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Unsaturated phosphinic analogues of γ-aminobutyric acid as $GABA_C$ receptor antagonists

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Abstract

The phosphinic and methylphosphinic analogues of γ-aminobutyric acid (GABA) are potent $GABA_C$ receptor antagonists but are even more potent as $GABA_B$ receptor agonists. Conformationally restricted unsaturated phosphinic and methylphosphinic analogues of GABA and some potent $GABA_B$ receptor phosphonoamino acid antagonists were tested on $GABA_C$ receptors in *Xenopus* oocytes expressing human retinal ρ_1 mRNA. 3-Aminopropyl-*n*-butyl-phosphinic acid (CGP36742), an orally active $GABA_B$ receptor antagonist, was found to be a moderately potent $GABA_C$ receptor antagonist ($IC_{50} = 62$ μM). The unsaturated methylphosphinic and phosphinic analogues of GABA were competitive antagonists of the $GABA_C$ receptors, the order of potency being [(*E*)-3-aminopropen-1-yl]methylphosphinic acid (CGP44530, $IC_{50} = 5.53$ μM) > [(*E*)-3-aminopropen-1-yl]phosphinic acid (CGP38593, $IC_{50} = 7.68$ μM) > [(*Z*)-3-aminopropen-1-yl]methylphosphinic acid (CGP70523, $IC_{50} = 38.94$ μM) > [(*Z*)-3-aminopropen-1-yl]phosphinic acid (CGP70522, $IC_{50} > 100$ μM). This order of potency differs from that reported for these compounds as $GABA_B$ receptor agonists, where the phosphinic acids are more potent than the corresponding methylphosphinic acids. © 1997 Elsevier Science B.V.

Keywords: $GABA_C$ receptor; GABA (γ-aminobutyric acid); $GABA_B$ receptor agonist; $GABA_B$ receptor antagonist; *Xenopus* oocyte; *trans*-4-Aminocrotonic acid; *cis*-4-Aminocrotonic acid

1. Introduction

γ-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the central nervous system (CNS) and activates three major subtypes of GABA receptors, the $GABA_A$, $GABA_B$ and $GABA_C$ receptors. $GABA_A$ receptors are ligand-gated Cl^- channels which are inhibited by the alkaloid, bicuculline (Johnston, 1996a). These are heterooligomeric receptors made up of α, β, γ, and δ subunits. $GABA_B$ receptors are transmembrane receptors coupled to second messenger systems and Ca^{2+} and K^+ channels via G-proteins. These receptors are not blocked by bicuculline but are activated by (−)-baclofen and 3-aminopropylphosphinic acid (CGP27492) and blocked by phaclofen and saclofen (Kerr and Ong, 1995).

$GABA_C$ receptors (sometimes called $GABA_{NANB}$ and ρ receptors) were first proposed when a series of conformationally restricted GABA analogues, including *cis*-4-aminocrotonic acid (CACA), that had bicuculline-insensitive depression actions on neuronal activity, showed no affinity for [^{3}H]baclofen binding sites in rat cerebellar membranes (Drew et al., 1984). $GABA_C$ receptors with similar pharmacology were first found in neurons from rat retina (Feigenspan et al., 1993) and white perch retina (Qian and Dowling, 1993). In rat retina, rod bipolar cells contain bicuculline-insensitive, baclofen-insensitive receptors that were activated by CACA (Feigenspan et al., 1993). These were detected by the co-application of GABA with 100 μM bicuculline to abolish the $GABA_A$ component (Feigenspan et al., 1993). In white perch retina, rod-driven horizontal cells (H4) and not bipolar cells showed $GABA_C$ receptor-like pharmacology. Application of GABA on bipolar cells showed rapid desensitisation while on rod-driven horizontal cells, desensitisation was not observed (Qian and Dowling, 1993). Subsequently, $GABA_C$ receptors were found on cone-driven horizontal cells in catfish (Dong et al., 1994) and bipolar terminals in tiger salamander (Lukasiewicz et al., 1994).

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The expression of mRNA from bovine retina into *Xenopus* oocytes showed that GABA activated two distinct GABA receptors. Both receptors activated Cl^- currents. One was mediated by $GABA_A$ receptors and was blocked by bicuculline and the other was mediated by $GABA_C$ receptors and was insensitive to both bicuculline and baclofen (Polenzani et al., 1991). Subsequently, two cDNAs that have 30–38% sequence identity with $GABA_A$ receptor subunits were cloned from human retinal mRNA (Cutting et al., 1991, 1992). These subunits have been termed ρ_1 and ρ_2 and have 74% sequence identity (Cutting et al., 1991, 1992).

The species equivalent of the human ρ_1 and ρ_2 subunits have been cloned from rat (Enz et al., 1995). These show 88–99% homology with the respective human sequences. The use of PCR and in situ hybridisation have shown high expression of both the ρ_1 and ρ_2 subunits in rod bipolar cells. However, only the ρ_2 subunit is expressed in the CNS, particularly in the hippocampus and cortex (Enz et al., 1995). Recently, a third ρ subunit was cloned from rat retina cDNA (Ogurusu and Shingai, 1996). This subunit exhibits 63% and 61% sequence homology to the human ρ_1 and rat ρ_2 sequences, respectively (Ogurusu and Shingai, 1996).

