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GENERAL ANAESTHETICS AND THE ACETYLCHOLINE-SENSITIVITY OF CORTICAL NEURONES

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1 The effects of general anaesthetics on neuronal responses to iontophoretically-applied acetylcholine have been examined in slices of guinea-pig olfactory cortex maintained *in vitro*.

2 Acetylcholine excited 61% of the prepiriform neurones tested. The excitation was blocked by atropine, but not by dihydro-β-erythroidine or gallamine.

3 Alphaxalone reversibly depressed the acetylcholine-sensitivity of prepiriform neurones. Pentobarbitone did not consistently depress the acetylcholine sensitivity of these cells.

4 Ether, methoxyflurane, trichloroethylene and halothane caused a dose-related augmentation of acetylcholine-induced firing.

5 These results show that general anaesthetics do not necessarily depress the sensitivity of nerve cells to all excitatory substances and that different anaesthetics may affect a particular excitatory process in various ways.

Introduction

Previous studies have shown that general anaesthetics depress excitatory synaptic transmission in isolated slices of the guinea-pig olfactory cortex (Richards, 1974), and that the sensitivity of prepiriform neurones to iontophoretically-applied L-glutamate is depressed by similar concentrations of anaesthetic (with the exception of halothane; Richards & Smaje, 1976). The properties of glutamate resemble those of the natural transmitter at synapses between the lateral olfactory tract (l.o.t.) and prepiriform pyramidal cells. Thus, depression of synaptic transmission by anaesthetics other than halothane was attributed (at least in part) to an action on the postsynaptic sensitivity of prepiriform cells to the l.o.t. transmitter substance. The question arises whether the receptivity of these neurones to other excitatory substances will be affected in the same manner, or whether the depressant action of anaesthetics is specific for the excitation produced by glutamate and the l.o.t. transmitter.

Iontophoretic application of acetylcholine to olfactory cortex neurones causes excitation that is slow in both onset and decline, having the pharmacological properties of a predominantly muscarinic process (Legge, Randić & Straughan, 1966). Muscarinic excitation of cortical neurones is thought to result from a decrease in membrane permeability to potassium ions (Krnjević, Pumain & Renaud, 1971); the actions of acetylcholine, therefore, could be quite different in nature from the rapid actions of glutamate and the l.o.t. transmitter substance, which may be mediated by an increase in the membrane permeability to sodium (Richards, Russell & Smaje, 1975; Richards & Smaje, 1976).

This paper describes the actions of six general anaesthetics on the acetylcholine-sensitivity of prepiriform neurones. It will be shown that the four volatile agents tested (ether, methoxyflurane, trichloroethylene and halothane) caused an increased sensitivity of prepiriform neurones to acetylcholine. Pentobarbitone had no consistent effect on the sensitivity of neurones in the prepiriform cortex to acetylcholine. The steroid anaesthetic alphaxalone depressed the sensitivity of these neurones to acetylcholine in a similar manner to its action on their sensitivity to glutamate.

Methods

The methods were substantially those of the preceding paper (Richards & Smaje, 1976), with the following modifications.

Saline solutions

Standard saline had the following composition (mM): NaCl 134, KCl 5, KH_2PO_4 1.25, $MgSO_4$ 2, $CaCl_2$ 1,

$NaHCO_3$ 16 and glucose 10. High Mg^{2+}-low Ca^{2+} saline had the same composition as standard but with $MgSO_4$ 10 mM and $CaCl_2$ 0.75 mM, or as standard but with $MgSO_4$ 4 mM and $CaCl_2$, 0.5 mM.

Drugs

The following drugs were used, dissolved in the appropriate saline solution: atropine sulphate (BDH), dihydro-β-erythroidine hydrobromide (DHBE; kindly donated by Merck, Sharpe & Dohme), gallamine triethiodide (May & Baker), physostigmine sulphate (Wellcome).

