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The effects of cyclopentane and cyclopentene analogues of GABA at recombinant $GABA_C$ receptors

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Abstract

The pharmacological effects of the enantiomers of *cis*-3-aminocyclopentanecarboxylic acids ((+)- and (−)-CACP), the enantiomers of *trans*-3-aminocyclopentanecarboxylic acids ((+)- and (−)-TACP), and the enantiomers of 4-aminocyclopent-1-ene-1-carboxylic acids ((+)- and (−)-4-ACPCA) were studied on human homomeric ρ_1 and ρ_2 $GABA_C$ receptors expressed in *Xenopus* oocytes using two-electrode voltage clamp methods. These compounds are conformationally restricted analogues of γ-aminobutyric acid (GABA) held in a five-membered ring. (+)-TACP (EC_{50} (ρ_1) = 2.7 ± 0.2 μM; EC_{50} (ρ_2) = 1.45 ± 0.22 μM), (+)-CACP (EC_{50} (ρ_1) = 26.1 ± 1.1 μM; EC_{50} (ρ_2) = 20.1 ± 2.1 μM) and (−)-CACP (EC_{50} (ρ_1) = 78.5 ± 3.5 μM; EC_{50} (ρ_2) = 63.8 ± 23.3 μM) were moderately potent partial agonists at ρ_1 and ρ_2 $GABA_C$ receptors, while (−)-TACP (100 μM inhibited 56% and 62% of the current produced by 1 μM GABA at ρ_1 and ρ_2 receptors, respectively) was a weak partial agonist with low intrinsic activity at these receptors. In contrast, (+)-4-ACPCA (K_i (ρ_1) = 6.0 ± 0.1 μM; K_i (ρ_2) = 4.7 ± 0.3 μM) did not activate $GABA_C$ ρ_1 and ρ_2 receptors but potently inhibited the action of GABA at these receptors, while (−)-4-ACPCA had little effect as either an agonist or an antagonist. The affinity order at both $GABA_C$ ρ_1 and ρ_2 receptors was (+)-TACP > (+)-4-ACPCA ≫ (+)-CACP > (−)-CACP ≫ (−)-TACP ≫ (−)-4-ACPCA. This study shows that the cyclopentane and cyclopentene analogues of GABA affect $GABA_C$ receptors in a unique manner, defining a preferred stereochemical orientation of the amine and carboxylic acid groups when binding to $GABA_C$ receptors. This is exemplified by the partial agonist, (+)-TACP, and the antagonist, (+)-4-ACPCA.

Keywords: γ-Aminobutyric acid (GABA); $GABA_C$ receptor; Structure–activity relationship; Cyclopentane and cyclopentene analogues of GABA; *Xenopus* oocyte

1. Introduction

γ-Aminobutyric acid (GABA) receptors are ubiquitous throughout the mammalian central nervous system (CNS). Three major classes of receptors have been identified and termed: $GABA_A$, $GABA_B$ and $GABA_C$ receptors. $GABA_A$ and $GABA_C$ receptors are members of the nicotinicoid superfamily of receptors that include nicotinic acetylcholine, strychnine-sensitive glycine, serotonin type 3 and some invertebrate anionic glutamate receptors. Both $GABA_A$ and $GABA_C$ receptors are Cl^- ion channels producing fast synaptic inhibition when activated by GABA (Fig. 1). In contrast, $GABA_B$ receptors are members of the G-protein coupled receptor superfamily. These receptors produce slow, longer-lasting inhibition and function to inhibit neurotransmitter release (see reviews, Kerr and Ong, 1995). All three classes of GABA receptors are pharmacologically, physiologically and biochemically distinct (see reviews, Bormann, 2000; Chebib and Johnston, 2000).

$GABA_C$ receptors are not blocked by the alkaloid, bicuculline, nor modulated by benzodiazepines, barbiturates and steroids, which typically affect $GABA_A$ receptors, nor are they activated by (−)-baclofen or inhibited by (−)-phaclofen, which typically affect $GABA_B$ receptors. Instead, $GABA_C$ receptors are selectively activated by (+)-*cis*-2-aminomethylcyclopropanecarboxylic acid ((+)-CAMP) (Fig. 1; Duke et al., 2000) and blocked by (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid (TPMPA) (Fig. 1; Murata et al., 1996; Ragozzino et al., 1996).

