Identification of Novel Specific Allosteric Modulators of the Glycine Receptor Using Phage Display*

**Megan E. Tips**‡, Jessica E. Lawshe‡, Andrew D. Ellington‡‡, and S. John Mihic‡¶

From the **Section of Neurobiology, ‡Division of Pharmacology and Toxicology, ‡‡Department of Chemistry and Biochemistry, \‡¶Waggoner Center for Alcohol and Addiction Research, and Institutes for \‡Neuroscience and \‡Cell and Molecular Biology, University of Texas, Austin, Texas 78712

The glycine receptor (GlyR) is a member of the Cys-loop superfamily of ligand-gated ion channels and the major mediator of inhibitory neurotransmission in the spinal cord and brainstem. Many allosteric modulators affect the functioning of members of this superfamily, with some such as benzodiazepines showing great specificity and others such as zinc, alcohols, and volatile anesthetics acting on multiple members. To date, no potent and efficacious allosteric modulator acting specifically at the GlyR has been identified, hindering both experimental characterization of the receptor and development of GlyR-related therapeutics. We used phage display to identify novel peptides that specifically modulate GlyR function. Peptide D12-116 markedly enhanced GlyR currents at low micromolar concentrations but had no effects on the closely related \( \gamma \)-aminobutyric acid type A receptors. This approach can readily be adapted for use with other channels that currently lack specific allosteric modulators.

The glycine receptor (GlyR)\(^2\) is a member of the Cys-loop superfamily of ligand-gated ion channels, including also the \( \gamma \)-aminobutyric acid type A (GABA\(_A\)) and serotonin-3 receptors. They share a number of structural features, including ligand-binding sites in the extracellular N-terminal domain and a transmembrane domain consisting of four segments, with a large intracellular loop connecting segments 3 and 4. Individual channels consist of five subunits co-localized with the transmembrane domain 2 segment of each subunit lining the anion-conducting pore (1, 2). Two classes of GlyR subunits have been identified: the \( \alpha \) subunits, of which there are four subtypes, and a single \( \beta \) subunit (3). Most native GlyRs in adult animals consist of heteromeric \( \alpha \beta \) subunits, although homomeric \( \alpha \)2 receptors are the predominant form found prenatally (4). GlyRs constitute the major inhibitory neurotransmitter receptor system in the brainstem and spinal cord (5), where they are thought to play a role in the modulation of pain signals and in the effects of volatile anesthetics (1). Some GlyR mutations result in the startle disorder hyperekplexia. GlyRs are also found throughout the brain, including the thalamus, hippocampus, and nucleus accumbens, where they were recently shown to be involved in the reinforcing properties of ethanol (6). The GlyR is only one of multiple ion channels and receptors thought to play a role in pain perception and alcohol and volatile anesthetic effects and in determining the state of neuronal excitability. The isolation of the role of the GlyR is hindered by the fact that, to date, no potent and efficacious allosteric modulator acting specifically at the GlyR has been identified. Phage display involves the expression of a random library of peptides on the coat proteins of bacteriophage. This method has long been used to identify peptides that can bind with high affinity to selected targets and to aid in identifying binding motifs (7). We combined phage display technology with standard electrophysiological testing to identify peptides that allosterically modulate GlyR function without affecting two closely related GABA receptors.

**EXPERIMENTAL PROCEDURES**

**Materials**—All chemicals were obtained from Sigma. *Xenopus laevis* was purchased from Xenopus Express (Homosassa, FL).

**Human Embryonic Kidney (HEK) Cell Culture and Expression of GlyRs**—HEK 239 cells obtained from American Type Culture Collection were grown according to standard procedures (8). Cells were cultured at 37 °C in a 5% CO\(_2\) atmosphere in Dulbecco’s modified Eagle’s medium with \( L \)-glutamine, sodium pyruvate, and 10% fetal bovine serum (Invitrogen). Cell lines were split every 5 days with trypsin/EDTA in Hanks’ balanced salt solution (Invitrogen) for 25 cycles, after which new aliquots of early passage cells were started. Cells were transfected with 4 \( \mu \)g of GlyR \( \alpha 1 \) subunit cDNA using PolyFect reagent (Qiagen). Control cells (untransfected HEK cells) were exposed to PolyFect and then split with no cDNA exposure. All cells were incubated for at least 48 h before use in panning.

