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Bicuculline antagonizes 5-HT_{3A} and $\alpha 2$ glycine receptors expressed in *Xenopus* oocytes

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Abstract

The present study examined the effects of bicuculline on the mouse 5-hydroxytryptamine$_{3A}$ receptor (5-HT_{3A} receptor and the human $\alpha 2$ subunit of the glycine receptor. Bicuculline antagonized both the 5-HT_{3A} receptor ($IC_{50} = 20.12 \pm 0.39$ μM) and the $\alpha 2$ glycine receptor ($IC_{50} = 169.40 \pm 1.73$ μM). A competitive form of antagonism by bicuculline was suggested by experiments in which the EC_{50}s for 5-HT and glycine were increased in the 5-HT_{3A} and $\alpha 2$ glycine receptors, respectively, as bicuculline concentrations were increased. A competitive nature of antagonism by bicuculline at the 5-HT_{3A} receptor was also suggested by displacement of the competitive antagonist, [^{3}H]GR65630 in SF21 insect cells expressing the 5-HT_{3A} receptor ($K_i = 19.01 \pm 0.71$ μ M). Our data and that of others reveal that bicuculline, a purported selective antagonist of the $GABA_A$ receptor, antagonizes at least one receptor subclass in every member of the superfamily of ligand-gated ion channels.

Keywords: 5-HT(5-hydroxytryptamine, serotonin); 5-HT_3 receptor; glycine receptor; $GABA_A$; Nicotinic acetylcholine receptor; Bicuculline

1. Introduction

The 5-hydroxytryptamine$_3$ (5-HT_3) receptor and glycine receptor, together with γ-aminobutyric acid$_A$ ($GABA_A$) receptor and the nicotinic acetylcholine form an evolutionary superfamily of ligand-gated ion channels responsible for fast excitatory (nicotinic acetylcholine receptor and 5-HT_3 receptor) and inhibitory ($GABA_A$ and glycine receptors) synaptic transmission (Grenningloh et al., 1987; Schofield et al., 1987; Noda et al., 1983; Maricq et al., 1991). The 5-HT_{3A} receptor, cloned from NCB20 cells (Maricq et al., 1991), is present in many regions of the central and peripheral nervous systems, but in relatively low abundance. The highest density of 5-HT_{3A} receptors is found in the area postrema and the nucleus of the solitary tract (Gehlert et al., 1991). In contrast, glycine receptors are mainly detected at high levels in the spinal cord and medulla, at lower levels in the midbrain, hypothalamus, and thalamus, and are virtually absent in the higher brain (see for review, Rajendra et al., 1997). Within the spinal cord, glycine receptors are present in the gray matter, but not the white columns. The most well-established role of the 5-HT_{3A} receptors is in regulating gastrointestinal motility and the vomiting reflex (Aapro, 1991). Currently, 5-HT_{3A} receptor antagonists, such as ondansetron, are approved for treatment of nausea and vomiting. 5-HT_{3A} receptors have been implicated in the production of pain, anxiety, depression, schizophrenia, dementia, and drug abuse (Greenshaw, 1993; Gyermek, 1995; Jackson and Yakel, 1995). Disruption of inhibitory pathways gated by glycine receptors can result in abnormal reflexes, loss of muscle control, convulsions, and even death (Rajendra et al., 1997). Therefore, the discovery of the compounds, which act as ligands to modulate the function of 5-HT_{3A} receptors and/or glycine receptors, allows for investigation of novel pharmacological approaches to treat numerous disease states.

