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ANAESTHETICS DEPRESS THE SENSITIVITY OF CORTICAL NEURONES TO L-GLUTAMATE

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1 The effects of general anaesthetics on the responses of neurones to iontophoretically applied L-glutamate have been examined in slices of the guinea-pig olfactory cortex *in vitro*.

2 Concentrations of pentobarbitone, ether, methoxyflurane, trichloroethylene and alphaxalone that are known to depress synaptic transmission in the prepiriform cortex also depressed the sensitivity of prepiriform neurones to L-glutamate.

3 Halothane, in concentrations that depress synaptic transmission (< 1%) did not alter the sensitivity of neurones to glutamate. Higher concentrations (> 1%) produced a dose-related depression of the glutamate sensitivity of neurones.

4 All four volatile anaesthetics tested caused some cells to alter their glutamate-evoked firing pattern to one in which the spike discharges were more closely grouped. Pentobarbitone and alphaxalone had no such effect.

5 If the sensitivity of the neurones to the endogenous excitatory transmitter is affected by anaesthetics in the same way as the glutamate-sensitivity, these results suggest that halothane depresses synaptic transmission by decreasing the amount of transmitter released from the nerve terminals, whereas the other anaesthetics depress the sensitivity of the post-synaptic membrane to the released transmitter.

Introduction

General anaesthetics in concentrations less than, or similar to, those required for the maintenance of anaesthesia depress excitatory synaptic transmission at a number of sites within the central nervous system, including the olfactory cortex (see Richards, 1974); this suggests that depression of excitatory synaptic transmission occurs during anaesthesia, and that it may be one of the cellular events responsible for the production of the anaesthetic state. A previous series of studies on the evoked field potentials of the olfactory cortex has shown that low concentrations of anaesthetics do not affect the electrical excitability of the fibres of the lateral olfactory tract (l.o.t.) or that of neurones of the prepiriform cortex, but they do depress the excitatory post-synaptic potentials (e.p.s.ps) that occur in the neurones of the prepiriform cortex following stimulation of the l.o.t. These results imply that anaesthetics decrease the amount of transmitter released by a nerve impulse, or they depress the sensitivity of the post-synaptic membrane to the transmitter, or both. However, field potential analysis could not by itself provide sufficient evidence to determine which of these alternatives was responsible for the observed depression of the e.p.s.ps (Richards, 1972, 1973a; Richards, Russell & Smaje, 1975). A definitive answer requires that the identity of the transmitter substance be known.

Unfortunately, the identity of the transmitter released from the l.o.t. is not established. Nonetheless, nerve cells in the prepiriform cortex can be excited by a number of substances including acetylcholine and glutamate (Legge, Randić & Straughan, 1966). While these two excitants are both thought to act directly on receptors located on the post-synaptic membrane, rather than by causing the release of other excitants, they excite neurones by quite different mechanisms. The excitation that is produced by acetylcholine is characteristically slow in onset and decline and results from a decrease in the resting membrane conductance to potassium (Krnjević, Pumain & Renaud, 1971), whereas the excitation produced by glutamate is rapid in onset and decline and mimics that of the naturally occurring transmitter (see Discussion in Richards *et al.*, 1975). In this paper we have examined the actions of six general anaesthetics on the sensitivity of neurones in the prepiriform cortex to iontophoretically applied L-glutamate. Brief accounts of some of these experiments have already been published (Richards & Smaje, 1974;

Richards *et al.*, 1975). In the accompanying paper (Smaje, 1976) the actions of general anaesthetics on the sensitivity of prepiriform neurones to iontophoretically applied acetylcholine are described.

Methods

Details of the methods of preparation, incubation, stimulation and field potential recording have already been described in detail (Yamamoto & McIlwain, 1966; Richards *et al.*, 1975). Guinea-pigs were killed by a blow on the back of the neck, the skull was opened and the brain removed. A thin tangential slice of the olfactory cortex was then made with a razor strip and a glass template. The slices so prepared had a nominal thickness of 410 μm and were incubated in a chamber (described by Doré & Richards, 1974) at 37°C. In this chamber the slice rested on a platinum grid with its cut surface bathed by artificial c.s.f. and the uncut, pial surface exposed to a humidified atmosphere of 95% O_2 and 5% CO_2. For the experiments with the steroid anaesthetic alphaxalone, a modified chamber was used which allowed both surfaces of the slice to be superfused with saline.

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+} saline had a similar composition but contained 10 mM $MgSO_4$ rather than 2 mM $MgSO_4$. No osmotic compensation was made. The solutions were saturated with a mixture of 95% O_2 and 5% CO_2 before use, and had a pH of 7.2–7.4.

Anaesthetics

The following anaesthetics were used: sodium pentobarbitone (Abbott Laboratories); diethyl ether (Macfarlan Smith), trichloroethylene (BDH 'Aristar' grade); halothane (redistilled from Fluothane, ICI Ltd.) and alphaxalone (Glaxo Laboratories). Sodium pentobarbitone was dissolved in the bathing saline solutions before being applied to the tissue. No pH correction was required as its concentration was very low (0.05–0.4 mM) in relation to that of the bicarbonate buffer (16 mM). The volatile anaesthetics were applied in the gas phase mixed with the stream of 95% O_2 and 5% CO_2 that superfused the upper surface of the slice. The exact concentration administered was estimated automatically by an on-line gas chromatograph (see Richards, 1973a; Richards *et al.*, 1975). Because of its highly hydrophobic nature, alphaxalone was applied as the steroid component of phospholipid vesicles (liposomes) suspended in the saline solution (see Richards & Hesketh, 1975); standard saline containing liposomes without alphaxalone was administered as a control.

