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THE ACTIONS OF VOLATILE ANAESTHETICS ON SYNAPTIC TRANSMISSION IN THE DENTATE GYRUS

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SUMMARY

1. The actions of four volatile anaesthetics on the evoked synaptic potentials of *in vitro* preparations of the hippocampus were examined.

2. All four anaesthetics (ether, halothane, methoxyflurane and trichloroethylene) depressed the synaptic transmission between the perforant path and the granule cells at concentrations lower than those required to maintain anaesthesia in intact animals.

3. The population excitatory post-synaptic potential (e.p.s.p.) and massed discharge of the cortical cells (population spike) were depressed at concentrations of the anaesthetics lower than those required to depress the compound action potential of the perforant path nerve fibres. None of the anaesthetics studied increased the threshold depolarization required for granule cell discharge. Furthermore, frequency potentiation of the evoked cortical e.p.s.p.s was not impaired by any of the anaesthetics studied.

4. It is concluded that all four anaesthetics depress synaptic transmission in the dentate gyrus either by reducing the amount of transmitter released from each nerve terminal in response to an afferent volley, or by decreasing the sensitivity of the post-synaptic membrane to released transmitter or by both effects together.

INTRODUCTION

There is evidence to support the idea that many general anaesthetics depress excitatory synaptic transmission during anaesthesia. Moreover, it has often been assumed that this is one of the mechanisms whereby anaesthetics produce their characteristic behavioural effects (see Somjen, 1967; Wall, 1967). However, until recently, this idea was based almost entirely on the actions of anaesthetics on synaptic transmission in the spinal cord or in sympathetic ganglia (see Larrabee & Posternak, 1952; Løyning,

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Oshima & Yakota, 1964; Somjen, 1963; Somjen & Gill, 1963; Weakly, 1969). Unequivocal evidence that general anaesthetics can depress excitatory synaptic transmission in the brain itself has only been obtained within the last four years in experiments with *in vitro* preparations of the olfactory cortex (Richards 1971, 1972, 1973*b*, 1974; Richards, Russell & Smaje, 1975). In contrast, Nicoll (1972) has shown that the excitatory transmission between the mitral cells and granule cells of the olfactory bulb is relatively insensitive to anaesthetics. Thus, two areas of the olfactory brain show different sensitivities to general anaesthetics. It is, therefore, necessary to ask whether excitatory synapses in other parts of the brain are readily depressed by general anaesthetics. If so, are they depressed by mechanisms similar to those responsible for the depression of synaptic transmission in the olfactory cortex?

To provide a partial answer to these questions we chose to investigate the actions of ether, halothane, methoxyflurane and trichloroethylene on excitatory synaptic transmission in *in vitro* preparations of the dentate gyrus of the hippocampus. A preliminary description of the action of halothane has been presented to the Society (Richards & White, 1974).

METHODS

The dentate gyrus was chosen for this study, because, like the olfactory cortex, it has a stereotyped laminated anatomical organization with a well defined afferent input, the perforant path.

Perforant path fibres arise from the entorhinal cortex, cross the hippocampal fissure and make synapses with the middle third of the apical dendrites of the granule cells in the dentate gyrus (see Fig. 1B). In addition, the cells and fibres are in discrete layers within the dentate gyrus (Lømo, 1971, Nafstad, 1967). As the granule cells of the lower blade of the dentate gyrus are superficial, a convenient *in vitro* preparation of this area can be made by taking a tangential slice across the regio inferior of the hippocampus and then incubating this slice of tissue in a suitable environment (see Yamamoto & Kawai, 1968; Bliss & Richards, 1971). The preparation so made consists of a single densely packed layer of granule cells together with the terminal lengths of their afferent perforant path fibres. Following stimulation of the perforant path fibres, stable and characteristic field potentials can be evoked (see Bliss & Richards, 1971). The general shape and size of the evoked potentials in the *in vitro* preparations is similar to those seen in intact animals by Lømo (1971).

Guinea-pigs were killed by a blow on the back of the neck; the skull was opened and the brain removed after section of the olfactory bulbs. The brain was divided down the mid line and the subcortical structures scooped out with a spatula to expose the ventral aspect of the hippocampus. The hippocampus was then removed by freeing it from its connexions with the cortex and was laid on the cutting platform, ventral face (regio inferior) uppermost. The slice of the dentate area was taken tangential to the pial surface with the aid of a razor strip and glass template. These preparations included part of the entorhinal area, the lower blade of the dentate gyrus and part of the *CA*3 region of the hippocampus (see Fig. 1). The slices so prepared had a nominal thickness of 410 μm.