The 5-HT_{3A} receptor and $\alpha 2$ glycine receptor share significant structural similarities and pharmacological characteristics with the muscle nicotinic acetylcholine receptor and $GABA_A$ receptor. All are composed of five subunits, which form a central pore that is lined by the second transmembrane domains. Hydropathy plots of the 5-HT_{3A} receptor and $\alpha 2$ glycine receptor predict four transmembrane domains, with long extracellular N-terminal do-

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mains, large intracellular loops between transmembrane 3 and transmembrane 4, and short extracellular C-terminals (Maricq et al., 1991; Handford et al., 1996). Despite the significant degree of structural homology, clear differences in channel properties, including ion selectivity, are found in superfamily members. For example, a chimeric receptor consisting of the extracellular, agonist-binding, N-terminal domain of the neuronal α7 nicotinic acetylcholine receptor and the corresponding C-terminal region of the 5-HT_{3A} receptor, forms functional channels that are gated by Ach and have permeability properties similar to that of the 5-HT_{3A} receptor (Eisele et al., 1993). Due to sequence and structural similarity, the ligand-gated ion channels share some ligands that bind to the agonist recognition sites. For example, the competitive antagonist, D-tubocurarine, binds with nanomolar affinity to both the muscle nicotinic acetylcholine receptor and the mouse 5-HT_{3A} receptor (Peters et al., 1990; Yan et al., 1998). The microtubule depolymerizing drug, colchicine, is a competitive antagonist at both $GABA_A$ receptors (Bueno and Leidenheimer, 1998) and glycine receptors (Machu, 1998). Recently, we have found that colchicine also competitively antagonizes the mouse 5-HT_{3A} receptor (Sun and Machu, unpublished data). The ''glycine receptor specific'' ligand, strychnine, has actions at the α7 nicotinic acetylcholine receptor and $GABA_A$ receptor in the nanomolar and micromolar range, respectively (Rothlin et al., 1999; Xu, 1999). Bicuculline, a phthalide isoquinoline derivative isolated from fumaraceous plants, has been used as a selective, potent, and competitive antagonist of $GABA_A$ receptors; concentrations inhibiting 50% of GABA-induced responses (IC_{50}s) are ~1–2μ M (Shirasaki et al., 1991; Green and Halliwell, 1997). Bicuculline is a potent convulsant in animals (Welch and Henderson, 1971). As an inhibitor of $GABA_A$ receptors, bicuculline has been well characterized and used extensively in pharmacological studies (reviewed in Rabow et al., 1995). However, bicuculline lacks selectivity, as is evidenced by its actions on nicotinic acetylcholine receptors. For instance, bicuculline, in the micromolar range, blocks nicotinic acetylcholine receptors in isolated pig pituitary intermediate lobe cells (Zhang and Feltz, 1991) and in cultured embryonic rat skeletal muscle (Liu et al., 1994), as well as α9 nicotinic acetylcholine receptors expressed in *Xenopus* oocytes (Rothlin et al., 1999). Furthermore, bicuculline has effects at glycine receptors, as is evidenced in displacement studies of [^{3}H] strychinine binding from the glycine receptor in various neuronal preparations (Goldinger et al., 1980; Braestrup et al., 1986; Marvizon et al., 1986). In contrast, bicuculline has been reported to have minimal effects on glycine-induced responses in rat hippocampal pyramidal cells (Shirasaki et al., 1991) and rat brain synaptoneurosomes (Engblom et al., 1996). The effect of bicuculline on the function of 5-HT_{3A} receptors is unknown. In the present study, we determined the actions of bicuculline on the α2 glycine receptor and 5-HT_{3A} receptor. Using a combination of electrophysiological approaches and radioligand binding, we found that bicuculline competitively inhibits the 5-HT_{3A} receptor with a slightly lower potency than that at the $GABA_A$ receptor while it weakly antagonizes the α2 glycine receptor.