Iontophoresis

Conventional five-barrelled electrodes with tip diameters of 3–8 μm were used; three barrels contained 3.3 M NaCl and two 0.5 M Na L-glutamate (BDH Ltd.) (pH 7.1–7.3). One NaCl-filled barrel was used to record the activity evoked by the ejection of glutamate or the electrical stimulation of the l.o.t. (Richards & Sercombe, 1968), while another served to balance out the net currents through the electrode (Salmoiraghi & Steiner, 1963). This barrel could also be used to check for artifacts caused by the passage of current. Diffusion of glutamate from the electrode tip was prevented by retaining currents (electrode tip positive, 10–20 nA; del Castillo & Katz, 1955).

Neurones in brain tissue slices are not generally spontaneously active so single units were isolated during excitation by continuous ejection of glutamate (electrode tip negative, 10–50 nA). Thereafter, glutamate was ejected from one barrel in pulses according to a fixed schedule; the pulse duration and current remained constant throughout each trial. The most useful schedule comprised an ejection period of 10 s followed by a retention period of 20 seconds. A fixed ejection schedule is desirable as the successive pulses are alike and a constant amount of drug is given at each application.

Recording

The evoked units were recorded through one NaCl-filled barrel of the iontophoretic pipette; the indifferent electrode was placed in the saline that bathed the lower surface of the slice. The recording barrel was coupled to a voltage follower or preamplifier through Ag-AgCl wires which were connected in turn to an oscilloscope and FM tape recorder. The overall flat band width of the recording system extended from 3 Hz to beyond 2.5 kHz. During the experiment the spike activity was amplified and filtered (pass band 300 Hz–10 kHz, centre frequency 2.5 kHz) and then fed into a discriminator counter which registered only those spikes whose amplitude fell between two adjustable voltage levels. The noise level of the recording system was approximately 30 μV peak-to-peak. Satisfactory discrimination of the spike discharges was generally achieved when the amplitude of a spike was greater than 100 μV.

Protocol

All experiments were restricted to the prepiriform area of the olfactory cortex, i.e. the area that lies between the lateral edge of the l.o.t. and the medial border of

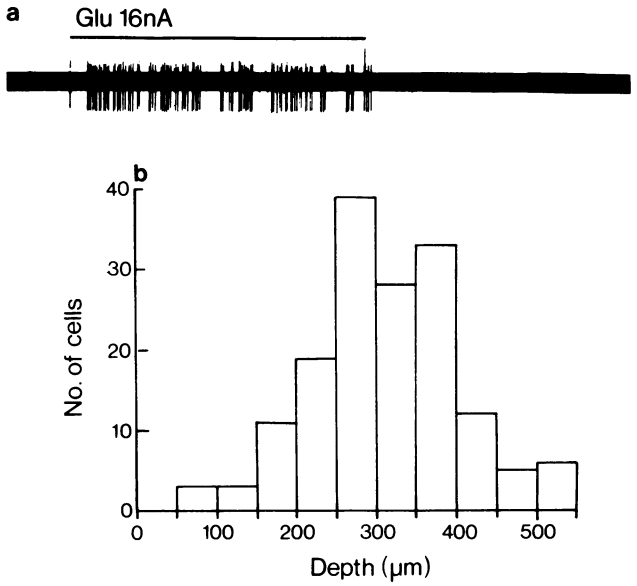

Figure 1 Characteristics of glutamate-sensitive neurones in the prepiriform cortex. (a) An original record of a neurone responding to iontophoretically applied glutamate. This cell was located 430 μm below the pia and was not spontaneously active. Note that cell discharge starts within 600 ms of applying the glutamate and there is little after-discharge. Calibration: horizontal bar 10 seconds. (b) The depth distribution of glutamate-sensitive cells in the prepiriform cortex. These cells were not spontaneously active but could be excited by stimulation of the l.o.t.

the rhinal fissure. The small pyramidal cells that comprise layer II of this area receive a massive projection from the fibres of the l.o.t. as do the deep pyramidal cells (see Shepherd, 1974). About half the cells located by application of glutamate were also tested to see whether l.o.t. stimulation excited them; in all cases it did so. It was impossible to apply this test when synaptic transmission was blocked by high Mg^{2+} saline.

If the responses of a unit to fixed pulses of glutamate were consistent for four or more minutes, the anaesthetic was introduced and the responses were followed for up to 30 min before the anaesthetic was removed and the unit allowed to recover from the anaesthetic. Before exposing a preparation to high Mg^{2+} saline, it was first incubated in standard saline. Then, while the evoked field potentials were monitored, the preparation was exposed to high Mg^{2+} saline. Synaptic transmission was rapidly blocked but to ensure total synaptic blockade the experiment was performed only when the evoked population e.p.s.p. was less than 5% of its initial size.

