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Short communication

Felbamate is a subunit selective modulator of recombinant γ-aminobutyric acid type A receptors expressed in *Xenopus* oocytes

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Abstract

Felbamate (2-phenyl-1,3-propanediol dicarbamate) is clinically available for the treatment of refractory epileptic seizures, and is known to modulate several ion channels including γ-aminobutyric acid type A ($GABA_A$) receptors. To determine felbamate subunit selectivity for $GABA_A$ receptors we expressed 15 different $GABA_A$ receptor combinations in *Xenopus laevis* oocytes. Felbamate positively modulated GABA-currents of $\alpha_1\beta_2\gamma_{2S}$, $\alpha_1\beta_3\gamma_{2S}$, $\alpha_2\beta_2\gamma_{2S}$ and $\alpha_2\beta_3\gamma_{2S}$, whereas felbamate was either ineffective or negatively modulated the other 11 receptor combinations. Regional distributions of $GABA_A$ receptor subunits suggest that felbamate may differentially modulate distinct inhibitory circuits, a possibility that may have relevance to felbamate efficacy in refractory epilepsies.

Keywords: Felbamate; $GABA_A$ receptor; Epilepsy; Anticonvulsant; Subunit selectivity

1. Introduction

Felbamate (2-phenyl-1,3-propanediol dicarbamate) was successfully introduced into clinical practice for the treatment of epilepsy in 1993. Unfortunately, the general use of felbamate has been curtailed since it is associated with increased incidences of aplastic anemia (Pellock, 1999); however, felbamate remains useful for patients with refractory seizure conditions and Lennox–Gastaut syndrome (Borowicz et al., 2004). Felbamate has varied activity at multiple channel types, including blockade of sodium currents (White et al., 1992; Pisani et al., 1995; Taglialatela et al., 1996) and inhibition of NMDA-evoked currents (Rho et al., 1994; Subramaniam et al., 1995), specifically NMDA receptors comprised of NR1 and NR2B subunits (Kleckner et al., 1999; Harty and Rogawski, 2000). Felbamate potentiated γ-aminobutyric acid type A ($GABA_A$) receptor-mediated chloride currents in cultured cortical and hippocampal neurons (Rho et al., 1994, 1997; Kume et al., 1996), but was ineffective on GABA-currents of cultured spinal cord neurons (Ticku et al., 1991). Additionally, felbamate inhibited the binding of [^{3}H]*t*-butylbicycloorthobenzoate in rat brain slices suggesting an effect near the picrotoxin-binding site on the $GABA_A$ receptor (Kume et al., 1996), but did not alter the binding of another picrotoxin-binding site specific compound, [^{35}S]*t*-butylbicyclophosphorothionate, in rat cerebral cortical membranes (Ticku et al., 1991).

The present study has found differences in the ability of felbamate to modulate native GABA-evoked currents in two types of cultured neurons. These differences may be explained by regional differences in the subtypes of $GABA_A$ receptor expressed. In an effort to determine the subunit specificity of felbamate modulation of GABA-evoked currents, this study investigated a wide range of recombinant $GABA_A$ receptors expressed in *Xenopus laevis* oocytes. Felbamate modulation proved to be extremely dependent on the subtypes of α, β, and γ subunits expressed in the receptor.

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2. Methods

All procedures involving animals were in accordance with the National Institutes of Health guidelines and were approved by the University of Utah Institutional Animal Care and Use Committee.

2.1. Dissociated cell culture

Neuronal cultures were prepared from 8-day-old Swiss Webster mouse pups under two separate conditions: 1) the entire hindbrain was isolated and prepared according to methods previously described by Skeen et al. (1993) and 2) cerebellar granule cells were obtained from dissected cerebellum. Culture media contained modified Eagle's medium (Sigma, St. Louis, MO, USA) supplemented with 25 mM KCl, 2 mM L-glutamine, 10% fetal calf serum and 1% penicillin/streptomycin. Twenty-four hours after plating, culture media was changed to DMEM (Sigma) supplemented with 25 mM KCl, 10% horse serum, 30 mM glucose, and 2 mM L-glutamine.

2.2. Whole-cell electrophysiology

Recordings were obtained 7–21 days after plating, using the whole-cell patch clamp technique. Glass capillaries (World Precision Instruments, Sarasota, FL, USA) were pulled to 3–6 MΩ resistance using a micropipette electrode puller (Sutter Instrument Co., Novato, CA, USA). For all data acquisition, Axopatch 200 Series amplifiers and pClamp 8.0 software were utilized (Axon Instruments, Union City, CA, USA). Signals were filtered at 5 kHz. Extracellular solution contained, in mM: 142 NaCl, 20 Sucrose, 10 Glucose, 10 HEPES, 1.5 KCl, 1 $CaCl_2$, 1 $MgCl_2$ (pH 7.34, 315 mOsm). The internal electrode solution contained, in mM: 140 CsCl, 10 HEPES, 5 EGTA, 4 $MgCl_2$ (pH 7.3, 290 mOsm=290). All drug solutions were prepared fresh before each recording session. Cells were voltage-clamped at −70 mV and perfused with 1 μM GABA ($EC_{2.5–6}$) for 2.5 s as a control, then stepped into 1 μM GABA+300 μM felbamate for 2.5 s using a 3-barrel puffer connected to a piezoelectric translator. Peak currents were measured and responses to GABA co-applied with felbamate were compared to control GABA-currents. Data is reported as the mean percent control GABA response±S.E.M.