**Phage Display**—On panning day 1, a plate of control HEK cells was washed three times with 0.01 M phosphate-buffered saline (PBS) containing 8.2 mM NaPO\(_4\), 1.5 mM K\(_2\)HPO\(_4\), 137 mM NaCl, and 2.7 mM KCl with 1.5% bovine serum albumin (BSA) and 0.1% Tween (PBS/BSA + T). Next, an aliquot containing 2 \( \times 10^{11} \) phage from the D12 phage library (New England Biolabs) was diluted in 1 ml of PBS/BSA + T. Phage were then applied to blank (control) HEK 293 cells and rocked gently at room temperature for 30 min. Phage that did not bind in this...
negative selection procedure were removed from the plate with a pipette, applied to the plate of GlyR-expressing cells, and rocked gently at room temperature for 60 min. Non-binding phage were discarded, and the plate was washed five times with PBS/BSA + T. At this time, positive selection was complete, and the only step remaining was the isolation of phage. Elution of the bound phage was performed by lowering the pH using 0.2 M glycine HCl (10 mM HCl buffered to pH 2.2 with glycine) plus 1 mg/ml BSA and rocking at room temperature for 10 min. Eluate was removed and neutralized with 150 μl of 1 M Tris-HCl (pH 9.0). Titering was performed, and the remainder of the eluate was added to the 20 ml of Escherichia coli in LB broth.

After 4.5 h of incubation, the culture was transferred to a 50-ml Falcon centrifuge tube and spun at 10,000 rpm for 10 min at 4 °C. The supernatant was transferred to a fresh tube and resuspended. The upper 80% of the supernatant was again transferred to a fresh tube, and a one-sixth volume of polyethylene glycol/NaCl (20% (w/v) polyethylene glycol 8000 and 2.5 mM NaCl) was added. Phage were allowed to precipitate overnight at 4 °C.

On panning day 2, polyethylene glycol precipitates were spun at 10,000 rpm for 15 min at 4 °C. The supernatant was decanted, and the precipitate was spun again. Residual supernatant was removed using a pipette. The pellet was resuspended in 1 ml of Tris-buffered saline (50 mM Tris-HCl (pH 7.5) and 150 mM NaCl), transferred to a 1.7-ml microcentrifuge tube, and spun at 10,000 rpm for 5 min at 4 °C. In a fresh microcentrifuge tube, the suspended phage was reprecipitated with polyethylene glycol/NaCl on ice for 60 min. After spinning at 10,000 rpm for 10 min at 4 °C, the supernatant was discarded. The pellet was resuspended in Tris-buffered saline and spun again for 1 min, and the supernatant was then transferred to a fresh tube and stored at 4 °C. The amplified phage were titered.

For successive panning rounds, the amplified phage were diluted in PBS/BSA + T so that the input concentration was always 2 \times 10^{11} virions. At the end of five rounds of panning, individual plaques from the most recent titer plates were isolated and incubated overnight in LB broth at 37 °C with agitation. The overnight culture was then purified using the S.N.A.P. MiniPrep kit (Invitrogen), and phage DNA was sequenced in-house using a –96gIII sequencing primer (New England Biolabs). Individual peptide sequences were sent to Peptide 2.0 Inc. (Chantilly, VA) for synthesis.

