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Prolongation of inhibitory postsynaptic currents by pentobarbitone, halothane and ketamine in CA1 pyramidal cells in rat hippocampus

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1 Spontaneous inhibitory postsynaptic currents (i.p.s.cs) were recorded in voltage-clamped CA1 neurones in rat hippocampal slices.

2 The exponential decay of i.p.s.cs was prolonged by concentrations of sodium pentobarbitone as low as 50 μM. With concentrations up to 100 μM, there was no change in the amplitude or rise time of the currents but current amplitude was depressed at 200 μM. The prolongation of currents increased with drug concentration within the range tested (50 to 200 μM).

3 Halothane, at concentrations from 1 to 5%, also increased the time constant of decay of i.p.s.cs. The effect increased with concentration and was fully reversible.

4 Ketamine, at a concentration of 0.5 mM, increased the time constant of decay of i.p.s.cs by 50 to 80% and the effect was reversible.

5 Ethanol (10–200 mM), nitrous oxide (75–80%), and caffeine (10 μM–5 mM) had no detectable effect on the i.p.s.cs.

6 It is suggested that pentobarbitone, halothane and ketamine increase the time constant of decay of the i.p.s.cs by stabilizing the open state of channels activated by γ-aminobutyric acid.

Introduction

Although anaesthetic drugs (e.g. ethanol) have been used for centuries to allow otherwise painful surgical procedures, the mechanisms involved in producing anaethesia are still uncertain. It has been known for some time that anaesthetics decrease the effectiveness of excitatory synaptic transmission (Sherrington 1906; Winterstein 1919; Larabee & Posternak 1952) at doses lower than those required to block axonal conduction. More recently, use of voltage clamp and patch clamp techniques at the neuromuscular junction has revealed that a variety of general anaesthetics depress transmission at this synapse by reducing channel open time (for a review, see Gage & Hamill, 1981). At least part of the depressant action of anaesthetics at central excitatory synapses is likely to be due to a similar mechanism. There is also compelling evidence that some general anaesthetics alter transmission at inhibitory synapses. For instance, early studies on the actions of pentobarbitone (Eccles & Malcolm 1946; Eccles *et al.*, 1963) suggested that this anaesthetic enhances γ-aminobutyric acid (GABA)-mediated inhibitory neurotransmission.

It has been difficult to use biophysical techniques similar to those used at the neuromuscular junction to study the effect of anaesthetics on synaptic transmission in the mammalian central nervous system. However, voltage clamp studies of cultured neurones have given useful information about possible synaptic actions of anaesthetics (for a review, see Mathers & Barker, 1982) and now, with the advent of single electrode voltage clamp techniques and isolated brain slice preparations, it has become possible to examine the effects of anaesthetics on postsynaptic conductance changes at central synapses. We have employed these techniques to study the effects of several anaesthetics on GABA-mediated synaptic currents in rat hippocampal slices. Some of these results have been presented briefly elsewhere (Robertson & Gage, 1984).

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Methods

Spontaneous inhibitory postsynaptic currents (i.p.s.cs) were recorded in the stratum pyramidale region of rat hippocampal slices using methods described previously (Collingridge *et al.*, 1984). Briefly, cells were voltage-clamped with a single microelectrode voltage clamp (ASFI, Axon Instruments Ltd) using switching rates between 10 and 16 kHz. Adequate settling of the current injection before voltage sampling was ensured at all times by continuously monitoring the headstage voltage at high gain. Currents were recorded digitally with a sample rate of 6.25 kHz and the decay of each current was fitted with a single exponential, as described elsewhere (Collingridge *et al.*, 1984). The rise time was measured from 20 to 80% of the peak amplitude by fitting a line (least squares fit) to the data points on the rising phase from 10 to 90% of peak amplitude. The temperature in all experiments was 20–25°C.

Drugs and anaesthetics used in this study were made up fresh during each experiment and dissolved in standard physiological salt solution (Collingridge *et al.*, 1984) immediately before use. The inhalational anaesthetic halothane was administered with a vaporizer (Fluotec 3, Cyprane) placed in the O_2/CO_2 supply circuit, so that the gas was administered both in the atmosphere above the hippocampal slices and in the inflow reservoir allowing the gas to dissolve in the perfusion solution. Nitrous oxide was supplied in a similar manner.