Data analysis

The total number of spikes generated by a single unit in response to a given pulse of glutamate was taken as an index of a cell's sensitivity to glutamate (see Discussion section). The spike activity from representative portions of the data recorded on magnetic tape was subjected to interspike interval analysis using the special purpose computer described by Lewin (1974). A minimum of 250 spikes was used to compile each interval histogram; records obtained from cells which had a very slow firing rate during exposure to anaesthetic were generally unsuitable for interval analysis.

Results

Excitation of prepiriform neurones by L-glutamate

Neurones in the isolated olfactory cortex were normally electrically silent. Neuronal discharge began within 100–1000 ms of the iontophoretic application of glutamate (2–80 nA), continued as long as the glutamate was being applied and stopped within 500 ms of the ending of the application (see Figure 1). These characteristics of the glutamate-sensitivity of prepiriform neurones are similar to those of neurones in other areas of the brain (Phillis, 1970) including the cat piriform cortex *in vivo* (Legge *et al.*, 1966).

In a series of 22 experiments, 159 cells were located

by iontophoretic application of L-glutamate. All of these cells could also be made to discharge in response to electrical stimulation of the l.o.t.; 75% were located 200–400 μm below the pial surface (Figure 1). This is the depth at which the dense layer of small pyramidal cells is found (layer II, see Lohman, 1963; Price, 1973).

Effects of anaesthetics on glutamate-sensitivity in standard saline

A total of 104 cells were studied in 71 preparations of the olfactory cortex; 76 of these cells (50 preparations) were examined in standard saline, the remainder were examined in high Mg^{2+} saline. These cells were stimulated by amounts of glutamate sufficient to evoke a steady rate of firing of about 30–40 spikes per 10 s ejection period (mean 36 spikes per 10 s application; range 10–150 spikes per 10 seconds). In standard saline this firing rate was achieved with currents of 2–80 nA. The amplitude of the spikes found suitable for study with anaesthetics ranged from 100–500 μV (mean 180 μV).

Pentobarbitone. Twelve cells were tested for changes in their sensitivity to glutamate before, during and after exposure to concentrations of pentobarbitone within the range 0.05–0.3 mM. At 0.05 mM pentobarbitone depressed the glutamate-sensitivity of 1 cell of 6 tested, but the higher concentrations used (0.1–0.3 mM) consistently depressed the glutamate-sensitivity of all the neurones that were tested. The depression developed within 3–4 min of exposing the tissue to a saline solution containing the drug and was dose-related and reversible (Figure 2). The rate at which the neurones recovered their normal sensitivity to glutamate depended on the duration of the exposure to pentobarbitone and on the concentration applied.

Alphaxalone. The glutamate-sensitivity of all 11 cells studied was depressed by alphaxalone. Of these 11 cells, 7 had their glutamate-evoked activity totally suppressed and the remaining 4 cells had their sensitivity to glutamate reduced by 60–90% when the tissue was exposed to a suspension of liposomes containing alphaxalone (50 μM per l of saline). The onset of the depression was characteristically abrupt and occurred within 1–6 min of exposing the tissues to alphaxalone (Figure 3). When the liposomes containing alphaxalone were replaced by pure phospholipid vesicles, the cells recovered their sensitivity to glutamate, although full recovery was often protracted. Phospholipid vesicles without alphaxalone had no depressant action.

Ether and methoxyflurane. A total of 28 cells were tested with these anaesthetics, 11 with ether and 17 with methoxyflurane. Both anaesthetics caused a dose-related depression of the glutamate-evoked activity of neurones in the prepiriform cortex (see Figure 5a,b and Figure 8 of Richards *et al.*, 1975).

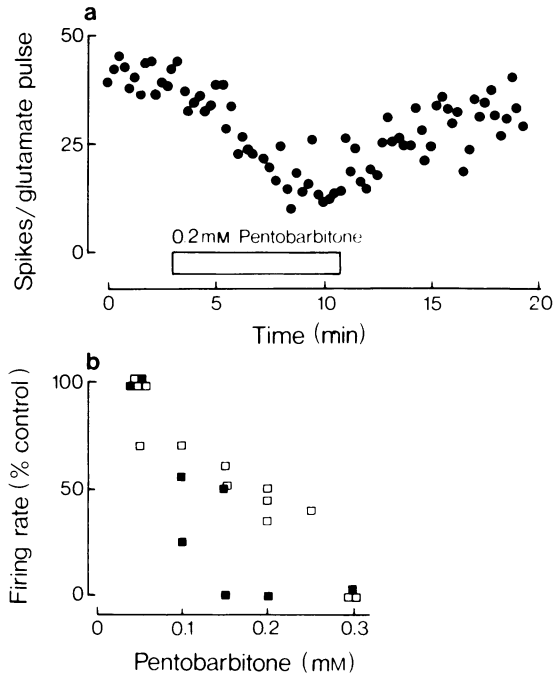

Figure 2 The action of pentobarbitone on the sensitivity of cells in the prepiriform cortex to glutamate. (a) Shows the time course of the action of 0.2 mM pentobarbitone on the glutamate-sensitivity of a cell 210 μm deep in the prepiriform cortex. The cell was excited by a 12 nA pulse of glutamate of 10 s duration every 30 seconds. In this and subsequent figures the ordinate is the total number of spikes generated by each pulse of glutamate. (b) The relationship between the glutamate sensitivity of prepiriform neurones and the concentration of pentobarbitone applied to them. Each point represents the glutamate-evoked firing rate of a cell expressed as a percentage of the firing rate in the absence of anaesthetic. The determinations were made after equilibration with anaesthetic. (□) Experiments conducted in standard saline; (■) experiments conducted in high Mg^{2+} saline.