Expression of human ρ subunits in *Xenopus* oocytes generates homooligomeric GABA receptors with intrinsic Cl^- channels. These receptor ion channels are activated by GABA and CACA but are insensitive to both bicuculline, (−)-baclofen, barbiturates and benzodiazepines. They have been shown to be sensitive to picrotoxin and have been classified as $GABA_C$ receptors (Cutting et al., 1991, 1992; Polenzani et al., 1991; Shimada et al., 1992; Kusama et al., 1993a,b; Wang et al., 1994; Bormann and Feigenspan, 1995; Johnston, 1996b).

The most potent $GABA_C$ receptor agonists are *trans*-4-aminocrotonic acid (TACA, $K_d = 0.6$ μM) and GABA ($K_d = 1.7$ μM) (Woodward et al., 1993). TACA, a conformationally restricted analogue of GABA in an extended conformation, is also a $GABA_A$ receptor agonist (Johnston, 1996a). CACA, a conformationally restricted analogue of GABA in a folded conformation, has moderate partial agonist activity at $GABA_C$ receptors ($K_d = 74$ μM) and may be the most selective agonist for this receptor subtype (Johnston, 1996b). A series of GABA analogues were tested for agonist and antagonist activity at $GABA_C$ receptors using poly(A)$^+$ RNA from mammalian retina injected in *Xenopus* oocytes (Woodward et al., 1993). Several potent $GABA_C$ receptor antagonists were identified including (3-aminopropyl)methylphosphinic acid (CGP35024; $K_B = 0.8$ μM), 3-aminopropylphosphinic acid (CGP27492; $K_B = 1.8$ μM), and 3-aminopropylphosphonic acid (3-APA, $K_B = 10$ μM) (Woodward et al., 1993). CGP35024 and CGP27492 are not selective for $GABA_C$ receptors as they are also very potent $GABA_B$ receptor agonists while 3-APA is a $GABA_B$ receptor antagonist. A more recently synthesised compound, 1,2,5,6-tetrahydropyridine-4-yl)methylphosphinic acid (TPMPA), does show potent and selective $GABA_C$ receptor antagonist activity ($K_d = 2.1$ μM) (Murata et al., 1996; Ragozzino et al., 1996). In this study, we demonstrate that the phosphinic and methylphosphinic acid derivatives of CACA and TACA, and 3-aminopropyl-*n*-butyl-phosphinic acid (CGP36742), an orally active $GABA_B$ receptor antagonist, are $GABA_C$ receptor antagonists (Bittiger et al., 1992, 1993; Froestl et al., 1992, 1995a; Olpe et al., 1993).

2. Materials and methods

2.1. Materials

[(*E*)-3-Aminopropen-1-yl]methylphosphinic acid (CGP44530), [(*E*)-3-aminopropen-1-yl]phosphinic acid (CGP38593), [(*Z*)-3-aminopropen-1-yl]methylphosphinic acid (CGP70523), [(*Z*)-3-aminopropen-1-yl]phosphinic acid (CGP70522), 3-aminopropyl-*n*-butyl-phosphinic acid (CGP36742), 3-aminopropyl(diethoxymethyl)phosphinic acid (CGP35348), 3-aminopropyl(cyclohexylmethyl)phosphinic acid (CGP46381), (2*S*)-3-amino-2-hydroxypropyl(cyclohexylmethyl)phosphinic acid (CGP51176) and (2*R*,1′*S*)-(3-*N*-[1′(3,4-dichlorophenyl)ethyl])amino-2-hydroxypropyl)benzylphosphinic acid (CGP55845A) were synthesised previously by Froestl et al. (1992, 1995a,b). CACA and TACA were prepared as previously described (Johnston et al., 1975) by Dr. K.N. Mewett (Department of Pharmacology, The University of Sydney, Sydney, Australia). GABA was purchased from Sigma (St. Louis, MO, USA).

2.2. Electrophysiological recording

Human ρ_1 cDNA in pcDNA (Invitrogen, San Diego, CA, USA) was obtained from Dr. George Uhl (National Institute for Drug Abuse, Baltimore, MD, USA). The plasmid was linearized with *Xba*I and cRNA made using the 'Mmessage Mmachine' kit from Ambion (Austin, TX, USA). 50 ng of cRNA was injected into defolliculated Stage V *Xenopus* oocytes. Two to seven days later, receptor activity was measured by two-electrode voltage-clamp recording using a Geneclamp 500 amplifier (Axon Instruments, Foster City, CA, USA) and a MacLab 2e recorder (ADInstruments, Sydney, NSW, Australia). Oocytes were voltage clamped at −60 mV and continuously superfused with ND96 buffer (96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$ and 5 mM HEPES, pH 7.5). For receptor activation measurements, the indicated concentrations of agonist and antagonist were added to ND96.