There are significant problems with the diffusion of drugs to cells in the slice preparation (see Nicholson & Hounsgaard, 1983), particularly with slices maintained at an interface between the bathing solution and a humidified atmosphere. The slices used in this study were 400 μm thick, and solution flow rates were 1 to 2 ml min^{-1}. The effects of any drug were always detected within 10 min of changing the solution and the maximum, stable effect was usually seen after approximately 20 min. Except for halothane and N_2O, all of the results presented were obtained from cells that had been exposed to a drug for at least 30 min. Even if diffusional barriers were still a significant problem after this time, drug concentrations at the cell membrane could only have been lower than the bath concentration.

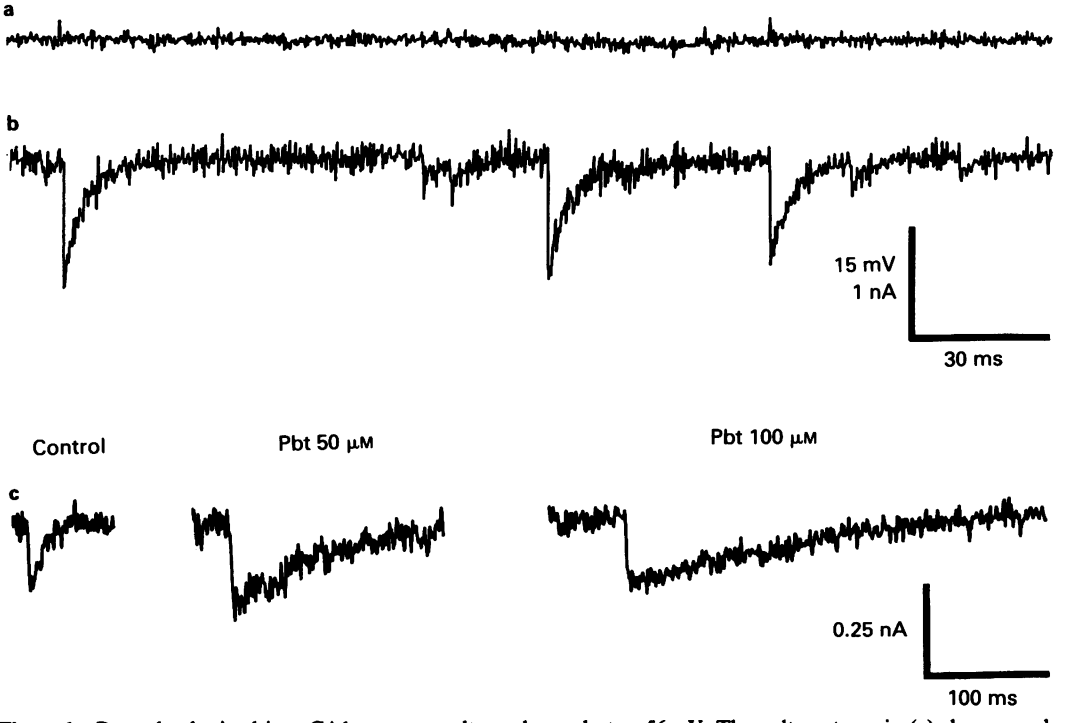

Figure 1 Records obtained in a CA1 neurone voltage clamped at − 56 mV. The voltage trace in (a) shows good control during the inhibitory postsynaptic currents (i.p.s.cs) in (b). The clamp switching rate was 13.5 kHz. The effect of pentobarbitone (Pbt) on the decay of i.p.s.cs recorded in another cell is shown in (c). Typical i.p.s.cs, recorded in control solution and solutions containing 50 μM and 100 μM pentobarbitone, illustrate the striking lengthening of the decay phase produced by this drug. Clamp potential, − 41 mV; clamp switch rate, 11.5 kHz.