Iontophoresis

At least two barrels of the five-barrelled microelectrodes contained 3.3 M NaCl and at least one contained Na L-glutamate (0.5 M, pH 7.1–7.3; BDH). In addition, at least one barrel contained acetylcholine chloride (0.2 M, pH 4–5; Roche). The electrodes were used within 24 h of filling. Current balancing was used in all experiments, except in some in which neurones were deliberately excited by anionic current passed from one or two NaCl-filled barrels.

Results

Acetylcholine-sensitivity of prepiriform neurones

In a series of 159 prepiriform neurones in 22 preparations located at random with glutamate, 97 (61%) could also be excited by acetylcholine (10–160 nA). The effect of acetylcholine was slow in onset (usually between 2 and 120 s) and persisted for a similar period after cessation of ejection (see Figure 2a). A temporary increase in firing rate often followed the termination of acetylcholine application, and a few units did not respond at all until this time. The depth distribution and spike amplitudes of cholinoceptive cells were not significantly different from those of cells responsive only to glutamate (Smaje, 1976).

When acetylcholine was ejected against a background of continuous glutamate excitation, an inhibitory effect starting within 2 s was revealed in all but one of 22 cells so examined. The characteristic action of acetylcholine (18/22 cells) was biphasic, the initial inhibition being followed by excitation within 2 s to several minutes.

In agreement with earlier field potential studies in the olfactory cortex (see Richards & Sercombe, 1970), the muscarinic blocking agent atropine (5 μM) blocked the excitatory effect of acetylcholine (but not that of glutamate) whereas the nicotinic blocking agents DHBE and gallamine (both 10 μM) did not. The excitatory action of acetylcholine was therefore considered to be muscarinic.

Frequently, a neurone that had been sensitive to acetylcholine would cease to be excited by acetylcholine. Sensitivity could occasionally be restored if a brief pulse of glutamate was used to initiate firing, or if the acetylcholine ejecting current was increased. However, approximately one in every six experiments was abandoned because the neurone became refractory to acetylcholine during the initial few minutes of the control period. Neither excitation by glutamate nor inhibition by acetylcholine were lost in this manner.

Effects of anaesthetics on acetylcholine-sensitivity

This was investigated on 61 cells in 43 preparations in standard saline. Acetylcholine was expelled with pulses of current, usually lasting 10 s and repeated every 30 s throughout an experiment. The mean number of spikes generated in response to each 10 s pulse (including after-discharge) was 85 (range 40–200 spikes), and was achieved with currents in the range 14–140 nA; mean 74 nA. Anaesthetics were delivered in concentrations known to depress excitatory synaptic transmission and glutamate sensitivity in the olfactory cortex (Richards & Smaje, 1976).

Pentobarbitone. Pentobarbitone (0.05–0.40 mM) had little or no effect on the responses of almost half (8/17) of the cells excited by acetylcholine. The sensitivity of the other 9 cells to acetylcholine was totally lost 2–22 min after the beginning of the exposure to pentobarbitone. This loss of sensitivity to acetylcholine was independent of the concentration of pentobarbitone applied. When a cell became unresponsive to acetylcholine, it was excited by applying glutamate to confirm that it was still near the electrode. Only three of the cells tested in this manner recovered their sensitivity to acetylcholine within 30 min of washing out the pentobarbitone.

Alphaxalone. Alphaxalone (50 μM) was delivered to the preparations as a component of phospholipid vesicles suspended in standard saline (Richards & Hesketh, 1975). The acetylcholine-evoked activity of all 5 neurones studied was abolished 1–6 min after the introduction of alphaxalone. Recovery was observed within 20 min in four cases, one such experiment being shown in Figure 1, but was negligible at 30 min in the fifth. Phospholipid suspension without the anaesthetic component served as the control solution in these experiments, and had no depressant effect on the acetylcholine responses of prepiriform neurones.

