Medicinal Chemistry and Molecular Pharmacology of $GABA_C$ Receptors

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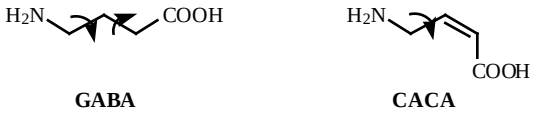
Abstract: $GABA_C$ receptors belong to the nicotinicoid superfamily of ionotropic receptors that include nicotinic acetylcholine receptors, bicuculline-sensitive $GABA_A$ receptors, strychnine-sensitive glycine receptors and 5HT3 serotonin receptors. The $GABA_C$ receptor concept arose from medicinal chemical studies of a conformationally restricted analog of GABA. Receptors matching the predicted properties of $GABA_C$ receptors were cloned from the retina. Post cloning studies revealed the unique physiology and pharmacology of these relatively simple homomeric receptors. Three subtypes of $GABA_C$ receptors have been cloned from mammalian sources and pharmacological differences between the ρ1, ρ2 and ρ3 $GABA_C$ receptors have been described. There is evidence for functional $GABA_C$ receptors in the retina, spinal cord, superior colliculus, pituitary and the gut and their involvement in vision, aspects of memory and sleep-waking behaviour. This review concentrates on the medicinal chemistry and molecular pharmacology of $GABA_C$ receptor subtypes emphasising possible new investigational tools with which to investigate further $GABA_C$ receptor function. The most useful currently available ligands that show some $GABA_C$ receptor subtype selectivity are TPMPA, P4PMA, imidazole-4-acetic acid, 2-methyl-TACA and (±)-TAMP.

Key Words: GABA receptors – chloride channels – CACA – CAMP – TPMPA

INTRODUCTION

The GABA molecule can adopt a variety of different low energy conformations. The design and development of GABA analogs of restricted conformation showed that different conformations of GABA were likely to interact with different macromolecules that recognise GABA in its function as an inhibitory neurotransmitter [1]. A key compound was cis-4-aminocrotonic acid (CACA), a conformationally restricted analog of GABA held in a range of folded conformations (Fig. **1**). In 1975, CACA was shown to have an inhibitory action on the firing of neurons in the spinal cord that was pharmacologically different to the predominant action of GABA on these neurons in that the



GABA **CACA**

Fig. (1). Structures of GABA and the prototype $GABA_C$ receptor agonist, CACA.

action of CACA was insensitive to the GABA antagonist bicuculline [2]. In 1981, Hill and Bowery [3] showed that another GABA analog, baclofen, also had a bicuculline-insensitive action on neurons, and proposed that there were two types of GABA receptors: $GABA_A$ receptors that were bicuculline-sensitive, and $GABA_B$ receptors that were bicuculline-insensitive and activated by baclofen.

It is now known that $GABA_A$ and $GABA_B$ receptors are fundamentally different types of receptors. $GABA_A$ receptors are ligand-gated ion channels made up of a pentameric mixture of protein subunits [4], while $GABA_B$ receptors are heterodimeric G-protein coupled receptors [5]. But what of CACA, the GABA analog that apparently did not interact with either $GABA_A$ or $GABA_B$ receptors, described as a 'disturber of the peace' by Krogsgaard-Larsen and colleagues [6]? The concept of a third major class of GABA receptors arose in 1984 from studies on the lack of effect of CACA on the binding of radioactive baclofen to rat cerebellar membranes: "folded analogs of GABA may interact with a class of binding site ($GABA_C$?) insensitive to (-)-baclofen and bicuculline." [7]

The expression of GABA receptors with novel properties in oocytes injected with $polyA^+$ RNA from mammalian retina [8], and the cloning of ρ1- and ρ2-subunit cDNAs from a human retinal library [9,10], represented major breakthroughs in the understanding of $GABA_C$ receptors. Expressed in *Xenopus* oocytes, the ρ-subunits formed homooligomeric GABA-activated chloride channels insensitive to bicuculline but sensitive to CACA. The expression of retinal $polyA^+$ RNA in oocytes led to GABA-activated chloride currents made up of two distinct components, one mediated by $GABA_A$ receptors and the other mediated by $GABA_C$ receptors, thus providing evidence that the protein subunits making up these different receptor populations associate independently of each other. Subsequent studies on the self-associating ρ-subunits

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expressed in oocytes and COS cells have provided extensive evidence that these subunits form GABA receptors with physiological and pharmacological properties distinct from those of heterooligomeric $GABA_A$ receptors [11-15].