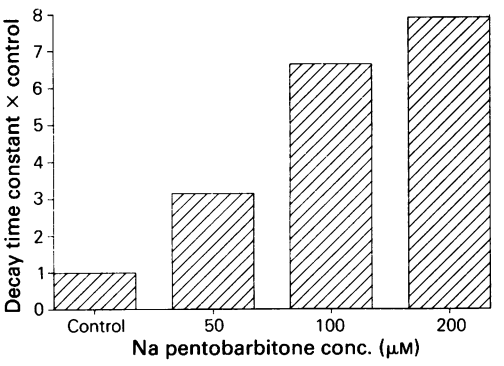

Figure 2 The increase in the average time constant of decay of inhibitory postsynaptic currents produced by pentobarbitone. The results were obtained in 5 voltage clamped cells exposed to 2 or more concentrations of pentobarbitone. The s.e. means for 50 μM and 100 μM pentobarbitone were 0.4 and 1.1 respectively. The two values at 200 μM pentobarbitone were the same (7.9).

Results

Pentobarbitone

As has been described previously (Collingridge *et al.*, 1984), the decay of spontaneous i.p.s.cs in hippocampal CA1 neurones is exponential with a single time constant that is presumed to be determined by the open time of ion channels, as at the endplate (Anderson & Stevens, 1973). It was also noted that sodium pentobarbitone increased the time constant of decay of the i.p.s.cs, but the effect was not examined over a range of concentrations. This observation has been extended in further experiments.

Examples of i.p.s.cs recorded from a cell voltage clamped at − 56 mV in control solution are shown in Figure 1b. The upper trace (Figure 1a) shows the voltage control during the currents. Typical examples of i.p.s.cs recorded from a cell before and after exposure to pentobarbitone can be seen in Figure 1c. The decay of the currents could be well fitted with a single exponential (Collingridge *et al.*, 1984) but the decay time constant was increased by 50 μM pentobarbitone and even more so by 100 μM pentobarbitone. Similar prolongation of i.p.s.cs in solutions containing pentobarbitone (50 to 200 μM) was seen in 15 cells. The effect could be fully reversed by perfusing the slice with control solution for more than 30 min.

The relationship between the average decay time constant and pentobarbitone concentration, recorded in 5 cells in which it was possible to change solutions without dislodging the electrode, is illustrated in Figure 2. It can be seen that pentobarbitone had a very large effect on the decay time constant which was increased on average more than three fold by a concentration as low as 50 μM and almost eight fold by

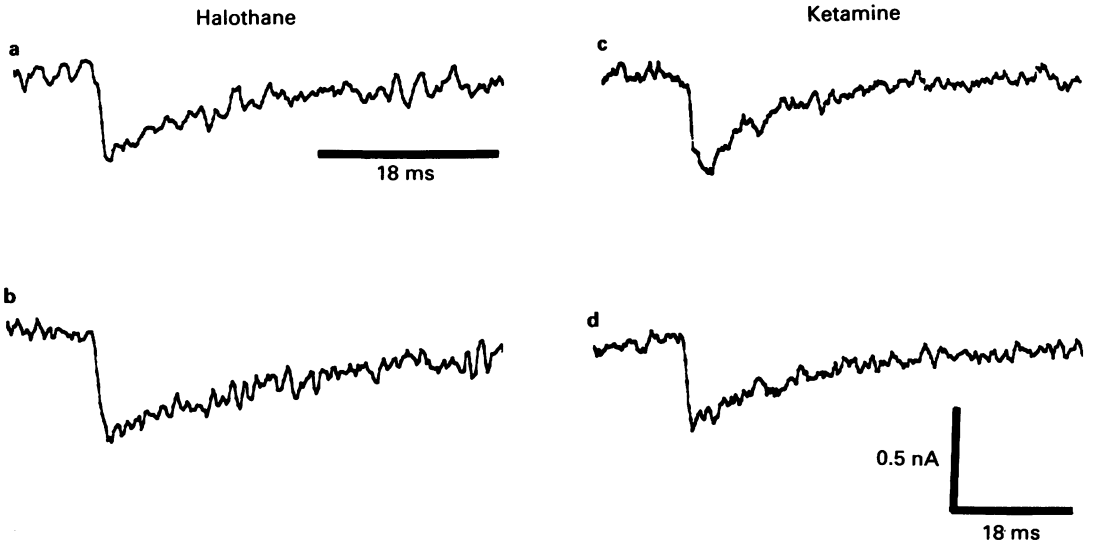

Figure 3 Slowing of the decay of inhibitory postsynaptic currents (i.p.s.cs) produced by halothane and ketamine. The decay of a typical i.p.s.c. in control solution (a) was clearly prolonged in the presence of 3% halothane (b). Note the difference in time scale for (a) and (b). In this cell, the clamp potential was − 54 mV and the switching rate 11.5 kHz. Similarly, the control i.p.s.c. (c) recorded in another cell was prolonged by ketamine 0.5 mM (d). Clamp potential, − 53 mV; switching rate, 11.3 kHz. The horizontal calibration bars denote 18 ms.

