The action of conformationally restricted analogues of GABA on *Limulus* and *Helix* central neurones

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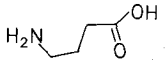
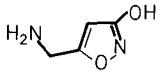
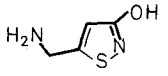
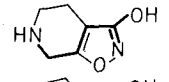
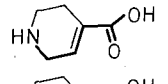
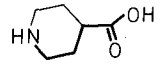
Summary. Intracellular recordings have been made from GABA sensitive neurones in the central nervous system of *Limulus* and *Helix*. The following conformationally restricted analogues of GABA all possessed GABA-like activity on *Limulus* neurones and *Helix* excitatory GABA receptors: muscimol, thiomuscimol, THIP, isoguvacine and piperidine-4-carboxylic acid. It is suggested that GABA interacts with these receptors in a partially extended and almost planar conformation.

Gamma-aminobutyric acid (GABA) has been shown to have a potent, mainly inhibitory effect, on a wide range of central neurones and peripheral muscle[1-3]. Studies have shown that there are differences between the inhibitory (H) and excitatory (D) receptors on *Helix* neurones[4], in particular muscimol being more potent on D cells than H cells. Recently certain new conformationally restricted analogues of GABA, THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol), isoguvacine and piperidine-4-carboxylic acid have been tested on cat spinal interneurones[5]. These compounds have been found to depress the firing rate of spinal neurones with a potency similar to that of GABA, and this action was blocked by bicuculline methochloride. These findings suggest that GABA may well interact with its receptor in the mammalian spinal cord in a partially extended and almost planar conformation. It was felt of interest to investigate the actions of these compounds, together with thiomuscimol, on invertebrate GABA receptors in order to elucidate possible differences between the 'active conformations' of GABA with respect to its receptors on invertebrate neurones and on vertebrate spinal cord neurones.

Materials and methods. Intracellular recordings were made from unidentified cells in the abdominal ganglion of *Limulus polyphemus* and from 2 identified cells in the visceral ganglion of *Helix aspersa*. The *Limulus* were dissected from the ventral surface and the isolated ganglia placed on a slide in a bath of volume 20 ml and viewed with an Olympus binocular microscope. The *Helix* brain was prepared as previously described[6] and viewed in the same manner as the *Limulus* ganglia. Potentials were amplified using conventional methods and displayed on a Hewlett-Packard or Brush pen recorder. The *Limulus* Ringer had

Compound	Structure	Equipotent molar ratio (EPMR)
GABA		1.0
Muscimol		0.06±0.03
Thiomuscimol		3.5 ±1.2
THIP		1.3 ±0.5
Isoguvacine		0.53±0.16
Piperidine-4-carboxylic acid		0.4 ±0.07

A comparison of the potencies of a number of cyclic GABA analogues on *Limulus* neurones. Where the EPMR is less than 1 then the analogue is more potent than GABA while where it is greater than 1 then the analogue is less active than GABA. In all cases n = 7.