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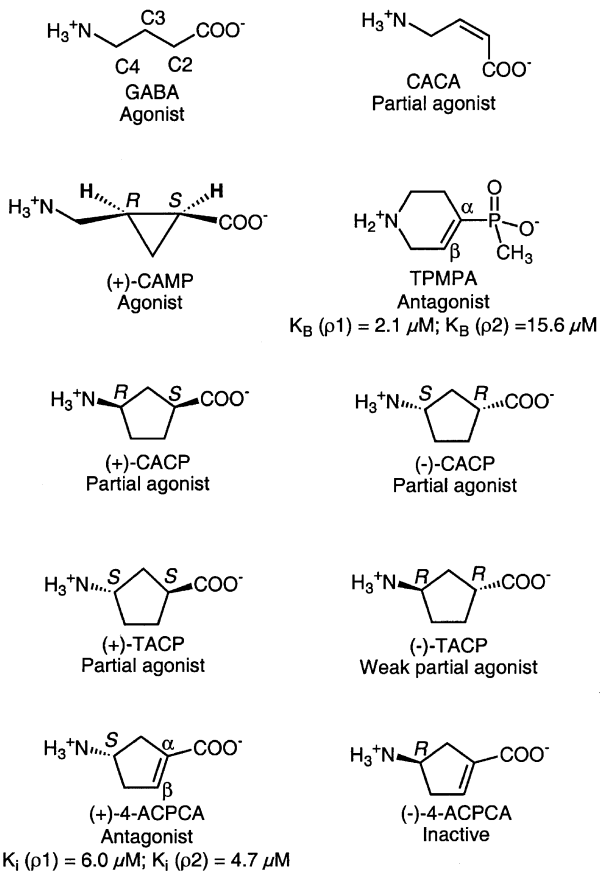

Fig. 1. Structures of key GABA analogues that show agonist, partial agonist and antagonist effects at $GABA_C$ receptors.

$GABA_C$ receptors are believed to be homomeric or pseudoheteromeric pentamers comprising ρ-subunits. A number of ρ-subunits have been cloned including two from human (ρ_1 and ρ_2) (Cutting et al., 1991, 1992), three from rat (ρ_{1-3}; Wang et al., 1994; Zhang et al., 1995; Ogurusu et al., 1995; Ogurusu and Shingai, 1996) and five from perch (ρ_{1-5}; Qian et al., 1997). The human ρ-subunits do not assemble with the classical α and β subunits of the $GABA_A$ receptor (Hackam et al., 1998), although some evidence exists for heteromeric assembly of perch ρ-subunits with the γ2-subunit of the $GABA_A$ receptor (Qian and Ripps, 1999). In general, $GABA_C$ receptors are formed from either ρ_1 or ρ_2 subunits or a mixture of ρ_1 and ρ_2 subunits when expressed in vitro, such as *Xenopus leavis* oocytes (Kusama et al., 1993a,b; Chebib et al., 1997a,b, 1998; Duke et al., 2000) or mammalian cell expression systems (Enz and Bormann, 1995; Enz and Cutting, 1998). These recombinant receptors have similar physiological and pharmacological properties to $GABA_C$ receptors found on native cells, indicating that these combinations may exist in vivo.

$GABA_C$ receptor responses have been recorded from a number of neurones including rod bipolar cells from rat retina (Feigenspan et al., 1993) and thyrotropin-secreting cells in the pituitary gland of the rat and guinea pig (Boue-Grabot et al., 2000). $GABA_C$ receptors detected on rod bipolar cells in the rat retina were insensitive to both bicuculline and baclofen but were activated by *cis*-4-aminocrotonic acid (CACA, Fig. 1) after co-application of GABA with bicuculline (100 μM) to abolish the $GABA_A$ component (Feigenspan et al., 1993). Electrophysiological recordings on thyrotropin-secreting cells, showed that GABA induced a bicuculline-insensitive Cl^- current that was readily desensitised (Boue-Grabot et al., 2000), indicating that $GABA_C$-like receptors also exist on thyrotropin-secreting cells in the pituitary glands of rat and guinea pig (Boue-Grabot et al., 2000). Further studies using northern blot and RT-PCR in the pituitary indicated that this area is enriched with ρ_1 mRNA (Boue-Grabot et al., 2000).

Structure–activity relationship studies on $GABA_C$ receptors have been carried out in a variety of systems including bovine retinal poly$(A)^+$ RNA expressed in *Xenopus* oocytes (Woodward et al., 1993) and human homooligomeric ρ_1 and ρ_2 cRNAs expressed in *Xenopus* oocytes (Kusama et al., 1993a,b; Ragozzino et al., 1996; Chebib et al., 1997a,b, 1998; Duke et al., 2000). These studies have led to the discovery of a variety of useful compounds, including TPMPA (Fig. 1; K_B $(\rho_1) = 2.1$ μM; Murata et al., 1996; Ragozzino et al., 1996) and (+)-CAMP (Fig. 1; K_D $(\rho_1) = 40$ μM and K_D $(\rho_2) = 17$ μM; Duke et al., 2000), that are used as pharmacological tools to study $GABA_C$ receptors.

TPMPA was the first selective $GABA_C$ receptor antagonist that differentiated $GABA_A$ and $GABA_B$ receptors from $GABA_C$ receptors. TPMPA is at least 100 times more potent as an antagonist at $GABA_C$ ρ_1 receptors than at $GABA_A$ receptors and is 500 times more potent at $GABA_C$ ρ_1 receptors than at $GABA_B$ receptors (Murata et al., 1996; Ragozzino et al., 1996). However, at $GABA_C$ ρ_2 receptors, TPMPA was approximately 8 times weaker than at $GABA_C$ ρ_1 receptors (Chebib et al., 1998).

(+)-CAMP was shown to be a selective agonist at both ρ_1 and ρ_2 $GABA_C$ receptors while its enantiomer, (−)-CAMP was a weak antagonist at these receptors (Duke et al., 2000). This compound appears to be a better tool than CACA because it had little effect on GABA transporters (Duke et al., 2000), while CACA was shown to be a substrate for a GABA transporter that was sensitive to nipecotic acid and β-alanine (Chebib and Johnston, 1997).

