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Enantioselective actions of 4-amino-3-hydroxybutanoic acid and (3-amino-2-hydroxypropyl)methylphosphinic acid at recombinant $GABA_C$ receptors

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Abstract—The *R*- and *S*-enantiomers of 4-amino-3-hydroxybutanoic acid (GABOB) were full agonists at human recombinant ρ1 $GABA_C$ receptors. Their enantioselectivity (*R* > *S*) matched that reported for their agonist actions at $GABA_B$ receptors, but was the opposite to that reported at $GABA_A$ receptors (*S* > *R*). The corresponding methylphosphinic acid analogues proved to be ρ1 $GABA_C$ receptor antagonists with *R*(+)-CGP44533 being more potent than *S*(−)-CGP44532, thus showing the opposite enantioselectivity to the agonists *R*(−)- and *S*(+)-GABOB. These studies highlight the different stereochemical requirements for the hydroxy group in these analogues at $GABA_A$, $GABA_B$ and $GABA_C$ receptors.

Receptors for GABA, the principal mediator of neuronal inhibition in mammalian brain, may be grouped into three major classes: $GABA_A$, $GABA_B$ and $GABA_C$ receptors.[1] $GABA_A$ and $GABA_C$ receptors belong to the nicotinicoid superfamily of ligand-gated ion channels,[2] while $GABA_B$ receptors are G-protein coupled receptors.[3] GABA is a conformationally flexible molecule that acts on receptors in a chiral environment and thus enantiomers of GABA analogues might be expected to show some enantioselective actions where the chiral environments confer some preference for one enantiomer over another.[4,5] Extensive studies have been carried out on the enantioselective actions of GABA analogues on $GABA_A$ and $GABA_B$ receptors,[4,6–8] while only limited studies have been made on enantioselective actions on $GABA_C$ receptors.[9–11] $GABA_C$ receptors are drug targets for the treatment of visual, sleep and cognitive disorders.[12]

In the present study, we examine the effects of enantiomers of 4-amino-3-hydroxybutanoic acid (GABOB) and (3-amino-2-hydroxypropyl)methylphosphinic acid (*S*(−)-CGP44532 and *R*(+)-CGP44533) on human ρ1 $GABA_C$ receptors expressed in *Xenopus* oocytes.[13] The structures of these enantiomers are shown in Figure 1. As noted by other authors, the carboxylic and phosphinic acids have opposite *R* and *S* assignments because in the Cahn Ingold Prelog nomenclature the phosphinic acid assumes a higher priority than the carboxylic acid.[7,8] Studies on $GABA_A$ receptors have reported *S*(+)-GABOB to be about twice as potent *R*(−)-GA-

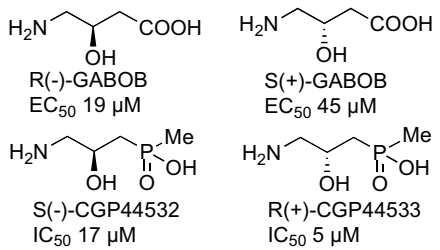

Figure 1. Structures of *R*(−)- and *S*(+)-GABOB, *S*(−)-CGP44532 and *R*(+)-CGP44533 together with their EC_{50} values as agonists or their IC_{50} values as antagonists as human recombinant ρ1 receptors expressed in *Xenopus* oocytes.

Keywords: GABA; γ-aminobutyric acid: GABOB, 3-hydroxyGABA; CGP44532 *S*(−)-(3-amino-2-hydroxypropyl)methylphosphinic acid; CGP44533 *R*(+)-(3-amino-2-hydroxypropyl)methylphosphinic acid; $GABA_C$ receptors; Oocytes.
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BOB as an agonist,[6,14] while on $GABA_B$ receptors *R*(−)-GABOB is the more potent agonist[14] with *S*(+)-GABOB acting as a partial agonist.[8] *S*(−)-CGP44532 and *R*(+)-CGP44533 have no reported action on $GABA_A$ receptors. They are agonists at $GABA_B$ receptors with *S*(−)-CGP44532 being more potent than *R*(+)-CGP44533 consistent with the enantioselectivity shown by GABOB.[7] The relative potency of *S*(−)-CGP44532 and *R*(+)-CGP44533 varies in different $GABA_B$ receptors suggesting that they may distinguish between different subtypes of $GABA_B$ receptors.[8]