Oocyte Isolation and cDNA Nuclear Injection—Oocytes were surgically removed from X. laevis housed at 19 °C on a 12-h light/dark cycle. Stage V and VI oocytes were selected and 150 mM NaCl), transferred to a 1.7-ml microcentrifuge tube, the suspended phage was reprecipitated with polyethylene glycol/NaCl (20% (w/v) polyethylene glycol 8000 and 2.5 mM NaCl) was added. Phage were allowed to precipitate overnight at 4 °C. The pellet was resuspended in Tris-buffered saline and spun at 10,000 rpm for 10 min at 4 °C. The supernatant was transferred to a fresh tube and respun. The upper 80% of the supernatant was again transferred to a fresh tube, and a one-sixth volume of polyethylene glycol/NaCl (20% (w/v) polyethylene glycol 8000 and 2.5 mM NaCl) was added. Phage were allowed to precipitate overnight at 4 °C. The pellet was resuspended in 1 ml of Tris-buffered saline (50 mM Tris-HCl (pH 7.5) and 150 mM NaCl), transferred to a 1.7-ml microcentrifuge tube, and spun at 10,000 rpm for 5 min at 4 °C. In a fresh microcentrifuge tube, the suspended phage was reprecipitated with polyethylene glycol/NaCl on ice for 60 min. After spinning at 10,000 rpm for 10 min at 4 °C, the supernatant was discarded. The pellet was resuspended in Tris-buffered saline and spun again for 1 min, and the supernatant was then transferred to a fresh tube and stored at 4 °C. The amplified phage were titered. For successive panning rounds, the amplified phage were diluted in PBS/BSA + T so that the input concentration was always 2 \times 10^{11} virions. At the end of five rounds of panning, individual plaques from the most recent titer plates were isolated and incubated overnight in LB broth at 37 °C with agitation. The overnight culture was then purified using the S.N.A.P. MiniPrep kit (Invitrogen), and phage DNA was sequenced in-house using a –96gIII sequencing primer (New England Biolabs). Individual peptide sequences were sent to Peptide 2.0 Inc. (Chantilly, VA) for synthesis.

Oocyte Electrophysiological Recording—Oocytes were im- paled in the animal poles with two high resistance (0.5–10 megohms) glass electrodes filled with 3 mM KCl. Using a Warner Instruments OC-725C oocyte clamp, oocytes were voltage-clamped at −70 mV while modified Barth’s saline was perfused over them at a rate of 2 ml/min using a Masterflex peristaltic pump (Cole Parmer Instrument Co., Vernon Hills, IL) through 18-gauge polyethylene tubing. All drug solutions were prepared in modified Barth’s saline. Drug applications (5–60 s) were followed by 5–15-min washout periods as appropriate.

Statistics—Current peak responses were measured from chart recorder tracings. Peptide effects were calculated as percent changes compared with the effects produced by glycine or GABA in the absence of peptide. In all cases, oocyte data were obtained from at least two different frogs. The percent enhancement or inhibition values obtained in the presence of peptide were compared by either one- or two-way analysis of variance, as indicated, to determine statistical significance, with a criterion of p < 0.05 required.

RESULTS

We used a commercially available phage display library, expressing more than two billion unique sequences, to isolate peptides that bind to GlyR α1 expressed in HEK 293 cells. Homomeric GlyR α1 was chosen as the target rather than heteromeric GlyR α1β because of its invariant stoichiometry: heteromeric α1β receptors contain αα, αβ, and βα intersubunit interfaces. In addition, we wanted to avoid the possible complication that some receptors in our population were α1 homomers while others were α1β heteromers. A modified phage display protocol (11) involved first applying phage to untransfected HEK 293 cells in the negative selection step to remove those phage that bound to various endogenous tar-gets that were not of interest. Phage that did not bind in this negative selection procedure were then applied to HEK 293 cells that expressed the GlyR α1 subunit, and this constituted the positive selection portion of the procedure. After five rounds of panning, 35 individual colonies were sequenced to identify the peptides inserted in the phage pIII protein that resulted in phage binding (Table 1). Three sequences were harvested from multiple colonies, implying selective enrichment of our phage pool. No clear homology was seen among the collected sequences. Because of the size of the extracellular domain of the GlyR and thus a large number of potential binding sites, this diversity of peptide sequences was not unexpected.

