needed to discuss the steric requirements for GABA antagonism reliably.

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1 Curtis, D. R., Duggan, A. W., Felix, D., and Johnston, G. A. R., *Nature*, **226**, 1222 (1970).
2 Beart, P. M., Curtis, D. R., and Johnston, G. A. R., *Nature new Biol.*, **234**, 80 (1971).
3 Steward, E. G., Player, R., Quilliam, J. P., Brown, D. A., and Pringle, M. J., *Nature new Biol.*, **233**, 87 (1971).
4 Steward, E. G., and Player, R. B., *Acta Cryst.*, **B28**, 1313 (1972).
5 Karle, J., and Karle, I. L., *Acta Cryst.*, **21**, 849 (1966).
6 Tomita, K., *Tet. Lett.*, **1971**, 2587 (1971).
7 Takenaka, A., Oshima, E., Yamada, S., and Watanabe, T., *Acta Cryst.*, **B29**, 503 (1973).
8 Brehm, L., Hjeds, H., and Krogsgaard-Larsen, P., *Acta Chem. Scand.*, **26**, 1298 (1972).
9 Giglio, E., *Nature*, **222**, 339 (1969).

DR STEWARD replies: Reporting a crystal structure determination of bicuculline, Gilardi[1] points out that in discussions of the conformation of the molecule in relation to its action as a specific antagonist to γ-aminobutyric acid (GABA), an incorrect configuration has been used[2,3]. The configuration was, in fact, consistent with that given in *Chemical Abstracts* at that time. Subsequently, we suspected that the wrong diastereoisomer was portrayed. Confirmed by the full structure determination (D. Moss, personal communication), this did not affect the content of our discussion[3] which drew attention to the possible congruence of N and O=C−O in both GABA and bicuculline, compared with Curtis's suggested[2] correspondence of N and O=C−O in GABA to N and C−C=O in bicuculline.

In a more detailed discussion[4] of the structure and activity of central inhibitory transmitters, we have made use of the crystal structure data.

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1 Gilardi, R. D., *Nature new Biol.*, **245**, 86 (1973).
2 Curtis, D. R., Duggan, A. W., Felix, D., and Johnston, G. A. R., *Nature*, **226**, 1222 (1970).
3 Steward, E. G., Player, R. B., Quilliam, J. P., Brown, D. A., and Pringle, M. J., *Nature new Biol.*, **233**, 87 (1971).
4 Warner, D., Player, R. B., and Steward, E. G., *Int. Union of Crystallography, First European Meeting*, B4 (1973).

Analogues of Gamma-Aminobutyrate on Rat Hippocampal Neurones

CONFORMATIONALLY restricted analogues of various small organic compounds utilizing ring structures have been employed with great success in biochemistry[1–5], but only a few reports have dealt with the effects of spatial conformers on brain physiological functions[6,7]. A four carbon amino acid with an acetylenic bond in the 2,3 carbon position (4-aminotetrolic acid) has been reported to mimic the physiological action of gamma-aminobutyrate (GABA) on spinal cord motorneurones[8].

This study compares the effect of GABA with that of a series of aminocyclohexane carboxylic acid compounds on hippocampal neurones. These compounds offer a systematic variation of the distance between amino and carboxyl functional groups and possess the advantage of lacking the electromeric changes of amino and carboxy groups which are present in unsaturated aliphatic or aromatic ring analogues of amino acids. Their synthesis has been previously described[9–12].

In our experiments Sprague–Dawley rats, weighing about 200 g and anaesthetized with halothane, were used. For recordings of unit activity, a five barrel micropipette was directed stereotactically toward the dorsal hippocampus. Almost all the cells tested were therefore located within the pyramidal layer of areas CA1 or CA3[14,15]. Of the four outer barrels, one contained 1.0 M GABA, two contained one or more of the aminocyclohexane carboxylic acids (0.5 M), acetylcholine (0.5 M), or bicuculline (saturated solution, approximately 15 mM) and the fourth barrel was used to neutralize any current effects[13]. The centre barrel, containing 3.0 M NaCl, was used for recording the unit extracellular action potentials. The spontaneous firing pattern of monitored cells was always assessed for several minutes before application of drugs, and recordings were only made from those cells that exhibited potentials of at least 100 μV.

The conformers possess identical molecular weights and net charge at *p*H 6.0 The 1,2- and 1,3-cyclohexane analogues, however, are racemic and, most probably, only one of the two enantiomers is physiologically active, so the conformers were therefore iontophoresed at effective concentrations approximately one-fourth that of GABA.

Sixty-two cells were tested for their responses to the conformers and each was inhibited by GABA as previously reported[16]. The response of hippocampal neurones to iontophoretic application of the GABA conformers varied from a complete blockade of spike discharge (Fig. 1*B* and *C*) to a moderate decrease in firing rate (Fig. 1*A*, *C* and *D*) and to no effect at all. The first response (complete blockade) was produced only when the 1,2-*trans* or the 1,3-*cis* isomers of aminocyclohexane carboxylic acid were applied. Out of the fourteen cells tested with the 1,2-*trans* compound, thirteen were completely inhibited or exhibited substantial decreases in their firing rate with a median threshold of 85 nA. Of the nineteen cells tested with the 1,3-*cis* derivative, seventeen were significantly slowed or completely inhibited (median threshold=75 nA) (Fig. 1*C*). The second response (diminished firing rate only) was found most frequently following the application of the 1,2-*cis* derivative (twelve out of fourteen cells studied with this compound exhibited this pattern, 100 nA median threshold). Responses of this type occurred only occasionally after application of the 1,3-*trans*, 1,4-*cis*, or the 1,4-*trans* aminocyclohexane carboxylic acids. For example, three of six cells tested with the 1,3-*trans* amino acid responded with a minimal decrease in firing rate (Fig. 1*D*) and no response was observed in the other three units. Of the nine units tested with the 1,4 substituted compounds, only one responded to the 1,4-*cis* conformer (Fig. 1*E*) and only two units to the 1,4-*trans* compound. For both, the responses were characterized by a minimal slowing of the firing rate, a requirement for large iontophoretic currents (125 nA for minimal response), prolonged delay in onset of effect relative to that of GABA or the 1,2-*trans* or 1,3-*cis* conformers and the lack of reproducibility (Fig. 1*E*). The cells that did not respond were tested with currents up to 150 nA, sustained for periods as long as 20 s.

Statistical comparison of the effects of the six conformers using the extended median test[17] revealed that significant differences exist among them ($\chi^2=17.6$, $P<0.001$). These comparisons corroborate the overall finding that the 1,2-*trans* and 1,3-*cis*-aminocyclohexane carboxylic acid isomers are markedly more effective in inducing complete or obvious slowing of the average firing rate of hippocampal neurones. The effect of the 1,3-*cis* conformer was markedly potentiated in the presence of GABA "leak" (no holding current, Fig. 1*F*). The effect of 1,3-*cis* was also reduced by simultaneous