Trichloroethylene. Low concentrations of trichloroethylene (0.05–0.3%) caused a dose-related depression of the glutamate-evoked activity of all 13 cells tested (Figures 4a, 5c). However, increasing the trichloroethylene concentration above 0.3% did not cause a further decrease in the responsiveness to glutamate but caused an apparent reversal of this trend until at 0.7–1% trichloroethylene the firing rate

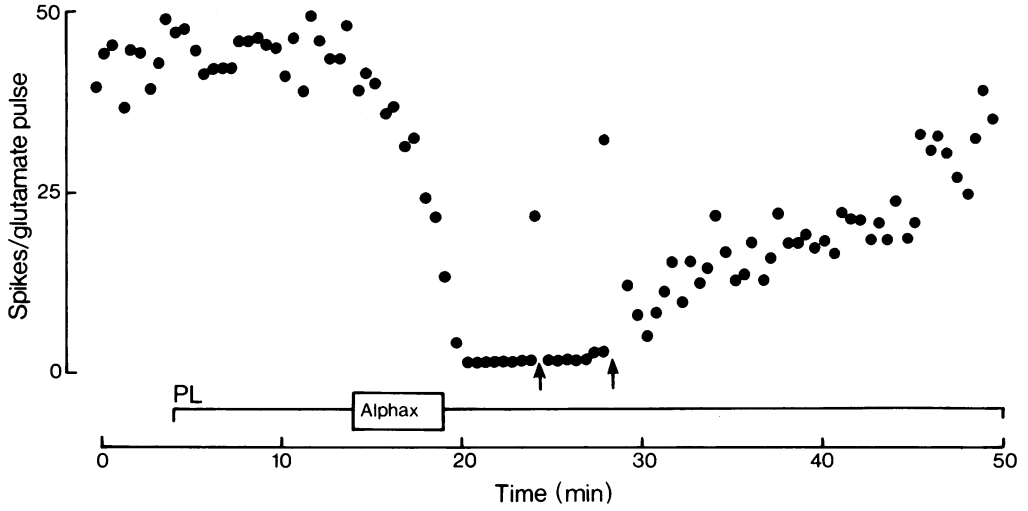

Figure 3 The action of 50 μM alphaxalone on the glutamate-sensitivity of a neurone in the prepiriform cortex. This cell was 300 μm deep and was excited by a 16 nA pulse of glutamate of 10 s duration every 30 seconds. Abbreviations: PL, suspension of phospholipid liposomes was superfused as a control. Alphax, liposomes containing alphaxalone replaced the phospholipid liposomes. The arrows indicate 32 nA pulses of glutamate which were used to check that contact with the cell had not been lost.

equalled or exceeded that recorded in the absence of anaesthetic (3 cells). Increasing the trichloroethylene concentration still further to 1.5% resulted in the complete loss of activity.

These effects were reversible and were consistent with the idea that trichloroethylene has two actions, a depressant action on the glutamate-sensitivity and a direct stimulatory effect on the cell membrane. Evidence in favour of a direct stimulatory action was obtained from 3 cells which were exposed to 0.65–0.9% trichloroethylene; during exposure to such high concentrations all three cells began to discharge spontaneously during the periods between applications of glutamate and the spike amplitude was reduced by 10–50%.

Halothane. Unlike the other anaesthetics studied, halothane had no obvious depressant action on the glutamate-sensitivity of neurones in the prepiriform cortex even at concentrations known to depress synaptic transmission by 50% or more (up to 1%) (Richards, 1973a). This result was found with all 14 cells studied. One example is shown in Figure 4b. Above 1.2%, however, a depressant effect was seen which was dose-related and reversible (10 cells; Figure 5d). As with trichloroethylene, exposure to concentrations of halothane greater than 1.2% caused 3 cells (out of 10) to fire spontaneously between the iontophoretic pulses of glutamate; under these conditions the spike amplitude was reduced.

Effects of anaesthetics on glutamate-sensitivity in high Mg^{2+} saline

Glutamate is able to diffuse at least 300 μm through cortical tissue (Herz, Zieglgansberger & Färber, 1969) and will therefore excite a number of cells in addition to the one under observation. It is already known that anaesthetics depress synaptic transmission in the cortex (see Richards, 1974; Richards *et al.*, 1975). Therefore, the depression of the glutamate-evoked activity of a cell that results from exposure to anaesthetic might not be due to a direct effect on the postsynaptic membrane of the cell but to a depressant action on synapses of adjacent cells which could modify the activity of the cell under observation via intracortical connexions. This possibility could be excluded if similar results were obtained under circumstances that did not permit synaptic transmission. Increasing the level of Mg^{2+} in the bathing medium depresses synaptic transmission between the l.o.t. and the cells of the prepiriform cortex until at 10–13 mM Mg^{2+} transmission is substantially blocked (Richards & Sercombe, 1970). The same is presumably true for other synaptic contacts within the slice. Accordingly, a proportion of the experiments were conducted in high Mg^{2+} saline.

