ACCELERATED COMMUNICATION

Molecular Cloning and Pharmacological Characterization of a New Galanin Receptor Subtype

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SUMMARY
Galanin, a 29–30-amino acid neuropeptide, is widely distributed in central and peripheral systems and mediates a variety of physiological functions. Pharmacological studies have suggested the existence of multiple receptor subtypes but only the type I (GalR1) galanin receptor has been cloned. Now we report the cloning by a combination of sib selection and rapid amplification of cDNA ends of a cDNA encoding a new galanin receptor (GalR2) from rat hypothalamus. The receptor is 372 amino acids in length and shares only 40% homology with the rat GalR1 receptor. It contains seven putative transmembrane domains with the amino and carboxyl termini being least identical to GalR1. Northern blot analyses revealed a 2-kilobase pair mRNA species distributed in several tissues, suggesting a broader functional spectrum than GalR1. 125I-Labeled human galanin binding to rat GalR2 receptor expressed in COS-1 cells was saturable (Kd = 0.59 nM) and could be displaced by galanin, several galanin fragments, and chimeric peptides. The pharmacological profiles of GalR1 and GalR2 receptors were distinguishable by galanin fragment(2–29), which bound the cloned GalR2 receptor with markedly higher affinity than the GalR1 receptor. Activation of the cloned receptor by galanin led to inhibition of forskolin-stimulated intracellular cAMP production. The cloning of this new receptor subtype should provide further insights into the mechanisms by which galanin mediates its diverse physiological functions. The identification of galanin(2–29) as a receptor-specific ligand should enhance the understanding of specificity of galanin-receptor interactions.

Galanin, a neuropeptide 29–30 amino acids in length, mediates diverse and crucial physiological functions (1, 2). Centrally it effects cognition, nociception, and feeding behavior (3–5), and peripherally, it mediates regulations of insulin release and muscle contraction (6, 7). Galanin exerts its function through binding to its receptors. Biochemically, a galanin receptor has been characterized as a glycoprotein with an apparent molecular mass of 54 kDa (8) and a signaling pathway linked through G proteins to the inhibition of adenyl cyclase through a pertussis-toxin sensitive mechanism (9).

Pharmacological studies with several peptidic agonists and antagonists of galanin receptor suggest the existence of more than one receptor subtypes (10–12). The galanin GalR1 receptor has been cloned from human melanoma cells (13), rat Rin1B insulinoma cells (14), and rat brain (15). The receptor belongs to the G protein-coupled receptor superfamily, characterized by seven hydrophobic transmembrane domains (16), and displays inhibitory effects on forskolin-stimulated intracellular cAMP production in cells either stably or transiently expressing GalR1 receptor. In this report, we describe the cloning of a novel galanin receptor subtype by a combination of rib selection of a rat hypothalamus cdNA library and RACE. The receptor has been expressed in COS-1 cells and displays a distinct pharmacological profile that is distinguished from that of GalR1 by its high affinity for the fragment galanin(2–29).

Materials and Methods
125I-Labeled human-galanin (2200 Ci/mmol) and α-[32P]dATP (5000 Ci/mmol) were purchased from DuPont/New England Nuclear (Boston, MA). Oligonucleotides used in this study were custom-synthesized by Life Technologies (Grand Island, NY), and their sequences are: oligo97, 5’-caccaccaacctgttcatcctsaacctg; oligo98, 5’-gggcagmaggtanccraasac-gaargtgca; oligo112, 5’-gccagat-cctgcgtgcctttccaggccaccatc; oligo113, 5’-gccagat-

ABBREVIATIONS: GalR, galanin receptor; RACE, rapid amplification of cDNA ends; DMEM, Dulbecco’s modified Eagle’s medium; PBS, phosphate-buffered saline; GMAP, galanin messenger-associated peptide(1–41); PCR, polymerase chain reaction; bp, base pair; kb, kilobase pair; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; PMSF, phenylmethylsulfonyl fluoride.
acgtgccagggagc; oligo124, 5'-gacctggcaggtctgaacagc; oligo129, 5'-tgatgtctgtgcgctactat; oligo139, 5'-gggggatccattcgtttgatac; and oligo137, 5'-aattcttttttgacccagg. Rat multiple tissue Northern and rat genomic DNA blots were obtained from Clontech (Palo Alto, CA).