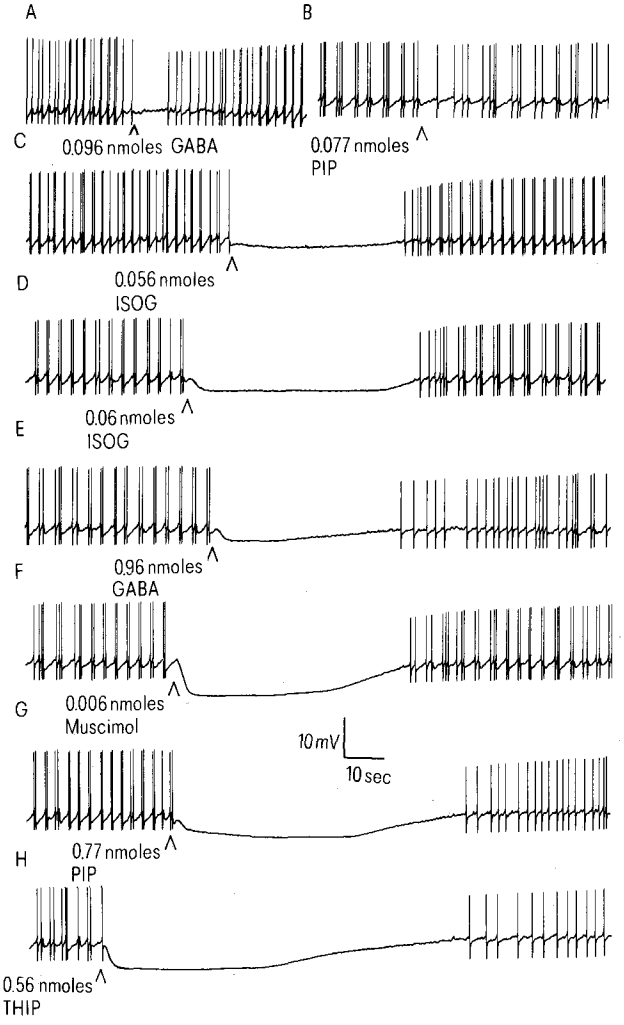


Fig. 1. These are intracellular pen recording traces from a neurone in the abdominal ganglion of *Limulus*. *A* shows a threshold inhibition to 0.096 nmoles GABA; *B* shows a threshold inhibition to 0.077 nmoles piperidine-4-carboxylic acid; *C* shows a 2 mV hyperpolarisation to 0.056 nmoles of THIP; *D* shows a 3 mV hyperpolarisation to 0.06 nmoles isoguvacine; *E* shows a 3 mV hyperpolarisation to 0.96 nmoles of GABA; *F* shows an 8.5 mV hyperpolarisation to 0.006 nmoles of muscimol; *G* shows a 5.5 mV hyperpolarisation to 0.77 nmoles piperidine-4-carboxylic acid; *H* shows a 7 mV hyperpolarisation to 0.56 nmoles THIP. All the traces are from the same cell and the voltage and time calibration are given.