Functional evidence for the existence of native $GABA_C$ receptors came in 1993 with back-to-back papers in Nature reporting that bicuculline-insensitive actions of GABA were mimicked by CACA in rat retinal bipolar cells [16] and in white perch rod-driven retinal horizontal cells [17]. The pharmacological properties of these responses closely resembled those observed for recombinant $GABA_C$ receptors resulting from retinal mRNA expressed in oocytes.

$GABA_C$ receptors belong to the nicotinicoid superfamily of ligand-gated ion channels that include nicotinic acetylcholine receptors, $5\text{-}HT_3$ receptors, $GABA_A$ receptors, strychnine-sensitive glycine receptors and some invertebrate anionic glutamate receptors [18]. The most studied and best understood of this class of receptors is the nicotinic acetylcholine receptors, while the $GABA_A$ receptors appear to be the most complex and the $GABA_C$ receptors the simplest. Members of the nicotinicoid superfamily are considered to be pentamers made up of protein subunits likely to have been derived from a common ancestor [19].

Recent reviews of $GABA_C$ receptors have emphasised their structure and function from a molecular biological perspective highlighting their structural characteristics, identifying structural domains, noting the chromosomal localisation of $GABA_C$ subunit genes and resulting link to diseases, identifying $GABA_C$ receptor associated proteins and cloning new splice variants [20-22]. This review concentrates on the medicinal chemistry and molecular pharmacology of $GABA_C$ receptor subtypes emphasising possible new investigational tools with which to investigate further $GABA_C$ receptor function.

SIMILARITIES AND DIFFERENCES BETWEEN $GABA_A$ AND $GABA_C$ RECEPTORS

$GABA_A$ and $GABA_C$ receptors are subfamilies of the nicotinicoid superfamily of ligand gated ion channels [18]. The protein subunits that make up the $GABA_A$ and $GABA_C$ receptor subfamilies show up to 36% optimised sequence identity overall and up to 73% sequence identity in the membrane spanning regions. They are clearly derived from common ancestral proteins in the nicotinicoid superfamily. The $GABA_A$ and $GABA_C$ subfamilies can be distinguished on the basis of their different channel properties, the availability of selective agonists, antagonists and modulators, the different chromosomal localisation of the genes coding for the protein subunits of the two subfamilies and the reluctance of these subunits to co-express in functional receptors, and the different proteins that anchor the protein complexes to the cytoskeleton [21,23].

$GABA_C$ RECEPTOR SUBTYPES

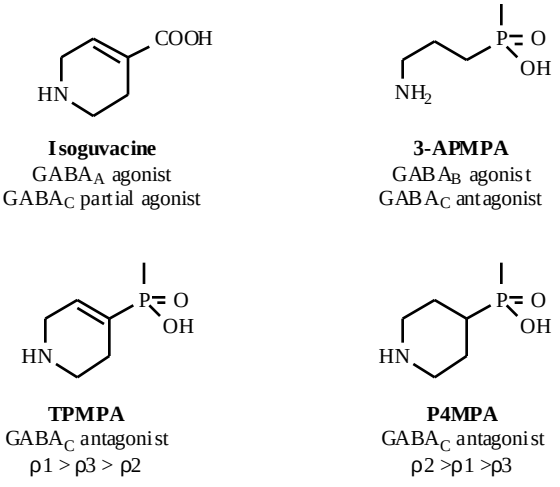
$GABA_C$ receptors are considered to be both homo-oligomeric and pseudohetero-oligomeric receptors comprised of ρ-subunits. To date, at least five different ρ−subunits have been cloned, including three from humans (ρ1, ρ2 and ρ3)[9,10,24] with two ρ1 splice variants [25], three from rat (ρ1−3) [26-29], two from chick (ρ1 and ρ2) [30], five from white perch (ρ1A, ρ1B, ρ2A, ρ2Band ρ3) [31], and two from mouse (ρ1 and ρ2) retinae [32].

The use of reverse transcriptase PCR and *in situ* hybridisation techniques has shown that ρ1 mRNA is located predominantly in the retina, whereas ρ2 mRNA is more widely distributed in the CNS. In humans, the ρ2 subunit is present in most brain regions, including the cerebellum, thalamus and temporal and frontal cortices [33]. In rat brain, ρ3 mRNA expression pattern is different to the expression pattern of ρ1 and ρ2 mRNA's with lower expression levels of ρ3 mRNA in the retina and stronger expression levels in the hippocampus [34]. Outside the CNS, $GABA_C$ ρ1 and ρ2 receptors have also been found associated with specific neurons in the gut [35,36]. Truncated ρ1 and ρ2 mRNAs have been described in thyrotropin-secreting cells in the pituitary together with $GABA_C$-like receptors that differ from those found in the retina [37].

Pharmacological differences between recombinant ρ1, ρ2 and ρ3 $GABA_C$ receptors are emerging which may aid in assessing the functional relevance of these $GABA_C$ receptor subtypes in various tissues.