CACA and (+)-CAMP are conformationally restricted analogues of GABA, restricted about the C2 and C3 positions (Fig. 1). These compounds have been very useful in studying the conformation(s) of GABA that bind to $GABA_C$ receptors, and in identifying GABA receptor heterogeneity. However, these compounds are still quite flexible about the C4 position, allowing the aminomethyl group to freely rotate. This makes it difficult to predict the position of the amine group when it binds to the agonist/antagonist binding site of the $GABA_C$ receptor.

By restricting the C2 and C4 positions of GABA in the form of a five-membered ring, one can determine the stereochemical orientation of both the amine and carboxylic acid moieties, which preferentially bind to $GABA_C$ receptors. Such compounds include the cyclopentane and cyclopentene analogues of GABA, *cis*-3-aminocyclopentanecarboxylic acids (CACP), *trans*-3-aminocyclopentanecarboxylic acids (TACP) and 4-aminocyclopent-1-ene-1-carboxylic acids (4-ACPCA). These compounds have little flexibility. They are chiral, and resolving the enantiomeric pairs of these compounds allows one to study the position of both the amine and carboxylic acid moieties required for binding to the agonist/antagonist binding site of the $GABA_C$ receptor. Although these compounds are known to have a number of pharmacological actions on GABA systems such as inhibiting the firing of cat spinal interneurons in a bicuculline sensitive manner (Johnston et al., 1979), affecting $GABA_A$ receptors both centrally and in the periphery (Allan et al., 1986), inhibiting GABA transaminase (Qiu and Silverman, 2000) and inhibiting GABA transport (Allan et al., 1986), their effects on $GABA_C$ receptors are unknown. Such studies are required, as much of the early work evaluating the effects of CACP and TACP on inhibiting the firing of cat spinal interneurons indicates that not all the effects could be completely blocked by bicuculline (Johnston et al., 1979). In this study, we show that the enantiomers of CACP, (+)- and (−)-CACP (Fig. 1), the enantiomers of TACP, (+)- and (−)-TACP (Fig. 1), and the enantiomers of 4-ACPCA, (+)- and (−)-4-ACPCA (Fig. 1) also affect $GABA_C$ receptors. These studies contribute to the structure–activity profiles of $GABA_C$ receptors and provide leads for the design and development of selective $GABA_C$ receptor ligands.

2. Materials and methods

2.1. Materials

(+)- and (−)-CACP, (+)- and (−)-TACP, (+)- and (−)-4-ACPCA were prepared according to the literature (Allan and Twitchin, 1980; Allan and Fong, 1986). GABA was purchased from Sigma (St. Louis, MO, USA). Human ρ_1 cDNA subcloned into pcDNA1.1 (Invitrogen, San Diego, CA, USA) was kindly provided by Dr. George Uhl (National Institute for Drug Abuse, Baltimore, MD, USA). Human ρ_2 cDNA subcloned in pKS (Invitrogen) was a gift from Dr. Garry Cutting (Center for Medical Genetics, Johns Hopkins University School of Medicine, Baltimore, MD, USA).

2.2. Electrophysiological recording

X. laevis were anaesthetised with 0.17% ethyl 3-aminobenzoate and a lobe of the ovaries was removed. The lobe of the ovary was rinsed with OR2 (82.5 mM NaCl, 2 mM KCl, 1 mM $MgCl_2.6H_2O$, 5 mM HEPES, pH 7.5) and treated with collagenase A (2 mg/ml in OR2, Bohringer Manheim) for 2 h. Released oocytes were then rinsed in frog Ringer solution (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2 \cdot 6H_2O$, 1.8 mM $CaCl_2$, 5 mM HEPES, pH 7.5) supplemented with 2.5 mM pyruvate, 0.5 mM theophylline and 50 μg/ml gentamycin, and stage V–VI oocytes were collected.

Human ρ_1 cDNA subcloned in pcDNA1.1 and ρ_2 cDNA subcloned in pcKS were linearised using the restriction enzymes, *Xba*1 and *ECO*RV, respectively. Capped RNAs were synthesised from linearised plasmid containing ρ_1 and ρ_2 cDNAs using the "mMESSAGE mMACHINE" kit