2. Materials and Methods

2.1. Isolation of Xenopus laevis oocytes

Frogs were kept in tanks of dechlorinated tap water on a 12-h light/dark cycle at 19°C and fed a diet of dehydrated liver in *Xenopus* I chow three times per week. Frogs were anesthetized by immersion in cold 0.12% 3-aminobenzoic acid ethyl ester for 20 min. After removal through a small surgical incision in the frog's abdomen, ovarian lobes were placed in modified Barth's solution (MBS) containing (in mM): NaCl 88, KCl 1, $NaHCO_3$ 2.4, HEPES 10, $MgSO_4$ 0.82, $Ca(NO_3)_2$ 0.33, and $CaCl_2$ 0.91 (pH 7.5). Ovarian lobes were manually dissected into clumps of four to ten oocytes and were then subjected to chemical separation and defolliculation. Clumps of oocytes were placed in medium containing 2 mg/ml collagenase Type 2 and (in mM): NaCl 83, KCl 2, $MgCl_2$ 1, and HEPES 10 (pH 7.5) and gently rocked for 2 h. Oocytes were then removed to fresh collagenase medium and rocked gently for an additional 2 h. Lastly, oocytes were rinsed with MBS and stored in incubation media composed of ND96, containing (in mM): NaCl 96, KCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1, and HEPES 5 (pH 7.5), plus 10 mg/l streptomycin, 50 mg/l gentamicin, 10,000 units/l penicillin, 96 mg/l sulfamethoxazole, 19 mg/l trimethoprim, 0.5 mM theophylline, and 2 mM sodium pyruvate.

2.2. Transcription of cDNA to cRNA

Mouse 5HT_{3A}-R cDNA (obtained from Dr. David Julius, UCSF) was subcloned into pCR-Script™ Amp SK(+)$^-$ (Stratagene, La Jolla, CA). The cDNA was linearized with *Bam*HI, extracted with phenol–chloroform, precipitated with sodium acetate and ethanol, and resuspended in diethyl pyrocarbonate treated water. The cDNA was then transcribed with T3 mMESSAGE mMACHINE (Ambion, Austin, TX).

2.3. Microinjection of oocytes with 5-HT_{3A} Receptor cRNA and α2 Glycine Receptor cDNA

An aliquot of 5-HT_{3A} receptor cRNA was centrifuged at 15,000 × g and ethanol was removed a tuberculin syringe. After air drying, the pellet was resuspended in a volume of diethylpyrocarbonate treated water to yield a

concentration of 5–30 ng of cRNA/50 nl. The aliquots of 5-HT_{3A} receptor cRNA was drawn up into a micropipette (10–20 μm tip size). Fifty nanoliters of cRNA was injected into the animal/vegetal pole equator. Oocyte nuclei were microinjected with cDNAs encoding α2 glycine receptor (provided by H. Betz, Max-Plank-Institut fur Hirnforschung, Frankfurt, Germany). The ''blind'' method of Colman, (1984) was used to inject 50 pg to 5 ng of receptor cDNA dissolved in 30 nl of diethylpyrocarbonate-treated water per oocyte.

Oocytes were stored in incubation medium in Corning cell well plates (Corning Glass Works, Corning, NY) at room temperature. Incubation medium was changed daily. Oocytes were recorded from day 1 through day 7 following injection.

2.4. Electrophysiological recordings

Oocytes were perfused in a 100-μl volume chamber with MBS via a roller pump (Cole-Parmer Instrument, Chicago, IL) at 2 ml/min. Oocytes were impaled with two glass electrodes (1.2 mm outside diameter and 1–10 MΩ resistance) filled with 3 M KCl. Oocytes were voltage clamped to −70 mV with a Warner Instruments Model OC-725C oocyte clamp (Hamden, CT). Clamping currents were plotted on a strip chart recorder (Cole Parmer Instrument,). Serotonin (0–50 μM) or glycine (0–2000 μM), in the absence or presence of bicuculline (0–1000 μM), was dissolved in MBS and applied to the oocytes for 30 s. Serotonin HCl, glycine, and (−)-bicuculline methobromide were purchased from Sigma Chemical (St. Louis, MO) and were stored as 1 mM (5-HT) or 10 mM (glycine and bicuculline) stocks at −20° C.

2.5. [^{3}H]GR65630 binding assay

A full-length mouse 5-HT_{3A} receptor cDNA was cloned into the baculovirus transfer vector pBacHis-3. Production of recombinant baculovirus and viral infections were conducted by using the media and protocols described in the BacPAK™ baculovirus Expression System of ClonTech (Clontech Laboratories, Palo Alto, CA). Briefly, the host cell line, SF21, was infected with recombinant baculovirus. Recombinant baculovirus was then plaque purified, propagated, and thereafter used to infect SF21 insect cells growing in Grace's insect cell medium containing 10% fetal bovine serum.