TPMPA - THE FIRST SELECTIVE $GABA_C$ ANTAGONIST

TPMPA, (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid (Fig. **2**), was the first selective $GABA_C$ antagonist to be developed [38,39]. It arose from the finding that isoguvacine has activity at both $GABA_A$ and $GABA_C$ receptors but no activity at $GABA_B$ receptors, indicating that the tetrahydropyridine ring of isoguvacine (Fig. **2**) distinguishes $GABA_B$ from $GABA_C$ receptors but does not distinguish $GABA_A$ from $GABA_C$ receptors. Combining this with the knowledge that a methylphosphinic acid group,



Isoguvacine
$GABA_A$ agonist
$GABA_C$ partial agonist

3-APMPA
$GABA_B$ agonist
$GABA_C$ antagonist

TPMPA
$GABA_C$ antagonist
ρ1 > ρ3 > ρ2

P4MPA
$GABA_C$ antagonist
ρ2 >ρ1 >ρ3

Fig. (2). Structures of substances that led to the development of the first selective $GABA_C$ receptor antagonist, TPMPA.

as in the potent $GABA_B$ agonist 3-APMPA (Fig. **2**) [40] interacts with $GABA_C$ [12] but not with $GABA_A$ receptors led to TPMPA, which incorporates both the tetrahydropyridine ring system and a methylphosphinic moiety [38,39].

TPMPA is more that 100 times more selective in blocking ρ1 $GABA_C$ receptors than in blocking $GABA_A$ receptors from rat cerebral cortex polyA+ RNA expressed in *Xenopus* oocytes [39]. At $GABA_B$ receptors, TPMPA is a weak agonist when measured by whole-cell patch recordings from pyramidal neurons in rat hippocampal slices [39]. TPMPA is greater than 500 times more selective in blocking ρ1 $GABA_C$ receptors expressed in *Xenopus* oocytes than in activating $GABA_B$ receptors [39].

TPMPA has been shown to block responses in the neonatal rat spinal cord providing evidence for the involvement of $GABA_C$ receptors in synaptic transmission in this tissue [41].

TPMPA shows some subunit selectivity being 8-times weaker at human recombinant ρ2 than at ρ1 $GABA_C$ receptors expressed in *Xenopus* oocytes [42]. At rat recombinant ρ3 $GABA_C$ receptors, TPMPA was 2-times weaker than at human recombinant ρ1 $GABA_C$ receptors [43]. The ρ1δ51 splice variant of ρ1 $GABA_C$ receptors was more sensitive to TPMPA than are ρ1 $GABA_C$ receptors [44].

The piperidine analog of TPMPA, P4MPA ((piperidine-4-yl)methylphosphinic acid) is a more potent antagonist of human recombinant ρ2 $GABA_C$ receptors than is TPMPA [45,46], but weaker than TPMPA at human recombinant ρ1 and rat recombinant ρ3 $GABA_C$ receptors [43].

TPMPA has been used to study $GABA_C$ receptor function in the retina [47,48], spinal cord [41], superior colliculus [49], and gut [35], and the involvement of $GABA_C$ receptors in memory consolidation [45,46] and sleep-waking behaviour [50].

CACA - THE PROTOTYPE $GABA_C$ AGONIST

CACA, *cis*-4-aminocrotonic acid, was the prototype $GABA_C$ agonist, shown to be an inhibitor of neuronal firing insensitive to the $GABA_A$ antagonist bicuculline [2] that did not influence the binding of the $GABA_B$ agonist baclofen to brain membranes [7]. Functional studies of the effects of CACA in a variety of neuronal preparations linked CACA to receptors that are activated by GABA and are distinct from the classical $GABA_A$ and $GABA_B$ receptors and led to widespread interest in $GABA_C$ receptors [11,12,16,17]. CACA was shown to be a moderately potent partial agonist at $GABA_C$ receptors. TACA, the *trans*-isomer of CACA, was considerably more potent as an agonist at $GABA_C$ receptors but less selective in that TACA was also a potent agonist at $GABA_A$ receptors, and a substrate for GABA-T [2]. CACA showed similar potency as a partial agonist at human ρ1 and ρ2 receptors expressed in *Xenopus* oocytes (EC_{50} (ρ1) = 74 μM; EC_{50} (ρ2) = 70 μM) and showed 70-80% of the efficacy of GABA [13,51]. It appeared to be weaker (EC_{50} 139 μM) and less efficacious (57%) at rat ρ3 receptors expressed in *Xenopus* oocytes [43].