PCB sib selection. PCR and galanin receptor-specific primers were used to screen pools of a rat hypothalamus cDNA library. When positive pools were identified they were subcloned and screened similarly by the PCR screening until the final round of screening in which individual colonies were selected by analysis of plasmid minipreps. The rat cDNA library was constructed as follows. Total RNA from rat hypothalamus was extracted with Tri-reagent-RNA/DNA/protein Isolation Reagent (Molecular Research Center, Cincinnati, OH). Poly(A)+ RNA from the total RNA was purified with a mRNA purification kit that employs oligo(dT)-cellulose chromatography (Pharmacia, Piscataway, NJ). Double strand cDNA was synthesized from the poly(A)+ RNA with a Marathon cDNA amplification kit (Clontech). A portion of the cDNA was blunt-end ligated with adapter containing a Bst XI restriction site. The Bst XI adaptor-linked cDNA was then ligated into pCDNA3 vector predigested with Bst XI.

Cyclic AMP analysis. COS-1 cells grown in 150-mm diameter dishes were transfected as described above. After a 3-day post-transfection growth, the cells were washed with PBS, resuspended on the plates, and resuspended at a density of 2 x 10^5 cells/100 µl of buffer containing 75 mM Tris-Cl, pH 7.4, 250 mM sucrose, 12.5 mM MgCl₂, 1.5 mM EDTA, and 0.2 mM sodium metabisulfite. Cells were incubated in DMEM for 1 hr at 22° in 130 µl of the buffer plus 0.1 mM forskolin and 0.2 mM 3-isobutyl-1-methylxanthine with rat galanin or peptides at the indicated concentrations. The cells were then lysed by boiling for 5 min and chilled on ice. The concentration of cAMP in the cell lysates was determined by scintillation proximity assay (Perkin Elmer, Branchburg, NJ). The data were analyzed with the Prism nonlinear regression method to obtain maximum inhibition and EC₅₀ values.

Purification kit that employs oligo(dT)-cellulose chromatography (Pharmacia, Piscataway, NJ). Double strand cDNA was synthesized from the poly(A)+ RNA with a Marathon cDNA amplification kit (Clontech). A portion of the cDNA was blunt-end ligated with adapter containing a Bst XI restriction site. The Bst XI adaptor-linked cDNA was then ligated into pCDNA3 vector predigested with Bst XI.

PCR and RACE amplification of cDNA fragment. Unless otherwise specified, PCR was always run with KlenTaq polymerase that possesses proof reading activity (Clontech) and a cycling profile of 94° for 1 min, 65° for 1 min, and 72° for 2 min (40 cycles). For RACE, nested oligonucleotides specific to the rat GalR2 cDNA and nested adaptor primers were used. Approximately 0.1 µg of cDNA from tissues was used as template in PCR and RACE, and 1 µl of overnight Escherichia coli cell culture was used in PCR sib selection.

DNA sequencing and analysis. DNA sequencing was performed with ABI Prism dye termination DNA sequencing reagents and an ABI automated sequencing apparatus (Perkin Elmer, Branchburg, NJ). DNA and protein sequence comparisons were performed with the DNA* software (DNAstar, Madison, WI).