2.3. Analysis of kinetic data

Current (I) as a function of agonist concentration ([A]) was fitted by least squares to $I = I_{max}[A]^{n_H}/(EC_{50}^{n_H} +$

$[A]^{n_H}$), where I_{max} is the maximal current, the EC_{50} is the effective dose that activates 50% of the maximal current and n_H is the Hill coefficient. EC_{50} values are expressed as mean ± S.E.M. ($n = 3–6$) and are determined by fitting data from individual oocytes using Kaleidagraph 2.1 (1990). Current (I) as a function of antagonist concentration ([Ant]) was fitted by least squares to $I = I_{max} - \{I_{max}[Ant]^{n_H}/(IC_{50}^{n_H} + [Ant]^{n_H})\}$, where the IC_{50} is the inhibition dose that blocks 50% of the current generated by 1 μM GABA and n_H is the Hill coefficient. IC_{50} values are expressed as mean ± S.E.M. ($n = 3–6$). K_B values are the apparent dissociation constants for the antagonists and were determined using Schild plot analysis (Arunlakshana and Schild, 1959). $-\log K_B$ values were determined using the following equation: $\log\{(A)/(A^*) - 1\} = m\log[Ant] - \log K_B$, where A is the EC_{50} of GABA in the presence of a known antagonist concentration, A^* is the EC_{50} of GABA in the absence of an antagonist, [Ant] is the concentration of the antagonist, and m is the slope of the curve. For simple competitive antagonism, m is 1. $-\log K_B$ values were determined by fitting data to the above function using Kaleidagraph 2.1 (1990). Schild analyses were carried out for compounds that had IC_{50} values of less than 30 μM.

3. Results

Expression of human ρ_1 mRNA in *Xenopus* oocytes generated $GABA_C$ receptors which showed a dose-dependent GABA-activated inward current when the cell was voltage clamped at −60 mV. This could be blocked by compounds such as CGP44530, CGP38593, CGP70523, CGP70522 and CGP36742 as shown in Fig. 1. The structures of the compounds are shown in Figs. 2 and 3. These compounds were first screened at 100 μM to determine agonist activity, by activation of Cl^- channels, or antagonist activity, by blocking the activation of the channels by 1 μM GABA. Fig. 2 shows the active compounds that had some effect at 100 μM as agonists (Fig. 2A) or antagonists (Fig. 2B) at $GABA_C$ receptors and Fig. 3 shows the compounds that had no effect at 100 μM as agonists or antagonists at $GABA_C$ receptors. Only the carboxylic acids, TACA, GABA and CACA activated the Cl^- channel. TACA was more potent than GABA with an EC_{50} of 0.44 ± 0.02 μM and was almost a full agonist with a maximal TACA dose generating 95% of the maximal GABA-activated current. GABA was found to have an EC_{50} value of 0.82 ± 0.09 μM. CACA was less potent than GABA. The EC_{50} was 37.4 ± 6.1 μM and was a partial agonist with a maximal CACA dose generating 75% of the maximal GABA-activated current (Table 1). The Hill coefficients (n_H), as shown in Tables 1 and 2, were greater or equal to 2 which suggests that more than one molecule of the agonist is required to bind before the Cl^- channels can open. These findings are in agreement with those of Woodward et al. (1993).

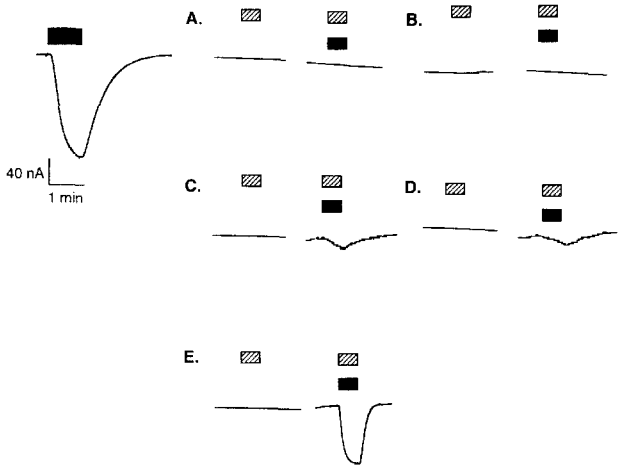

Fig. 1. Expression of human ρ_1 receptors in *Xenopus* oocytes produce homooligomeric GABA receptors ($GABA_C$ receptors) with intrinsic Cl^- channels. GABA (1 μM) activates the Cl^- channels (duration indicated by filled bar) and produces an inward current when the oocyte is clamped at −60 mV. (A) CGP38593 (100 μM), (B) CGP44530 (100 μM), (C) CGP70523 (100 μM), (D) CGP36742 (100 μM), and (E) CGP70522 (300 μM) do not activate the receptor (duration indicated by hatched bar). However, when (A) CGP38593 (100 μM), (B) CGP44530 (100 μM), (C) CGP70523 (100 μM), (D) CGP36742 (100 μM), and (E) CGP70522 (300 μM) are co-applied with GABA (1 μM), the GABA response is blocked or reduced.

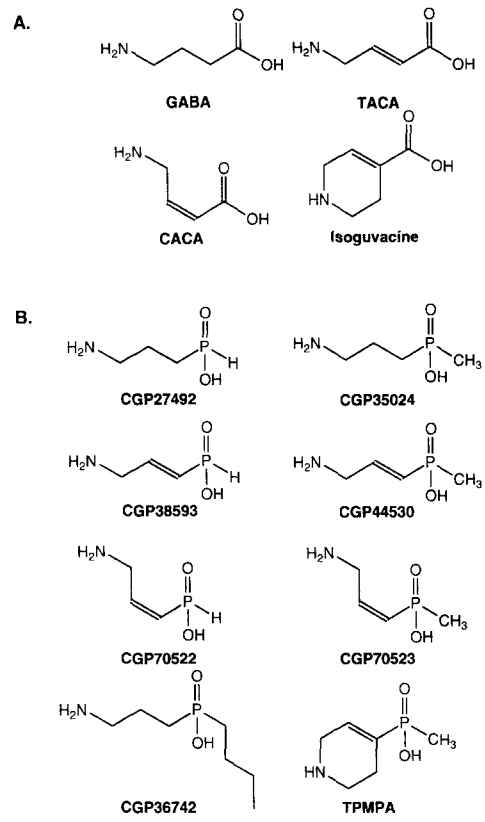

Fig. 2. (A) Structures of compounds that show agonist activity at $GABA_C$ receptors. (B) Structures of compounds that show antagonist activity at $GABA_C$ receptors.