It is now known that CACA has other actions in addition to being a partial agonist at $GABA_C$ receptors. Uptake studies have shown that CACA acts as a weak inhibitor of β-alanine transport in rat cerebral cortex slices [52]. It appears to be a weak substrate for a transporter that transports GABA, β-alanine and nipecotic acid in glial cells isolated from guinea-pig retina [53]. CACA stimulates the passive release of [^{3}H]GABA and [^{3}H]β-alanine by hetero-exchange from slices of rat cerebellum, cerebral cortex and spinal cord, without influencing potassium-evoked release [54]. CACA is only ten times weaker as a substrate for the transporter than as a partial agonist at the $GABA_C$ receptors. This transporter may be related to the GAT-3 protein, a GABA transporter located predominantly on glial cells in the CNS [54].

In addition to its agonist action on $GABA_C$ receptors in the retina, CACA has been shown to influence calcium currents at lower concentrations than those that activate GABA-evoked chloride currents [55].

CACA has been shown to weakly activate $GABA_A$ receptors that contain α6 subunits on rat cerebellar granule cells and on recombinant α6β2γ2S $GABA_A$ receptors expressed in HEK cells [56]. This action of CACA was apparent at 30 μM CACA, did not saturate at 1 mM, was abolished by 100 μM bicuculline and was insensitive to 100 μM TPMPA.

Thus it appears that studies using CACA as a selective $GABA_C$ receptor partial agonist should be interpreted with caution.

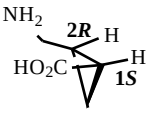
(+)-CAMP - A SELECTIVE $GABA_C$ RECEPTOR AGONIST

The (+)-isomer of CAMP, (*1S,2R*)-2-aminomethylcyclopropanecarboxylic acid, may be a superior $GABA_C$ receptor agonist to CACA [57]. Racemic CAMP has been widely investigated for its effects on a variety of GABA receptors. CAMP is a conformationally restricted analog of GABA held in a range of folded conformations similar to those available to CACA. Conformational restriction in CAMP is achieved via a cyclopropane ring, rather than a *cis* double bond as in CACA; this has less influence on the ionisation of the carboxyl group, which in CACA is made more acidic by the double bond.

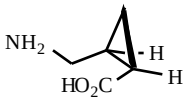
CAMP is a moderately potent full agonist at human ρ1 and ρ2 $GABA_C$ receptors (Kd 65 μM at ρ1 and Kd 104 μM at ρ2) [57] and a partial agonist (EC_{50} 53 μM, I_{max} 57%) at rat ρ3 $GABA_C$ receptors [43]. CAMP has no effect in inhibiting [^{3}H]GABA uptake in cortical slices and thus may be the most selective agonist at $GABA_C$ receptors currently available [58].

Resolution of CAMP into its optical isomers by separation of diastereoisomeric esters [59] afforded (+)- and (-)-CAMP (Fig. **3**), which surprisingly showed opposite

pharmacology at $GABA_C$ receptors [57]. (+)-CAMP was a relatively potent full agonist (Kd 40 μM at ρ1 and Kd 27 μM at ρ2), while (-)-CAMP was a weak antagonist (IC_{50} 900 μM at ρ1 and 400 μM at ρ2). Rarely do enantiomers have identical biological properties, but it is also relatively uncommon to find them exerting opposite pharmacological effects. Neither isomer of CAMP influenced GABA transport [57].



(+)-CAMP
Selective $GABA_C$ agonist



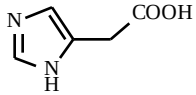
(-)-CAMP
Weak $GABA_C$ antagonist

Fig. (3). Structures of (+)-CAMP, a selective $GABA_C$ receptor agonist and of (-)-CAMP, a $GABA_C$ receptor antagonist.

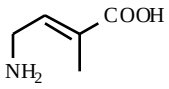
(+)-CAMP is a weak antagonist at human α1β2γ2L $GABA_A$ receptors expressed in oocytes, 1 mM producing 30% inhibition of the current produced by 10 μM GABA [57]. It has yet to be tested on recombinant α6β2γ2S $GABA_A$ receptors that are activated by CACA [56].

IMIDAZOLE-4-ACETIC ACID

Imidazole-4-acetic acid is a naturally occurring metabolite of histidine that activates $GABA_A$ receptors [60]. It is an analog of GABA where the amino group of GABA has been replaced by an imidazole ring (Fig. **4**). It penetrates the blood-brain barriers on systemic administration and reduces blood pressure and heart rate by stimulating central $GABA_A$ receptors [61]. Inhibitors of histamine methylation in the brain promote formation of imidazole-4-acetic acid [62].