Transfection of COS-1 cells. COS-1 cells grown in DMEM supplemented with 10% fetal calf serum (FCS) in were split 1:6 into 150-mm dishes (Nunc) 3 days before transfection. On the day of transfection, the cells were grown in 150-mm diameter dishes (Nunc) 3 days before transfection. On the day of transfection, the cells were washed three times with PBS (no Ca²⁺ and Mg²⁺). The cell pellet was resuspended in 2 ml of 25 mM HEPES buffer, pH 7.4, 0.1 mM PMSF, and 0.1 mg/ml bacitracin were added and incubated at room temperature for 10 min. The cells were centrifuged and resuspended at 10,000 × g for 15 min at 4°. The cell pellet was resuspended in 2 ml of 25 mM Tris-Cl, pH 7.4, 0.2 mM phenylmethyisulfonyl fluoride by vortexing and dispersed with a syringe attached with a 23-gauge needle. Protein concentrations were determined with a BCA method (Pierce, Rockford, IL). Results and Discussion

Galanin serves as a neurotransmitter and hormone that mediates diverse physiological functions in the central and peripheral systems. Pharmacological studies suggest that more than one receptor subtype exists, raising the possibility that multiple galanin receptors are involved in these various functions. To directly test this hypothesis, we used a molecular cloning approach to identify a non-GalR1 receptor from rat. Several PCR primers, either identical or degenerate, corresponding to the transmembrane regions of rat GalR1 cDNA, were used in PCR with rat lung cDNA as template, which was generated by reverse-transcription from rat lung poly(A)+ mRNA. Rat lung was previously shown to contain no detectable GalR1 mRNA (14) thus was used to avoid preferred amplification of rat GalR1 cDNA. PCR with two primers, oligo97 and oligo98, yielded a band of approximately 420 bp. This band was cloned and found to contain rat GalR1 cDNA and various non-GalR1 sequences. One of the non-GalR1 clones (9798sw8) possessed highest homology to rat GalR1 cDNA at both nucleotide (68%) and amino acid levels.

125I-Galanin binding assay. Binding of 125I-human galanin to the membranes was performed in a buffer containing 25 mM Tris-Cl (pH 7.4), 1% bovine serum albumin (radioimmunoassay grade), 0.1% bacitracin, 2 µg/ml leupeptin, 0.1 mM PMSF, and 10 mM MgCl₂. Ligand saturation plots were performed using 10 µg of the membrane protein in a total volume of 200 µl using 3 µl cold galanin to determine nonspecific binding. Peptide competition studies were performed in a total volume of 200 µl, containing 10 µg of membrane protein and 0.3 nM 125I-human galanin. Incubations were at room temperature for 1 hr and terminated by rapid vacuum filtration through Multiscreen FB Filter Plates (Millipore, Bedford, MA) that had been pretreated with 0.3% polyethyleneimine. The filters were then washed three times with 100 µl of PBS (pH 7.4). All data were analyzed using nonlinear regression software (Prism, GraphPad, San Diego, CA) and the Kᵣ calculated according to the method of Cheng and Prusoff (17).
Fig. 1. Molecular cloning of a rat GalR2 cDNA. A, Isolation of the rat GalR2 cDNA. Left, five steps involved in the cloning; long boxes, clones obtained at each step by PCR, RACE, or by PCR-based sib selection. Pairs of primers used in the cloning steps are shown on the top (forward primer) and bottom (reverse primer) of each clone accompanied by their names. The lengths and relative positions of the clones are shown approximately to scale. ATG and stop codon indicate the initiation and termination sites for the translation of the receptor. A cycling profile of 92°C for 0.5 min, 58°C for 0.5 min, and 72°C for 1 min (40 cycles) was used in step 1, and standard cycling was used in other PCRs (see Materials and Methods). B, Nucleic acid and amino acid sequences of the rat GalR2 receptor. Shown are the coding nucleotide sequence of the rat GalR2 cDNA, and underneath is the deduced amino acid sequence. Right, numbering of the nucleotide sequence; underlined Roman letters, putative transmembrane regions I–VII, assigned based on comparison with the rat GalR1 sequence (15) and hydrophobicity analysis (27). p, Potential N-linked glycosylation site; F, two Cys residues that may form a disulfide bridge. C, Alignment of the amino acid sequence of rat GalR2 receptor with rat GalR1 receptor (15). The top sequence pCR3.1-rGalR#20 shows the translated rat GalR2 receptor encoded by clone pCR3.1-rGalR#20, and the amino acid residues in rat GalR1 different from the GalR2 receptor are shown beneath. Dotted lines, amino acid residues identical to the GalR2 sequence. Gaps (-) are introduced to optimize the alignment.
Two primers specific to the rat GalR2 cDNA were designed (oligo112 and oligo113) and used in PCR to screen cDNA pools of a rat hypothalamus cDNA library (5000 clones/pool). After four rounds of PCR sib selection, a clone, C27#33, with comparable size and considerable sequence overlap with clone 9798sw8, was identified from pool C27 (Fig. 1A). The 3′ end of the clone was unique to rat GalR2 cDNA and allowed generation of another rat GalR2 specific primer (oligo124). Three rounds of PCR sib selection on the rat hypothalamus cDNA pools with primers oligo112 and oligo124 yielded a positive clone (B45-11-16) from pool B45 that had an insert size of 1.3-kb (Fig. 1A) and contained primer oligo129 and the inner one of the two RACE adaptors. The final 3′ RACE product (Fig. 1A), obtained with primer oligo129 and the inner one of the two nested adaptor primers, overlapped the 3′ portion of clone C27#33. A search of the GenBank database revealed that an open reading frame in this clone possessed highest homology to rat GalR1 (≈90% of the length of GalR1 cDNA) but contained no stop codon (Fig. 1A). To obtain the 3′ end, RACE was performed by using a rat brain cDNA library linked with the RACE adaptor on the two ends as template (Clontech) and nested primers specific to rat GalR2 cDNA and the RACE adaptors. The final 3′ RACE product (Fig. 1A), obtained with primer oligo129 and the inner one of the two nested adaptor primers, overlapped the 3′ portion of clone B45-11-16 (Fig. 1A).