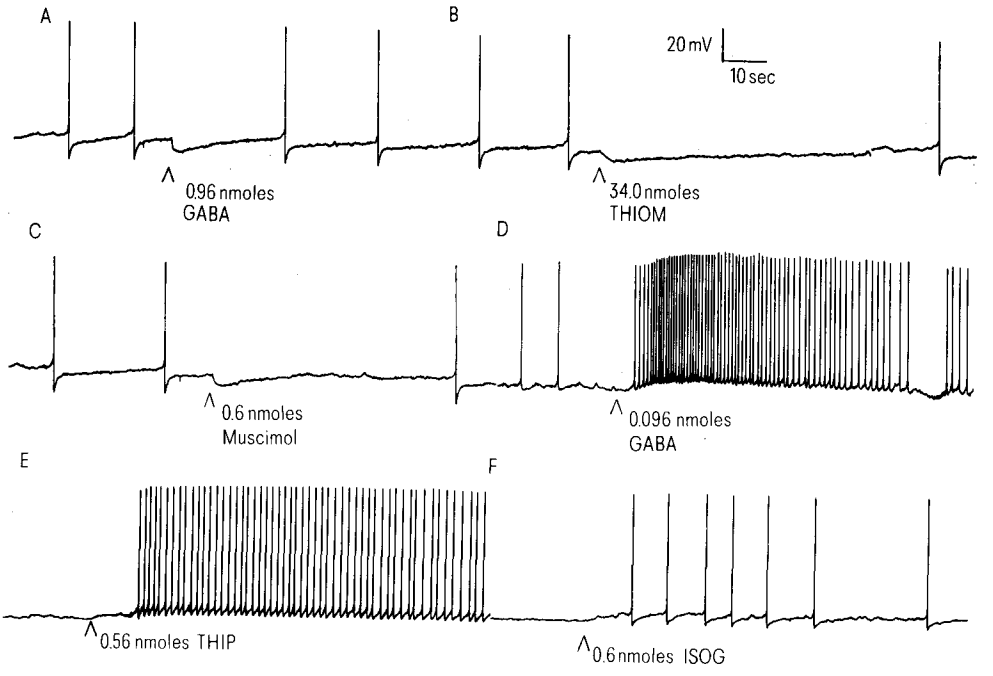


Fig. 2. These are intracellular pen recording traces from 2 neurones in the visceral ganglion of *Helix*. *A* shows a 6 mV hyperpolarisation to 0.96 nmoles of GABA; *B* shows a 6 mV hyperpolarisation to 34 nmoles thiomuscimol; *C* shows a 6 mV hyperpolarisation to 0.6 nmoles muscimol; *D* shows a 4–6 mV depolarisation to 0.096 nmoles GABA; *E* shows a 4–6 mV depolarisation to 0.56 nmoles THIP; *F* shows a 4 mV depolarisation to 0.6 nmoles isoguvacine. *A*, *B* and *C* are from the same E4 cell and traces *D*, *E* and *F* are from the same E13 cell. The voltage and time calibrations are given.

the following composition: NaCl 444 mM; KCl 9 mM; $CaCl_2$ 37 mM; Tris HCl buffer 15 mM; final pH 7.4. The *Helix* Ringer had the following composition: NaCl 80 mM; KCl 4 mM; $CaCl_2$ 7 mM; $MgCl_2$ 5 mM; Tris HCl buffer 5 mM; final pH 7.4. All the drugs used in this study were dissolved in the appropriate Ringer and applied directly over the preparation. The equipotent molar ratios (EPMR) for the analogues under test were obtained of nmoles of GABA required to produce the same response. Analogues which were more potent than GABA had an EPMR of less than 1 while compounds which were less active than GABA had a value greater than 1. The following compounds were used in this study: gamma-aminobutyric acid (GABA) (BDH); 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP) hydrobromide[7]; isoguvacine hydrobromide[8]; piperidine-4-carboxylic acid; muscimol hydrobromide[9]; thiomuscimol dihydrobromide[10].

Results and discussion. GABA is a potent inhibitory agent on *Limulus* neurones (figure 1, traces A and E). The table compares the EPMRs for GABA, muscimol, thiomuscimol, THIP, isoguvacine and piperidine-4-carboxylic acid. THIP, isoguvacine and piperidine-4-carboxylic acid are approximately equipotent with GABA while muscimol is about 20 times more potent than GABA. Replacement of the heterocyclic oxygen atom of muscimol by sulphur has a dramatic effect on the biological activity, thiomuscimol being 2 orders of magnitude weaker than muscimol.

GABA excites cell E13 and inhibits cell E4 activity, the 2 neurones in the visceral ganglion of *Helix* used in this study[11]. Figure 2, traces B and C, compares the potency of muscimol and thiomuscimol on cell E4 while traces D, E and F compare the actions of GABA, THIP and isoguvacine, respectively, on cell E13. In this preparation thiomuscimol is about 50 times weaker than muscimol on cell E4. As in the case of *Limulus*, preliminary studies show that THIP, isoguvacine and piperidine-4-carboxylic acid are 1–10 times less potent than GABA on cell E13. However on cell E4 there appears to be a striking difference between the responses to THIP, isoguvacine and piperidine-4-carboxylic acid, compared to cell E13 in *Helix* and the *Limulus* neurones. At doses of up to 100 times greater than GABA, THIP, isoguvacine and piperidine-4-carboxylic acid had little or no GABA-like activity on E4 cells in most preparations. Glycine had no GABA-like effect on either *Limulus* or *Helix* neurones.

These results indicate that the 'active conformation' of GABA with respect to *Limulus* inhibitory receptors, the *Helix* excitatory receptors, and the cat spinal cord receptors are similar. The results provide indirect evidence that GABA interacts with the receptors concerned in a partially extended and almost planar conformation. Still muscimol is about 50 times more potent than GABA on *Helix* excitatory receptors[4] and about 20 times more potent than GABA on *Limulus* neurones. Thus muscimol seems to be able to adopt the optimum conformation with respect to these receptors, and this conformation may be different from those represented by the rigid molecules of THIP and isoguvacine. Muscimol is equipotent with GABA on *Helix* inhibitory GABA receptors while THIP and isoguvacine appear to have little GABA-like activity on this receptor. Thus the structural requirements of the *Helix* inhibitory receptor with respect to agonists appears to be radically different and requires further study.

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