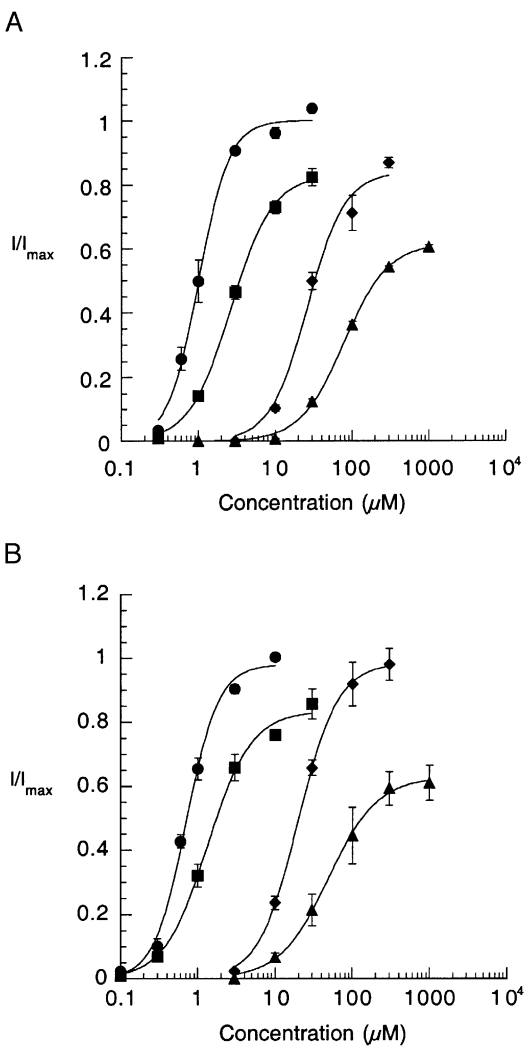

Fig. 2. (A) Agonist dose response curves for GABA (●), (+)-TACP (■), (+)-CACP (♦) and (−)-CACP (▲) at human $GABA_C$ ρ_1 receptors expressed in *Xenopus* oocytes. Data are the mean ± S.E.M. ($n = 3–6$ oocytes). (B) Agonist dose response curves for GABA (●), (+)-TACP (■), (+)-CACP (♦) and (−)-CACP (▲) at human $GABA_C$ ρ_2 receptors expressed in *Xenopus* oocytes. Data are the mean ± S.E.M. ($n = 3–6$ oocytes).

Table 1
Effects of (+)- and (−)-TACP, (+)- and (−)-CACP and (+)- and (−)-4-ACPCA at $GABA_C$ receptors expressed in *Xenopus* oocytes

Compound	Human $GABA_C$ ρ_1 receptors			Human $GABA_C$ ρ_2 receptors		
	EC_{50} (μM)[a]	n_H[b]	Im (%)[c]	EC_{50} (μM)[a]	n_H[b]	Im (%)[c]
GABA	1.01 ± 0.06	2.2 ± 0.3	100	0.48 ± 0.03	1.7 ± 0.2	100
(+)-TACP	2.7 ± 0.2	1.6 ± 0.1	83 ± 2	1.45 ± 0.22	1.7 ± 0.4	85 ± 5
(+)-CACP	26.1 ± 1.1	2.0 ± 0.1	84 ± 6	20.1 ± 2.1	1.8 ± 0.2	99 ± 7
(−)-CACP	78.5 ± 3.6	1.5 ± 0.1	62 ± 1	63.8 ± 23.3	1.5 ± 0.3	65 ± 3
(−)-TACP (100 μM)	2% activation (56% blockade of 1 μM GABA)			4.5% activation (62% blockade of 1 μM GABA)		
(+)-4-ACPCA	K_i = 6.0 ± 0.1 μM[d]			K_i = 4.7 ± 0.3 μM[d]		
(−)-4-ACPCA (100 μM)	1% activation			no effect as agonist or antagonist		

[a] EC_{50} is the concentration that activates 50% of receptors. Data are the mean ± S.E.M. (n = 3–6 oocytes).
[b] n_H is the Hill coefficient. Data are the mean ± S.E.M. (n = 3–6 oocytes).
[c] Im is the intrinsic activity calculated as a percentage of the maximum whole cell current produced by a maximum dose of GABA which is assigned as 100%. Data are the mean ± S.E.M. (n = 3–6 oocytes).
[d] K_i is the inhibition constant of the antagonist. Data are the mean ± S.E.M. (n = 3–6 oocytes).

from Ambion (Austin, TX, USA). Approximately 10 ng/50 nl of ρ_1 cRNA and 50 ng/50 nl of ρ_2 cRNA were injected into defolliculated stage V–VI *Xenopus* oocytes. Oocytes containing ρ_1 and ρ_2 cRNAs were stored at 18 °C. Two to ten days later, receptor activity was measured by two-electrode voltage clamp recordings using a Geneclamp 500 amplifier (Axon Instruments, Foster City, CA, USA), a MacLab 2e recorder (AD Instruments, Sydney, NSW, Australia), and the program, Chart version 3.5. Oocytes injected with ρ_1 cRNAs were voltage clamped at

