stimuli regularly produced small (<1 mV) depolarizations of the cells lasting much longer than a synaptic potential (1-5 s). With repetitive orthodromic stimulation at quite low frequencies (2-8 Hz) these depolarizations summed to give a steady depolarization of 3-10 mV, declining slowly after cessation of stimulation with a half-time of 5-20 s. Such responses were not seen after withdrawing the electrode.

The membrane potential was reduced in a repeatable manner by raising the K^+ concentration of the perfusing solution. Within the range 6-24 mM $[K^+]$ the membrane potential appeared to be a linear function of log $[K^+]_{out}$, with a slope of 47 mV per log unit.

Neither GABA (100 μM) nor carbachol (180 μM) altered the membrane potential: both depolarize ganglion neurones (Adams & Brown, 1973).

The properties of these cells appear to resemble those previously described for glial cells (see Kuffler & Nicholls, 1966; Lasansky, 1971), but differ from the 'unresponsive cells' of the cerebral cortex described by Krnjevic & Schwarz (1967) in their lower input resistance and failure to respond to cholinomimetic stimulation or to GABA.

Glial cells in sympathetic ganglia can accumulate exogenous γ-amino-butyric acid (GABA) (Young, Brown, Kelly & Schon, 1973); this may be released again by raising $[K^+]$ or by electrical stimulation (Bowery & Brown, 1972). The present observations might be relevant to the mechanism of this release and to possible interactions of neurones and glia.

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A comparison of the rate of onset of excitation and inhibition by decamethonium acting at frog endplate receptors

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del Castillo & Katz (1957) found that pulses of decamethonium (DECA) produced slower depolarizations when applied iontophoretically to endplate receptors than did acetylcholine or carbachol (CARB). They suggested that as DECA appeared to reach the receptors quickly (as judged by the slowing in rate of rise of a simultaneously elicited CARB response) this slow onset of depolarization might reflect slow activation of the DECA-receptor complex. Since these observations might have general implications for receptor theory, this problem has been re-examined, with particular attention to the rate of onset of antagonism by DECA of CARB responses.

The methods were similar to del Castillo & Katz (1957) except that triple-barrelled micropipettes were sometimes used, a saline-filled barrel then being available for control of electrical artefacts. The pipette was manipulated in the vicinity of an endplate region, during intracellular recording, until rapid CARB responses (<20 ms rise-time) were obtained. A short pulse to the DECA barrel then gave a slow depolarization (Figure 1). A long pulse (0.5-1.0 s) to the CARB barrel was then applied and DECA reapplied during the CARB depolarization (Figure 1). Since DECA is a partial agonist, it inhibited CARB, and the membrane hyperpolarized (del Castillo & Katz, 1957).

In 12 experiments the time to peak of the DECA excitation was 88 ± 9.6 ms (mean ± s.e.)

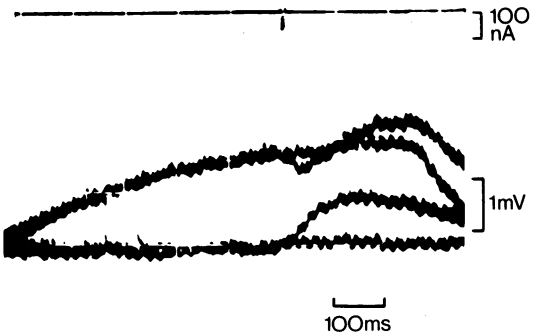

Fig. 1 Intracellular record of the action of iontophoretically applied DECA and CARB on a frog sartorius muscle fibre endplate. Four traces were superimposed: a baseline; a response to DECA alone; a response to CARB alone; a response to DECA during the action of CARB. The iontophoretic current pulses, 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