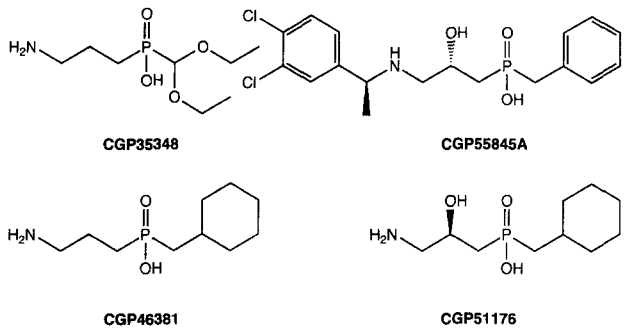

Fig. 3. Structures of orally active $GABA_B$ receptor antagonists with no cognitive enhancement effects. These compounds show no affinity for $GABA_C$ receptors as either agonists or antagonists when tested at 100 μM.

CGP35024, CGP27492, CGP44530, CGP38593, CGP70523 and CGP70522 did not activate any current on their own (Fig. 1). They acted as $GABA_C$ receptor antagonists, inhibiting the current activated by 1 μM GABA (Fig. 1). IC_{50} values were obtained for these compounds (Table 1) and Schild analyses were carried out for the active compounds (Table 2). K_B (binding constant) values for CGP27492, CGP44530, CGP35024, and CGP38593 are shown in Table 1.

The methylphosphinic analogue, CGP44530, and phosphinic analogue, CGP38593, of TACA were antagonists with IC_{50} values of 5.5 ± 1.2 μM and 7.7 ± 0.7 μM respectively. These compounds had lower affinity for the $GABA_C$ receptor expressed in *Xenopus* oocytes than the corresponding methylphosphinic analogue, CGP35024, and phosphinic analogue, CGP27492, of GABA. CGP35024 had an IC_{50} of 0.75 ± 0.07 μM and CGP27492 had an IC_{50} of 2.47 ± 0.04 μM. The methylphosphinic analogue, CGP70523 and phosphinic analogue, CGP70522, of CACA were antagonists with IC_{50} values of 38.9 ± 4.9 μM and > 100 μM respectively. These compounds had lower affinity for $GABA_C$ receptors than the methylphosphinic and phosphinic analogues of GABA and TACA. The order of potency of the methyl phosphinic acids and phosphinic acids is CGP35024 > CGP27492 > CGP44530 > CGP38593 > CGP70523 ≫ CGP70522. The new compounds CGP44530, CGP38593, CGP70523 and CGP70522 were weaker at the $GABA_C$ receptor than the existing phosphinic acid, CGP27492 and the methylphosphinic acid, CGP35024.

CGP35024, CGP27492, CGP44530 and CGP38593 were found to be competitive antagonists. The gradients of the Schild regression plots were not significantly different from 1 over the concentrations tested, indicating that these compounds compete for the same site as GABA.

CGP36742 was found to be an antagonist with moderate potency at the $GABA_C$ receptor with an IC_{50} value of 62.5 ± 0.5 μM. This compound is orally active showing cognitive enhancement effects. Other related compounds such as CGP35348, CGP46381, CGP51176 and CGP55845A (Fig. 3) are also orally active but do not show cognitive enhancement effects as observed for CGP36742. These were screened at 100 μM and had no effect as

Table 1
Summary of EC_{50}, IC_{50}, K_B and Hill coefficients of various agonists and antagonists at the $GABA_C$ receptor expressed in *Xenopus* oocytes

	EC_{50} (μM)	IC_{50} (μM)	n_H	K_B (μM)
GABA	0.82 ± 0.09		2.6 ± 0.2	
TACA	0.44 ± 0.02		2.4 ± 0.2	
CACA	37.4 ± 6.1		2.2 ± 0.3	
Isoguvacine [a]	99			
CGP35024		0.75 ± 0.07	1.8 ± 0.1	0.58 ± 0.14
CGP44530		5.5 ± 1.2	2.4 ± 0.5	8.6 ± 1.6
CGP70523		38.9 ± 4.9	1.6 ± 0.1	
CGP27492		2.47 ± 0.04	1.9 ± 0.2	3.2 ± 1.0
CGP38593		7.7 ± 0.7	1.8 ± 0.4	15.5 ± 1.7
CGP70522		> 100		
CGP36742		62.5 ± 0.5	3.0 ± 0.4	
TPMPA [a]				2.1

EC_{50} is the effective dose that activates 50% of the maximal current when tested at ρ_1 receptors expressed in *Xenopus* oocytes. IC_{50} is the concentration that inhibits 50% of the response produced by 1 μM GABA. Data are the mean ± S.E.M. ($n = 3$–6 oocytes). n_H is the Hill coefficient. K_B is the binding constant for the antagonist. These were determined using Schild plot analysis assuming competitive antagonism over the tested concentrations (Table 2).