IMIDAZOLE-4-ACETIC ACID
$GABA_A$ agonist
ρ1 $GABA_C$ antagonist/partial agonist
ρ2 $GABA_C$ partial agonist
ρ3 $GABA_C$ antagonist



2-Methyl-TACA
$GABA_A$ agonist
ρ1 $GABA_C$ antagonist
ρ2 $GABA_C$ partial agonist

Fig. (4). Structures of imidazole-4-acetic acid and 2-methyl-TACA, lead compounds for the development of subtype specific $GABA_C$ receptor ligands.

It is a partial agonist at $GABA_C$ receptors showing a 12-fold higher intrinsic activity at ρ2 than at ρ1 receptors (EC_{50} 16 μM, I_{max} 3% at ρ1; EC_{50} 3 μM, I_{max} 38% at ρ2) [13,42,51]. It is a potent antagonist at ρ1 $GABA_C$ receptors (K_B1.5 μM), with an affinity similar to that of GABA [42]. It is also an antagonist (K_B13 μM) at ρ3 $GABA_C$ receptors [43].

Imidazole-4-acetic acid may be a useful compound for distinguishing between ρ1, ρ2 and ρ3 $GABA_C$ receptors when used in conjunction with other substances. Although it has a complex pharmacology [63], imidazole-4-acetic acid has been used to characterise $GABA_C$ receptors in rat pelvic ganglia [64]. Species differences have been noted in the effects of imidazole-4-acetic acid on $GABA_C$ receptors in the retina [65].

2-METHYL-TACA

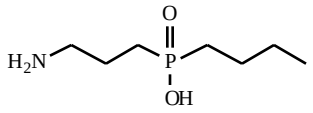
2-Methyl-TACA, *trans*-4-amino-2-methylbut-2-enoic acid (Fig. **4**), shows contrasting pharmacological properties at ρ1 and ρ2 $GABA_C$ receptors [42]. It is a competitive antagonist at human ρ1 $GABA_C$ receptors (Kb 45 μM) and a partial agonist at human ρ2 $GABA_C$ receptors (Kd 101 μM, I_{max} 34%) and thus may be useful in differentiating between homo-oligomeric ρ1 and ρ2 receptors in native tissues.

A number of analogs of GABA and TACA substituted in the 2-position act as $GABA_C$ receptor antagonists [66]. 2-Methyl-TACA is an open chain analog of isoguvacine (Fig. **2**), a $GABA_A$ receptor agonist [67] and $GABA_C$ receptor partial agonist. The increased conformational flexibility of 2-methyl-TACA may be a factor in the ability of 2-methyl-TACA to stimulate human ρ2 $GABA_C$ receptors.

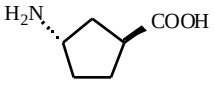
TAMP, (+)-TACP AND (+)-4-ACPCA

TAMP, *trans*-2-aminomethylcyclopropanecarboxylic acid (Fig. **5**), in its racemic form has long been known as a $GABA_A$ [58] and $GABA_C$ receptor partial agonist [13]. Separated into its optical isomers, (-)-TAMP is more potent against ρ2 $GABA_C$ receptors (Kd 3 μM, I_{max} 50%) than against ρ1 $GABA_C$ receptors (Kd 9 μM, I_{max} 40%) or

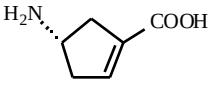
(±)-TAMP
$GABA_A$ agonist
$GABA_C$ ρ1, ρ2 partial agonist
$GABA_C$ ρ3 antagonist



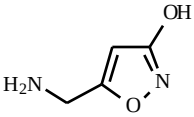
CGP36742
$GABA_B$ antagonist
$GABA_C$ antagonist



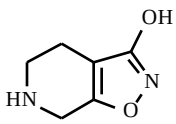
(+)-TACP
$GABA_A$ agonist
$GABA_C$ partial agonist



(+)-4-ACPCA
$GABA_A$ agonist
$GABA_C$ antagonist



Muscimol
$GABA_A$ agonist
$GABA_C$ partial agonist



THIP
$GABA_A$ partial agonist
$GABA_C$ antagonist

Fig. (5). Structures of (±)-TAMP, CGP36742, (+)-TACP, (+)-4-ACPCA, muscimol and THIP, compounds having interesting actions on $GABA_C$ receptors.

α1β2γ2L $GABA_A$ receptors (Kd 50 μM, I_{max} 50%), while (+)-TAMP is weaker than (-)-TAMP at all 3 receptors (Kd 30 μM, I_{max} 60% at ρ2; Kd 60 μM, I_{max} 40% at ρ1; and Kd 500 μM, I_{max} 50% at α1β2γ2L) [57]. It has been shown recently that (±)-TAMP has no agonist properties at rat ρ3 $GABA_C$ receptors, but it is a potent antagonist (Kb 5 μM) at these receptors [43]. Thus (-)-TAMP may be a useful agent with which to distinguish ρ3 from ρ1 and ρ2 $GABA_C$ receptors, when used in combination with other agents.