The glutamate ejecting current was increased in 10 mM Mg^{2+} saline in order to keep the control rates of firing comparable to those in standard saline (*ca* 35 spikes per 10 s glutamate pulse). The mean current

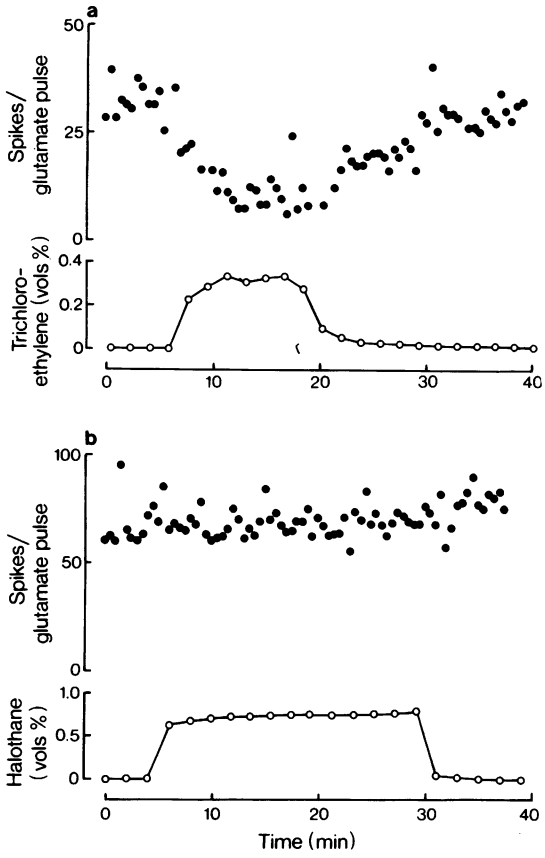

Figure 4 The actions of two volatile anaesthetics on the glutamate-sensitivity of neurones in the prepiriform cortex. (a) The depressant action of trichloroethylene. This cell was 300 μm deep and was excited by a 20 nA pulse of glutamate for 10 s every 30 seconds. (b) Lack of depressant effect of halothane. This cell was 280 μm deep and was excited by a 8 nA pulse of glutamate for 10 s every 30 seconds.

required was 39 nA compared to a mean of 25 nA required to give comparable firing rates in 2 mM Mg^{2+} saline. This increase in current reflects the increase in the threshold depolarization required for spike initiation which is known to occur when Mg^{2+} concentrations are elevated (Kelly, Krnjević & Somjen, 1969; Richards & Sercombe, 1970). Nonetheless, anaesthetics depressed the sensitivity of neurones in the prepiriform cortex to iontophoretically applied glutamate in high Mg^{2+} saline. This result was obtained with all five anaesthetics so examined (alphaxalone was not tested): pentobarbitone 0.05–0.3 mM, 6 units; ether 1.2–6%, 5 units; methoxyflurane 0.04–0.35%, 6 units; trichloroethylene 0.09–0.42%, 6 units; halothane 1.4–5%, 2 units (but no depression was observed at concentrations below 1%–5 units).

The actions of pentobarbitone and the volatile anaesthetics on the glutamate-sensitivity of neurones in the prepiriform cortex is summarized in Figure 2b and Figure 5 for experiments in standard and high Mg^{2+} saline. It is clear from these graphs that the depressant action of anaesthetics on glutamate-sensitivity was not reduced under conditions of synaptic blockade.

Interspike interval distribution

Neurones in the prepiriform cortex excited by glutamate had a variable temporal pattern of impulse generation. About 20% of the glutamate-excited units displayed a regular train of impulses; in the remainder, the impulses were grouped. Interspike interval analysis showed a single broad peak with a bias towards short intervals; the modal interval ranged from 10–200 milliseconds.

Exposure to pentobarbitone caused an increase in the modal interval commensurate with the fall in mean firing frequency (9 cells). Analysis of the four experiments with alphaxalone, in which this anaesthetic did not cause a total suppression of the glutamate responses, yielded similar results.