[a] Data from Murata et al. (1996).

Table 2
Results of Schild analyses of CGP35024, CGP27492, CGP44530 and CGP38593 at the $GABA_C$ receptor expressed in *Xenopus* oocytes

Antagonist (μM)	EC_{50} of GABA (μM)	n_H	Slope of Schild plot
CGP35024			
3	4.5 ± 0.1	2.3 ± 0.1	1.14
10	10.0 ± 1.4	2.2 ± 0.2	
30	28.8 ± 4.2	1.9 ± 0.2	
CGP27492			
10	3.2 ± 0.2	2.3 ± 0.1	0.99
30	9.3 ± 1.4	2.4 ± 0.3	
100	25.7 ± 0.1	2.5 ± 0.2	
CGP44530			
10	1.85 ± 0.04	2.6 ± 0.2	1.01
30	3.2 ± 0.2	3.0 ± 0.1	
100	10.7 ± 0.5	3.7 ± 0.4	
CGP38593			
30	2.5 ± 0.1	2.7 ± 0.1	0.95
60	4.1 ± 0.2	2.5 ± 0.4	
100	6.8 ± 0.3	3.0 ± 0.1	

EC_{50} is the effective dose that activates 50% of the maximal current when tested at ρ_1 receptors expressed in *Xenopus* oocytes. EC_{50} values are expressed as mean ± S.E.M. ($n = 3$–6) and are determined by fitting data from individual oocytes using Kaleidagraph 2.1 (1990). EC_{50} values of GABA have shifted to the right in the presence of a known concentration of the antagonist. $-\log K_B$ values were determined as described in Section 2. The K_B values are shown in Table 1. n_H is the Hill coefficient. These are greater than 1, indicating that more than 1 molecule of GABA is required for the channel to open. Slope of Schild plot analysis indicating competitive antagonism over the tested concentrations.

either agonists or antagonists at $GABA_C$ receptors. These compounds show high selectivity as $GABA_B$ receptor antagonists.

4. Discussion

Selective agonists and antagonists are needed to determine the physiological role of $GABA_C$ receptors. GABA is a flexible compound due to rotation about the C2–C3 and C3–C4 bonds. It can exist in a range of low-energy conformations (Johnston et al., 1978; Allan and Johnston, 1983). Two of these conformations have been restricted by the introduction of unsaturation in the form of a double bond at the C2–C3 position. The two compounds that represent these restricted conformations are CACA and TACA (Johnston et al., 1975). CACA and TACA have fewer degrees of rotational freedom than GABA and can only rotate about the C3–C4 bond (Johnston et al., 1978; Allan and Johnston, 1983). CACA is a partially folded analogue of GABA. It has moderate activity at $GABA_C$ receptors expressed in *Xenopus* oocytes and is to date the most selective, albeit weaker agonist at these receptors having minimal activity on $GABA_A$ and $GABA_B$ receptors (Johnston, 1996b). TACA is an extended analogue of GABA. It has potent agonist activity at $GABA_C$ receptors expressed in *Xenopus* oocytes; however, it is not selective as it is also a potent $GABA_A$ receptor agonist (Johnston, 1996b).

Woodward et al. (1993), using poly(A)$^+$ RNA from mammalian retina expressed in *Xenopus* oocytes, tested many $GABA_A$ and $GABA_B$ receptor agonists and antagonists to determine a pharmacological profile for $GABA_C$ receptors. From this study, it was found that the phosphinic and methylphosphinic analogues of GABA, which are known potent $GABA_B$ receptor agonists, were potent antagonists at $GABA_C$ receptors. As a result of the structure-activity relationship study and the selectivity of CACA for $GABA_C$ receptors, we have investigated the methylphosphinic acid and phosphinic acid analogues of CACA and the closely related analogue, TACA, as potential $GABA_C$ receptor antagonists.

The development of many alkylphosphinic and phosphinic analogues of GABA has yielded novel $GABA_B$ receptor agonists and antagonists (Olpe et al., 1990, 1993; Bittiger et al., 1992, 1993; Froestl et al., 1992, 1995a,b), including the methylphosphinic and phosphinic analogues of TACA and CACA, i.e., CGP44530, CGP38593, CGP70522 and CGP70523. In this study, we tested these compounds on $GABA_C$ receptors expressed in *Xenopus* oocytes, and found them to be competitive antagonists. The antagonist potencies of CGP44530, CGP38593, CGP70522 and CGP70523 were found to be lower than that of the methylphosphinic and phosphinic analogues of GABA, CGP35024 and CGP27492.

Table 3
A summary of the affinities of the compounds used in this study at GABA receptors

Compound	Receptor affinity [a] (μM)		
	$GABA_A$ [a]	$GABA_B$ [c]	$GABA_C$ [d]
GABA	0.128 [k]	0.033	$EC_{50} = 0.82$ [e]
CGP27492	1.7 [k]	0.005	2.47
CGP35024	Inactive at 10 [k]	0.016	0.75
CGP36742	508	38	62
TACA	0.14 [f,k]	Inactive at 100 [g]	$EC_{50} = 0.44$ [e]
CGP38593	6.8	0.28	7.68
CGP44530	Inactive at 100	0.65	5.53
CACA	25 [f,k]	Inactive at 100 [g]	$EC_{50} = 37$ [e]
CGP70522	6.6	4.4	> 300
CGP70523	242	16	38
Isoguvacine	1.4 [f,k]	Inactive at 500 [h]	$EC_{50} = 99$ [i]
TPMPA	$K_b = 320$ [j]	$EC_{50} \sim 500$ [h]	$K_b = 2.1$ [i]