Early studies on (+)-TACP, (+)-*trans*-3-aminocyclopentanecarboxylic acid (Fig. **5**), showed that it was a potent inhibitor of the firing of cat spinal interneurones whose inhibitory action could not be blocked completely by the $GABA_A$ receptor antagonist bicuculline [68]. Studies on radiolabelled (+)-TACP binding to rat brain membranes showed a high affinity binding component that could be inhibited by CACA suggesting that (+)-TACP could bind to $GABA_C$ receptors [69]. Recent studies have shown that (+)-TACP is a potent partial agonist at ρ1, ρ2 and ρ3 receptors (EC_{50}s 2.7, 1.5 and 2.7 μM; I_{max} 83, 85 and 27% respectively) [43,70].

(+)-4-ACPCA, (+)-4-aminocyclopent-1-ene-1-carboxylic acid (Fig. **5**), a $GABA_A$ receptor agonist [71] is an antagonist at ρ1 and ρ2 $GABA_C$ receptors (Ki 6 and 5 μM respectively) [70].

The structures of these and related compounds provide clues as to how they bind to $GABA_C$ receptors to exert their agonist and antagonist actions [43,70,72].

MUSCIMOL AND THIP

Muscimol and THIP (Fig. **5**) are widely used as selective $GABA_A$ receptor agonists [4]. However they have potent actions on $GABA_C$ receptors which means that interpretation of studies with these agents should be treated with some caution. No "selective" $GABA_A$ receptor agonist is known that does not have significant action on either $GABA_B$ and/or $GABA_C$ receptors.

Muscimol, a conformationally restricted analog of GABA in which a hydroxyisoxazole moiety replaces the carboxyl group of GABA [1], is a potent partial agonist at human ρ1 and ρ2 $GABA_C$ receptors (Kd 2.3 and 1.4 μM respectively) expressed in oocytes [13,51]. It shows similar potency (EC_{50} 1.9 μM) at rat ρ3 $GABA_C$ receptors [43]. Muscimol appears to be more potent at $GABA_C$ receptors than at $GABA_A$ receptors [12].

THIP, 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol (Fig. **5**), is a conformationally restricted analog of muscimol [1]. It is a potent $GABA_A$ receptor partial agonist of high efficacy [67], but has proved to be a moderately potent $GABA_C$ receptor antagonist (Kb 32 μM at $GABA_C$ receptors produced from bovine retinal poly$(A)^+$ RNA expressed in oocytes [12] and Kb 10 μM at rat ρ3 $GABA_C$ receptors [43]).

Unlike GABA, both muscimol and THIP pass the blood-brain barriers on systemic administration [73]. Muscimol is psychoactive, while THIP is a potent analgesic. Side effects of THIP (including sedation, dizziness, and blurred vision) meant that it had too low a therapeutic index to be therapeutically useful as an analgesic [74,75]. There is renewed interest in THIP with respect to sleep therapy as it produces slow wave sleep and reduces spindling activity in non rapid eye movement sleep in humans [76].

THIP-induced analgesia is not sensitive to bicuculline indicating that $GABA_A$ receptors are not involved [77]. Perhaps the $GABA_C$ receptor antagonist action of THIP contributes to its analgesic action? Benzodiazepine-sensitive $GABA_A$ receptors do not appear to be involved in the effects of THIP on sleep patterns [76]. As noted above, the $GABA_C$ receptor antagonist TPMPA has been used to probe the involvement of $GABA_C$ receptors in sleep-waking behaviour [50].

CGP36742

CGP36742, 3-aminopropyl-*n*-butylphosphinic acid (Fig. **5**) is one of a range of phosphinic acid analogs of GABA that act as $GABA_C$ receptor antagonists [72]. CGP36742 was developed as an orally active $GABA_B$ receptor antagonist [40,78]. It inhibited the binding of CGP27492 to $GABA_B$ receptors on rat brain membranes with an IC_{50} of 38 μM [40,78] and had much weaker effects on binding to $GABA_A$ receptors (IC_{50} 500 μM) [79]. Furthermore, CGP36742 had no effect on a range of other receptors at 1 mM, including benzodiazepine, muscarinic cholinergic, adrenergic, serotonergic, histaminergic, NMDA, quisqualate and kainate receptors.

Functional studies on ρ1 $GABA_C$ receptors expressed in oocytes showed however that CGP36742 acted as a $GABA_C$ receptor antagonist with an IC_{50} = 62 μM, i.e. approximately half as potent as against $GABA_B$ receptors [72].