SF21 insect cells growing in complete Grace's insect cell medium were infected with recombinant virus for 2 days. Cells were then harvested, and the pellets were resuspended in 10 ml of HEPES buffer (50 mM, pH 7.4). Cell membranes were prepared as described previously (Sun et al., 1999). The protein concentrations were determined with the BSA protein assay reagents (Pierce, Rockford, IL). Radioligand binding was accomplished according to the method of Sun et al. (1999), with modifications as described in the following. Binding reactions consisted of crude cell membrane proteins (75 μg/tube), the radiolabelled 5-HT_{3A} receptor antagonist, [^{3}H]GR65630 (1 nM), and bicuculline (0.1 nM–3.0 mM) and were incubated for 15 min in a final volume of 250 μl HEPES buffer (50 mM, pH 7.4) at room temperature. Nonspecific binding was measured in the presence of 50 μM MDL-72222, and ranged from 75–125 dpm. Incubation was terminated by filtering the reaction mixtures through WHATMAN GF/B filters (presoaked for 30 min in 0.3% polyethyleneimine) with a BRANDEL M-24 Cell Harvester (Brandel, Gaithersburg, MD). Filters were then washed four times with 10 ml HEPES buffer (50 mM, pH 7.4) at 4°C in the cell harvester. Radioactivity was counted in a Packard scintillation counter. The counting efficiency for tritium was approximately 48%. All analyses were performed in duplicate. Specific binding was determined by subtracting

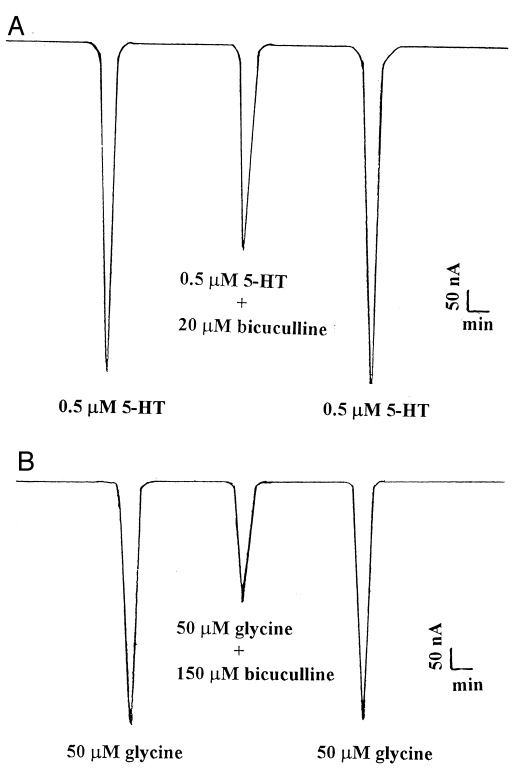

Fig. 1. Representative tracings of currents produced in a single oocyte expressing the mouse 5-HT_{3A} receptor or human α2 glycine receptor. (A) 5-HT_{3A} receptor: Serotonin, 0.5 μM was applied for 30 s to obtain a baseline current. Bicuculline (20 μM) was coapplied with serotonin, 0.5 μM, for 30 s. (B) α2 glycine receptor: glycine, 50 μM, was applied for 30 s to obtain a baseline current. Bicuculline (150 μM) was coapplied with glycine (50 μM) for 30 s. An inhibition of ~50% of the baseline current was observed for each receptor. Washout of bicuculline inhibition was observed with the next application of 5-HT or glycine 5 min later.

nonspecific binding from total binding. Specific binding values were used for analysis.

2.6. Data analysis

Nonlinear regression curve fitting was conducted with Graphpad Prism (San Diego, CA), and EC_{50}s, IC_{50}s, and Hill coefficients were derived.