Full-length rat GalR2 cDNA was obtained by PCR with primers oligo139 and oligo137 and rat hypothalamus cDNA as a template. A strong single band PCR product (~1.2-kb, Fig. 1A) was obtained and ligated into a TA cloning vector, pCR3.1 (Invitrogen, San Diego, CA). The clone was named pCR3.1-rGalR#20. Fig. 1B shows the open reading frame and the deduced amino acid sequence for the encoded rat GalR2 receptor. The receptor is 372 amino acids in length, has a calculated molecular mass of 40.7 kDa, and contains seven putative transmembrane spanning regions typical of G protein-coupled receptors. Other features common to the G protein-coupled receptor superfamily are also present in the rat GalR2 amino acid sequence. There is a single potential N-linked glycosylation site in the amino-terminal region, two Cys residues in the first and second extracellular loops that form a putative disulfide bond in these receptors, and two Cys residues in the carboxy-terminal region that may be involved in palmitoylation (Fig. 1B). The overlapping sequences between the independent clones B45-11-16 (cDNA), the 3′ RACE product in cloning step 4, and pCR3.1-rGalR#20 (Fig. 1A) are identical, indicating the absence of PCR artifacts in the coding sequence.

The amino acid sequence of the rat GalR2 receptor is significantly different from that of rat GalR1. The overall homology is 40% at the amino acid level as analyzed by the Jutun Hein method (18) (Fig. 1C). A search of the SwissProt databank showed similarity to rat cholecystokinin receptor (38%) (19), the human somatostatin receptor (37%) (20), and the human K opioid receptor (31%) (21). Sequence alignment between rat GalR1 and rat GalR2 amino acid sequences shows the greatest similarity in the second, third, and seventh transmembrane regions, being 63, 52, and 52% identical, respectively. Least conserved regions appear at the amino- and the carboxy-terminal regions, being 16% and 13% identical, respectively (Fig. 1C).