Volatile anaesthetics. Exposure to volatile agents in low to moderate concentrations caused a prompt and sustained augmentation of the acetylcholine-induced

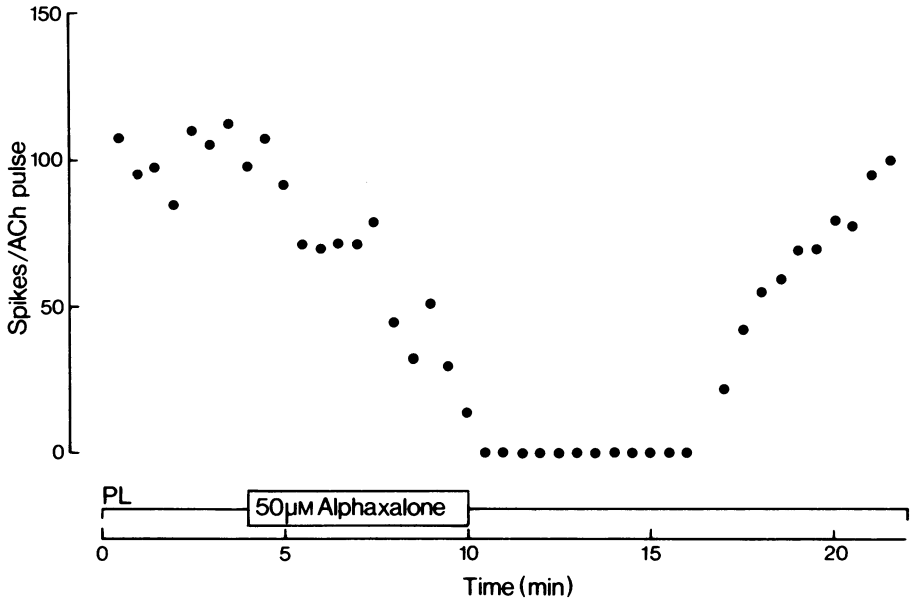

Figure 1 The effect of alphaxalone on the acetylcholine-sensitivity of a neurone in the prepiriform cortex. This cell was 390 μm deep and was excited by a 75 nA pulse of acetylcholine for 10 s every 30 seconds. PL, phospholipid liposome suspension superfused as a control; Alphaxalone, liposomes containing alphaxalone in a 1 : 1 molar ratio with phospholipid. The final molarity of the alphaxalone in the suspension was 50 μM.

firing of all cells studied (ether 0.5–2.9%, 10 units; methoxyflurane 0.05–0.35%, 11 units; trichloroethylene 0.03–0.40%, 11 units; halothane 0.09–1.0%, 6 units). Figure 2 presents a typical experiment in which a 14 min exposure to 0.4–0.5% halothane caused the mean firing rate of the neurone to be approximately doubled. The delay in the onset of cholinergic excitation was reduced, the steady level of firing increased, and the after-discharge lengthened. Although action potentials could sometimes not be recorded in the presence of high concentrations of anaesthetic, this may have been due to depolarization block, rather than to depression of sensitivity to acetylcholine. This is illustrated in Figure 3, in which the firing frequency of a cell excited by acetylcholine rapidly dropped when the concentration of trichloroethylene was increased from 0.2 to 0.5%; the spike amplitude was considerably diminished when the cell was discharging at its maximum rate. Figure 4 summarizes the results obtained with each volatile anaesthetic (open squares): the dose-related nature of the effects is evident. Refractoriness to the excitatory action of acetylcholine never developed during the delivery of a volatile anaesthetic.

Four possible explanations for these results were considered: first, anaesthetics may have depressed inhibitory influences from adjacent neurones in synaptic contact with the cell under observation. This was excluded on the basis of experiments performed under conditions of synaptic blockade. Increase of the magnesium and reduction of the calcium content of the bathing saline combine to suppress synaptic transmission in the olfactory cortex slice, probably by reducing transmitter output, as their effects on post-synaptic threshold tend to cancel (Richards & Sercombe, 1970). Accordingly, three experiments with trichloroethylene and five with halothane were performed in preparations equilibrated with high Mg^{2+}-low Ca^{2+} saline; the results were similar to those in standard saline (filled squares, Figure 4).