S(+)-GABAOB and *R*(−)-GABOB acted as full agonists as shown in Figure 2. Dose–response analyses were conducted on three cells, with both drugs being tested on the same cell at concentrations of 0.3, 1, 3, 10, 30, 100, 300 and 600 μM. The EC_{50}s determined from these curves were 44.8 ± 7.4 μM for *S*(+)-GABOB and 19.1 ± 3.3 μM for *R*(−)-GABOB. A two-way paired *t*-test showed that the EC_{50}s were significantly different ($t_{0.025,2} = 6.795$, $p = 0.021$). The EC_{50} for GABA under these conditions was 1.19 ± 0.02 μM.[11]

In contrast to the enantiomers of GABOB, the corresponding methylphosphinic acid analogues showed no agonist activity and proved to be ρ1 $GABA_C$ receptor antagonists when tested on further three cells. *R*(+)-CGP44533, an analogue of *S*(+)-GABOB, was more potent than *S*(−)-CGP44532, an analogue of *R*(−)-GABOB, with IC_{50}s of 5.4 ± 0.5 and 17.1 ± 1.2 μM respectively. A two-way paired *t*-test showed that the EC_{50}s were significantly different ($t_{0.025,2} = 7.833$, $p = 0.0014$) (see Fig. 3).

Thus the enantiomers of GABOB were full agonists at ρ1 $GABA_C$ receptors while *S*(−)-CGP44532 and *R*(+)-CGP44533 were antagonists. Furthermore, the GABOB enantiomers showed the opposite enantiomeric preference of *R*(−) > *S*(+) to that shown by the methylphosphinates *R*(+) > *S*(−).

The enantioselectivity (*R* > *S*) of the enantiomers of GABOB in their agonist action at human recombinant ρ1 $GABA_C$ receptors found in the present study matched that reported for their agonist actions at

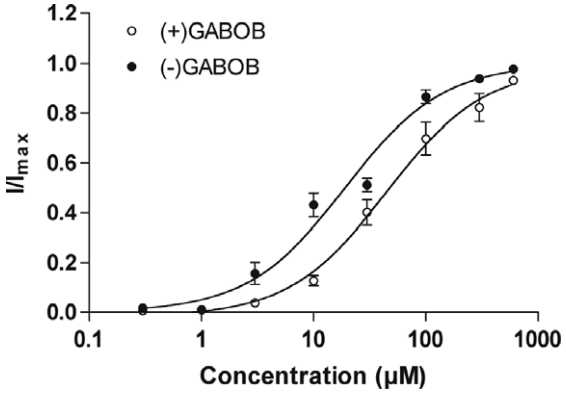

Figure 2. Dose–response curves for *S*(+)-GABOB and *R*(−)-GABOB at recombinant $GABA_C$ receptors formed from ρ1 subunits. Values are expressed as means ± SE mean ($n = 3$).

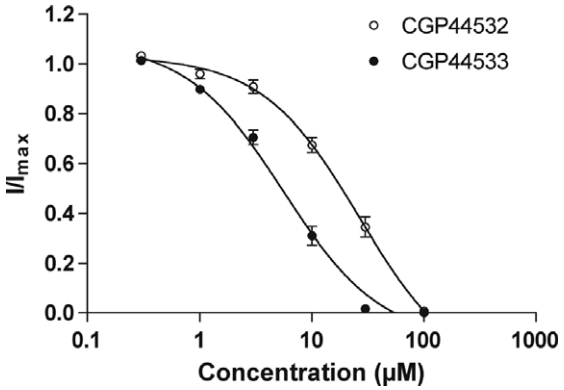

Figure 3. Effects of *S*(−)-CGP44532 and *R*(+)-CGP44533 as antagonists of responses to 1 μM GABA. Values are expressed as means ± SE mean ($n = 3$).