Peptides corresponding to 10 sequences were synthesized for characterization using two-electrode voltage-clamp electrophysiology. A maximally effective concentration of glycine (10 mM) was first applied to Xenopus oocytes to determine their levels of expression of the GlyR 2 days after GlyR α1 subunit

Specific Peptide Modulators of the Glycine Receptor

We used a commercially available phage display library, expressing more than two billion unique sequences, to isolate peptides that bind to GlyR α1 expressed in HEK 293 cells. Homomeric GlyR α1 was chosen as the target rather than heteromeric GlyR α1β because of its invariant stoichiometry: heteromeric α1β receptors contain αα, αβ, and βα intersubunit interfaces. In addition, we wanted to avoid the possible complication that some receptors in our population were α1 homomers while others were α1β heteromers. A modified phage display protocol (11) involved first applying phage to untransfected HEK 293 cells in the negative selection step to remove those phage that bound to various endogenous targets that were not of interest. Phage that did not bind in this negative selection procedure were then applied to HEK 293 cells that expressed the GlyR α1 subunit, and this constituted the positive selection portion of the procedure. After five rounds of panning, 35 individual colonies were sequenced to identify the peptides inserted in the phage pIII protein that resulted in phage binding (Table 1). Three sequences were harvested from multiple colonies, implying selective enrichment of our phage pool. No clear homology was seen among the collected sequences. Because of the size of the extracellular domain of the GlyR and thus a large number of potential binding sites, this diversity of peptide sequences was not unexpected.

Peptides corresponding to 10 sequences were synthesized for characterization using two-electrode voltage-clamp electrophysiology. A maximally effective concentration of glycine (10 mM) was first applied to Xenopus oocytes to determine their levels of expression of the GlyR 2 days after GlyR α1 subunit
Specific Peptide Modulators of the Glycine Receptor

TABLE 1
List of peptide sequences identified after panning with the D12 phage library and how often each sequence was identified

| Phage | Sequence | Identification frequency |
|-------|----------|--------------------------|
| 12-100 | TSYTTSTIFOPRA | 1 |
| 12-101 | ILANDLFAPGPR | 1 |
| 12-104 | NYWSAITATSSL | 1 |
| 12-105 | KPNLTLTSPVQP | 1 |
| 12-106 | LLAATTPHRPP | 1 |
| 12-107 | I SMRPRRQLOQP | 1 |
| 12-108 | SIIVSTQPSLP | 1 |
| 12-109 | SMTSHQNWLLA | 1 |
| 12-110 | ANPSSTNHLLTP | 1 |
| 12-111 | TTTTIZATQPHH | 1 |
| 12-112 | YPSFTHSAPSL | 1 |
| 12-113 | FQNSRLKVISSP | 1 |
| 12-114 | QDVHLITQGQRYT | 1 |
| 12-115 | NLHIERQWQLM | 1 |
| 12-116 | YESRIGQVAPSQ | 1 |
| 12-117 | ERVMLPPAPQPA | 2 |
| 12-119 | IPHTNHQHPSPM | 1 |
| 12-120 | TNSTSWMTAMPPP | 1 |
| 12-121 | THTTNAQGSPV | 1 |
| 12-122 | TMQPTAPRFPHY | 1 |
| 12-124 | SVSVGKFLPSPPR | 1 |
| 12-125 | SNWQHRAISIK | 1 |
| 12-126 | GIQLANPRLYD | 1 |
| 12-127 | QDSMLKYPVDPHL | 1 |
| 12-128 | SHHIYPSQWPL | 1 |
| 12-130 | WAETNPLAQRP | 1 |
| 12-131 | SNGQDRPPLLT | 1 |
| 12-133 | SSSLPWRHDTSSR | 2 |
| 12-136 | EOWLAYDRIYAS | 1 |
| 12-138 | ETLPIFTTIAST | 1 |

cDNA injection. A low concentration of glycine eliciting a response equal to ~10% of the maximal current (EC10 where EC is the effective concentration) was identified in each oocyte and applied several times to ensure stability of responses. The oocyte was then incubated with a peptide at 30 μM concentration of peptide D12-106 on GlyR function. D12-106 inhibited the effects of glycine when it was applied alone for 30 s. However D12-106 inhibited the effects of glycine when it was co-applied with EC10 glycine. D12-105 also did not affect the holding currents of oocytes but potentiated GlyR α1 function when it was co-applied with EC10 glycine. c, percent changes in EC10 glycine responses produced by 30 μM concentrations of D12 peptides pre-applied for 30 s before also being co-applied with EC10 glycine. These peptides exhibited varying degrees of potentiation or inhibition at the GlyR, but none acted as agonists in directly activating the receptor. Data are expressed as the mean ± S.E. of four to eight oocytes obtained from at least two frogs.