The slices were incubated at 37–38° C in the chamber described by Doré & Richards (1974). Stimulation was achieved by manoeuvering a glass micropipette (2–4 μm tip diameter) filled with 20 % (w/v) NaCl into the region occupied by the perforant path just after it had traversed the hippocampal fissure; the stimuli were monopolar, with the electrode as the cathode. The rate of stimulation was usually 0·2 Hz. The pulses were derived from an isolated stimulator triggered by a Digitimer. The evoked field potentials were recorded monopolarly by glass micropipettes

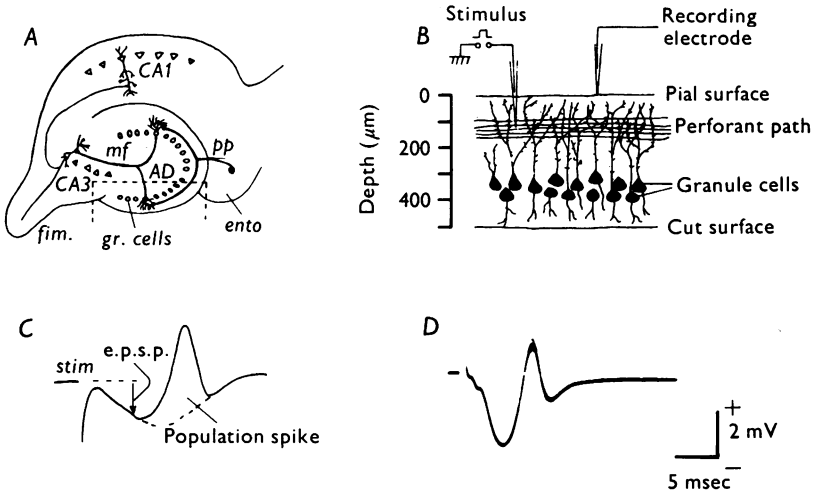

Fig. 1. Diagrammatic representation of the anatomical organization of the dentate gyrus together with examples of the potentials elicited by perforant path stimulation. *A*, schematic drawing of a transverse section through the hippocampus to show the area from which the preparations were made (indicated by the dashed lines). *CA* 1, *CA* 3, pyramidal cells of the hippocampus: *fim*, fimbria; *mf*, mossy fibres; *AD*, area dentata; *gr.* cells, granule cells; *pp*, medial perforant path; *ento*, entorhinal area. *B*, schematic drawing of a section through a preparation of the dentate gyrus to show the innervation of the granule cells by the medial perforant path and the electrode placements. *C*, drawing of the characteristic potential evoked by perforant path stimulation labelled to show the nomenclature, *stim*, stimulus artifact; e.p.s.p., population e.p.s.p. *D*, photograph of ten faint superimposed sweeps to show the small variation in the responses evoked by normal afferent stimulation at 1 Hz.

(1–2 μm tip diameter, 1–2 MΩ resistance) filled with 20 % (w/v) NaCl: the indifferent electrode was placed in the saline that bathed the lower surface of the slice. (The upper surface was exposed to a humidified atmosphere containing 95 % O_2, 5 % CO_2.) The recording electrode was coupled to a voltage follower constructed from a high input-impedance operational amplifier (Analog Devices Type 41L) through silver-silver chloride wires. The voltage follower was connected to an oscilloscope and a FM tape recorder. The over-all flat band width of the recording system extended from 3 Hz to beyond 2·5 kHz.

Saline solutions. The saline solutions used to bathe the preparations had the following composition: control saline NaCl, 134 mM; KCl, 5 mM; KH_2PO_4, 1·25 mM;

$MgSO_4$, 2 mM; $CaCl_2$, 1 mM; $NaHCO_3$, 16 mM; glucose 10 mM. The solutions were saturated with 95% O_2, 5% CO_2 before use and had a pH of 7·2–7·4.

Administration of anaesthetics. Four volatile anaesthetic agents were used in this study. Diethyl ether (MacFarlane Smith Ltd), halothane (redistilled from Fluothane^R I.C.I. Ltd), methoxyflurane (Penthrane^R Abbott Laboratories Ltd), and trichloroethylene (B.D.H. Ltd., 'Aristar' grade). They were all applied to the slices in the gas phase by mixing them with the O_2, CO_2 gas mixture that superfused the upper surface of the slice. The exact concentration of anaesthetic administered was continually monitored every 2 min by a gas chromatograph fitted with an automatic sampling device. Further details of the analytical methods can be found in the papers by Richards (1973*b*), and Richards *et al.* (1975).

