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ORIGINAL PAPER

Unsaturated Analogues of the Neurotransmitter GABA: *trans*-4-Aminocrotonic, *cis*-4-Aminocrotonic and 4-Aminotetrolic Acids

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Abstract Analogues of the neurotransmitter GABA containing unsaturated bonds are restricted in the conformations they can attain. This review traces three such analogues from their synthesis to their use as neurochemicals. *trans*-4-Aminocrotonic acid was the first conformationally restricted analogue to be extensively studied. It acts like GABA across a range of macromolecules from receptors to transporters. It acts similarly to GABA on ionotropic receptors. *cis*-4-Aminocrotonic acid selectively activates bicuculline-insensitive $GABA_C$ receptors. 4-Aminotetrolic acid, containing a triple bond, activates bicuculline-sensitive $GABA_A$ receptors. These findings indicate that GABA activates $GABA_A$ receptors in extended conformations and $GABA_C$ receptors in folded conformations. These and related analogues are important for the molecular modelling of ionotropic GABA receptors and to the development of new agents acting selectively on these receptors.

Keywords GABA · *trans*-4-Aminocrotonic acid · *cis*-4-Aminocrotonic acid · 4-Aminotetrolic acid · $GABA_A$ receptors · $GABA_C$ receptors

Special Issue: In Honor of Dr. Philip Beart.

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Introduction

The neurotransmitter, GABA, is a highly flexible molecule so it is possible that different conformations bind to its receptive macromolecules such as transporters, receptors or catabolising enzymes. Structural analogues of the neurotransmitter GABA that contain double or triple bonds are limited in the shapes they can attain in contrast to GABA being a highly flexible molecule. Such conformationally restricted analogues are useful neurochemicals with which to probe the different shapes that GABA may adopt in its interactions with receptive macromolecules. The simple unsaturated analogues—*trans*-4-aminocrotonic acid and 4-aminotetrolic acid—were the subject of Philip Beart's PhD thesis in 1972 (The Neurochemistry of Amino Acid Transmitters, Australian National University). Subsequently 4-aminotetrolic acid was converted into *cis*-4-aminocrotonic acid and this led to the discovery of a new subtype of ionotropic GABA receptors (Fig. 1).

trans-4-Aminocrotonic Acid

trans-4-Aminocrotonic acid (TACA, *E*-4-aminobut-2-enoic acid) was the first conformationally restricted analogue of GABA to be studied extensively. The constraints of the double bond held the four carbon atoms in a plane, thus preventing free rotation around the C2-C3 bond in GABA.

TACA had been prepared in 1954 by dehydration of the readily available 4-amino-3-hydroxybutyric acid [1], and is now available commercially. Crystal structure analysis showed its similarity to GABA [2, 3] as did pKa measurements—GABA 4.04, 10.71; TACA 3.55, 9.46 [4, 5] with TACA being a somewhat stronger acid than GABA due to the electron withdrawing double bond.

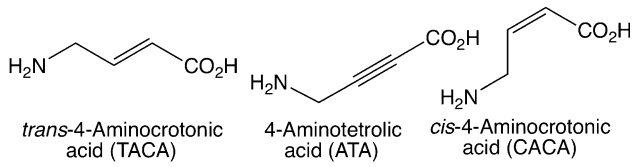


Fig. 1 Structures of simple unsaturated analogues of GABA

The action of TACA on nervous system function was first studied in 1960 by Purpura who described a GABA-like depressant action on neuronal firing [6]. Then Krnjevic and Phillis in 1963 described TACA as having an inhibitory activity comparable with that of GABA on neuronal firing in the mammalian cerebral cortex [7].

With the advent of agents to distinguish different subtypes of GABA postsynaptic receptors, TACA was found to have a more potent action than that of GABA on the ionotropic $GABA_A$ and $GABA_C$ receptors [8] and a much less potent action on the metabotropic $GABA_B$ receptors [9].

TACA was also found to be a potent competitive inhibitor of GABA uptake into rat brain slices [5] and a weak substrate for GABA transaminase activity in rat brain extracts [10].