2.3. Oocyte isolation and injection

X. laevis oocytes were isolated as described by Cartier et al. (1996). The available $GABA_A$ receptor subunits were circular pCIS2 plasmids containing cDNA encoding the human α_1, α_2, γ_{2S}, and γ_{2L}, and rat α_4, α_6, β_1, β_2, β_3, and ρ_1 subunits that were generously provided by Dr. Roy Twyman while at the University of Utah and Dr. Neil Harrison (Cornell University). cDNA was injected into oocytes 24 h after isolation. Glass capillary tubes (World Precision Instruments) were pulled to a fine tip on a micropipette puller (Sutter Instrument Co.) and broken back to an outside diameter of 21 μm. Combinations of subunit cDNA stocks were diluted to 0.2–2 ng/nl in a 1:1 ratio for αβ combinations and 1:1:1 for αβγ combinations (except for $\alpha_4\beta\gamma$ combinations where the ratio was 5:1:1). The combinations were injected into the nucleus of the oocyte (~37 nl) with a nano-injector (World Precision Instruments).

2.4. Oocyte recordings

Electrophysiological recordings were performed 1–5 days following injection as previously described (Simeone et al., 2006). Briefly, recordings were conducted at room temperature in a 100 μl chamber continuously perfused (6 ml/min) with a Ringer's solution containing, in mM: 115 NaCl, 2.5 KCl, 1.0 $BaCl_2$, and 10 HEPES (pH 7.4). Two-electrode voltage-clamp recordings were obtained with a GeneClamp 500 amplifier (Axon Instruments) using 3 M KCl-filled microelectrodes (1–5 MΩ). Recordings were performed at a holding potential of −60 mV and captured with pClamp6 Fetchex and

Table 1
Felbamate modulation of $GABA_A$ receptor subtypes

Modulation	Subunit combination	+300 μM FBM	*n*	+1 μM DZP	*n*	+50 μM FMZ	*n*
		% Control		% Control		% Control	
Positive	$\alpha_1\beta_2\gamma_{2S}$	150.8±6.1[c]	9	295.2±45.2[c]	7	n.d.	
	$\alpha_1\beta_3\gamma_{2S}$	137.1±4.4[c]	14	386.2±59.2[c]	6	n.d.	
	$\alpha_2\beta_2\gamma_{2S}$	119.1±3.5[c]	6	260±43.4[b]	4	n.d.	
	$\alpha_2\beta_3\gamma_{2S}$	124.3±3.5[c]	8	381.2±60.6[c]	7	n.d.	
Negative	$\alpha_1\beta_1$	61.2±11.4[a]	3	99±0.6	3	n.d.	
	$\alpha_1\beta_3\gamma_{2L}$	9.5±34.3[b]	5	170±18.8[b]	4	n.d.	
	$\alpha_4\beta_1\gamma_{2S}$	35.8±18.1[a]	3	n.d.		162.2±12.8[b]	3
	$\alpha_4\beta_3\gamma_{2S}$	−182.1±79.8[b]	7	n.d.		157.1±25.4[a]	6
	$\alpha_6\beta_1\gamma_{2S}$	47.3±2.7[c]	6	n.d.		161.3±5.0[b]	6
No significant modulation	$\alpha_1\beta_1\gamma_{2S}$	87.8±2.1	2	235.4±27.8	2	n.d.	
	$\alpha_1\beta_3$	66.5±21.5	3	104.9±4.9	2	n.d.	
	$\alpha_2\beta_1$	94.9±3.4	4	102±2.8	4	n.d.	
	$\alpha_2\beta_1\gamma_{2S}$	94.0±5.0	5	281.1±66.6[a]	4	n.d.	
	$\alpha_6\beta_3\gamma_{2S}$	93.6±5.3	6	n.d.		147.1±11.6[b]	5
	ρ_1	82.3±6.5	2	95.8±2.6	2	n.d.	

Data presented as the mean percent of control GABA response±S.E.M. and grouped according to statistically significant felbamate modulation. Significance vs. control [a]$P \leq 0.05$, [b]$P \leq 0.01$ and [c]$P \leq 0.001$. Abbreviations: felbamate, FBM; diazepam, DZP; flumazenil, FMZ; not determined, n.d.