![Figure 1](image-url)
Specific Peptide Modulators of the Glycine Receptor

FIGURE 2. Minimal effects of peptides as allosteric modulators at the GABA receptor. a, sample two-electrode voltage-clamp tracing showing the effects of a 30 μM concentration of peptide D12-106 on GABA, α1β2γ2S receptor function. D12-106 had no effect on the holding currents of oocytes expressing the GABA α1 receptors. b, sample tracing showing a lack of effect of a 30 μM concentration of peptide D12-133 on GABA, α1β2γ2S receptor function. D12-133 also did not affect the holding currents of oocytes in the absence of GABA and had no effect when it was co-applied with EC10 GABA. c, percent changes in EC10 GABA responses produced by 30 μM concentrations of D12 peptides pre-applied for 30 s before also being co-applied with EC10 GABA to α1β2γ2S receptors. None of these acted as agonists in directly activating the receptor and had minimal to no effects in the presence of EC10 GABA. Data are expressed as the mean ± S.E. of two to six oocytes obtained from at least two frogs.

This peptide exist on the GlyR. We then showed that 30 μM D12-116 acted to left-shift the glycine concentration-response curve without any effect at saturating glycine concentrations (Fig. 4b). This result is in concordance with previous studies showing that other allosteric modulators of the GlyR, such as alcohols, volatile anesthetics, and low concentrations of zinc, also act by left-shifting glycine concentration-response curves (15–17).

DISCUSSION

One of the most pharmacologically relevant of the proposed roles of the GlyR in vivo is the one it may play in alcohol-induced behaviors. Many cell-surface proteins, such as voltage- and ligand-gated ion channels, enzymes, and transporters, have been implicated as possible mediators of the effects of ethanol, but the relative importance of each putative target to the effects of alcohol in vivo remains poorly understood (18). A major impediment to the rational identification of compounds to treat alcohol abuse is this lack of understanding of which of these many possible molecular targets mediate which specific in vivo effects of alcohol, such as reinforcement, ataxia, or the
Specific Peptide Modulators of the Glycine Receptor

a) oocytes expressing homomeric GlyR α1 were preincubated with 1–100 μM peptide D12-116 for 30 s before EC₁₀ glycine was co-applied with peptide for a further 30 s. No peptide concentration tested elicited currents in the absence of glycine. The threshold peptide concentration at which GlyR function was enhanced appears to be between 3 and 10 μM. Data are expressed as the mean ± S.E. of three to four oocytes obtained from at least two frogs. b) D12-116 acts as an allosteric modulator of GlyR function by shifting the glycine concentration-response curve to the left. ○, glycine applied alone; ●, glycine plus 30 μM D12-116. Curves were fit using a four-parameter logistic equation. The EC₅₀ of the glycine curve was 796 μM, and the peptide decreased it to 196 μM. Two-way analysis of variance showed significant effects of glycine concentration (F(7, 63) = 205, p < 0.001) as well as the presence of peptide (F(1, 63) = 150, p < 0.001). Data are expressed as the mean ± S.E. of four oocytes obtained from at least two frogs.

FIGURE 4. Characterization of peptide D12-116. a, oocytes expressing homomeric GlyR α1 were preincubated with 1–100 μM peptide D12-116 for 30 s before EC₁₀ glycine was co-applied with peptide for a further 30 s. No peptide concentration tested elicited currents in the absence of glycine. The threshold peptide concentration at which GlyR function was enhanced appears to be between 3 and 10 μM. Data are expressed as the mean ± S.E. of three to four oocytes obtained from at least two frogs. b, D12-116 acts as an allosteric modulator of GlyR function by shifting the glycine concentration-response curve to the left. ○, glycine applied alone; ●, glycine plus 30 μM D12-116. Curves were fit using a four-parameter logistic equation. The EC₅₀ of the glycine curve was 796 μM, and the peptide decreased it to 196 μM. Two-way analysis of variance showed significant effects of glycine concentration (F(7, 63) = 205, p < 0.001) as well as the presence of peptide (F(1, 63) = 150, p < 0.001). Data are expressed as the mean ± S.E. of four oocytes obtained from at least two frogs.