All the volatile anaesthetics caused a decrease in the modal interval of the interspike interval histogram in a proportion of the cells studied, despite the ability of three of them (ether, methoxyflurane and trichloroethylene) to depress the firing rate. The decrease in the modal interval reflected a tendency for the spikes to occur in tight groups or bursts; within a burst of spikes the modal intervals ranged from 4–25 milliseconds. Reducing the discharge rate merely by lowering the glutamate ejection current was never accompanied by such a change (Figure 6). Increasing the concentration of anaesthetic usually caused the grouping of impulses to become tighter. For example, one neurone exposed to 0.1% trichloroethylene fired in bursts of 2–6 spikes with a modal interval of 22.5 ms; on increasing the concentration to 0.2% the spikes then occurred in pairs or triplets with a shorter modal interval of 7.5 milliseconds. Not all cells showed changes in their firing pattern even when they were exposed to high concentrations of anaesthetic. The proportion of cells showing burst firing during exposure to anaesthetic was as follows: ether 4 of 6 cells that could be satisfactorily analysed; methoxyflurane 3/6; trichloroethylene 6/8; halothane 4/7. In the case of methoxyflurane, however, three additional units fired in bursts of spikes at the onset of exposure to anaesthetic but not while a steady concentration was maintained. This tendency for volatile anaesthetics to induce cells to fire in groups of spikes was found even in the presence of 10 mM Mg^{2+}; ether

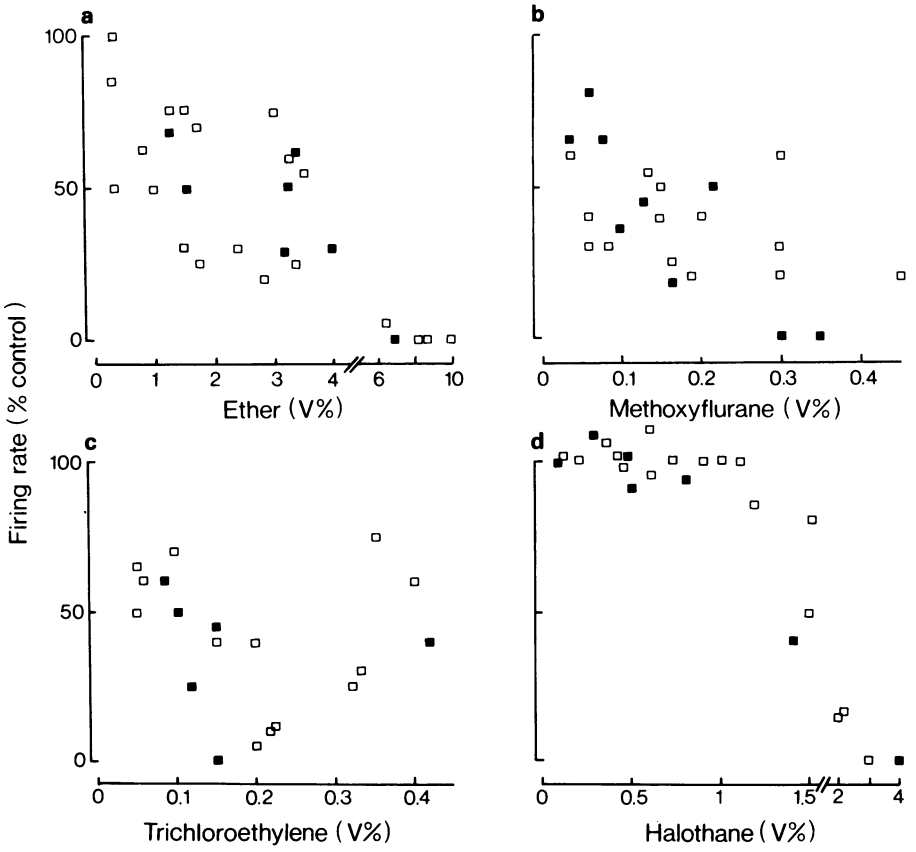

Figure 5 Summary of the relationship between the glutamate-sensitivity of neurones in the prepiriform cortex and the concentrations of volatile anaesthetics to which they were exposed: (□) experiment conducted in standard saline; (■) experiment conducted in high Mg^{2+} saline. Each symbol shows the firing rate of a cell during exposure to anaesthetic expressed as a percentage of the firing rate before exposure to anaesthetic. Some cells were exposed to two or three concentrations of anaesthetic.

0/2 cells; methoxyflurane 1/5; trichloroethylene 6/7; halothane 1/5.

Action of anaesthetics on the glutamate-sensitivity of cells in the guinea-pig neocortex

To test the general validity of our results, we also examined the effects of two general anaesthetics, halothane and methoxyflurane, on the sensitivity of neocortical cells to iontophoretically applied glutamate.

A total of 14 cells were examined in 7 coronal slices of neocortex maintained *in vitro*. All 5 cells exposed to 0.03–0.3% methoxyflurane showed a dose-related depression of glutamate-evoked activity. By contrast, none of the 6 cells exposed to halothane (0.23–1.2%) showed a depression of glutamate-evoked activity at concentrations below 0.9%. Two cells showed a depression in sensitivity to glutamate when exposed to halothane concentrations between 0.9 and 1.2%.

A further 3 cells were tested in high Mg^{2+} saline for changes in glutamate sensitivity during exposure to methoxyflurane. The glutamate-evoked activity of all 3 cells was depressed in a dose-related manner by 0.08–0.2% methoxyflurane.

None of the neocortical cells exposed to methoxyflurane showed any increased tendency to fire in groups of spikes whereas 3 of the 6 cells exposed to halothane did so.