3. Results

Fig. 1 illustrates typical responses of the 5-HT_{3A} and $\alpha 2$ glycine receptors to bicuculline. Bicuculline clearly inhibited both 5-HT and glycine-mediated responses in oocytes expressing the 5-HT_{3A} receptor (Fig. 1A) or the $\alpha 2$ glycine receptor (Fig. 1B), respectively. Bicuculline quickly washed out, as baseline responses were obtained with the next application of agonist alone. Bicuculline concentration response curves were generated for oocytes expressing the 5-HT_{3A} receptor or the $\alpha 2$ glycine receptor (Fig. 2). Bicuculline was more potent at the 5-HT_{3A} receptor than at the $\alpha 2$ glycine receptor. Inhibition was observed at bicuculline concentrations ranging from 3–300 μM for the 5-HT_{3A} receptor (Fig. 2a) and from 15–1000 μM for the $\alpha 2$ glycine receptor (Fig. 2b). Bicuculline IC_{50}s were 20.12 ± 0.39 μM for 5-HT_{3A} receptors and 169.40 ± 1.73 μ M for $\alpha 2$ glycine receptors. Hill coefficients were ~ 1 for both receptors.

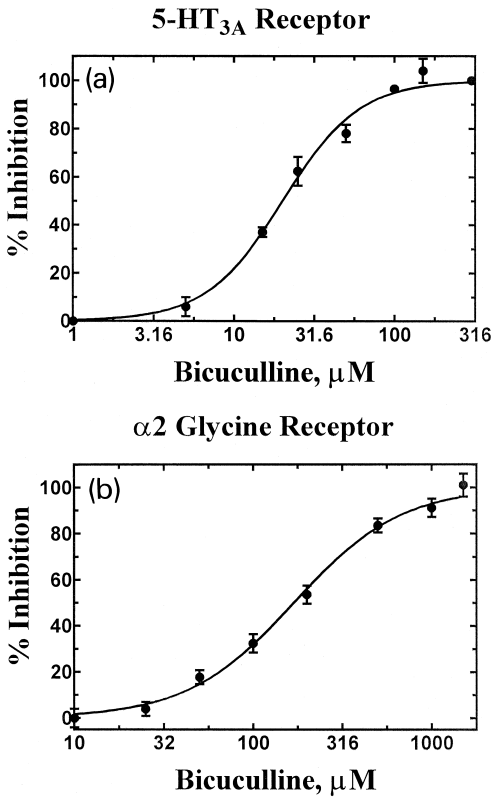

Fig. 2. Concentration–response curves for bicuculline generated in oocytes expressing either mouse 5-HT_{3A} receptors or human $\alpha 2$ glycine receptors. (a) Bicuculline (0–300 μM) was coapplied with 0.5 μM 5-HT for 30 s ($n = 3$–5). An IC_{50} of 20.12 ± 0.39 μM was obtained (b) Bicuculline (0–1000 μM) was coapplied with 50 μM glycine for 30 s ($n = 3$–4). An IC_{50} of 169.4 ± 1.73 μM was obtained.