Second, a direct stimulatory action of the anaesthetics was considered. A control level of firing, without the intermediary of a chemical excitant, was provided by extracellular anionic current stimulation (range 120–240 nA, mean 180 nA; 10 s pulses every 30 s) (Krnjević & Phillis, 1963a). All the experiments were conducted in high Mg^{2+}-low Ca^{2+} saline to prevent the release of transmitter substances from other depolarized structures. Six units were studied with trichloroethylene (0.06–0.60%) and six with halothane (0.15–1.3%). Both anaesthetics had a mild stimulatory effect in high concentration (trichloroethylene >0.4%, halothane >0.9%), presumably corresponding to that seen in previous experiments

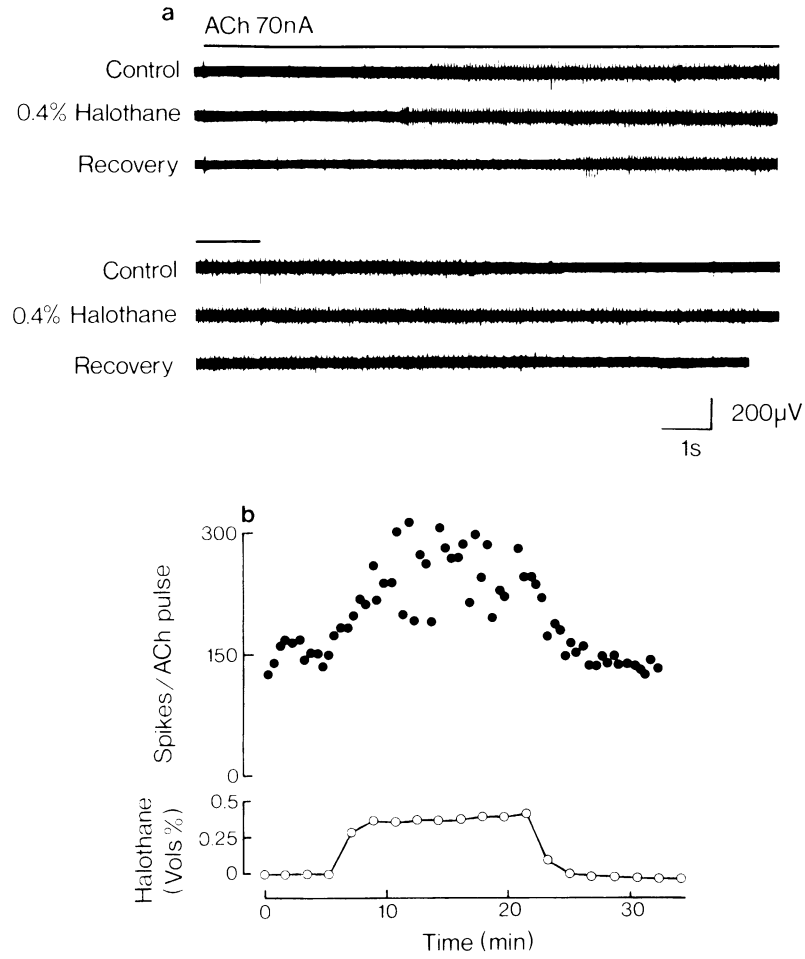

Figure 2 The action of halothane on the sensitivity of a neurone to iontophoretically-applied acetylcholine. (a) The discharge of a neurone in response to a 15 s 70 nA pulse of acetylcholine (ACh, black bar) before, during and after exposure to halothane. The records in the two panels are continuous. This cell was 290 μm deep in the prepiriform cortex. (b) Summary of the time course of the action of halothane on the cell shown in (a).