Discussion

The interpretation of our experiments rests on the assumption that the firing rate of a cell in response to a

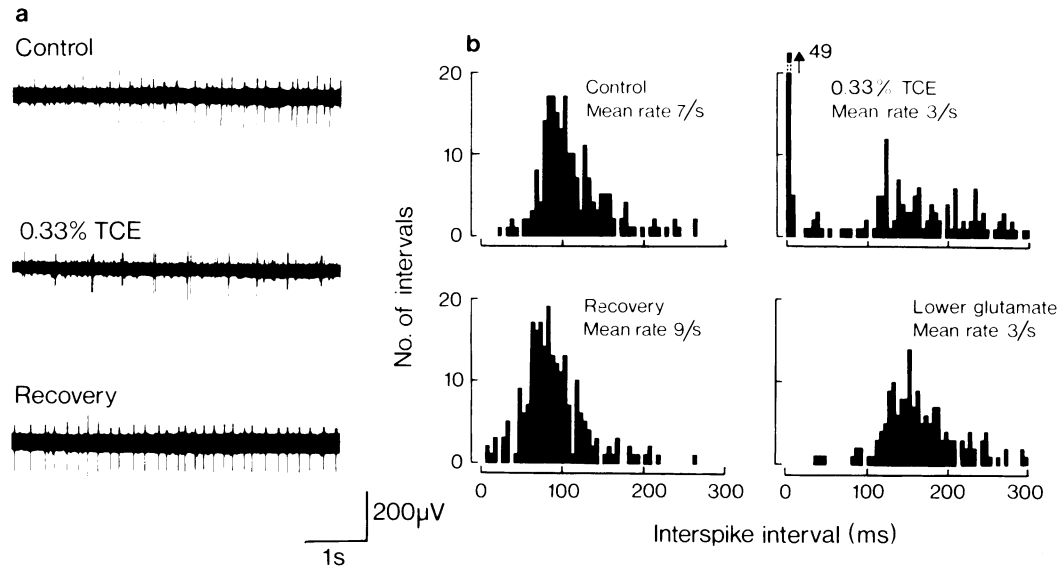

Figure 6 Changes in the interspike interval distribution caused by exposure to trichloroethylene (TCE). (a) Filmed record of a cell firing in response to a 38 nA, 10 s pulse of glutamate in the presence and absence of trichloroethylene. This cell was 300 μm deep. Note that the cell discharges tend to occur as pairs of spikes in the presence of trichloroethylene. (b) Interspike interval histograms taken from an extended record of the same cell. Each histogram was compiled from the intervals between 250 spikes. Note the increase in the proportion of short intervals which reflects the tendency for this cell to fire in pairs of spikes during exposure to trichloroethylene. This effect could not be mimicked by reducing the glutamate excitation of the cell (30 nA).

given pulse of glutamate reflects the sensitivity of that cell to glutamate. This will only be true if there is no change in the coupling between the glutamate-induced depolarization and the generation of the action potential, if subliminal excitation and inhibition of the cell are either constant or insignificant, and if glutamate acts directly on the post-synaptic membrane and not by causing the release of some other excitatory substance. The first of these conditions is satisfied as it has already been shown that concentrations of general anaesthetics sufficient to depress synaptic transmission by 50% or more do not increase the action potential threshold, or otherwise impair the coupling between the population e.p.s.p. and the discharge of neurones in the prepiriform cortex (Richards, 1972, 1973a and unpublished data; Richards *et al.*, 1975). As the results of experiments performed on preparations bathed in standard saline were similar to those obtained from preparations in which synaptic transmission was blocked by high Mg^{2+} saline (see Results section) subliminal excitation and inhibition cannot have influenced our results. Furthermore, these experiments also exclude the possibility that glutamate causes the release of some other substances from the nerve terminals as transmitter release is inhibited by high Mg^{2+} saline (Richards & Sercombe, 1970; Rubin, 1970).

The reasons for choosing glutamate as an analogue for the unknown endogenous transmitter have been set out in detail elsewhere (Richards *et al.*, 1975) and may be summarized by saying that, like the transmitter itself, glutamate excites nerve cells by a sodium-dependent mechanism following its interaction with a specific receptor site. There is even some circumstantial evidence to suggest that glutamate itself may be the transmitter released from the l.o.t. nerve terminals (see Bradford & Richards, 1976). Although we confined our experiments to neurones in the prepiriform cortex, chiefly those of layer II, which are innervated by the l.o.t., it is possible that our results reflect the properties of receptors other than those responsible for synaptic transmission between the l.o.t. and the pyramidal cells of the prepiriform cortex.

All six anaesthetics that were studied depressed the firing rate of cortical neurones to iontophoretically-applied L-glutamate. For the reasons discussed earlier, these results suggest that general anaesthetics depress the sensitivity of the post-synaptic membrane itself to glutamate. As anaesthetics do not alter the coupling between the population e.p.s.p. and the discharge of the pyramidal cells, this depression is not the result of

a generalized increase in the resting membrane permeability to potassium or chloride such as that seen following the iontophoretic application of 2, 4 dinitrophenol to cortical neurones (Godfraind, Krnjević & Pumain, 1970). It is more likely that this depression could reflect a decrease in the affinity of the receptor for glutamate, or a decrease in the efficacy of glutamate due to a change in receptor conformation, or a decrease in the ability of the ionophore linked to the glutamate receptor to admit ions across the membrane. At present there is no evidence which would allow us to distinguish between these possibilities.