To study the distribution of the mRNA of the rat GalR2 receptor, Northern blot analysis was performed by hybridization of blotted poly(A)⁺ RNA from several rat tissues (Fig. 2A) to 32P-labeled 9798sw8 fragment (~420 bp). A single 2-kb mRNA transcript was detected in all the tissues with somewhat stronger signals in kidney, testis, skeletal muscle, and liver than in brain, heart, spleen, and lung. The strikingly wide distribution of rat GalR2 mRNA is in contrast to the distribution of rat GalR1 mRNA, which has been detected at significant levels only in brain and spinal cord. The data suggest that rat GalR2 receptor may play a broader physiological role than GalR1 receptor, by acting not only in the central nervous system but also in peripheral systems. Southern blot hybridization to rat genomic DNA digested with restriction enzymes with 32P-labeled full-length rat GalR2 cDNA followed by high stringency washes shows a single band of 15, 4, and 2.1 kb with EcoRI, HindIII, and BglII, respectively (Fig. 2B). Two bands at 3.8 and 1.6 kb observed with BamHI, and three bands at 2.7, 1, and 0.7 kb observed with PstI, may have resulted from BamHI and PstI sites in the introns and exons in the coding region. The presence of one PstI site identified in the cDNA sequence (Fig. 1B) is consistent with this explanation. Thus, GalR2 appears to exist as a single copy in the rat genome.

Fig. 2. A, Northern blot analysis. Northern blot containing rat poly(A)⁺ mRNA isolated from several tissues was probed with a 32P-labeled 420-bp fragment within the rat GalR2 cDNA coding region. Lane 1, testis; lane 2, kidney; lane 3, skeletal muscle; lane 4, liver; lane 5, lung; lane 6, spleen; lane 7, brain; and lane 8, heart. The blot was reprobed with 32P-labeled actin cDNA to ensure comparable loading of mRNA from the tissues (bottom). B, Southern blot analysis. A Southern blot containing Sprague-Dawley rat kidney genomic DNA at 4 μg/lane was hybridized with full-length rat GalR2 cDNA (excised out of pCR3.1-rGalR#20 and gel-purified) labeled with 32P. The lanes indicate the DNA digested with restriction enzymes: R, EcoRI; H, HindIII; Bm, BamHI; P, PstI; and Bg, BglII.
The ability of the expressed rat GalR2 receptor to bind radiolabeled galanin was examined by performing binding of \(^{125}\text{I}\)-human galanin to membranes of COS-1 cells transfected with pCR3.1-rGalR#20 plasmid. The binding was saturable with high affinity, showing a \(K_d\) value of 0.59 ± 0.27 nM and a \(B_{\text{max}}\) value of 461 ± 153 fmol/mg of membrane protein (mean ± standard deviation; 4 independent transfections). A representative binding curve is illustrated in Fig. 3. Binding of the radioligand to cell membranes transfected with vector alone was negligible. Nonlinear regression analyses with one or two site-fit suggest that the ligand binds to a single class of binding sites in COS-1 cells transfected with the rat GalR2 cDNA. Similar results were obtained when the saturation binding assay was performed with \(^{125}\text{I}\)-porcine galanin and rat GalR2 receptor prepared from COS-1 cells \((K_d = 0.78 \text{ nM} \text{ and } B_{\text{max}} = 595 \text{ fmol/mg of membrane protein}).\)