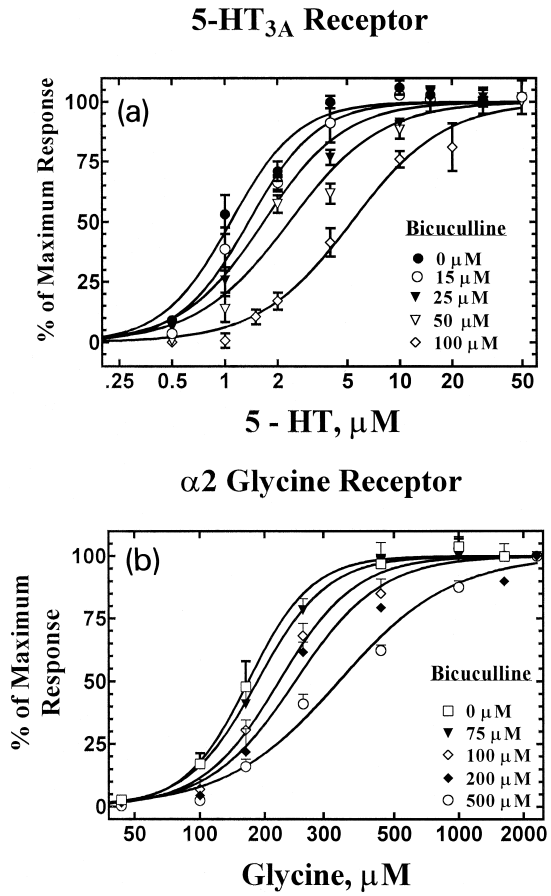

Fig. 3. Bicuculline shifts the EC_{50} values of 5-HT in 5-HT_{3A} receptors and of glycine in $\alpha 2$ glycine receptors. (a) 5-HT_{3A} receptor: 5-HT concentration response curves were generated in the presence of bicuculline, 0–100 μM ($n = 3$–5). (b) $\alpha 2$ glycine receptor: Glycine concentration response curves were generated in the presence of bicuculline (0, 75, 100, 200, and 500 μM) ($n = 3$–4). Parallel shifts in the agonist concentration response curves were observed in a and b, which is suggestive of a competitive form of inhibition.

In order to characterize the nature of the inhibitory effects of bicuculline, 5-HT and glycine concentration response curves were generated in the presence of increasing concentrations of bicuculline in oocytes expressing 5-HT_{3A} receptors and $\alpha 2$ glycine receptors, respectively. Bicuculline concentrations of 15, 25, 50 and 100 μM were used when the 5-HT (0.1–50 μM) concentration response curves were generated (Fig. 3a). On the other hand, 75, 100, 200, and 500 μM bicuculline were used when glycine (75–2000 μM) concentration response curves were generated. Parallel shifts to the right in both the 5-HT and the glycine concentration response curves were observed as the bicuculline concentrations increased. It is important to note that the inhibitory effect of each bicuculline concen-

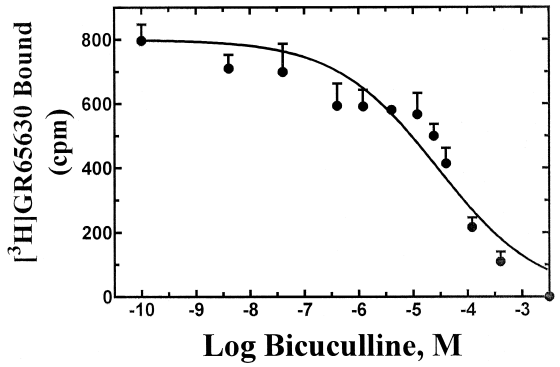

Fig. 4. Competition curves for [^{3}H]GR65630 and bicuculline. Crude cell membrane proteins isolated from SF21 insect cells infected with recombinant baculovirus containing a full-length mouse 5-HT$_{3A}$ receptor cDNA were used. Two independent binding assays were conducted, and all analyses were performed in duplicate. The calculated K_i value was 19.1 ± 0.71 μM.

tration was completely overcome by high concentrations of 5-HT (Fig. 3a) or glycine (Fig. 3b). As the concentration of bicuculline was increased from 0, 15, 25, 50 to 100 μM, the EC_{50}s for 5-HT increased from 1.11 ± 0.06, 1.40 ± 0.07, 1.60 ± 0.03, 2.21 ± 0.05 to 5.04 ± 0.22 μM for the 5-HT$_{3A}$ receptor. The Hill slopes were 2.03 ± 0.48, 2.06 ± 0.27, 1.74 ± 0.33, 1.54 ± 0.44, and 1.55 ± 0.28, respectively. For the α2 glycine receptor, the EC_{50}s for glycine were 154.0 ± 12.2, 166.9 ± 12.2, 202.5 ± 15.7, 233.6 ± 20.0 and 345.1 ± 23.5 μM, in the presence bicuculline, 0, 75, 100, 200, and 500 μM. The corresponding Hill slopes were 3.54 ± 0.35, 3.20 ± 0.05, 2.61 ± 0.43, 1.98 ± 0.20, and 2.91 ± 0.33 ($n = 3 - 7$). These results strongly suggest that bicuculline inhibits both 5-HT$_{3A}$ receptors and α2 glycine receptors in a competitive manner, within the tested concentration range.