CGP36742 has shown promising therapeutic potential for the treatment of cognitive deficits, petit mal epilepsy and depression [79-83]. As not all orally active $GABA_B$ antagonists show this therapeutic potential [78], it is possible that antagonism of $GABA_C$ receptors contributes to these effects [45,72].

BARBITURATES

Barbiturates have long been known to be able to enhance the action of GABA on $GABA_A$ receptors [84] but they appear to have no action on wild type $GABA_C$ receptors [13]. Indeed, chimeric constructs of β3 $GABA_A$ and ρ1 $GABA_C$ receptor subunits have been used to show that the residues of the β3 subunit involved in barbiturate binding to $GABA_A$ receptors are located downstream from the middle of the second membrane spanning region [85].

Splicing of the third membrane spanning region of a β2 $GABA_A$ subunit into a ρ1 $GABA_C$ receptor subunit produces a chimera that is sensitive to barbiturates [86]. Mutation of a single tryptophan residue at position 328

within the third membrane spanning domain of ρ1 $GABA_C$ receptor subunits to a variety of hydrophobic amino acids is sufficient to impart barbiturate sensitivity to ρ1 $GABA_C$ receptors [86].

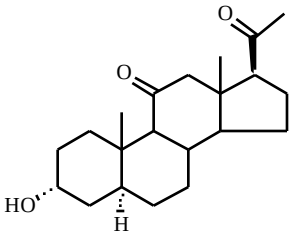
The second membrane spanning region of ρ1 $GABA_C$ receptors can also be modified to confer barbiturate sensitivity on these receptors. Mutation of an isoleucine residue at position 307 to serine results in a mutant ρ1 $GABA_C$ receptor that can be positively modulated by barbiturates [87].

Thus, it appears that two key amino acid residues (serine 307 and tryptophan 328) in ρ1 $GABA_C$ receptors are responsible for the lack of activity of barbiturates at these receptors, in contrast to the positive modulation and direct agonist action of barbiturates at $GABA_A$ receptors.

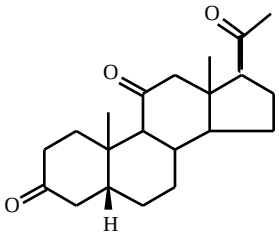
NEUROACTIVE STEROIDS

While early studies reported $GABA_C$ receptors to be insensitive to modulation by neuroactive steroids under conditions where such steroids at nM concentrations modulated $GABA_A$ receptors [88], it is now known that μM concentrations of steroids can modulate $GABA_C$ receptors [89]. The low affinity effects of neuroactive steroids on $GABA_C$ receptors are more apparent at low concentrations of GABA well below its EC_{50}. Under such conditions, the anaesthetic agent alphaxalone, and the neurosteroids allopregnanolone and allotetrahydroxydeoxycorticosterone (5α-THDOC) (Fig. **6**) enhanced GABA-evoked currents at ρ1 $GABA_C$ receptors expressed in oocytes and prolonged the decay time of these currents, i.e. they were positive modulators. At 20 μM, these steroids potentiated GABA-evoked currents by 96%, 42% and 198% respectively. These are all 5α-steroids and are the first examples of agents able to positively modulate $GABA_C$ receptors.

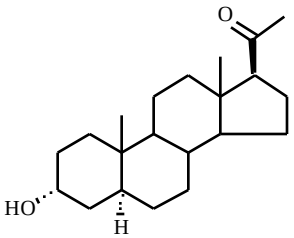
The related 5β-steroids (Fig. **6**) - 5β-dihydroprogesterone (IC_{50} 5 μM), pregnanolone (IC_{50} 0.55 M) and tetrahydrodeoxycorticosterone (5β-THDOC, IC_{50} 1 μM) – were negative modulators in that they inhibited GABA-evoked currents at these ρ1 $GABA_C$ receptors [89]. Thus the configuration of the A/B ring junction in these steroids influences their action on $GABA_C$ receptors, the *trans*-



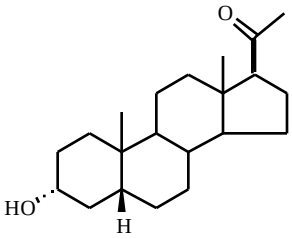
Alphaxolone
$GABA_A$ positive modulator
$GABA_C$ positive modulator



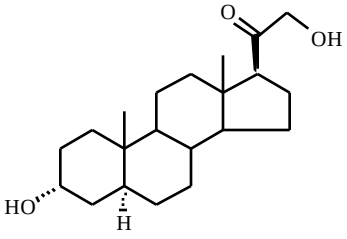
5β-Dihydroprogesterone
$GABA_A$ positive modulator
$GABA_C$ negative modulator