To characterize the pharmacology of the rat GalR2 receptor, \(^{125}\text{I}\)-human galanin binding to membranes prepared from COS-1 cells transfected with pCR3.1-rGalR#20 plasmid was competed with various galanin peptide analogs (Fig. 4). Rat galanin, galantide, galanin fragment(2–29), and galanin(1–13)-bradykinin(2–9) bound to rat GalR2 receptor at high affinity (Table 1 and Fig. 4). Lower affinity was found with galanin(1–13)-spantide (C7), galanin (1–16), and M40, with 16–32-fold lower affinities than native galanin. Two ligands, (D-Thr\(^6\),D-Trp\(^8,9\))galanin(1–15)\(\text{o}\) and GMAP, whose affinities have not been reported for cloned galanin receptors, showed almost no binding to rat GalR2 receptor (Table 1). For comparison, a few ligands were tested with membranes of rat GalR1 receptor prepared from transiently transfected COS-1 cells (Table 1). Native galanin, galantide, and galanin(1–16) bound GalR1 with high affinity, whereas galanin(2–29) bound with lower affinity (by ~260-fold), consistent with the reported profile for the cloned GalR1 receptor (13–15). Cholecystokinin, somatostatin and dynorphin did not displace the radioligand in the assay.

Although most ligands displayed similar affinities between rat GalR1 and GalR2 receptors, we noted that the affinity of galanin(2–29) for GalR2 receptor was considerably higher than that for GalR1 receptor. Direct comparison of affinities of galanin(2–29) for GalR1 and GalR2 revealed that the affinity for GalR1 was 42-fold lower than that for GalR2 (Table 1, Fig. 5). As controls in these experiments, galanin bound the two receptors with similar affinities (Fig. 5). The low affinity of galanin(2–29) for GalR1 indicates a stringent requirement for the amino-terminal Gly residue on the ligand for binding the receptor whereas this requirement is significantly lower for GalR2 receptor. Along with Trp2, Asp5, and Tyr9, the amino-terminal Gly residue of galanin has been identified as a key component of the pharmacocore for galanin binding to the GalR1 receptor (22). A hydrogen bond from the amino terminus of galanin (23). The high binding affinity of galanin(2–29) to GalR2 receptor suggests that amino-terminal glycine is not important for the interac-

Fig. 3. Saturation analysis of \(^{125}\text{I}\)-human galanin binding to membranes prepared from COS-1 cells transfected with clone pCR3.1-rGalR#20. Points, representative experiment performed in duplicate. Specific binding curve, fit with one-class binding sites on the receptor by nonlinear regression analysis. ○, total binding; Δ, nonspecific binding defined by the presence of 3 μM rat galanin; ●, rat GalR2 receptor-specific binding calculated as the difference between the total and nonspecific bindings.
TABLE 1
Pharmacological profile of the cloned rat GalR2 receptor

<table>
<thead>
<tr>
<th>Ligand</th>
<th>$K_i$ (nm)</th>
<th>$K_i$ (ligand)/$K_i$ (galanin)</th>
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<tr>
<td>Rat galanin(1-29)</td>
<td>0.56 ± 0.04 (3)</td>
<td>1.0</td>
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<tr>
<td>Galantide</td>
<td>1.17 ± 0.43 (4)</td>
<td>2.1</td>
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<tr>
<td>Rat galanin(2-29)</td>
<td>3.5 ± 0.5 (2)</td>
<td>6.3</td>
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<tr>
<td>Galanin(1-13)-bradykinin(2-9)</td>
<td>4.0 ± 3.0 (2)</td>
<td>7.1</td>
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<tr>
<td>Galanin(1-13)-spantide I, (C7)</td>
<td>9.1 ± 1.7 (2)</td>
<td>16.3</td>
</tr>
<tr>
<td>Rat galanin(1-16)</td>
<td>13 ± 12 (3)</td>
<td>23.2</td>
</tr>
<tr>
<td>M40</td>
<td>18 ± 18 (3)</td>
<td>32.1</td>
</tr>
<tr>
<td>(o-Thr$^5$, o-Trp$^9$)galanin(1-5)ol</td>
<td>&gt;1250 (4)</td>
<td>&gt;2200</td>
</tr>
<tr>
<td>GMAP (1-41)</td>
<td>&gt;840 (2)</td>
<td>&gt;1500</td>
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<tr>
<td>Rat GalR1 receptor</td>
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<td></td>
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<tr>
<td>Rat galanin(1-29)</td>
<td>0.33 ± 0.19 (2)</td>
<td>1.0</td>
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<tr>
<td>Galantide</td>
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<tr>
<td>Rat galanin(1-16)</td>
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<td>14.8</td>
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<tr>
<td>Rat galanin(2-29)</td>
<td>87 ± 28 (2)</td>
<td>263.6</td>
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Fig. 5. Competition of $^{125}$I-human galanin by galanin(2–29) for rat GalR1 and GalR2 receptors. Data are one representative experiment performed in duplicate in which rat GalR1 and GalR2 receptors from transfected COS-1 cells were directly compared. Symbols shown are rat galanin with GalR1 (○) and GalR2 (●), and galanin(2–29) with GalR1 (○) and GalR2 (●).