[a] Receptor affinities are IC_{50} values unless otherwise stated.
[b] IC_{50} values, i.e., concentration that inhibits 50% of the total [^{3}H]muscimol binding using rat cortical membranes (Froestl et al., 1995a,b).
[c] IC_{50} values for the inhibition of [^{3}H]CGP27492 binding using rat cortical membranes (Froestl et al., 1995a,b).
[d] IC_{50} values for the inhibition of the response of 1 μM GABA using human ρ_1 mRNA expressed in *Xenopus* oocytes as described in Section 2.
[e] EC_{50} values, i.e., the effective dose that activates 50% of the maximal current when tested at ρ_1 receptors expressed in *Xenopus* oocytes as described in Section 2.
[f] IC_{50} values for the inhibition of the total Na-independent [^{3}H]GABA binding using rat brain membranes (Johnston et al., 1978).
[g] Data from Kerr and Ong (1995) using guinea-pig ilium, in the presence of bicuculline, against baclofen-depression of twitch contractions.
[h] Data from Ragozzino et al. (1996) using whole-cell patch recordings from pyramidal neurons in hippocampal slices in the presence of bicuculline (20 μM).
[i] Data from Murata et al. (1996) using human ρ_1 mRNA expressed in *Xenopus* oocytes.
[j] Data from Ragozzino et al. (1996) using poly(A)$^+$ RNA from rat cortex expressed in *Xenopus* oocytes.
[k] EC_{50} values for GABA, CGP27492, CGP35024, TACA, CACA and isoguvacine using poly(A)$^+$ RNA from rat cortex expressed in *Xenopus* oocytes are 107 μM, 938 μM, inactive at 1 mM, 133 μM, inactive at 5 mM, and 305 μM, respectively (Woodward et al., 1993). These values are different from the values obtained from radioligand binding assays.

The relative effects of the compounds at $GABA_A$, $GABA_B$ and $GABA_C$ receptors are shown in Table 3. The compounds, CGP38593, CGP70522 and CGP27492, were moderately potent at $GABA_A$ receptors when tested using radioligand binding assays ($IC_{50} = 6.8$ μM; $IC_{50} = 6.6$ μM and $IC_{50} = 1.7$ μM, respectively) (Froestl et al., 1995a). However, the compounds were more potent at $GABA_B$ receptors than at $GABA_A$ receptors using this assay. Similarly, these compounds appear more potent at $GABA_B$ receptors than at $GABA_C$ receptors.

The unsaturated methylphosphinic acid analogues of TACA and CACA, CGP44530 and CGP70523, have weak effects at $GABA_A$ receptors when tested using radioligand binding assays ($IC_{50} = > 100$ μM and 242 μM, respectively). The methylphosphinic acid analogue of GABA,

CGP35024, showed no effects at 10 μM (Froestl et al., 1995a). This moiety appears to reduce the affinity of the compounds for $GABA_A$ receptors. In contrast, these compounds appear to be more potent at $GABA_B$ receptors than at $GABA_A$ receptors, and appear more potent at $GABA_B$ receptors than at $GABA_C$ receptors. Subsequently, introducing unsaturation to the phosphinic acid and methylphosphinic acid analogues of GABA reduces the potency of the compounds at $GABA_B$ receptors. Consequently, it maybe the combination of the methylphosphinic acid moiety and unsaturation that reduces the apparent selectivity for $GABA_B$ receptors and gains selectivity for $GABA_C$ receptors.

In the above discussion, we have compared the potencies and selectivity of a variety of compounds in which ligand binding assays on $GABA_A$ and $GABA_B$ receptors were compared to electrophysiological studies on $GABA_C$ receptors. However, it is not possible to make a quantitative comparison between receptor types because different techniques have been employed. The potencies, for example of GABA and TACA, are lower when electrophysiological methods are employed compared to radioligand binding assays.

CGP36742 was shown to be a moderately potent antagonist at $GABA_B$ receptors using a [^{3}H]CGP27492 binding assay (IC_{50} = 35 μM) (Bittiger et al., 1992; Olpe et al., 1993; Froestl et al., 1995a). It had weak effects at $GABA_A$ receptors (IC_{50} = 500 μM) (Bittiger et al., 1992) and had no effect at other receptor types including NMDA, benzodiazepine, quisqualate, kainate, muscarinic cholinergic, adrenergic, serotoninergic and histaminergic receptors (1 mM) (Bittiger et al., 1992; Froestl et al., 1995b). However, in the present study, CGP36742 showed moderate antagonist activity at $GABA_C$ receptors (IC_{50} = 62 μM) and its apparent selectivity for $GABA_B$ compared to $GABA_C$ receptors was approximately 2-fold. This compound has shown promising therapeutic potential in the treatment of cognitive deficits, petit mal epilepsy and depression (Bittiger et al., 1992). Therefore, it is possible that antagonism of $GABA_C$ receptors contributes to the cognitive enhancement potentiation of CGP36742 that is not shown by other orally active $GABA_B$ receptor antagonists (Froestl et al., 1995b).