(Richards & Smaje, 1976), but inadequate in itself to account for the acceleration of acetylcholine responses.

Third, a capacity of volatile anaesthetics to suppress cholinergic inhibition was examined in six preparations treated with atropine; cholinergic inhibition was then revealed against a background of glutamate excitation. As halothane does not depress the glutamate excitation of cortical neurones (Richards & Smaje, 1974, 1976) its action on cholinergic inhibition was studied. No significant effect on the inhibitory action of acetylcholine was observed with halothane administered in concentrations from 0.2 to 1.1% (7 units; Figure 5).

Finally, it was possible that volatile anaesthetics inhibited the hydrolysis of acetylcholine by acetylcholinesterase. This was tested in preparations whose cholinesterase activity was already abolished by treatment with physostigmine (7.8–78 μM). Difficulty was experienced in obtaining satisfactory responses to acetylcholine as refractoriness to the excitatory action of acetylcholine, or rapid depolarization block, commonly developed. As a result, only one neurone was successfully studied. The acetylcholine-induced firing rate of this cell rose during a 7 min exposure to trichloroethylene (0.2–0.35%) to over twice that in the control period; the neurone became refractory to acetylcholine at the termination of anaesthetic

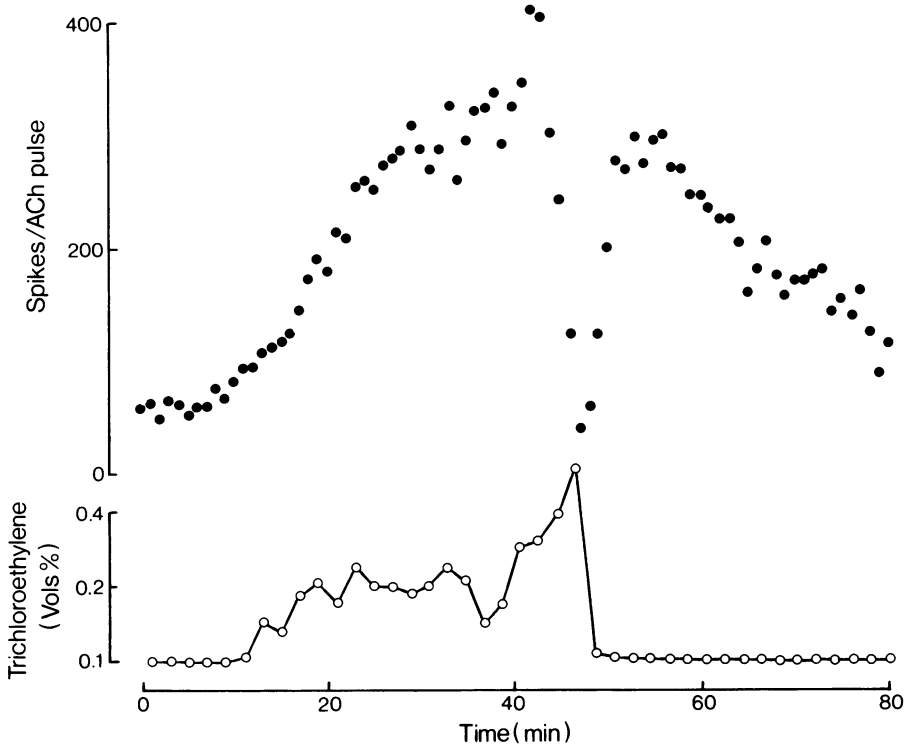

Figure 3 The actions of trichloroethylene on the sensitivity of a neurone to iontophoretically-applied acetylcholine. This cell was 310 μm deep and was excited by a 90 nA pulse of acetylcholine for 10 s every 30 seconds. Trichloroethylene (0.2%) caused the firing rate of the cell to be quadrupled but increasing the anaesthetic to 0.5% caused a block of the responsiveness to acetylcholine. Both effects were reversed as the anaesthetic was removed.

delivery. The acceleration of acetylcholine responses cannot have been due to inhibition of acetylcholinesterase in the absence of significant activity of this enzyme.

