have evidence for the presence of the gene analogous to the bovine α₁AR subtype in both the human and rat genome, a more extensive investigation by *in situ* hybridization of human and rat tissues will be required to elucidate the tissue distribution as well as the level of expression of this novel α₁AR subtype.

The structural features of the new α₁-receptor subtype are consistent with those of the other adrenergic receptors which have been cloned as well as with the wider family of G-protein-coupled receptors. The remarkable conservation of sequence within the presumed membrane-spanning domains between the two α₁-receptor subtypes is consistent with their very similar ligand binding properties. This sequence conservation extends to those regions of the third cytoplasmic loop and carboxyl-terminal cytoplasmic tail which are presumed to lie closest to the plasma membrane. Inasmuch as these regions of the receptor molecule have been suggested to be those responsible for coupling to G-proteins, this might suggest that the effector function of these receptor subtypes might also be similar. However it has been speculated that α₁-receptor subtypes might mediate distinct functions (19).
The availability of clones for distinct α1-adrenergic receptor subtypes should now facilitate refinement not only of the structural basis of adrenergic receptor ligand binding but also of the signal transduction mechanisms utilized by different α1-adrenergic receptor subtypes.

Acknowledgments—We gratefully acknowledge Sabrina Exum for assistance with tissue culture and ligand binding, K. Theisen for generously providing the bovine brain cDNA library in XZAP, and Drs. John Regan and Sheila Collins for helpful discussions.

References
Molecular cloning and expression of the cDNA for a novel alpha 1-adrenergic receptor subtype.
D A Schwinn, J W Lomasney, W Lorenz, P J Szklut, R T Fremeau, Jr, T L Yang-Feng, M G Caron, R J Lefkowitz and S Cotecchia


Access the most updated version of this article at http://www.jbc.org/content/265/14/8183

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/265/14/8183.full.html#ref-list-1