development of tolerance. Enhancement of inhibitory GlyR function by ethanol is consistent with some of the behavioral consequences of its administration (6, 19). However, because ethanol clearly affects multiple biochemical targets in addition to the GlyR, it has proved difficult to determine conclusively the roles that individual putative targets play in the various behavioral effects of this agent. There would thus be great utility in identifying compounds that can act as either ethanol mimics or ethanol antagonists but at only one putative alcohol target. For example, were we to administer our D12-116 peptide intrathecally to rats and observe ataxia, it would suggest that ataxia produced by ethanol is also mediated by enhancement of GlyR function. Although studies using knock-out and knock-in mice (20) have provided some successes, these methods, due to their nature, necessarily involve a departure from the wild-type tar-
bodies and show reduced interactions with the immune system (30). However, peptides have traditionally been discounted as viable drugs due to their low bioavailabilities and general inability to cross the blood-brain barrier (31). In recent years, this view has begun to change, as new technologies have arisen to overcome these limitations. Cell-penetrating peptides have long been used to deliver drugs into cellular cytoplasm, but recently, this technology has also been used to target bioactive peptides to brain tissue (32). Nanoparticles, a more recently developed technology, have also been highly successful in delivering systemically administered peptides into brain tissue at pharmacologically relevant concentrations (33). Efforts are also being made to improve the oral delivery of peptides to increase their marketability as therapeutic agents (34).

In our studies, we modified common phage display techniques to identify allosteric modulators of the GlyR. Our approach utilizes the cellular expression of the target protein rather than scaffolding of a purified target. This allows for selection in the native environment of the target and eliminates concerns about misfolding of the target protein. As a result, our peptides have the opportunity to bind only in the extracellular region of the target. Because none of the peptides tested thus far acted as agonists when applied alone, peptides may not be able to bind at the intersubunit orthosteric glycine-binding site (1) to directly activate the GlyR. In the positive selection portion of the phage display screen, we expressed the GlyR in HEK 293 cells in the absence of exogenously added glycine, so we assume that selection occurred against the GlyR in the closed channel state because these receptors do not activate spontaneously. To circumvent possible concerns about cell type-specific binding of our peptides, we used receptors expressed in HEK 293 cells for panning but switched to the GlyR expressed by Xenopus oocytes for the functional tests.

The wide diversity of peptides identified in our panning screen suggests that a more stringent negative control during phage panning might increase peptide specificity. For example, expressing one or both GABA ρ1 or α1/β2γ2S receptor subtypes in the negative selection portion of the panning procedure would be expected to remove those peptides that bind to both GABA and GlyRs, such as D12-106 and D12-124 (Figs. 1 and 2). Another possibility would be to attempt to identify peptides that bind only to specific GlyR subunits by expressing the GlyR present in adhering cell system and is particularly useful for channels or receptors that currently lack specific allosteric modulators.