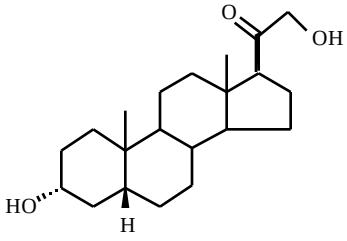
Allopregnanolone
$GABA_A$ positive modulator
$GABA_C$ positive modulator



Pregnanolone
$GABA_A$ positive modulator
$GABA_C$ negative modulator



Allotetrahydrodeoxycorticosterone (5α-THDOC)
$GABA_A$ positive modulator
$GABA_C$ positive modulator



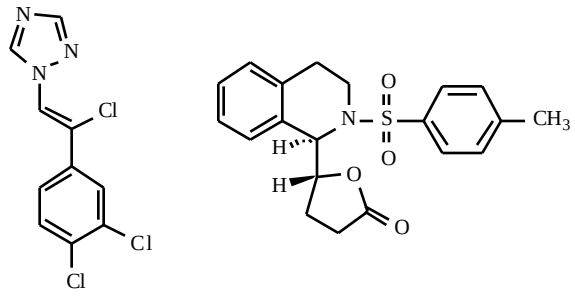
Tetrahydrodeoxycorticosterone (5β-THDOC)
$GABA_A$ positive modulator
$GABA_C$ negative modulator

Fig. (6). Structures of steroids that have low affinity modulatory actions on $GABA_C$ receptors.

configuration in the 5α-steroids being favourable to enhancing GABA action and the *cis*-configuration resulting in inhibition of GABA action. The low affinity effects of these neuroactive steroids on $GABA_C$ receptors are quite different from the high affinity effects typically observed with $GABA_A$ receptors [89].

LORECLEZOLE AND (+)-ROD188

There are other agents, in addition to the 5β-steroids shown in Fig. **6**, that enhance the action of GABA on $GABA_A$ receptors and reduce the action of GABA on $GABA_C$ receptors. The anticonvulsant drug, loreclezole (Fig. **7**), is a positive modulator of $GABA_A$ receptors containing a β2 or β3 subunit [90]. This selectivity is determined by a single amino acid residue in the second membrane spanning region (β2Asn289, β3Asn290) which when converted to a serine residue (as in β1 subunits) leads to a 300-fold loss of potency of loreclezole as a positive modulator of $GABA_A$ receptors. Against ρ1 $GABA_C$ receptors, loreclezole is a potent negative modulator (IC_{50} 0.5 μM) that has been described as a simple functional marker for $GABA_C$ receptors [91]. Another positive modulator of $GABA_A$ receptors, (+)-ROD188 that is known to have a different mode of action to that of loreclezole [92], is also a negative modulator (IC_{50} 100 μM) of ρ1 $GABA_C$ receptors [91].



Loreclezole
$GABA_A$ positive modulator
$GABA_C$ negative modulator

(+)-ROD188
$GABA_A$ positive modulator
$GABA_C$ negative modulator

Fig. (7). Structures of loreclezole and (+)-ROD188, $GABA_A$ receptor positive modulators and $GABA_C$ receptor negative modulators.

CONCLUSIONS

While medicinal chemical studies on $GABA_C$ receptors are just beginning, it is already clear that $GABA_C$ receptors are very different from $GABA_A$ receptors in terms of agonist and antagonist structural profiles. Functional studies indicate the involvement of $GABA_C$ receptors in the retina, spinal cord, superior colliculus, pituitary and the gut. $GABA_C$ receptors are involved in vision and have been implicated in aspects of memory and sleep-waking behaviour.

There are at least 3 major subtypes of $GABA_C$ receptors found in mammals: the ρ1 subtype is located predominantly in the retina; the ρ2 subunit is found in most brain regions, including the cerebellum, thalamus and temporal and frontal cortices; and the ρ3 subunit is found in the hippocampus.

Pharmacological differences between recombinant ρ1, ρ2 and ρ3 $GABA_C$ receptors are emerging which may aid in assessing the functional relevance of these $GABA_C$ receptor subtypes in various tissues. The structure-activity profiles of the $GABA_C$ receptor agonists and antagonists highlighted in this review provide leads for the design and development of subtype selective $GABA_C$ receptor ligands.

The most useful currently available $GABA_C$ subtype selective ligands appear to be: the $GABA_C$ antagonists TPMPA and P4MPA which show an order of potency ρ1 > ρ3 > ρ2 and ρ2 > ρ1 > ρ3 respectively; imidazole-4-acteic acid which is a potent partial agonist at ρ2 of moderate efficacy, a partial agonist of very low efficacy at ρ1 and an antagonist at ρ3; 2-methyl-TACA, which is an antagonist at ρ1 and a partial agonist at ρ2; and (±)-TAMP, which is a potent antagonist at ρ3 and a partial agonist at ρ1 and ρ2.

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