Measurement of wave forms. The general shape of the evoked potentials is described in the Results and can be seen in Fig. 1. Two technical points must be made: first, the stimuli delivered to the dentate gyrus were monopolar and capable of exciting only a narrow region of the perforant path, even at high stimulus intensities (40–100 V); second, the recording electrode was very near to the stimulating electrode (1–2 mm separation). Thus the stimulus artifact was troublesome as it was frequently superimposed on the early phase of the evoked potential.

The measurements of the evoked field potentials of the dentate gyrus were similar to those of the olfactory cortex (see Richards 1973*a*), the population e.p.s.p. was measured at a fixed latency from the stimulus artifact and the population spike measured by its area, except in those experiments in which the height of the population spike provided a sufficiently accurate measure of the response of the postsynaptic cells, e.g. during studies of the time course of the action of the anaesthetics. The presynaptic potential was not always recorded, but when it was, its area was taken as an index of the number of fibres contributing to it.

RESULTS

If the perforant path is stimulated with a weak shock, a negative wave can be recorded from the pial surface 1–2 mm away from the stimulating electrode along the axis of the perforant path. (There is very little lateral spread of excitation to localized perforant path stimulation.) If the shock is increased in intensity, this negative wave increases in amplitude and a positive peak becomes superimposed upon it. The evoked negative wave has been shown to be the field potential originating from the synchronous depolarization of the granule cells and so will be termed the population e.p.s.p.; the positive peak has been shown to reflect the synchronous discharge of the granule cells in response to the afferent volley and has been called the population spike. In favourable circumstances, with the recording electrode located in the perforant path itself, it is possible to record the compound action potential of the perforant path fibres preceding the population e.p.s.p. Evidence for this interpretation of the evoked field potentials can be found in the papers by Andersen, Bliss & Skrede (1971); Bliss & Richards (1971) and Lømo (1971). As with isolated preparations of the olfactory cortex, these evoked potentials showed little variation in size or shape during repetitive stimulation at low frequencies (0·2 Hz), see

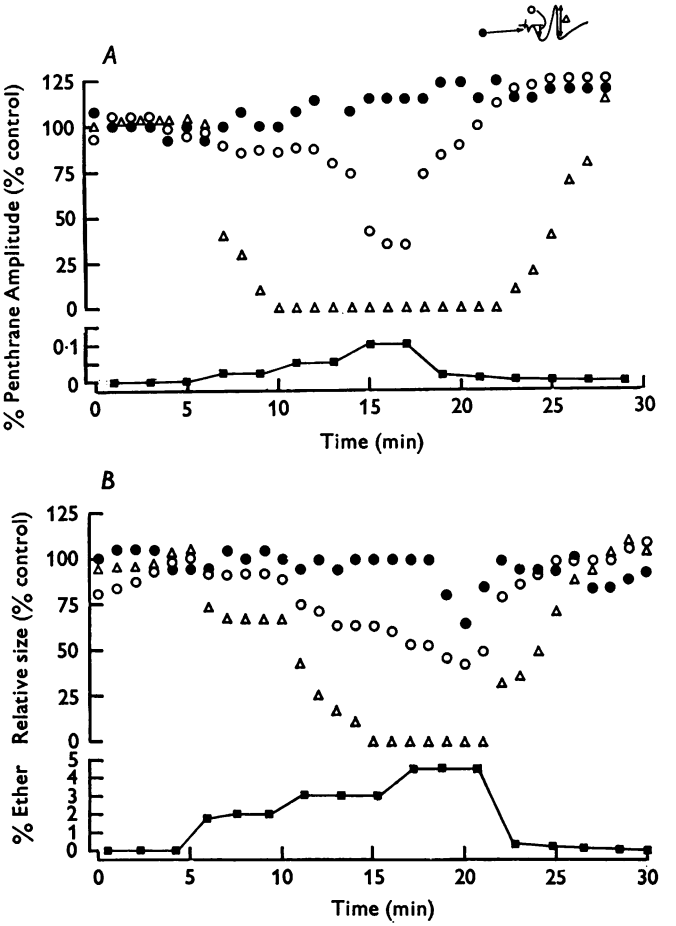

Fig. 2. The time course of the actions of two anaesthetics on the evoked potentials of the dentate gyrus. *A*, the action of methoxyflurane (Penthrane). *B*, the action of ether. Note that in this experiment the population e.p.s.p. was increasing in amplitude during the initial control period and that this trend was reversed following exposure of the preparation to 2 % ether. The perforant path compound action potential (●), population e.p.s.p. (○) and population spike (△) are plotted as percentages of their amplitude recorded just before the application of anaesthetic. In both *A* and *B* the population spike was the most readily depressed component of the evoked potentials, the population e.p.s.p. the next most susceptible components and the perforant path compound action potential the least susceptible, only being depressed by concentrations of ether greater than 4 %.