Fig. 6. Inhibition by galanin of forskolin-stimulated cAMP production in rat GalR2 receptor-expressing COS-1 cells. Transfected COS-1 cells were incubated in the presence of 0.1 mM forskolin alone (control) and rat galanin at indicated concentrations. Results shown are mean ± standard deviation from an experiment performed in duplicate. Similar results were obtained in a repeating experiment. Curve, best fit to all the data points by one-site nonlinear regression analysis. The basal and forskolin-stimulated intracellular cAMP levels were 55 ± 5 (n = 4) and 221 ± 26 (n = 4) pmol/10$^6$ cells, respectively. The basal cAMP level was not affected by incubation with galanin (1 μM).

Rat galanin caused concentration-dependent inhibition of forskolin-stimulated cAMP production in COS-1 cells transiently transfected with rat GalR2 cDNA. Nonlinear regression analysis of the data revealed a maximum inhibition of 31 ± 2% and an EC$_{50}$ value of 0.22 ± 0.44 nm (Fig. 6). The effect of galanin was completely blocked by overnight incubation with pertussis toxin (100 ng/ml) before the assay. Therefore, the cloned rat GalR2 receptor is functional in mediating intracellular signaling. Like the rat GalR1 receptor, activation of rat GalR2 receptor also leads to inhibition of forskolin-stimulated cAMP synthesis.

In summary, we have isolated a new galanin receptor subtype and characterized its pharmacological profile using galanin related fragments and chimeric peptides. When compared with the known GalR1 receptor, the new receptor, GalR2, possesses a wide tissue distribution and has a distinct pharmacological profile, characterized by a high affinity for galanin(2–29). A GalR2 receptor has been reported by two other groups. Forray et al reported a receptor that displayed inhibition of forskolin-stimulated cAMP production and similar wide distribution of GalR2 mRNA among central and peripheral tissues (24). A GalR2 receptor with 35% homology to the GalR1 receptor also inhibited forskolin-stimulated cAMP accumulation, and showed expression in brain regions and dorsal root ganglia of the spinal cord (25, 26). However, limited sequence information in these reports does not allow to determine whether these two receptors are identical to each other or to the receptor described in this report. In addition to the unambiguous identification of a new galanin receptor subtype, we have for the first time identified a ligand that differentiates between the two defined galanin receptor subtypes. These results using the two galanin receptor subtypes complement earlier observations of different pharmacological profiles in different tissue and cell preparations. Although galanin(2–29) is not known to be a natural ligand, the GalR2 receptor may mediate functions through variable expressions regulated by its own promoter with different tissue-specificity and strength. Alternatively, natural ligands for GalR2 with pharmacological properties distinct from that of the GalR1 receptor may exist and yet have to be identified. It is possible that more than two galanin receptors exist and account for the diverse physiological functions that galanin mediates. The cloning of GalR2 should aid in the cloning and identification of these putative new galanin receptors.
Galanin receptors. The availability of GalR2 receptor should also provide an additional tool to better define the roles of the galanin receptors in various physiological processes.

References