In an electroconvulsive threshold test in mice, TACA (at 15 and 25 mg/kg i.p.) significantly decreased the threshold indicating proconvulsant properties on systemic administration [11]. Such an effect was completely opposite to the authors' primary assumption and expectance. The authors suggested that TACA-induced stimulation of $GABA_C$ receptors, highly expressed in superior colliculus, may lead to the proconvulsant action. Further experiments are needed to clarify this finding.

Numerous analogues of TACA have been prepared. Perhaps the most interesting are analogues substituted in the 2-position. The 2-fluoro analogue, *trans*-4-amino-2-fluorobut-2-enoic acid, was found to be a potent agonist (K_D=2.43 μM) at human ρ1 $GABA_C$ receptors expressed in oocytes. In contrast, the 2-methyl analogue, *trans*-4-amino-2-methylbut-2-enoic acid, was found to be a moderately potent antagonist (IC_{50}=31.0 μM and K_B=45.5 - μM). These observations highlight the possibility that subtle structural substitutions may change an agonist into an antagonist when interacting with $GABA_C$ receptors [12] (Fig. 2).

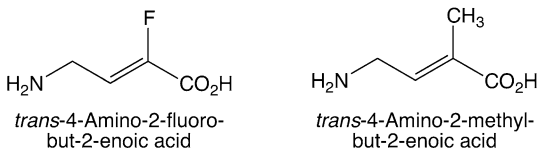


Fig. 2 Structures of some 2-substituted derivatives of 4-aminocrotonic acid

4-Aminotetrolic Acid

4-Aminotetrolic acid (ATA, 4-aminobut-2-ynoic acid) was first prepared by Philip Beart as part of his PhD studies in a hazardous multi-step synthesis [13]. The triple bond holds the four carbons atoms in a line thus representing a unique fully extended conformation of GABA with no possible folding. It is a much stronger acid than GABA with pKa values of 1.80 and 8.34 [14]. An X-ray crystal structure and molecular orbital calculations support the concept of ATA as a GABA analogue [15, 16]. It is not available commercially (Fig. 3).

Reduction of the triple bond in ATA and its derivatives with tritium has provided a convenient route to the preparation of GABA labelled to high specific activity due to the incorporation of four tritium atoms [17–20]. An improved synthesis of ATA has been reported [21].

ATA has an inhibitory action on the firing of a spinal interneuron in anesthetized cats 20–50 % as strong as that of GABA [13]. The inhibitory action of ATA, like that of GABA, could be reversibly antagonized by bicuculline whereas the glycine antagonist strychnine was ineffective as an antagonist. It has a similar action on bicuculline sensitive GABA receptors in the isolated superior cervical ganglion of the rat [22]. ATA has no activity on $GABA_B$ receptors [13]. Like TACA, ATA was found to be a competitive inhibitor of GABA uptake into rat brain slices [5]. ATA is also an inhibitor of GABA transaminase activity without influence on glutamate decarboxylase [23].

We wrote at the time "The successful interaction of 4-aminotetrolic acid with bicuculline-sensitive postsynaptic receptors supports the view that GABA acts on these receptors in an extended rather than a folded conformation,

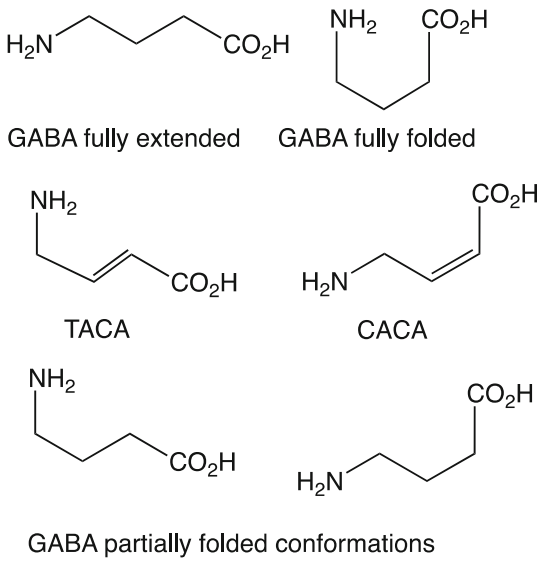


Fig. 3 Some conformations of GABA, TACA and CACA

and further supports our original suggestion regarding the structural similarities of bicuculline, GABA and muscimol" [13]. The actions of bicuculline and muscimol on GABA receptors have been recently reviewed [24, 25].

