Table 1 Effects of antagonists against GABA and glycine inhibition. Shift ratios (≥0.4 classified as a block) were averaged for each neurone studied. The numerator in the first column refers to the number of cells blocked and the denominator to the number of cells studied. The mean charge (current x time) passed through the antagonist barrel at the time an agonist inhibitory response was measured is given in the last column

	Blocked (cells)		*Mean shift ratio*		*Mean charge (coulombs x 10^{-5})*	
	GABA	*Glycine*	*GABA*	*Glycine*	*GABA*	*Glycine*
Strychnine	3/24	24/30	0.16	1.6	1.8	0.7
Picrotoxin	14/27	1/19	0.7	0.07	4.1	3.9
Bicuculline	13/23	6/19	1.0	0.55	3.6	3.8
N-methylbicuculline	39/46	5/32	1.6	0.15	1.5	1.7

antagonizing glycine rather than GABA depression. Conversely *N*-methylbicuculline showed similar qualities with regard to GABA. Though *N*-methylbicuculline usually increased the firing rate of the neurone being studied, this action appeared to be independent of GABA antagonism.

Both picrotoxin and bicuculline were found to be less consistent antagonists of GABA than *N*-methylbicuculline but this may be due to unresolved problems associated with their release from micropipettes.

It therefore appears that when applied microiontophoretically strychnine and *N*-methylbicuculline are the most useful antagonists available for studying glycine and GABA depression respectively in the CNS.

Financial support from the MRC is gratefully acknowledged.

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The effect of analogues of glutamic acid on the glutamate receptors of *Helix* neurones

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Glutamic acid has been shown to have both excitatory and inhibitory effects on *Helix* neurones (Kerkut & Walker, 1962; Gerschenfeld & Lasansky, 1964). Results are presented from three identifiable cells in the sub-oesophageal ganglia of *H. aspersa*; cell 3, 4 and 5 (Akhtar, Azanza, Kerkut, Piggott, Rasool, Walker & Woodruff, 1973). Cell 3 has a biphasic response to glutamate, hyperpolarization followed by depolarization; cell 4 is depolarized by glutamate while cell 5 is hyperpolarized.

The isolated snail brain was prepared according to Walker (1968) and placed in 10 ml of Ringer of following composition (mM): NaCl, 80; KCl, 4; $CaCl_2$, 7; $MgCl_2$, 5; Tris-chloride buffer, 5; pH 7.8. Cell activity was recorded using glass microelectrodes filled with molar potassium acetate. The potentials were amplified and displayed on a Tetronix 502A oscilloscope and permanently

recorded on a Watanabe pen recorder. The analogues, dissolved in Ringer, were added direct to the bath while glutamate was iontophoretically ejected as an anion through a second electrode positioned close to the experimental cell.

The analogues were tested for glutamate-like activity and glutamate-blocking action. The results are summarized in Table 1. All analogues were tested on at least six different cells at doses up to 10 μmoles. The following compounds were inactive: L and D α- and L and D γ-glutamyl-hydroxamic acid, diethyl-glutamic acid ester, DL-methionine-DL-sulfoxime, 1-amino-1-carboxy-cyclopentyl-2-acetate, γ-hydroxy-D-glutamate, *Trans* 1-amino-1-3-dicarboxy-cyclohexane, α-amino-γ-cyanobutric acid and *N*-chloro-, *N*-bromo-, and *N*-fluoro-acetyl-glutamic acid.

Addition or substitution on the glutamate molecule greatly reduces the potency on both excitatory and inhibitory receptors. The structural requirements appear equally specific on both type of receptor. The analogues with similar potency to glutamate are γ-methyl-glutamic acid, γ-fluoro-glutamate and Ibotenic acid. These compounds all involve additions on the γ carbon atom indicating that possibly less specificity is required in the interaction of the γ group to the receptor compared to the α carbon group.

S.M.P. was supported by an M.R.C. research fellowship.

Table 1 The effect of analogues of glutamic acid on the glutamate receptors of 3 neurones in the sub-oesophageal ganglia of *H. aspersa*

	Cell 3			*Cell 4*			*Cell 5*		
	Agonist		*Antag.*	*Agonist*		*Antag.*	*Agonist*		*Antag.*
Compound	*H*	*D*		*H*	*D*		*H*	*D*	
L-glutamic acid	+++++	+		–	+++++		+++++	–	
α-methyl-glutamate	+	+	–	–	+	–	+++	–	–
β-methyl-glutamate	–	+	–	–	++	A+	+	–	A+
γ-methyl-glutamate	++++	++	A+	–	+++	A+	+++	–	A+
β-phenyl-glutamate	+	–	–	–	+	–	–	–	–
N-methyl-glutamate	–	–	–	–	+	–	+	–	–
γ-fluoro-glutamate	+	++	–	–	+++	–	+++++	–	A+
γ-methyl-glut. ester	–	+	–	–	+	–	–	–	–
Di-methyl-glut. ester	–	–	–	–	+	–	–	–	–
Ibotenic acid	+++	+++	A+	–	+++	–	+++++	–	A+
l-amino-l-carboxy-cyclopentyl-2-carboxylate	–	+	–	–	+	–	+	–	–
Cis l-amino-l-3-di-carboxy-cyclohexane	–	+	–	–	+	–	–	–	–

+++++ Potency of glutamate on these cells. A+ Blocking action of glutamate response when compound acts as an agonist. H = hyperpolarization and D = depolarization by glutamate. – = no activity on the neurone.

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