Experiments with neocortical preparations

A small number of experiments was also performed with coronal slices of the guinea-pig sensorimotor neocortex. Five neurones in separate preparations were exposed to trichloroethylene (0.07–0.25%). The responses of these cells to acetylcholine were increased during exposure to this anaesthetic. This effect was very similar to that observed in the olfactory cortex preparation.

Discussion

The excitatory responses of isolated prepiriform neurones to acetylcholine closely resembled those seen in the cat piriform cortex *in vivo* (Legge *et al.* 1966). These authors do not mention the development of refractoriness to acetylcholine, although this does occur in the thalamus (Andersen & Curtis, 1964; McCance, Phillis & Westerman, 1968), and desensitization to acetylcholine is well known in other cholinoceptive tissues (Michelson & Zeimal, 1973). Expression of the phenomenon was modified by anaesthetics: no unit ceased responding to acetylcholine in the presence of a volatile agent, whilst several did so during exposure to pentobarbitone.

The assumption that the firing rate of a neurone in response to acetylcholine reflects the sensitivity of that cell to acetylcholine rests on several conditions. First, that there be no change in the coupling between depolarization and discharge in the presence of an anaesthetic; this has been considered in the accompanying paper (Richards & Smaje, 1976). Next, that subliminal excitation or inhibition from adjacent neurones (or from an intermediate substance that may be released) should be constant or insignificant; the

