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STRUCTURAL FACTORS GOVERNING AGONIST AND ANTAGONIST ACTIVITY IN THE $GABA_A$ SYSTEM

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Abstract—By comparing the structures of GABA and semi-rigid $GABA_A$ analogues, a distinction between structural requirements for agonist and antagonist activity at the $GABA_A$ receptor has been suggested, based on differences in arrangements of charge centres. However, additional structural distinction(s) appear to be necessary since the GABA molecule itself can attain the arrangements suggested for both agonist and antagonist activity, and GABA is not an antagonist. We now propose that a specifically located benzene ring and steric effects in the N^+ region are also involved in distinguishing between $GABA_A$-active compounds.

Comparison of the structure of γ-amino butyric acid (GABA) with that of 4,5,6,7-tetrahydroisoxazolo [5,4-c] pyridin-3-ol (THIP) and other semi-rigid $GABA_A$ agonists has suggested [1] that agonist activity at the $GABA_A$ receptor is associated with N^+ and COO^- (or equivalent) charge centres being in a Y-shaped arrangement (Fig. 1a). In contrast, for the specific antagonist bicuculline (BIC) [2] these charge centres are in an approximately linear arrangement (Fig. 1b) as deduced from the active conformation of BIC ($\theta = 270°$–$290°$—see Fig. 1c) determined by NMR spectroscopy [1, 3]. However, since both arrangements are accessible to GABA (Fig. 1a, b) additional structural features appear to be necessary for conferring on BIC its antagonist properties, an aspect that has received little attention

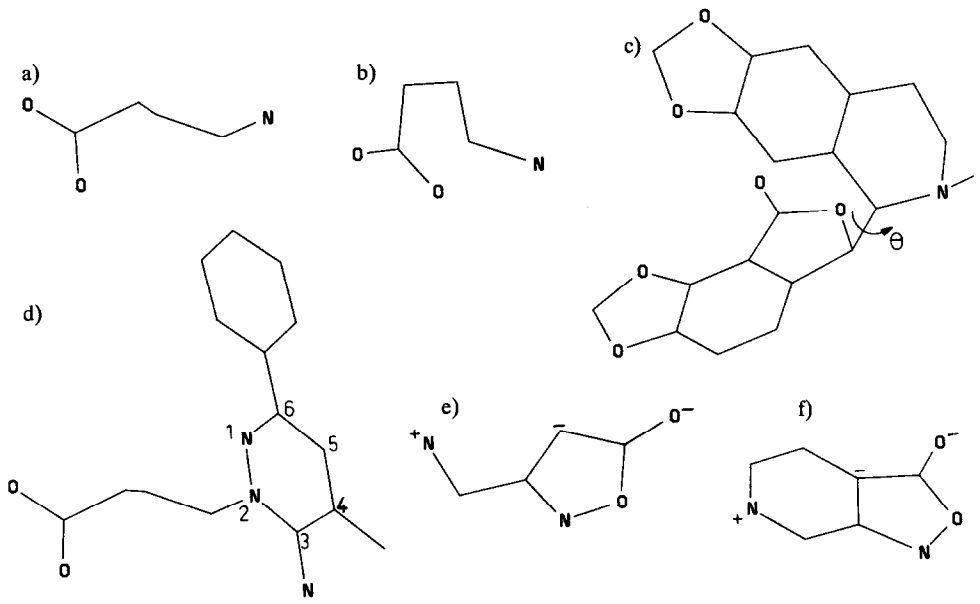

Fig. 1. Molecular structures. (a) GABA in the Y-shaped agonist conformation (from a comparison with THIP and other semi-rigid agonists). (b) GABA in the "linear" antagonist conformation (from a comparison with BIC [1]). (c) BIC in its active conformation, $\theta = 270°$–$290°$ (from NMR work on protonated BIC, the active form of BIC, and BIC methohalides [1]). The positive nitrogen-region and the negative COO region are sterically hindered by the presence of the phthalide and isoquinoline groups respectively. (d) SR95103—in a conformation which can be superimposed on the $GABA_A$ agonist conformation (Fig. 1a). Reasons for SR95103 not being an agonist are discussed in the text. Note that steric hindrance between the 3-amino group and the carboxypropyl side-chain will destabilize this conformation. (e) Iso-muscimol and (f) iso-THIP. In these "iso" molecules the oxazolo N and O atoms are interchanged, leading to a transference of negative charge to the oxazolo carbon atom as indicated [1], and the "linear" arrangement of charge centres. These molecules are not potent $GABA_A$ antagonists for reasons discussed in the text. Hydrogen atoms have been excluded for clarity.