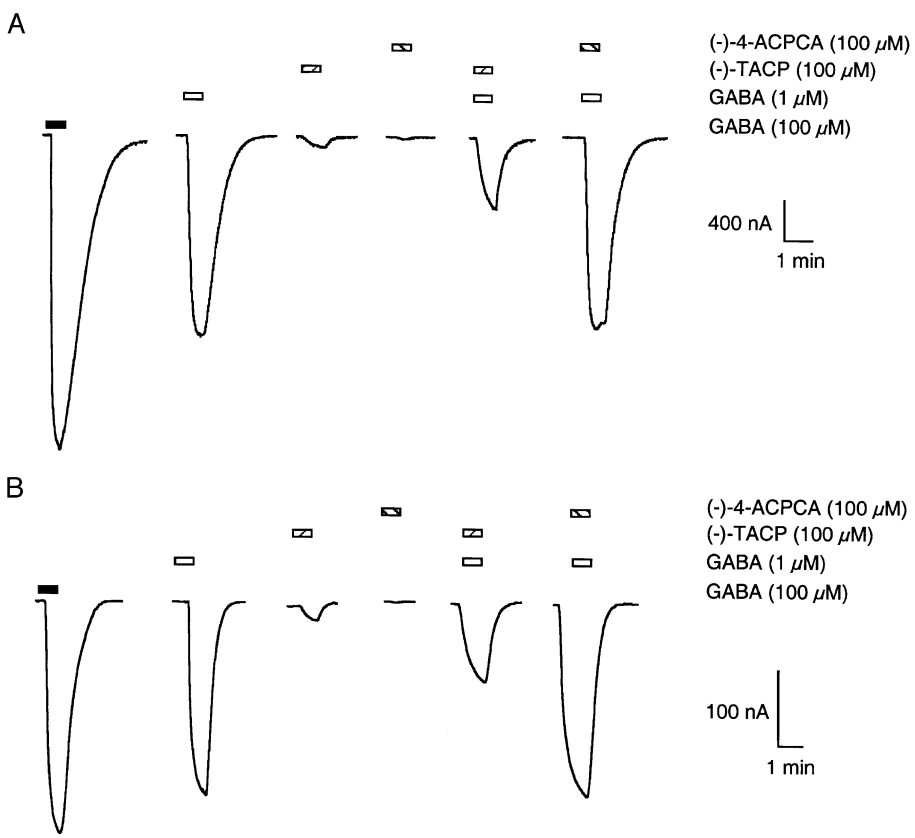

Fig. 3. (A) A maximal current is achieved by the addition of GABA (100 μM; duration indicated by solid bar) on *Xenopus* oocytes expressing human $GABA_C$ ρ_1 receptors. A submaximal current is achieved by the addition of GABA (1 μM; duration indicated by open bar) on the same oocyte. (−)-TACP (100 μM; duration indicated by the forward hatched bar) produced 2% of the total current but in the presence of GABA (1 μM) inhibited the response by 56%. (−)-4-ACPCA (100 μM; duration indicated by the backward hatched bar) had little effect alone and in the presence of GABA (1 μM). (B) A maximal current is achieved by the addition of GABA (100 μM; duration indicated by solid bar) on *Xenopus* oocytes expressing human $GABA_C$ ρ_2 receptors. A submaximal current is achieved by the addition of GABA (1 μM; duration indicated by open bar) on the same oocyte. (−)-TACP (100 μM; duration indicated by the forward hatched bar) produced 4.5% of the total current but in the presence of GABA (1 μM) inhibited the response by 62%. (−)-4-ACPCA (100 μM; duration indicated by the backward hatched bar) had little effect alone and in the presence of GABA (1 μM).

−60 mV while oocytes injected with ρ_2 were voltage clamped at −100 mV, and continuously superfused with frog Ringer solution. For receptor activation measurements, the indicated concentrations of drug were added to the buffer solution.

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 maximum current, EC_{50} is the effective concentration that activates 50% of the maximum current produced by a given drug and n_H is the Hill coefficient. EC_{50} values are expressed as mean ± S.E.M. ($n = 3$–6 oocytes) and are determined by fitting data from individual oocytes using Kaleidagraph 3.0 (1993). The intrinsic activity of partial agonists, I_m, is calculated as a percentage of the maximum whole cell current produced by a maximum dose of GABA. K_i values are the inhibitory constants for the antagonists and were determined using the following equation $\log\{(A)/(A^*) - 1\} = \log[\text{Ant}] - \log K_i$, 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 the antagonist and [Ant] is the concentration of the antagonist. K_i values are expressed as mean ± S.E.M. ($n = 3$–6 oocytes).

2.4. Molecular modelling

A computer-assisted study was carried out on (+)-CAMP, (+)- and (−)-CACP, (+)- and (−)-TACP, (+)- and (−)-4-ACPCA using Chem-3D Plus (Cambridge Scientific Computing, Cambridge, MA, USA) to determine the conformation of the ligands that may bind to the receptor site. The three-dimensional matrices of the compounds were optimised using the molecular mechanics optimisation routines in Chem-3D Plus. (+)- and (−)-CACP, (+)- and (−)-TACP, (+)- and (−)-4-ACPCA, were fitted against the antagonist, (+)-CAMP by superimposing the amine and the acidic groups of each compound.

3. Results

Expression of the human ρ_1 and ρ_2 cRNAs in *X. laevis* oocytes generated GABA gated ion channels with similar pharmacological profiles as previously described for $GABA_C$ receptors (Chebib et al., 1997a,b, 1998; Duke et al., 2000). With $GABA_C$ ρ_2 receptors, currents of 30–600 nA were recorded when the oocytes were held at −100 mV, whereas with $GABA_C$ ρ_1 receptors, currents of 200–2000 nA were recorded when the oocytes were held at −60 mV.

