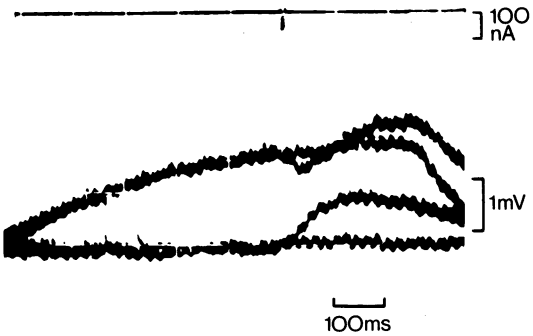

Fig. 1 Intracellular record of the action of iontophoretically applied DECA and CARB on a frog sartorius muscle fibre endplate. Four traces were superimposed: a baseline; a response to DECA alone; a response to CARB alone; a response to DECA during the action of CARB. The iontophoretic current pulses, monitored in the upper beam, were: DECA, 100 nA and 10 ms; CARB, 4 nA and 800 ms. The response to DECA in the presence of CARB is biphasic. Inhibition of CARB by DECA peaks before the depolarization produced by DECA alone.

and the time to peak of the inhibition was 38 ± 4.5 ms (the time being measured from the start of the DECA pulse). The ratio of the times to peak for excitation and inhibition was 2.4 ± 0.3. Since low doses of CARB potentiate low doses of DECA, adjustment of the CARB pulse could provide biphasic DECA responses (Figure 1).

Various artefacts may occur in this type of experiment: interbarrel coupling; direct effect of current on the fibre; desensitization; effects due to geometrical factors; changes in membrane time constant during agonist action. Suitable controls were made against these factors, the last being excluded by voltage clamp experiments.

Since active DECA-receptor complexes revert to inactive complexes with a time constant ~0.25 ms (Katz & Miledi, 1973) and maximal activation by DECA at equilibrium only involves about 0.7% of the available receptors (Adams, unpublished), the expected time constant for the inactive → active transition is ~35 ms, similar to the delay between peak inhibition and excitation. However, these results can also be explained by access delays without invoking slow receptor kinetics.

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Evaluation of iontophoretic *N*-methylbicuculline and other inhibitory amino-acid antagonists in rat brain stem

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Specific strychnine antagonism of neuronal depression by glycine has been well documented (Curtis, Hösli & Johnston, 1968; Hösli & Tebećis, 1970). However the specificity and effectiveness of picrotoxin and bicuculline as antagonists of γ-aminobutyric acid (GABA) mediated depression has been questioned (Godfraind, Krnjević & Pumain, 1970; Straughan, Neal, Simmonds, Collins & Hill, 1971). Recently *N*-methylbicuculline (bicuculline methochloride) has been reported to be a specific GABA antagonist in the spinal cord (Johnston, Beart, Curtis, Game, McCulloch & MacLachlan, 1972). We have evaluated this compound together with strychnine, picrotoxin and bicuculline as antagonists of inhibitory amino-acids in the medulla-pons of urethane anaesthetized rats.

Standard microiontophoretic techniques were used to study drug effects on spontaneously active cells. Consistent control depressant responses were obtained to consecutive applications of glycine and GABA and these agonists were retested during the continuous application of one of the antagonists. The responses were analysed and antagonism expressed as the ratio (shift ratio) between test and control agonist responses (Hill & Simmonds, 1973). A shift ratio of $\geqslant 0.4$ was regarded as significant and indicative of antagonism. These values have been related to the charge passed through the antagonist barrel (Table 1).

Strychnine was both potent and consistent in

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.

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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