cis-4-Aminocrotonic Acid

cis-4-Aminocrotonic acid (CACA, *Z*-4-aminobut-2-enoic acid) was first reported in 1975 and initially synthesised by partial hydrogenation of ATA by Philip Beart before undertaking postdoctoral research with Leslie Iversen at the University of Cambridge [8]. It was his last synthesis as a medicinal chemist before becoming a fully fledged neurochemist. Subsequently, an improved synthesis from a derivative of ATA was reported [17]. It has been available commercially since 1992. Radioactive CACA has been prepared by reduction of an acetylenic intermediate with tritium gas [18]. The resultant high specific activity preparation of [^{3}H]CACA was found to be toxic producing burning sensations to the face, eyes and hands of personnel handling the preparation, thus limiting the extent of the binding studies that can be carried out.

CACA was particularly important as an analogue of GABA in a partially folded conformation and this led to the discovery of a new subtype of ionotropic GABA receptor. CACA is close to TACA in pKa values (3.93 and 9.84) but has very different actions on GABA systems [8].

Light slowly converts CACA into the more thermodynamically stable TACA. Contamination of some samples of CACA with up to 0.1 % TACA as detected by nuclear magnetic spectroscopy can complicate interpretation of the effects of high concentrations of CACA, given the relatively high potency of TACA in many test systems compared to that of pure CACA [26].

CACA is a weak substrate for a transporter that transports GABA, β-alanine and nipecotic acid in glial cells isolated from guinea-pig retina [27]. It stimulates the release of GABA and β-alanine from slices of rat spinal cord and cerebellum [28]. These studies are consistent with the idea that CACA, β-alanine, nipecotic acid and GABA are substrates for a common transporter, that may be related to the GAT-3 transport protein cloned from rat CNS (Fig. 4).

In terms of shape, CACA represents more folded conformations of GABA while TACA represents more extended conformations. Importantly GABA and TACA can adopt similar partially folded conformations, but CACA cannot match the more extended conformations of GABA.

Although CACA has an inhibitory action of the firing of spinal neurones, this action is not blocked by the $GABA_A$ antagonist bicuculline under conditions whereby TACA had a potent bicuculline-sensitive action [8]. The inhibitory action of CACA is not blocked by the glycine antagonist

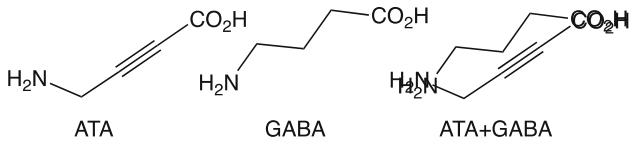


Fig. 4 Overlapping structures of ATA and GABA

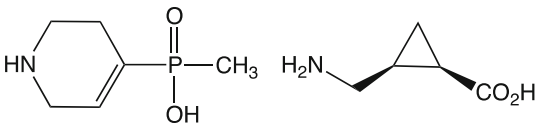
strychnine thus ruling out an interaction with glycine receptors. Unlike the $GABA_B$ agonist baclofen, CACA is equally potent as a inhibitor of the firing of Renshaw cells and spinal interneurones. These results indicated that CACA is not an agonist for $GABA_A$ or $GABA_B$ receptors on spinal neurones [8].

CACA was described as a 'disturber of the peace' by Krogsgaard-Larsen and colleagues [29]. 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" [30].