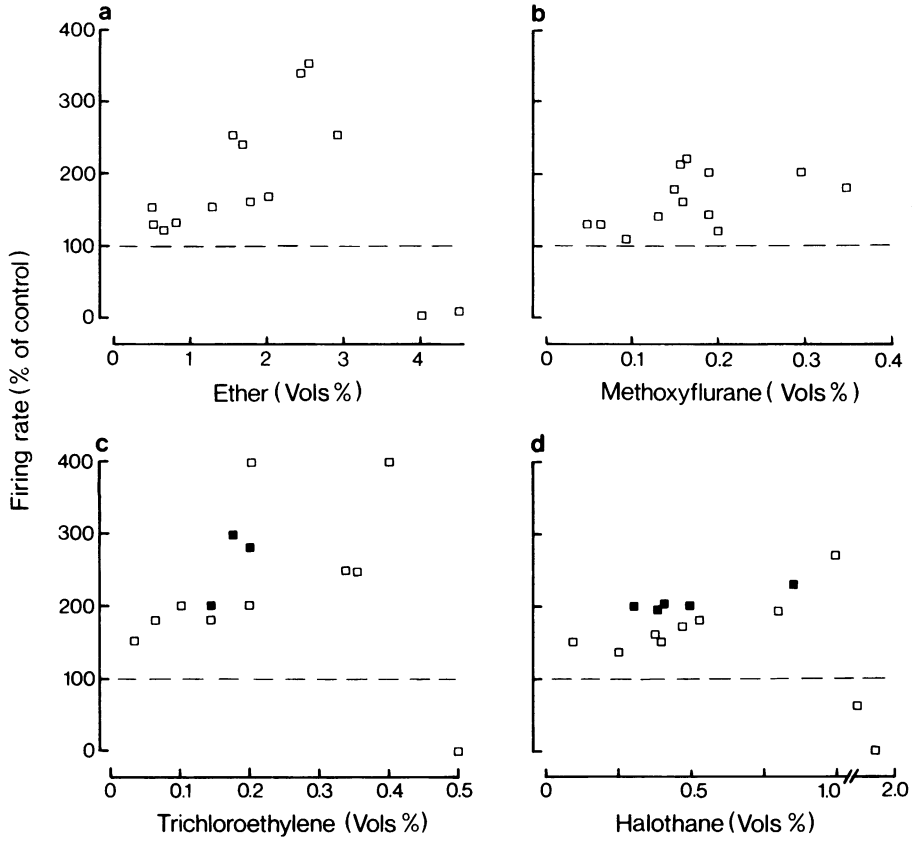

Figure 4 Summary of the relationship between the acetylcholine responses of neurones in the prepiriform cortex and the concentrations of volatile anaesthetics applied. The results are expressed as a percentage of the acetylcholine-evoked firing rate observed in the absence of anaesthetic. (The normalized control firing rate is indicated by the horizontal dotted line). (□) Experiments in standard saline; (■) experiments in high Mg^{2+}- low Ca^{2+} saline.

experiments with high Mg^{2+}-low Ca^{2+} saline suggest that this was true. Furthermore, the potentiating effect of volatile anaesthetics on the acetylcholine responses of prepiriform neurones was shown not to result from direct stimulation by the anaesthetics, or from reduction in cholinergic inhibition or cholinesterase activity. The remaining possibilities are that volatile anaesthetics increase the affinity of muscarinic receptors for acetylcholine, or that they increase the efficacy of acetylcholine in depolarizing the neuronal membrane through changes in the conformation of the receptor or receptor-linked ionophore. As the excitation produced by acetylcholine is thought to result from a decrease in the permeability of the resting membrane to potassium (Krnjević *et al.* 1971), one possible effect of volatile agents could be to decrease further the resting K^+ conductance. If these agents also interfered with the K^+ conductance increase that follows the entry of sodium during an action potential, the repolarizing phase of a spike would be prolonged; this could provide an explanation for the burst firing observed in glutamate-excited neurones (Richards *et al.*, 1975; Richards & Smaje, 1976). An intracellular investigation of *Aplysia* neurones has shown various agents (including the volatile anaesthetic chloroform, but also pentobarbitone) to cause changes in K^+ conductance of this sort (Barker, 1975b). Intracellular studies in the mammalian brain are required to explore this possibility further.

The effects of volatile anaesthetics on the sensitivity of neurones to acetylcholine may be contrasted with their depressant action on the glutamate-sensitivity of neurones in the same region of cortex.