The results summarized in Figures 2b and 5 and Table 1 show that pentobarbitone, ether, methoxyflurane and trichloroethylene all produced a dose-dependent depression of the glutamate-sensitivity of neurones within the concentration range required to depress synaptic transmission between the fibres of the l.o.t. and the neurones of the prepiriform cortex. Similarly, alphaxalone (50 μM) also depressed both sensitivity of neurones to glutamate and synaptic transmission. While our results do not exclude the possibility that these anaesthetics may depress the release of transmitter, they are consistent with the view that all five anaesthetics depress synaptic transmission, at least in part, by depressing the sensitivity of the post-synaptic membrane to endogenously released transmitter.

In contrast to the other anaesthetics studied, halothane did not depress the glutamate-sensitivity of cortical neurones until relatively high concentrations were reached (> 1%), whereas synaptic transmission became depressed at much lower concentrations (> 0.4%) (Richards, 1973a). Thus, with halothane there was little parallelism between the depression of synaptic transmission and the depression of glutamate-sensitivity. It is possible that halothane does not depress the glutamate-sensitivity of the cortical neurones because it has both a direct stimulatory action on the post-synaptic neurones as well as a depressant action on their sensitivity to glutamate. This seems unlikely as halothane does not cause the neurones to fire spontaneously until high concentrations are reached and at these concentrations (> 1.2%) their glutamate-sensitivity is depressed. Alternatively, low concentrations of halothane (< 1%) may depress synaptic transmission by reducing the output of transmitter from the nerve terminals.

The glutamate-sensitivity of neurones *in vitro* preparations of neocortex was depressed by low concentrations of methoxyflurane, but not by low concentrations of halothane, so it appears that our results do not merely reflect properties of glutamate receptors that are peculiar to the neurones of the prepiriform cortex. Furthermore, Ransom & Barker (1975) have found that the glutamate-sensitivity of spinal neurones grown in tissue culture is also depressed by pentobarbitone.

As changes in the responsiveness of a neurone to glutamate that are caused by anaesthetics *in vivo* may not only reflect changes in the sensitivity of that neurone to glutamate but also changes in background activity it is not easy to compare our results directly with those obtained in intact animals. However, there is a measure of agreement between our results and those of certain experiments conducted *in vivo*. For example, Crawford & Curtis (1966) and Johnson, Roberts & Straughan (1966) found that systemic administration of barbiturates depressed the sensitivity of cortical neurones to iontophoretically-applied glutamate and DL-homocysteic acid. Crawford (1970), working with the cerveau isolé preparation of the cat, found that halothane (< 1.5%) had no effect on the response of cells in the cat pericruciate gyrus but he also reported that methoxyflurane and trichloroethylene were without effect. Catchlove, Krnjević & Maretić (1972) using anaesthetized animals and a similar protocol to Crawford's also failed to find a depressant effect of volatile

Table 1 Comparison of the depressant effects of anaesthetics on the population e.p.s.p. and on the glutamate-sensitivity of prepiriform neurones

Anaesthetic	*Concentration required for anaesthesia*	*Depression of pop e.p.s.p. amplitude**	*Depression of glutamate firing rate†*
Pentobarbitone	0.2 mM	25–50%	50–100%
Alphaxalone	50 μM	25–100%	60–100%
Ether	3.0%	25–50%	25–80%
Methoxyflurane	0.25%	20–40%	40–90%
Trichloroethylene	0.3%	25–50%	70–100%
Halothane	0.9%	25–50%	0–10%

* Ranges taken from the following references: Richards (1972, 1973a), Richards *et al.*, (1975), Richards & Hesketh (1975) and unpublished data.
† Ranges taken from Figures 2b and 5.

anaesthetics on the sensitivity of cortical cells to glutamate. However, in the cuneate nucleus Galindo (1969) found that pentobarbitone depressed the sensitivity of neurones to glutamate whereas halothane did not, although both anaesthetics depressed synaptic transmission between the dorsal columns and the medial lemniscus.

All the volatile agents changed the temporal firing pattern of some neurones from a more or less regular train of impulses towards a more irregular pattern in which the impulses tended to be grouped. This change in firing pattern was observed both with volatile anaesthetics that depressed the glutamate-sensitivity of neurones and with halothane which did not. It was found in preparations in which synaptic transmission was blocked with high Mg^{2+} saline, and so does not depend upon the integrity of synaptic connexions but on some intrinsic property of the membrane. A similar change in the firing pattern of synaptically-evoked neurones in the prepiriform cortex has also been seen during administration of ether and trichloroethylene (Richards, 1973b; Richards *et al.*, 1975). It is reasonable to suppose that this effect is related to the burst firing of neurones seen *in vivo* during anaesthesia with volatile anaesthetics (see Goodman & Mann, 1967; Robson, 1967; Lee, 1970). This disruption of the normal temporal pattern of impulse generation may well compound the disorganizing influence of anaesthetics produced by their actions on synaptic transmission.

In conclusion, these experiments lend no support to the idea that there is a single unitary mechanism whereby anaesthetics produce their effects but rather suggest that general anaesthetics affect membrane-linked functions in a variety of ways.

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