Fig. 1*D*. They did, however, often show a slow, steady increase in their amplitude during a complete experiment of an hour's duration.

Effect on anaesthetics on evoked field potentials

The action of four general anaesthetics on the evoked field potentials of the dentate gyrus was examined in fifty-five preparations; eleven with ether, twelve with halothane, fifteen with methoxyflurane and seventeen with trichloroethylene. In all experiments the results were similar, all four

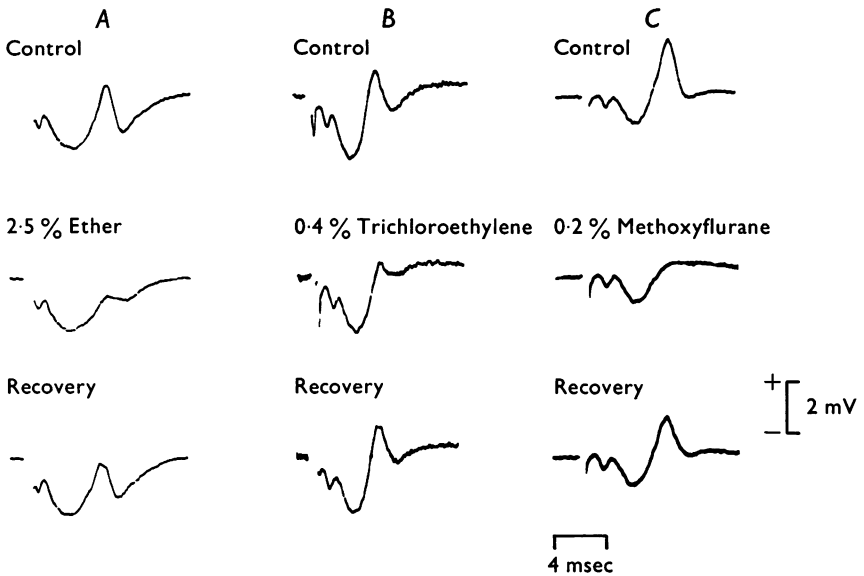

Fig. 3. Examples of the action of ether, trichloroethylene and methoxyflurane on the evoked potentials of the dentate gyrus. The potentials shown in columns *A*, *B* and *C* were recorded from different preparations. The calibrations at the right of the figure apply to all three sets of records.

anaesthetics depressed synaptic transmission measured by the size of the population spike (a measure of the post-synaptic cell discharge). The population spike was the most susceptible component, the population e.p.s.p. the next and the perforant path compound action potential the most refractory component to the action of these anaesthetics (see Figs 2, 3 of this paper and Fig. 1 of Richards & White, 1974). The depression of the population e.p.s.p. and population spike occurred at much lower concentrations of anaesthetic than the depression of these components of the evoked potentials in the olfactory cortex (see Table 1) and at concentrations of anaesthetic much lower than those required to maintain surgical anaes-

thesia in a variety of species (Eger, Brandstater, Saidman, Regan, Severinghaus & Munson, 1965, Halsey, 1974), as defined by the so-called minimum alveolar concentration (MAC) of anaesthetic required to prevent a motor reaction to an incision in the body wall in 50 % of the animals studied.

TABLE 1. A comparison of the depression of the synaptic transmission produced by volatile anaesthetics in the dentate gyrus and olfactory cortex.

		Dentate gyrus			Olfactory cortex		
Anaesthetic	MAC (dog) (%)	ED 50 for depression of pop e.p.s.p. (v/v %)	% Depression of pop spike at ED 50 for e.p.s.p. depression	No. of expts.	ED 50 for depression of pop e.p.s.p. (v/v %)	% Depression of pop spike at ED 50 for e.p.s.p. depression	No. of expts.
Ether	3	1·5–2·5	60–100	11	3·0–4·0	30–60	7
Halothane	0·9	0·3–0·4	90–100	12	0·8–1·0	40–60	11
Methoxyflurane	0·2	0·1–0·2	75–100	15	0·2–0·3	30–50	9
Trichloroethylene	*0·2–0·3	0·2–0·5	80–100	17	0·35–0·5	40–50	5

* No data available, values indicated were calculated from levels in the venous blood of human subjects under trichloroethylene anaesthesia (Clayton & Parkhouse, 1962). MAC values are from Eger *et al.* 1965. Olfactory cortex data from Richards 1973 *a*, *b*, Richards *et al.* 1975 and unpublished data.