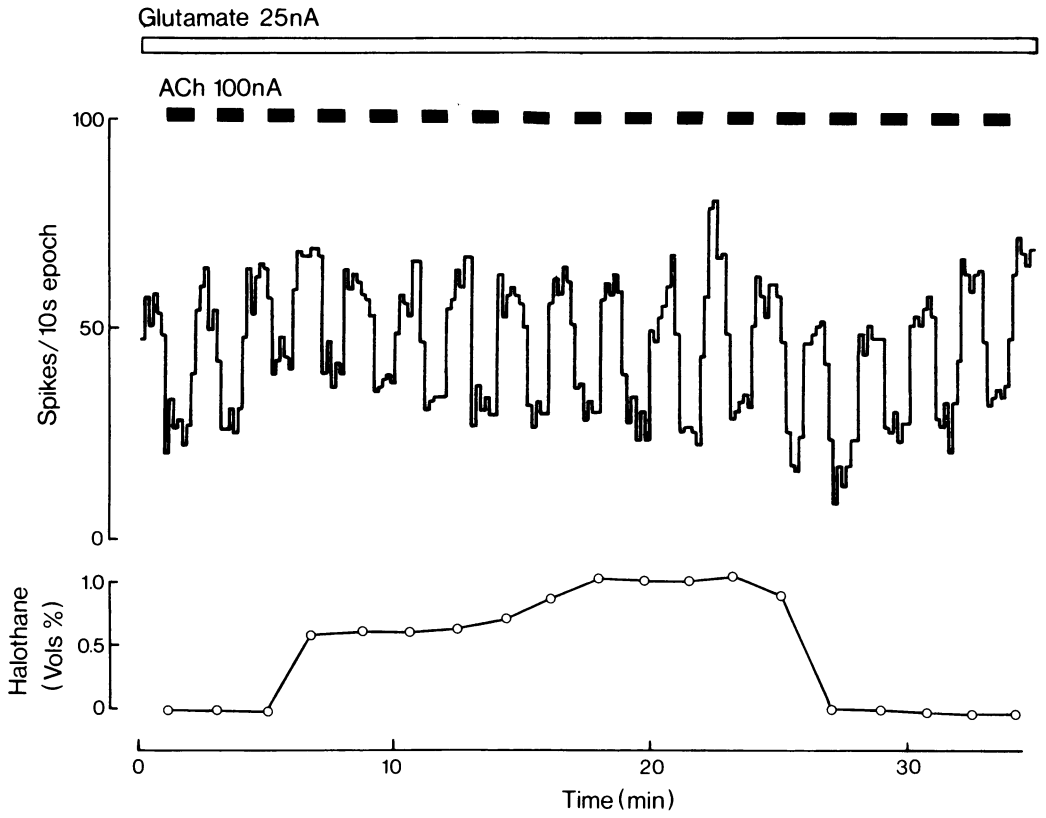

Figure 5 The effect of halothane on the inhibitory action of acetylcholine on a neurone in the prepiriform cortex. This cell was 400 μm deep in the prepiriform cortex and was excited by a continuous release of glutamate (25 nA). The acetylcholine (ACh, 100 nA) was released for 1 min periods every 2 min (indicated by black bars). The experiment was conducted in standard saline containing atropine (0.2 mM) in order to block the excitatory actions of acetylcholine. Halothane (up to 1.1%) had no effect on the inhibitory action of acetylcholine.

Although pentobarbitone depressed the glutamate-sensitivity of neurones, it failed to depress the acetylcholine-sensitivity of those cells that did not become totally refractory to acetylcholine. Only alphaxalone reversibly depressed both the glutamate-sensitivity and the acetylcholine-sensitivity of neurones. These findings show that different anaesthetics do not necessarily share a common mode of action.

A particular advantage of *in vitro* preparations of the central nervous system is the absence of spontaneous nerve cell activity. This has made possible the study of the action of anaesthetics on the sensitivity of individual nerve cells to excitants without the variations in subliminal excitation and inhibition that are characteristic of the intact animal. The fact that such influences have been removed should be considered when the present experiments are compared to those conducted *in vivo*. Systemic administration of barbiturates (Krnjević & Phillis, 1963b; Crawford & Curtis, 1966; Crawford, 1970; Catchlove, Krnjević & Maretić, 1972) and high concentrations of volatile anaesthetics (Catchlove *et al.*, 1972) have been shown to depress the sensitivity of cortical neurones to acetylcholine in parallel with their depression of spontaneous nerve cell activity. However, when Crawford (1970) applied pentobarbitone iontophoretically he found little depressant effect on the acetylcholine-sensitivity of cortical neurones; he also showed that methoxyflurane, trichloroethylene and halothane did not depress the sensitivity of cortical neurones to acetylcholine when delivered in moderate concentrations.

To conclude, the present experiments together with those of the previous paper (Richards & Smaje, 1976) have shown that the depolarizing action of glutamate

is depressed by general anaesthetics whereas that of acetylcholine was depressed by alphaxalone but not by other general anaesthetics. As muscarinic excitation of cortical neurones is thought to result from a decrease in the resting membrane permeability to potassium ions (Krnjević *et al.*, 1971) while glutamate excitation is thought to result from an increase in the sodium permeability (see Richards *et al.*, 1975), these results are consistent with the suggestion of Barker (1975a) that most general anaesthetics depress the sensitivity of the post-synaptic membrane to chemical transmitters only where the transmitters cause an increased membrane permeability to sodium.

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