The interaction between bicuculline and 5-HT$_{3A}$ receptors was further characterized with ligand displacement assays. The 5-HT$_{3A}$ receptor was overexpressed in SF21 insect cells with the baculovirus-insect cell expression system. Competition binding analyses were conducted with membranes obtained from the SF21 insect cells expressing 5-HT$_{3A}$ receptors. The results revealed a complete displacement of [^{3}H]GR65630 by bicuculline (Fig. 4). Bicuculline inhibited the binding of [^{3}H]GR65630 with an IC_{50} of 27.80 ± 1.41 μM. The calculated K_i for displacement of [^{3}H]GR65630 binding by bicuculline was 19.01 ± 0.71 μM. These results suggest that bicuculline competitively inhibits 5-HT$_{3A}$ receptors. Ligand displacement assays with bicuculline were not further pursued with the α2 glycine receptor, given that bicuculline was far less potent at α2 glycine receptors.

4. Discussion

Bicuculline is a well-characterized competitive antagonist of GABA$_A$ receptors, (Macdonald and Olsen, 1994) with an IC_{50} of ~1–2 μM (Shirasaki et al., 1991; Green and Halliwell, 1997). The antagonism by bicuculline of GABA$_A$ receptor-mediated responses has been extensively studied with various methods, both in vitro and in vivo, including measurements of GABA-gated Cl^- currents, cell firing, and specific radioligand binding (Curtis et al., 1970; reviewed by Bormann, 1988). For example, a recent study with isolated neurons indicated that bicuculline (1 μM) selectively reduced GABA-evoked responses by 50% (Sergeeva, 1998). Furthermore, physiological responses mediated by GABA$_A$-ergic synaptic transmission have been tracked with bicuculline in vivo. Application of bicuculline produces reversible red–green color blindness in goldfish (Wietsma and Spekreijse, 1991), and induces changes in blood pressure, heart rate (Martin et al., 1991), and transport processes in small intestine in rats (Hardcastle et al., 1991). These effects of bicuculline have been attributed solely to its actions at GABA$_A$ receptors.

However, many studies have questioned the specificity of bicuculline as a competitive antagonist of GABA$_A$ receptors. For example, it was reported that bicuculline blocks the response of nicotinic acetylcholine receptors in isolated pig pituitary intermediate lobe cells (Zhang and Feltz, 1991) and cultured embryonic rat skeletal muscle (Liu et al., 1994). The blocking effect of bicuculline at nicotinic acetylcholine receptors in these preparations was concentration dependent, with IC_{50}s ranging from 43.8 to 101.2 μM, and the mode of inhibition appeared to be noncompetitive. Recently, α9 nicotinic acetylcholine receptors expressed in oocytes were shown to be competitively blocked by bicuculline, with an IC_{50} of 0.8 μM (Rothlin et al., 1999). Bicuculline also displaced specific [^{3}H] strychnine binding to glycine receptors in spinal cord synaptosomes (Goldinger and Muller, 1980) and membranes (Marvizon et al., 1986). The reported inhibitory constants were similar to that determined for bicuculline-induced displacement of [^{3}H]-muscimol from GABA$_A$ receptors. In contrast, bicuculline was reported to have minimal effects on glycine-stimulated Cl^- flux. For instance, glycine-evoked currents measured with whole cell voltage clamp in the rat striatum were reduced 22% by 5 μM bicuculline (Sergeeva, 1998). In addition, bicuculline had no effect on glycine-induced Cl^- flux in rat forebrain synaptoneurosomes, as measured with a chloride-sensitive fluorescent indicator (Engblom et al., 1996). Previously, no experimental data had been reported on bicuculline effects at defined glycine receptors and 5-HT$_3$ receptors.

In the present study, α2 glycine receptors expressed in *Xenopus* oocytes were inhibited by bicuculline with an IC_{50} of ~169 μM. The parallel shift in the glycine concentration response curves as bicuculline concentrations were increased suggests that the mode of antagonism is competitive. Similar results suggestive of a competitive mode of inhibition were also obtained with the 5-HT$_{3A}$ receptor, but the IC_{50} of ~20 μM, calculated from the bicuculline inhibitory curve, was substantially lower. In

addition, it must be pointed out that another interpretation of the data is that bicuculline has an allosteric action at both glycine and 5-HT_{3A} receptors. If so, bicuculline would be an inverse agonist that is tightly coupled to the agonist recognition sites. To rule out this possibility, agonist concentrations response curves would have to be generated in both receptor constructs with a series of bicuculline concentrations in the millimolar range.