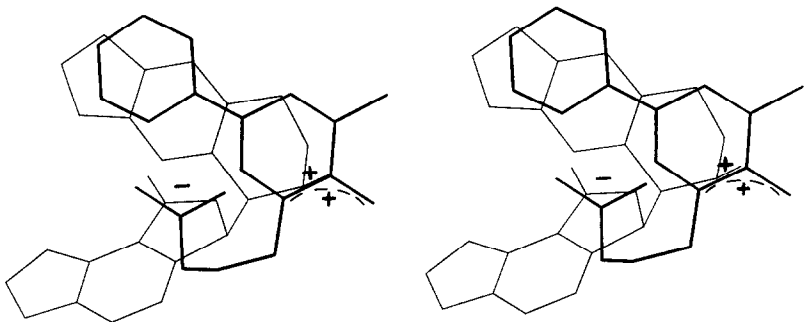

Fig. 2. Stereo view of match of BIC and SR95103. This 3-dimensional least-squares fit was obtained (using IMDAC*) by matching the COO oxygens in each molecule, the N in BIC with C3 in SR95103 (see below), and two benzene ring atoms (with a ring weighting factor of 1/2, to equalize the importance of each group being matched). The four SR95103 carboxypropyl torsion-angles were varied systematically by increments of between 5° and 20° for optimum overall fit. These atom reference points represent the negative and positive charge distribution in the COO and nitrogen-regions respectively, and the pi-electron clouds, all of which could be directly involved in binding the antagonist to a receptor. Atom C3 was chosen as a reference point because it is roughly in the centre of the positive charge distribution in the nitrogen region of SR95103. (The use of N2 as a reference point gives a poor overall fit, and the 3-amino nitrogen gives no benzene-ring overlap). The actual "points" of binding of the active moieties of these molecules at the receptor remain a matter for speculation.

so far. We have now examined the importance of the position of the benzene rings in BIC and another $GABA_A$ antagonist, (2-(carboxy-3'-propyl)-3-amino-4-methyl-6-phenyl pyridazium chloride (SR95103)), relative to the charge centres. The importance of the position of these rings has previously been only a matter of speculation [4]. In addition, we have examined the importance of steric bulk in the positive N-region of $GABA_A$ analogues, by comparing the effects of *N*-methylation on $GABA_A$ agonist and antagonist activity.

The potent and highly selective GABA antagonist SR95103 was discovered more recently [5] than BIC and contains a flexible carboxypropyl side chain (Fig. 1d). It can readily adopt the "linear" antagonist arrangement of charge centres as in BIC (though the positive charge in the nitrogen-region is delocalised—see Fig. 2) and, more importantly, its benzene ring can occupy a spatial position, relative to the charge centres, similar to that in BIC (Fig. 2). Furthermore, shortening or lengthening of the carboxyalkyl side chain displaces the relative position of the benzene ring and lower potencies are observed [5]. In addition, the 6-desphenyl analogue of SR95103 is completely inactive [5]. That the charge centres and benzene rings in BIC and SR95103 can be matched by having the correct length of alkyl chain, and the fact that actual removal of the benzene ring in SR95103 destroys its activity, imply that a benzene ring in the relative position found in BIC and in SR95103 is necessary for potent $GABA_A$ antagonist activity.

A further distinguishing structural feature between agonists and antagonists in the $GABA_A$ system is suggested by the opposing effects of steric bulk in the positive nitrogen region (N-bulk). With $GABA_A$ agonists, addition of extra methyl (or alkyl) groups at the positive nitrogen considerably reduces potency [6]. In contrast, in BIC and SR95103 there is considerable N-bulk (Fig. 1c), and *N*-methylation of BIC has only a small effect on potency (MeBIC is more potent *in vivo* [7] but less potent *in vitro* [8]). Compared with GABA agonists, therefore, it seems that N-bulk is associated with antagonist activity; this may explain why although SR95103 can also adopt the Y-shaped agonist arrangement of charge centres (Fig. 1c) it is devoid of agonist activity [5]. With regard to the negative (COO) region, whilst BIC is sterically hindered there, both SR95103 and GABA are unhindered, which implies that steric hindrance in that region is not important for $GABA_A$ antagonist activity.

That a sterically hindered positive nitrogen-region and a suitably located benzene ring are associated with $GABA_A$ antagonism offers an explanation for why certain GABA analogues with the required "linear" antagonist arrangement of charge centres are nevertheless inactive (e.g. iso-muscimol—Fig. 1e) or only weak antagonists (e.g. iso-THIP—Fig. 1f): they contain relatively unhindered nitrogen-regions and no benzene ring. Similarly, agonist activity appears to be precluded by the presence of these two features, since both SR95103 [5] and BIC are devoid of agonist activity.

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