Peace was restored in 1993 when, following the release of CACA commercially, three groups described the action of CACA on bicuculline-insensitive, baclofen-insensitive GABA receptors in the retina and receptors cloned from the retina [31–33]. These became known as $GABA_C$ receptors made up of ρ-subunits [26, 34], although he nomenclature of these ionotropic GABA receptors remains unsettled. This has been discussed in a comprehensive review of ionotropic GABA receptors [35]. It is important to distinguish between $GABA_A$ and $GABA_C$ receptors, as these receptors appear to have opposing roles in nervous system function [36, 37].

CACA is a partial agonist at human recombinant ρ1 and ρ2 $GABA_C$ receptors [26, 34]. It is much more selective as a $GABA_C$ receptor ligand than the more potent TACA, which interacts strongly with a variety of macromolecules that recognize GABA. Unlike TACA, CACA is, at best, a very weak $GABA_A$ receptor agonist and is neither a substrate for, nor an inhibitor of, GABA transaminase in extracts of rat brain mitochondria. In addition, it does not influence the activity of glutamate decarboxylase in rat brain extracts [8]. The *cis*-double bond in CACA appears to restrict the conformations available to CACA to those that interact with $GABA_C$ receptors and GAT3 transporters. CACA is tenfold weaker as a substrate for the transporter than as a partial agonist for $GABA_C$ receptors (Fig. 5).

Used in conjunction with TACA and the $GABA_C$ receptor antagonist TPMPA [38, 39], CACA has been used to aid in the characterisation of $GABA_C$ receptors in the retina [31, 33], hippocampus [40], colliculus [41, 42], pituitary [43], pelvic ganglia [44], and the gut [45–47]. CACA also has a peripheral anti-nociceptive effect in a paw pressure test [48] that may involve $GABA_C$ receptors.



TPMPA (+)-CAMP

Fig. 5 Structures of the selective $GABA_C$ antagonist TPMPA and the selective $GABA_C$ agonist (+)-CAMP

There is evidence for CACA activating receptors other than purely ρ-containing $GABA_C$ receptors. It activates α6 subunit-containing $GABA_A$ receptors but not $GABA_C$ receptors in cerebellar granule cells in a bicuculline-sensitive, TPMPA-insensitive manner [49]. In cerebellar Purkinje cells, CACA has a mixed action activating both bicuculline- and TPMPA-sensitive receptors [50]. CACA has been used to provide evidence for the co-assembly of $GABA_A$ and $GABA_C$ receptor subunits in the brain stem and hippocampus [51, 52].

Conclusion

The conformationally restricted unsaturated analogues of GABA introduced by Philip Beart and his colleagues continue to provide valuable information on the function of ionotropic GABA receptors. ATA shows selectivity for $GABA_A$ over $GABA_C$ receptors while CACA shows selectivity for $GABA_C$ over $GABA_A$ receptors. TACA activates both classes of ionotropic receptor. The structures of ATA, CACA and TACA are important for the molecular modelling studies of $GABA_A$ and $GABA_C$ receptors that aid in the discovery of drugs interacting with ionotropic GABA receptors [53, 54]. Unfortunately, the synthesis of pure CACA uncontaminated by TACA has remained difficult, limiting access to N-substituted analogues of CACA. Additionally, the unsaturated bond in these compounds alters the acidity in comparison to GABA. This can be avoided by using ring structures to restrict conformational freedom. Indeed the cyclopropane analogues of GABA have significant actions of $GABA_C$ receptors with *cis*-2-(aminomethyl)cyclopropanecarboxylic acid ((+)-CAMP) being a potent and selective full agonist at $GABA_C$ receptors [55, 56]. Neurochemicals such as these are important to the development of new agents acting selectively on ionotropic GABA receptors.

Acknowledgments It is a pleasure to contribute to this special issue honouring Philip Beart who was my first PhD student. We have maintained our collaboration over many years most recently on aspects of the history of the International Society for Neurochemistry. I am grateful to Philip Beart, Jane Hanrahan and Ken Mewett for their helpful comments on the manuscript.

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