Members of the ligand-gated ion channel superfamily are competitively antagonized by a broad range of compounds. For example, kinase inhibitors such as bisindolylmaleimide and H-7 antagonize 5-HT_{3A} receptors (Fan, 1994; Coultrap et al., 1999) or $GABA_A$ receptors (Leidenheimer et al., 1990). The microtubule depolymerizing agent, colchicine, competitively antagonizes $GABA_A$ receptors (Bueno and Leidenheimer, 1998), glycine receptors (Machu, 1998), and 5-HT_{3A} receptors (Sun and Machu, unpublished data). Numerous antagonists that have been deemed ''selective'' for a particular member of the ligand-gated ion channel superfamily have been shown to cross react with other receptors. For instance, D-tubocurarine competitively antagonizes both the muscle nicotinic acetylcholine receptor and mouse 5-HT_{3A} receptor with IC_{50}s in the nanomolar range (Peters et al., 1990; Yan et al., 1998). The widely used calcium channel blocker 3,4,5,-trimethoxybenzoic acid 8-(diethylamino)octyl ester (TMB-8) interacts with both muscle nicotinic acetylcholine receptors and $5HT_{3A}$ receptors (Sun et al., 1999). Picrotoxin, a potent $GABA_A$ receptor noncompetitive antagonist, blocks ACh-evoked cation channels in Aplysia neurons (Yarowsky and Carpenter, 1978) and competitively inhibits $\alpha 1$ glycine receptors (Lynch et al., 1995). The recently cloned $\alpha 9$ nicotinic acetylcholine receptor cross reacts with a number of ''selective'' ligands (Rothlin et al., 1999). The purported $GABA_A$ receptor antagonist, bicuculline, the glycinergic antagonist, strychnine, and 5-HT_{3A} receptor antagonist, ICS-205,950, all block ACh-evoked currents in oocytes expressing $\alpha 9$ nicotinic acetylcholine receptors. Interestingly, the respective potencies at which these drugs inhibit the $\alpha 9$ nicotinic acetylcholine receptors and the other receptors are quite similar, underscoring these compounds' lack of specificity (Rothlin et al., 1999). To our knowledge, bicuculline is the first ligand that antagonizes, with reasonable potency, a member of every receptor subclass in the ligand-gated ion channel superfamily. Bicuculline has a rank order of potency in the respective ligand-gated ion channels of: $GABA_A$ receptor $\simeq \alpha 9$ nicotinic acetylcholine receptor $>$ 5-HT_{3A} receptor $> \alpha 2$ glycine receptor. It appears, then, that bicuculline is a poor drug for discriminating $GABA_A$ receptor effects, especially where $GABA_A$ receptors are colocalized with $\alpha 9$ nicotinic acetylcholine receptors and/or the 5-HT_{3A} receptors. Although $\alpha 9$ nicotinic acetylcholine receptors have a limited distribution in outer hair cells in the ear (Rothlin et al., 1999), 5-HT_{3A} receptors are present on many GABA interneurons in the brain (Morales et al., 1996). Therefore, bicuculline-induced stimulation of a synaptic pathway containing a GABA interneuron could actually be the result of inhibition of 5-HT_{3A} receptors.

To summarize, our results clearly indicate that the $GABA_A$ receptor competitive antagonist, bicuculline, also antagonizes 5-HT_{3A} receptors and $\alpha 2$ glycine receptors. Bicuculline acts at the 5-HT_{3A} receptor with a slightly lower potency than that observed at $GABA_A$ receptors, but is much weaker at $\alpha 2$ glycine receptors. Bicuculline may be a useful tool to probe the agonist binding domain of ligand-gated ion channel receptors. However, bicuculline's cross-reactivity to and relative potencies at numerous ligand-gated ion channel receptors should be considered when it is used to identify and study either GABAergic, cholinergic, glycinergic or 5-HTergic transmission at central or peripheral synapses.

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