a concentration of 200 μM. At concentrations of 50 and 100 μM, pentobarbitone had little effect on the rise time or amplitude of i.p.s.cs: for example, in one cell, the average amplitude and rise time were 0.31 ± 0.1 nA and 0.76 ± 0.08 ms (n = 23) before, and 0.38 ± 0.03 nA and 0.83 ± 0.07 ms (n = 11) after, exposure to 100 μM pentobarbitone (means ± s.e.mean). With 200 μM pentobarbitone, the amplitude was invariably decreased.

Halothane

Halothane, like pentobarbitone, slowed the decay of i.p.s.cs. The effect, which was observed in all 15 cells studied, is illustrated in Figure 3. In this experiment, the average decay time constant before exposure to halothane was 9.9 ± 0.6 ms (mean ± 1s.e.mean, n = 15): one of the currents is shown in Figure 3a. Halothane (3%) was then applied directly in the atmosphere around the hippocampal slice and prolongation of currents was observed within a few seconds of exposure to the anaesthetic. Currents were captured for analysis when the effect had stabilized after 5 min in halothane. One of these currents is shown in Figure 3b. This i.p.s.c. was clearly prolonged. The average decay time constant had increased more than two fold to 21.5 ± 0.8 ms (mean ± 1s.e.mean, n = 30).

The sequential effects of a range of halothane concentrations on the decay time constant of i.p.s.cs are illustrated in two separate cells in Figure 4. Each point shows the mean from 9 to 35 i.p.s.cs: s.e.means varied from 0.3 to 1.2 ms. The effects of halothane obviously increased with concentration and were reversible. There was no significant change in the amplitude or rise time of i.p.s.cs. For example, in the experiment illustrated in Figure 4a, the average amplitude and rise time (mean ± 1 s.e.mean) were 0.45 ± 0.02 nA and 0.78 ± 0.07 ms (n = 15) in control solution, 0.51 ± 0.02 nA and 0.91 ± 0.04 ms (n = 30) with 3% halothane, 0.42 ± 0.02 nA and 0.45 ± 0.02 ms (n = 9) back in control solution, and 0.45 ± 0.02 nA and 0.92 ± 0.11 ms (n = 20) with 2% halothane. In most of the cells exposed to the anaesthetic, it was noticed that there was an increase in holding current indicating an increase in membrane conductance. This effect was not pursued, although the result was consistent with the increase in potassium conductance observed by Nicoll & Madison (1982). The effects of halothane were reversed within 5 min when a slice was flushed with control solution.

Ketamine

Previous studies have shown that ketamine, at low concentrations, reduces the time constant of decay of miniature endplate currents at the neuromuscular junction (Torda & Gage, 1977). In contrast, ketamine