Agonist dose response curves for human $GABA_C$ ρ_1 and ρ_2 receptors expressed in oocytes are shown in Fig. 2(A) and (B), respectively. The EC_{50} values, intrinsic activity (I_{max}, percentage of the maximal response of the agonist compared to the maximal response of GABA) and Hill coefficients (n_H) of all compounds in this study are summarised in Table 1. The EC_{50} and n_H of GABA were similar to the values reported for $GABA_C$ ρ_1 and ρ_2 receptors expressed in *Xenopus* oocytes (Kusama et al., 1993a,b). The effects of (+)- and (−)-CACP, (+)-and (−)-TACP, and (+)- and (−)-4-ACPCA are summarised in Table 1.

(+)-CACP is a moderately potent partial agonist at $GABA_C$ ρ_1 receptors ($I_{max} < 100\%$) and a moderately potent agonist, with similar affinity, at $GABA_C$ ρ_2 receptors ($I_{max} \approx 100\%$). It is approximately 26- and 42-times less active than GABA at $GABA_C$ ρ_1 and ρ_2 receptors, respectively. (−)-CACP is also a moderately potent partial agonist ($I_{max} < 100\%$) albeit 3 times weaker than (+)-CACP at both $GABA_C$ ρ_1 and ρ_2 receptors.

(+)-TACP is a more potent partial agonist than (+)-CACP at both $GABA_C$ ρ_1 and ρ_2 receptors ($I_{max} < 100\%$; Table 1). It is only 2.5-times less potent than GABA at $GABA_C$ ρ_1 receptors and only 3 times less potent than GABA at $GABA_C$ ρ_2 receptors. In contrast, (−)-TACP showed only weak activation of these receptors. (−)-TACP

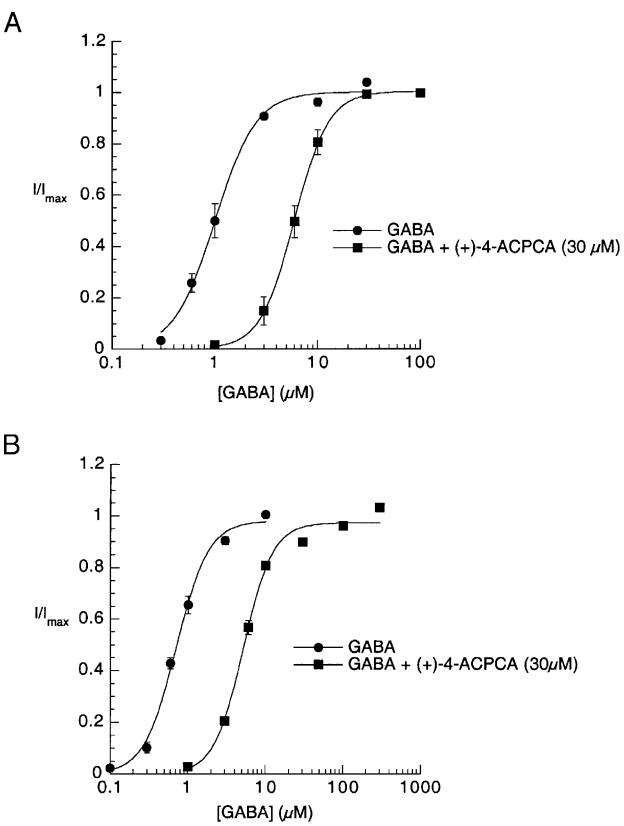

Fig. 4. Dose response curves of (A) GABA alone (●) and GABA in the presence of 30 μM (+)-4-ACPCA (■) at human $GABA_C$ ρ_1 receptors expressed in *Xenopus* oocytes. Data are mean ± S.E.M. ($n = 3$–6 oocytes); and (B) GABA alone (●) and GABA in the presence of 30 μM (+)-4-ACPCA (■) at human $GABA_C$ ρ_2 receptors expressed in *Xenopus* oocytes. Data are the mean ± S.E.M. ($n = 3$–6 oocytes).

(100 μM) activated the $GABA_C$ ρ_1 receptor by approximately 2% and the $GABA_C$ ρ_2 receptor by approximately 5% (Fig. 3(A) and (B), respectively). Furthermore, (−)-TACP (100 μM) blocked the effects of 1 μM GABA by 56% at $GABA_C$ ρ_1 and by 62% at $GABA_C$ ρ_2 receptors. This indicates that (−)-TACP is a weak partial agonist with low intrinsic activity.

Interestingly, (+)-4-ACPCA was a potent antagonist at both human $GABA_C$ ρ_1 and ρ_2 receptors (Fig. 4(A) and (B), respectively). In the presence of GABA, (+)-4-ACPCA (30 μM) shifts the agonist dose response curve to the right in a parallel manner indicating competitive antagonism at the concentration tested. In contrast, (−)-4-ACPCA (100 μM) had little effect alone and in the presence of GABA (1 μM) at both $GABA_C$ ρ_1 and ρ_2 receptors (Fig. 3(A) and (B), respectively).

4. Discussion

In this study, we investigated the effects of the enantiomers of CACP, (+)- and (−)-CACP, the enantiomers of TACP, (+)- and (−)-TACP, and the enantiomers of 4-ACPCA, (+)- and (−)-4-ACPCA on $GABA_C$ ρ_1 and ρ_2 receptors expressed in *Xenopus* oocytes using two-electrode voltage clamp methods. The affinity order of these compounds as agonists and antagonists was the same at both $GABA_C$ ρ_1 and ρ_2 receptors; (+)-TACP > (+)-4-ACPCA ≫ (+)-CACP > (−)-CACP ≫ (−)-TACP > (−)-4-ACPCA. This was similar to what was observed by Allan et al. (1986) on $GABA_A$ receptors using guinea pig ileum. However, the pharmacological properties of these compounds were different to their effects on $GABA_A$ receptors.