TPMPA was recently synthesised and tested at $GABA_A$, $GABA_B$, and $GABA_C$ receptors (Murata et al., 1996). It is a conformationally restricted analogue of CGP44530, and the methylphosphinic analogue of isoguvacine. It was found to be more than 100 times selective as an antagonist for the $GABA_C$ receptors than $GABA_B$ receptors and is 500 times more selective at $GABA_C$ receptors than $GABA_A$ receptors (Murata et al., 1996; Ragozzino et al., 1996). The relative lack of selectivity of the methylphosphinic and phosphinic analogues of TACA and CACA may be explained by the rotational freedom about the C3–C4 bond. TACA and its methylphosphinic and phosphinic analogues of these can attain two low-energy conformations about the C3–C4 bond as seen in Fig. 4. Restricting the rotation about the C2–C3 and C3–C4 bonds to one of the conformations, as with compounds like TPMPA, offers a folded conformation that is both potent and selective for the $GABA_C$ receptors compared to $GABA_A$ and $GABA_B$ receptors.

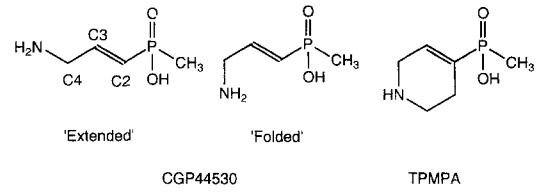

Fig. 4. The introduction of unsaturation in the form of a double bond at the C2–C3 position of CGP35024 restricts rotation at this position. CGP44530 has two low-energy conformations ('extended' or 'folded') that result from rotation about the C3–C4 bond. Restricting rotation about this bond, as with TPMPA, offers a 'folded' conformation that is both potent and selective for the $GABA_C$ receptor. This may explain why CGP44530 was not as selective as TPMPA.

This work has enhanced our understanding of the structural basis for the development of selective agonists and antagonists of the $GABA_C$ receptors. It has helped characterise these receptors by building onto the existing structure-activity relationship trends of phosphinic and methylphosphinic acid analogues of GABA.

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References

Allan, R.D., Johnston, G.A.R., 1983. Synthetic analogs for the study of GABA as a neurotransmitter. Med. Res. Rev. 3, 91–118.

Arunlakshana, O., Schild, H.O., 1959. Some quantitative uses of drug antagonists. Br. J. Pharmacol. 14, 48–58.

Bittiger, H., Bernasconi, R., Froestl, W., Hall, R., Jaekel, J., Klebs, K., Krueger, L., Mickel, S.J., Mondadori, C., Olpe, H.R., Pfannkuch, F., Pozza, M., Probst, A., Van Riezen, H., Schmutz, M., Schuetz, H., Steinmann, M.W., Vassout, A., Waldmeier, P., 1992. $GABA_B$ antagonists: potential new drugs. Pharmacol. Commun. 2, 70–74.

Bittiger, H., Froestl, R.W., Mickel, S.J., Olpe, H.R., 1993. $GABA_B$ receptor antagonists: from synthesis to therapeutic applications. Trends Pharmacol. Sci. 14, 391–393.

Bormann, J., Feigenspan, A., 1995. $GABA_C$ receptors. Trends Neurosci. 18, 515–519.

Cutting, G.R., Lu, L., O'Hara, B., Kasch, L.M., Donovan, D., Shimada, S., Antonarakis, S.E., Guggino, W.B., Uhl, G.R., Kazazian, H.H., 1991. Cloning of the GABA ρ_1 cDNA: a novel GABA subunit highly expressed in the retina. Proc. Natl. Acad. Sci. USA 88, 2673–2677.

Cutting, G.R., Curristin, S., Zoghbi, H., O'Hara, B., Seldin, M.F., Uhl, G.R., 1992. Identification of a putative GABA ρ_2 receptor subunit cDNA and co-localization of the genes encoding ρ_2 and ρ_1 to human chromosome 6q14–q21 and the mouse chromosome 4. Genomics 12, 801–806.

Dong, C.J., Picaud, S.A., Weblin, F.S., 1994. GABA transporters and $GABA_C$-like receptors on catfish cone- but not rod-driven horizontal cells. J. Neurosci. 14, 2648–2658.

Drew, C.A., Johnston, G.A.R., Weatherby, R.P., 1984. Bicuculline-insensitive GABA receptors: studies on the binding of (−)-baclofen to rat cerebellar membranes. Neurosci. Lett. 52, 317–321.

Enz, R., Brandstatter, J.H., Hartveit, E., Wassle, H., Bormann, J., 1995. Expression of GABA receptor rho1 and rho2 subunits in the retina and brain of rat. Eur. J. Neurosci. 7, 1495–1501.

Feigenspan, A., Wössle, H., Bormann, J., 1993. Pharmacology of GABA receptor Cl^- channels in rat retinal bipolar cells. Nature 361, 159–162.

Froestl, W., Mickel, S.J., Von Sprecher, G., Bittiger, H., Olpe, H.-R., 1992. Chemistry of new $GABA_B$ antagonists. Pharmacol. Commun. 2, 52–56.

Froestl, W., Mickel, S.J., Hall, R.G., Von Sprecher, G., Strub, D., Baumann, P.A., Brugger, F., Gentsch, C., Jaekel, J., Olpe, H.-R., Rihs, G., Vassout, A., Waldmeier, P.C., Bittiger, H., 1995a. Phosphinic acid analogues of GABA
1. New potent and selective $GABA_B$ receptor agonists. J. Med. Chem. 38, 3297–3312.