REFERENCES

1. Lynch, J. W. (2004) Physiol. Rev. 84, 1051–1095
2. Webb, T. I., and Lynch, J. W. (2007) Curr. Pharm. Des. 13, 2350–2367
3. Grenningloh, G., Pribilla, I., Prior, P., Multhaup, G., Beyreuther, K., Taleb, O., and Betz, H. (1990) Neuron 4, 963–970
4. Rajendra, S., and Schofield, P. R. (1995) Trends Neurosci. 18, 80–82
5. Betz, H. (1991) Trends Neurosci. 14, 458–461
6. Molander, A., and Söderpalm, B. (2005) Alcohol. Clin. Exp. Res. 29, 27–37
7. Azzazy, H. M., and Highsmith, W. E., Jr. (2002) Clin. Biochem. 35, 425–445
8. Freshney, R. I. (2002) Cytotechnology 39, 55–67
9. Mikic, S. I., Ye, Q., Wick, M. J., Kolchicine, V. V., Krasowski, M. D., Finn, S. E., Mascia, M. P., Valenzuela, C. F., Hanson, K. K., Greenblatt, E. P., Harris, R. A., and Harrison, N. L. (1997) Nature 389, 385–389
10. Colman, A. (1984) in Transcriptional Translation: A Practical Approach (Hames, B. D., and Higgins, S. J., eds) pp. 49–69, Oxford Press, Washington, D.C.
11. Hou, S. T., Dove, M., Anderson, E., Zhang, J., and MacKenzie, C. R. (2004) J. Neurosci. Methods 138, 39–44
12. Orteils, M. O., and Lunt, G. G. (1995) Trends Neurosci. 18, 121–127
13. Adamian, L., Gussin, H. A., Tseng, Y. Y., Muni, N. J., Feng, F., Qian, H., Pepperberg, D. R., and Liang, J. (2009) Proteins Sci. 18, 2371–2383
14. Barnard, E. A., Darlison, M. G., and Seeburg, P. (1987) Trends Neurosci. 10, 502–509
15. Mascia, M. P., Machu, T. K., and Harris, R. A. (1996) Br. J. Pharmacol. 199, 1331–1336
16. Krasowski, M. D., and Harrison, N. L. (2000) Br. J. Pharmacol. 129, 731–743
17. Bloomenthal, A. B., Goldwater, E., Pritchett, D. B., and Harrison, N. L. (1994) Mol. Pharmacol. 46, 1156–1159
18. Vengeliene, V., Bilbao, A., Molander, A., and Spanagel, R. (2008) Br. J. Pharmacol. 154, 299–315
19. Ye, H., Sokol, K. A., and Bhavsar, U. (2009) Alcohol. Clin. Exp. Res. 33, 1096–1074
20. Crabbe, J. C., Phillips, T. J., Harris, R. A., Arends, M. A., and Koob, G. F. (2006) Addict. Biol. 11, 195–269
21. Berg, T. (2003) Angew. Chem. Int. Ed. 42, 2462–2481
22. May, L. T., Avlani, V. A., Sexton, P. M., and Christopoulos, A. (2004) Curr. Pharm. Des. 10, 2003–2013
23. Mihic, S. J., Whiting, P. J., Klein, R. L., Wafford, K. A., and Harris, R. A. (1994) J. Biol. Chem. 269, 32768–32773
24. Jensen, A. A., and Brauner-Osborne, H. (2007) Curr. Neuropharmacol. 5, 180–186
25. Wilkemeyer, M. F., Chen, S. Y., Menkari, C. E., Brenneman, D. E., Sulik, K. K., and Charness, M. E. (2003) Proc. Natl. Acad. Sci. U.S.A. 100, 8543–8548
26. Guzman, L., Moraga-Cid, G., Avila, A., Figueroa, M., Yevenes, G. E., Fuentes-Alba, J., and Aguayo, L. G. (2009) J. Pharmacol. Exp. Ther. 331, 933–939
27. Doorbar, J., and Winter, G. (1994) J. Mol. Biol. 244, 361–369
28. Goodson, R. J., Doyle, M. V., Kaufman, S. E., and Rosenberg, S. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 7129–7133
29. Li, M., Yu, W., Chen, H. C., Cwirla, S., Whitehorn, E., Tate, E., Raab, R., Bremer, M., and Dower, B. (1996) Nat. Biotechnol. 14, 986–991
30. Ladner, R. C., Sato, A. K., Gorgelany, I., and de Souza, M. (2004) Drug Discov. Today 12, 525–529
31. Vlieghe, P., Lisowski, V., Martinez, J., and Khrestchatisky, M. (2010) Drug Discov. Today 15, 40–56
32. Morris, M. C., Deshayes, S., Heitz, F., and Divita, G. (2008) Biol. Cell 100, 201–217
33. Karatas, H., Aktas, Y., Gursoy-Ozdemir, Y., Bodur, E., Yemisci, M., Cabaiz, S., Vural, P., Panirbasli, O., Capan, Y., Fernandez-Megia, E., Novoa-Carballal, R., Riguera, R., Andrieux, K., Couvreur, P., and Dalkara, T. (2009) J. Neurosci. 29, 13761–13769
34. Hamman, J. H., Enslin, G. M., and Kotze, A. F. (2005) BioDrugs 19, 165–177
35. Gilbert, D. F., Islam, R., Lynagh, T., Lynch, J. W., and Webb, T. I. (2009) Front. Mol. Neurosci. 2, 1–10