At $GABA_A$ receptors, (+)- and (−)-CACP, (+)- and (−)-TACP, (+)- and (−)-4-ACPCA were all agonists (Allan et al., 1986). At $GABA_C$ receptors, (+)- and (−)-CACP, and (+)-TACP were potent partial agonists, while (−)-TACP was a weak partial agonist. Furthermore, (+)-4-ACPCA was a potent antagonist while (−)-4-ACPCA had little effect at $GABA_C$ receptors. (+)-4-ACPCA is the most potent antagonist possessing a carboxylic acid moiety at $GABA_C$ receptors. It is approximately equipotent at $GABA_C$ ρ_1 and ρ_2 receptors, while TPMPA has some selectivity for $GABA_C$ ρ_1 over ρ_2 receptors. Structurally, (+)-4-ACPCA and TPMPA are similar in that they are α,β-unsaturated acids, the double bond of each compound being in the same position (Fig. 1). Therefore, methylphosphinic acid analogues of (+)-4-ACPCA may lead to potent, subtype selective $GABA_C$ receptor antagonists.

A number of compounds exist like (+)-4-ACPCA that are agonists at $GABA_A$ receptors and antagonists at $GABA_C$ receptors including piperidine-4-sulphonic acid (P4S), 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP) and (*Z*)-3-[(aminoiminomethyl)thio]prop-2-enoic acid (ZAPA). Such pharmacological differences have been shown to depend on the subunit combinations particularly at $GABA_A$ receptors (Ebert et al., 1994). Thus, P4S, THIP, ZAPA and now (+)-4-ACPCA are important pharmacological tools because they can distinguish functionally between $GABA_A$ and $GABA_C$ receptors.

It is clear from this study that there exists a particular stereochemical relationship between the amine and carboxylic acid moieties in their interactions with GABA receptors. (+)-TACP and (+)-4-ACPCA exhibit higher affinity for $GABA_C$ ρ_1 and ρ_2 receptors than the corresponding enantiomers, (−)-TACP and (−)-4-ACPCA. This is similar to that observed at $GABA_A$ receptors where (+)-TACP and (+)-4-ACPCA are approximately 200- and 600-times more potent as agonists than the corresponding enantiomers, (−)-TACP and (−)-4-ACPCA, respectively.

In contrast, the stereochemical relationship between the amino and carboxylic acid moieties at GABA transporters is opposite to that observed at $GABA_A$ receptors and $GABA_C$ receptors. At GABA transporters, (−)-TACP and (−)-4-ACPCA were both approximately 300 times more potent at inhibiting GABA transport than the corresponding enantiomers, (+)-TACP and (+)-4-ACPCA, respectively. Thus, the stereochemical relationship between the amine and carboxylic acid moieties can be used to predict $GABA_A$ and $GABA_C$ receptor activity versus effects at GABA transporters.

Fig. 5 shows the superimposition of (+)- and (−)-CACP (Fig. 5(A) and (D), respectively), (+)- and (−)-TACP (Fig. 5(B) and (E), respectively), (+)- and (−)-4-ACPCA (Fig. 5(C) and (F), respectively) against (+)-CAMP, a selective $GABA_C$ receptor agonist. These superimpositions may indicate how compounds bind to $GABA_C$ receptors. In many cases, receptor binding of compounds is stereoselective and thus, the orientation of pharmacophores is important for selectivity and affinity for the receptor. (−)-CACP has a lower affinity for $GABA_C$ receptors than (+)-CACP. The amine and carboxylic acid moieties of (−)-CACP are in the opposite orientation to (+)-CACP, lowering its activity for $GABA_C$ receptors. Similarly, the amine and carboxylic acid moieties of (−)-TACP are in the opposite direction to (+)-TACP and therefore exhibits weaker binding at the agonist/antagonist binding site of the $GABA_C$ receptors. As (−)-TACP is a weak partial agonist, the area where the cyclopentane ring system lies when it binds to the receptor (Fig. 5(E)) may indicate an area of steric interaction. Similar observations were noted with (+)- and (−)-CAMP, where (+)-CAMP was the agonist and (−)-CAMP was the antagonist (Duke et al., 2000), indicating an area of steric interaction between the cyclopropyl group of (−)-CAMP and the receptor binding site. This area of steric interaction encountered by the cyclopentane ring of (−)-TACP is believed to be the same area as the area encountered by the cyclopropyl group of (−)-CAMP.