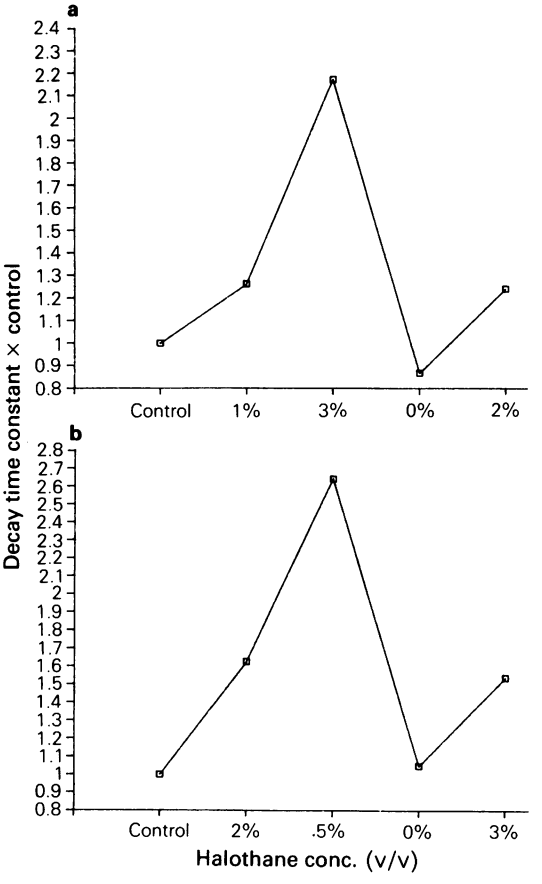

Figure 4 The effects of a range of halothane concentrations on the average time constant of decay of inhibitory postsynaptic currents (i.p.s.cs) in two cells. Each point shows the average decay time constant of 9 to 35 i.p.s.cs. In (a), the clamp potential was − 54 mV and switching rate 11.5 kHz. The control time constant was 9.9 ± 0.6 ms (mean ± 1 s.e.mean, n = 15). In (b), the clamp potential was − 72 mV and the switching rate 12.8 kHz. The control time constant was 11.5 ± 0.4 ms (mean ± 1 s.e.mean, n = 27).

potentiates inhibitory potentials in the olfactory cortex (Scholfield, 1980). Ketamine caused prolongation of i.p.s.cs, though to a lesser extent than pentobarbitone or halothane. The effect of ketamine (0.5 mM) on the decay of i.p.s.cs is illustrated in Figure 3. A current recorded after exposure to ketamine (Figure 3d) clearly decays more slowly than a current recorded before introduction of the drug (Figure 3c). In three experiments, 0.5 mM ketamine increased the average decay time constant of i.p.s.cs by 57%, 74% and 81%, while i.p.s.c. amplitude was not significantly affected. The increase in decay time constant was completely

reversible when slices were perfused with control solution for more than 30 min. No change in holding current (membrane conductance) was noted during exposure to ketamine.

Ethanol and nitrous oxide

Ethanol, at concentrations as high as 200 mM, caused no perceptible change in the decay time constant, rise time or amplitude of i.p.s.cs. Similarly, there were no detectable changes in i.p.s.cs in the presence of nitrous oxide (75–80% in carbon dioxide/oxygen).

Caffeine

Caffeine is a methylxanthine with central stimulatory activity (Snyder, 1981). Recent reports have suggested that this drug can selectively enhance or inhibit GABA responses, depending on the concentration applied (Nistri & Berti, 1983). Caffeine, at concentrations of 10 μM (10 cells), 50 μM (2 cells) and 500 μM (1 cell), had no effect on the time course or amplitude of i.p.s.cs. Furthermore, normal i.p.s.cs were observed in 7 cells exposed to 5 mM caffeine. The amplitude and time course of i.p.s.cs were within the normal range even after 90 min with this high concentration of caffeine. These results are in marked contrast to the effects of GABA, described by Nistri & Berti (1983), on the frog spinal cord. It is possible that the result may reflect a difference between the GABA receptors and channels of different species (as discussed by Nistri & Constanti, 1979).

Discussion

In this study, several general anaesthetics (pentobarbitone, halothane and ketamine), when applied at low, 'clinical' concentrations, were found to prolong spontaneous i.p.s.cs in hippocampal CA1 neurones by increasing their decay time constant. Such an effect would potentiate inhibition caused by GABA in these neurones. Pentobarbitone, in particular, must cause a profound potentiation of GABA-mediated inhibition in these cells when used clinically. It seems likely that similar effects may occur at GABA synapses elsewhere in the central nervous system. Indeed, it has been found previously that barbiturates and halothane potentiate GABA-mediated inhibition (Galindo, 1969; Nicoll, 1972; Nicoll *et al.*, 1975; Scholfield, 1980). On the other hand, ketamine has been found not to potentiate inhibition caused by GABA (Lodge & Anis, 1982; Anis *et al.*, 1983; but see Scholfield, 1980). However, it should be noted that the concentration used in our experiments may be somewhat higher than the concentration in rats anaesthetized with ketamine (Cohen *et al.*, 1973).