$GABA_B$ receptors,[6,14] but was the opposite to that reported at $GABA_A$ receptors (*S* > *R*).[6,14] The corresponding methylphosphinic acid analogues proved to be ρ1 $GABA_C$ receptor antagonists with *R*(+)-CGP44533 being more potent than *S*(−)-CGP44532, thus showing the opposite enantioselectivity to the agonists *R*(−)- and *S*(+)-GABOB.[7] These studies highlight the different stereochemical requirements for the hydroxy group in these analogues at $GABA_A$, $GABA_B$ and $GABA_C$ receptors and are indicative of different chiral environments in the GABA binding pocket of the different classes of GABA receptors.

Molecular modelling of ligand-gated ion channels is challenging.[15] While some progress has been made in modelling $GABA_C$ receptors[16,17] such studies have been hampered by the lack of data regarding chiral ligands having relatively potent and defined actions on these receptors. In earlier studies, we showed that the enantiomers of *cis*-2-aminocyclopropane carboxylic acid showed opposite pharmacology at ρ1 $GABA_C$ receptors, with the (1*S*,2*R*)-*cis* enantiomer ((+)-CAMP) being a relatively potent full agonist (EC_{50} 40 μM) and the (1*R*,2*S*)-cis enantiomer ((−)-CAMP) a weak antagonist (IC_{50} 890 μM).[9] Subsequently, studies were carried out on the enantiomers of the cyclopentane and cyclopentene analogues of GABA, most of which turned out to be partial agonists of varying potency and efficacy.[10] These studies were extended to the enantiomers of 4-amino-2-methylbutanoic acid where the (*S*)-enantiomer ((*S*)-2MeGABA) was a relatively potent agonist (EC_{50} 65 μM) and the (*R*)-enantiomer a potent antagonist (IC_{50} 16 μM).[11] To these chiral ligands we can now add *R*(−)- and *S*(+)-GABOB as potent agonists (EC_{50}s 19 and 45 μM, respectively), and *S*(−)-CGP44532 and *R*(+)-CGP44533 as potent antagonists (IC_{50}s 17 and 5 μM, respectively). The present findings are in general agreement with the rationale developed by Crittenden et al.[11] of competing steric interactions of methyl and methylene moieties at the GABA binding pocket on $GABA_C$ receptors, where steric bulk on one side of the ligand favours agonist action while steric bulk on the other side favours antagonist action. The hydroxyl moieties in the present series of chiral ligands have, however, quite different opportunities to the

methyl and methylene moieties for interaction with amino acids in the binding pocket via hydrogen bonding.

Acknowledgments

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13. The procedures involving the expression of human recombinant ρ1 $GABA_C$ receptors in *Xenopus laevis*, two-electrode voltage clamp recording from these oocytes and data analyses were carried out according to those described by Crittenden et al.[11] and were approved by the Animal Ethics Committee of the University of Sydney. Expression of the human ρ1 cRNA in *Xenopus oocytes* generated GABA-activated currents. Under two-electrode voltage clamp conditions at −60 mV, the currents ranged between 200 and 2000 nA with the maximum GABA response being obtained at 100 μM GABA. The enantiomers of GABOB and (3-amino-2-hydroxypropyl)methylphosphinic acid were kindly provided by Prof. Povl Krogsgaard-Larsen (University of Copenhagen, Denmark) and Dr. Wolfgang Froestl (Acimmune, Lausanne, Switzerland). Human ρ1 cDNA subcloned in pcDNA1.1 (Invitrogen, San Diego, CA, USA) was a gift from Dr. George Uhl (National Institute for Drug Abuse, Baltimore, MD, USA). All other reagents were purchased from Sigma (St. Louis, MO, USA). *Xenopus laevis* were obtained from an African *Xenopus* colony and housed in the Faculty of Veterinary Science at the University of Sydney.
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