Froestl, W., Mickel, S.J., Von Sprecher, G., Diel, P.J., Hall, R.G., Maier, L., Strub, D., Melillo, V., Baumann, P.A., Bernasconi, R., Gentsch, C., Hauser, K., Jaekel, J., Karlsson, G., Klebs, K., Maître, L., Marescaux, C., Pozza, M.F., Schmutz, M., Steinmann, M.W., Van Riezen, H., Vassout, A., Monadori, C., Olpe, H.-R., Waldmeier, P.C., Bittiger, H., 1995b. Phosphinic acid analogues of GABA
2. Selective, orally active $GABA_B$ antagonists. J. Med. Chem. 38, 3313–3331.

Johnston, G.A.R., 1996a. $GABA_A$ receptor pharmacology. Pharmacol. Ther. 69, 173–198.

Johnston, G.A.R., 1996b. $GABA_C$ receptors relatively simple transmitter-gated ion channels?. Trends Pharmacol. Sci. 17, 319–323.

Johnston, G.A.R., Curtis, D.R., Beart, P.M., Game, C.J.A., McCulloch, R.M., Twitchin, B., 1975. *cis*- and *trans*-4-aminocrotonic acid as GABA analogues of restricted conformation. J. Neurochem. 24, 157–160.

Johnston, G.A.R., Allan, R.D., Kennedy, S.M.E., Twitchin, B., 1978. Systematic study of GABA analogues of restricted conformation. In: GABA-Neurotransmitters. Alfred Benzon Symposium 12, Munksgaard, pp. 149–164.

Kerr, D.I.B., Ong, J., 1995. $GABA_B$ receptors. Pharmacol. Ther. 67, 187–246.

Kusama, T., Spivak, C.E., Whiting, P., Dawson, V.L., Schaeffer, J.C., Uhl, G.R., 1993a. Pharmacology of GABA ρ1 and GABA α/β receptors expressed in *Xenopus* oocytes and COS cells. Br. J. Pharmacol. 109, 200–206.

Kusama, T., Wang, T.-L., Guggino, W.B., Cutting, G.R., Uhl, G.R., 1993b. GABA ρ_2 receptor pharmacology profile: GABA recognition site similarities to ρ_1. Eur. J. Pharmacol. Mol. Pharmacol. Sect. 245, 83–84.

Lukasiewicz, P.D., Maple, B.R., Weblin, F.S., 1994. A novel GABA receptor on bipolar cell terminals in the tiger salamander retina. J. Neurosci. 14, 1202–1212.

Murata, Y., Woodward, R.M., Miledi, R., Overman, L.E., 1996. The first selective antagonist for a $GABA_C$ receptor. Bioorg. Med. Chem. Lett. 6, 2071–2076.

Ogurusu, T., Shingai, R., 1996. Cloning of a putative γ-aminobutyric acid GABA receptor subunit ρ_3 cDNA. Biochim. Biophys. Acta 1305, 15–18.

Olpe, H.-R., Karlsson, G., Pozza, M.F., Brugger, F., Steinmann, M.W., Van Riezen, H., Fagg, G., Hall, R.G., Froestl, W., Bittiger, H., 1990. CGP 35348: a centrally active blocker of $GABA_B$ receptors. Eur. J. Pharmacol. 187, 27–38.

Olpe, H.-R., Steinmann, M.W., Ferrat, T., Pozza, M.F., Greiner, K., Brugger, F., Froestl, W., Mickel, S.J., Bittiger, H., 1993. The actions of orally active $GABA_B$ receptor antagonists on GABAergic transmission in vivo and in vitro. Eur. J. Pharmacol. 233, 179–186.

Polenzani, L., Woodward, R.M., Miledi, R., 1991. Expression of mammalian γ-aminobutyric acid receptors with distinct pharmacology in *Xenopus* oocytes. Proc. Natl. Acad. Sci. USA 88, 4318–4322.

Qian, H., Dowling, J.E., 1993. Novel GABA responses from rod-driven retinal horizontal cells. Nature 361, 162–164.

Ragozzino, D., Woodward, R.M., Murata, F., Eusebi, F., Overman, L.E., Miledi, R., 1996. Design and in vitro pharmacology of a selective γ-aminobutyric $acid_C$ receptor antagonist. Mol. Pharmacol. 50, 1024–1030.

Shimada, S., Cutting, G., Uhl, G.R., 1992. γ-Aminobutyric acid A or C receptor? γ-Aminobutyric acid ρ_1 receptor RNA induces bicuculline-, barbiturate-, and benzodiazepine-insensitive γ-aminobutyric acid responses in *Xenopus* oocytes. Mol. Pharmacol. 41, 683–687.

Wang, T.-L., Guggino, W.B., Cutting, G.R., 1994. A novel γ-aminobutyric acid receptor subunit (ρ_2) cloned from human retina forms bicuculline-insensitive homooligomeric receptors in *Xenopus* oocytes. J. Neurosci. 14, 6524–6531.

Woodward, R.M., Polenzani, L., Miledi, R., 1993. Characterization of bicuculline/baclofen-insensitive (ρ-like) γ-aminobutyric acid receptors expressed in *Xenopus* oocytes
II. Pharmacology of γ-aminobutyric $acid_A$ and γ-aminobutyric $acid_B$ receptor agonists and antagonists. Mol. Pharmacol. 43, 609–625.