It is interesting that the three anaesthetics that potentiate inhibition by increasing the decay time constant of i.p.s.cs, depress excitatory transmission at the neuromuscular junction by reducing the decay time constant of excitatory currents. A drug that had both these effects (depression of excitation and potentiation of inhibition) at central synapses would have a powerful depressant action on transmission of signals in neuronal networks.

Ethanol has a depressant effect on the central nervous system but its mechanism of action is at present unknown. Previous results on the effects of ethanol on inhibitory transmission are conflicting. Nicoll (1972) found that ethanol, unlike several other anaesthetic substances tested, did not prolong synaptic inhibition in the rabbit olfactory bulb. Carlen *et al.* (1982) and Gruol (1982) have also shown that ethanol has no effect on GABA-mediated i.p.s.cs. However, Newlin *et al.* (1981) found that ethanol increased inhibition in the CA3 region of the hippocampus, and Nesteros (1980) has suggested that ethanol enhances GABA-mediated neurotransmission. Our results clearly show that ethanol has no effect on the GABA-activated inhibitory currents.

This lack of effect of ethanol was surprising, although Finger & Stettmeier (1984) have also found recently that ethanol has no effect on GABA-mediated i.p.s.cs in crayfish muscle. Ethanol increases channel open time at nicotinic neuromuscular junctions, where membrane hyperpolarization also increases channel open time, and has the opposite effect at glutaminergic junctions in crustacea where hyperpolarization decreases channel open time (Adams *et al.*, 1979). It has been suggested that all of these effects can be explained by the hypothesis that ethanol, by increasing the polarizability (dielectric constant) of lipids adjacent to receptor-channel complexes, stabilizes the state of the complex which is also stabilized by membrane hyperpolarization (Gage *et al.*, 1975). This hypothesis would predict that ethanol should reduce channel open time at GABA synapses where hyperpolarization reduces channel open time (Collingridge *et al.*, 1984). It could be argued that the decay of the i.p.s.cs is not determined by channel open time but this seems unlikely, as discussed below. The lack of any effect of ethanol on the decay of i.p.s.cs in which the decay time constant is voltage-sensitive weakens an attractive hypothesis.

It has been shown that many anaesthetics reduce the decay time constant of excitatory currents at the neuromuscular junction by reducing channel open time (for a review, see Gage & Hamill, 1981). Although there is no direct evidence, it seems likely that the increase in decay time constant of the i.p.s.cs described here is due to an increase in channel open time. However, it could be argued that the prolongation of the decay of the currents is due to inhibition of the reuptake of GABA: such an effect of pentobarbitone has

been demonstrated at inhibitory synapses on the crayfish stretch receptor (Aickin & Deisz, 1981). Nevertheless, there is much evidence against this possibility. Jessel & Richards (1977) found no effect of pentobarbitone, in the concentration range studied here, on GABA uptake in the hippocampus. Furthermore, in cultured hippocampal neurones, the increase in decay time constant of i.p.s.cs caused by barbiturates has been shown to be explained by an increase in channel open time (Segal & Barker, 1984). Finally, the GABA uptake inhibitor, nipecotic acid, at concentrations of 10 μM and 1 mM (Krogsgaard-Larsen & Johnston, 1975), causes no prolongation of spontaneous i.p.s.cs in rat hippocampal CA1 neurones (B. Robertson & I. Spence, unpublished observations). Pentobarbitone, halothane and ketamine could increase channel open time by stabilizing the open states of GABA-activated channels in many different ways e.g. by potentiating agonist binding (Willow & Johnston, 1980), by an allosteric effect on the receptor-channel complex or by changing the properties of 'boundary lipids'. There is, for instance, strong evidence that clinical concentrations of halothane can specifically disrupt the state of other protein complexes (Allison *et al.*, 1970; Hinkley & Telser, 1974).

It has been suggested that anaesthetics may decrease channel open time at the neuromuscular junction by blocking open channels (Adams, 1976) or by making the membrane more fluid (Gage & Hamill, 1975). Neither of these explanations can suitably account for an increase in channel open time at these inhibitory synapses. It seems more likely that anaesthetics can bind to hydrophobic sites on (or close to) receptor-channel complexes and, by so doing, stabilize channels in a closed (as at the motor endplate) or open (as at the GABA synapse) conformation.

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