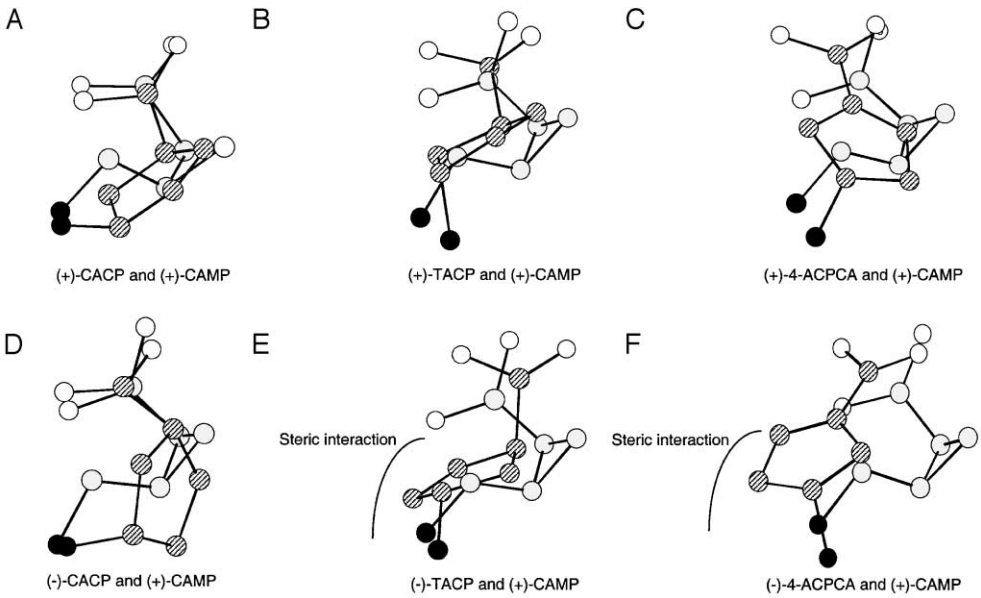

Fig. 5. The superimposition of (A) (+)-CACP (hatched circles), (B) (+)-TACP (hatched circles), (C) (+)-4-ACPCA (hatched circles), (D) (−)-CACP (hatched circles), (E) (−)-TACP (hatched circles), and (F) (−)-4-ACPCA (hatched circles) against (+)-CAMP (open circles), a selective $GABA_C$ receptor agonist. The amine and carboxylic acid moieties of (−)-CACP are in the opposite orientation to (+)-CACP, but are still able to activate $GABA_C$ receptors albeit 3 times weaker. Similarly, the amine and carboxylic acid moieties of (−)-TACP are in the opposite direction to (+)-TACP and therefore exhibit weaker effects at $GABA_C$ receptors. The area occupied by the cyclopentane ring of (−)-TACP may indicate an area of steric interaction. In the case of (−)-4-ACPCA, the amine moiety is in the opposite direction to (+)-4-ACPCA and as this compound has little activity at $GABA_C$ receptors; it is proposed that the cyclopentene ring of (−)-4-ACPCA may map an area of steric interaction. This area is equivalent to the area of steric interaction identified from studies using (−)-CAMP and (+)-CAMP (Duke et al., 2000).

(−)-4-ACPCA also had little effect at $GABA_C$ receptors. This can be explained, in part, by the fact that the amine group is in the opposite orientation to the amine group of (+)-4-ACPCA. This indicates that the amine group cannot interact with the binding site. To bind to the $GABA_C$ receptor, the cyclopentane group lies in a similar area to that occupied by the cyclopentane ring of (−)-TACP, which may map an area of steric interaction with the receptor (Fig. 5(F)). This area appears to be similar to the area occupied by the cyclopropyl ring of (−)-CAMP (Duke et al., 2000).

Radiolabelled (+)-TACP has been shown to bind to rat brain membranes with a lower binding density than radiolabelled muscimol, indicating that only a smaller number of GABA receptors is labelled with this compound (Dickenson et al., 1990). Furthermore, (+)-TACP bound to both a high (nanomolar range) and low affinity (micromolar range) binding site. The high affinity binding site was shown to be inhibited by bicuculline, CACA, which has been shown to be a selective $GABA_C$ receptor partial agonist, and $GABA_A$ receptor agonists, including muscimol, TACA, ZAPA, isoguvacine and THIP, which have now been shown to also affect $GABA_C$ receptors (Dickenson et al., 1990; Chebib and Johnston, 2000). Furthermore, the binding of radiolabelled (+)-TACP was not inhibited by the $GABA_B$ receptor agonist, baclofen. These results indicate that (+)-TACP binds with high affinity to a subset of $GABA_A$ receptors (Dickenson et al., 1990), which, as yet, have not been identified, and also to $GABA_C$ receptors in rat brain membranes.

In conclusion, the pharmacological profile of these compounds at the $GABA_C$ receptor is different from GABA transporters and $GABA_A$ receptors. This is highlighted by the fact that (+)-4-ACPCA is an antagonist at $GABA_C$ receptors while an agonist at $GABA_A$ receptors. Furthermore, there is a distinct stereochemical preference for the amine and carboxylic acid moieties when binding to $GABA_C$ receptors as indicated by preferred *S*-configuration of the amino and carboxylic acid groups of (+)-TACP and the *S*-configuration of the amino group of (+)-4-ACPCA. This preference is similar to that observed at $GABA_A$ receptors but is the opposite to that for GABA transporters. These studies contribute to the structure–activity profiles of $GABA_C$ receptors and provide leads for the design and development of selective $GABA_C$ receptor ligands.

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