In agreement with results obtained in earlier studies on the olfactory cortex, the presynaptic perforant path compound action potential was unaffected by concentrations of the anaesthetics that abolished the population spike and depressed the population e.p.s.p. by 50 % or more. However, in some experiments in which the effects of higher concentrations of anaesthetics were explored, it was found that the compound action potential was depressed by concentration of methoxyflurane and trichloroethylene greater than 0·5–0·6 % and by concentrations of ether greater than 4 % (see Fig. 2*B*). Thus, the compound action potential of the perforant path was depressed by concentrations of volatile anaesthetics similar to those required by depress the compound action potential of the lateral olfactory tract (Richards *et al.* 1975).

Effects on cell discharge

If the interpretation advanced for the population spike is correct, namely that it reflects the discharge of the post-synaptic cells, then the discharge of individual cells in the cortex will also be depressed by general anaesthetics. This point was examined with thirty-four cells; eight cells were

exposed to ether, eight to halothane, eight to methoxyflurane and ten to trichloroethylene. None were spontaneously active and all activity was evoked by perforant path stimulation. As predicted, the evoked discharge of all thirty-four cells was depressed by concentrations of the anaesthetics

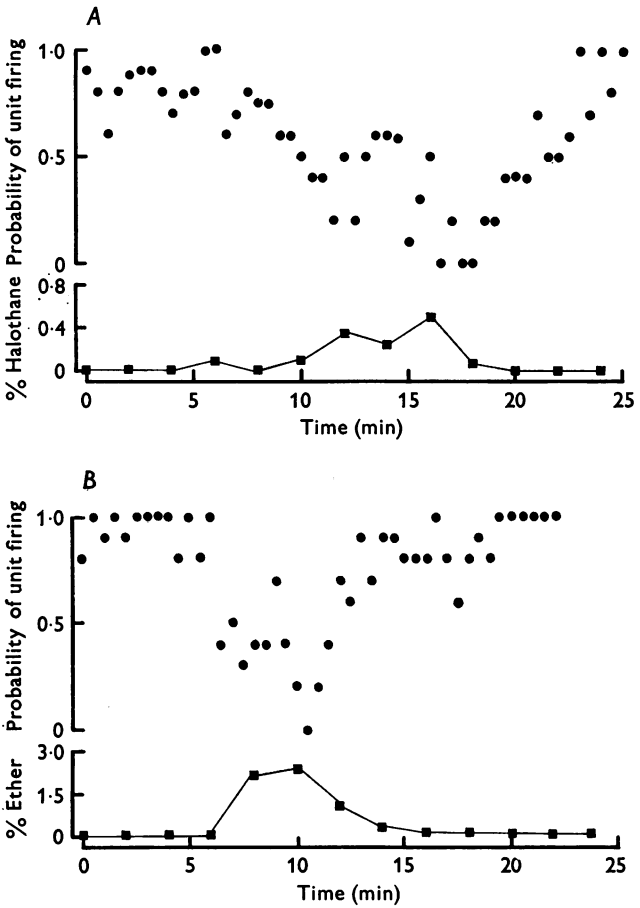

Fig. 4. The time course of action of halothane (*A*) and ether (*B*) on the evoked discharge of two granule cells (recorded from different preparations). The probability of discharge of each cell was depressed by anaesthetic in a dose-related manner.

similar to those required to depress the population spikes. The degree of depression was related to the concentration of anaesthetic applied. The results of two experiments can be seen in Fig. 4.

All the cells that were studied discharged in response to a perforant path volley with latencies corresponding to those of the population spike. Presumably, the records were thus obtained only from granule cells and

not from basket cells which are excited by granule cells. When the population spike was depressed by anaesthetic there was a small increase in its latency before it was abolished (see Fig. 3). Similarly, there was a small increase in the latency of discharge of the individual granule cells when their discharge was depressed by anaesthetics (see Fig. 5). In contrast to the results obtained with ether and trichloroethylene in the olfactory cortex, no anaesthetic caused any tendency for the granule cells to discharge several spikes in response to a single afferent volley. (Compare Fig. 5 with Fig. 1 of Richards, 1973*b* and Fig. 7 of Richards *et al.* 1975.)

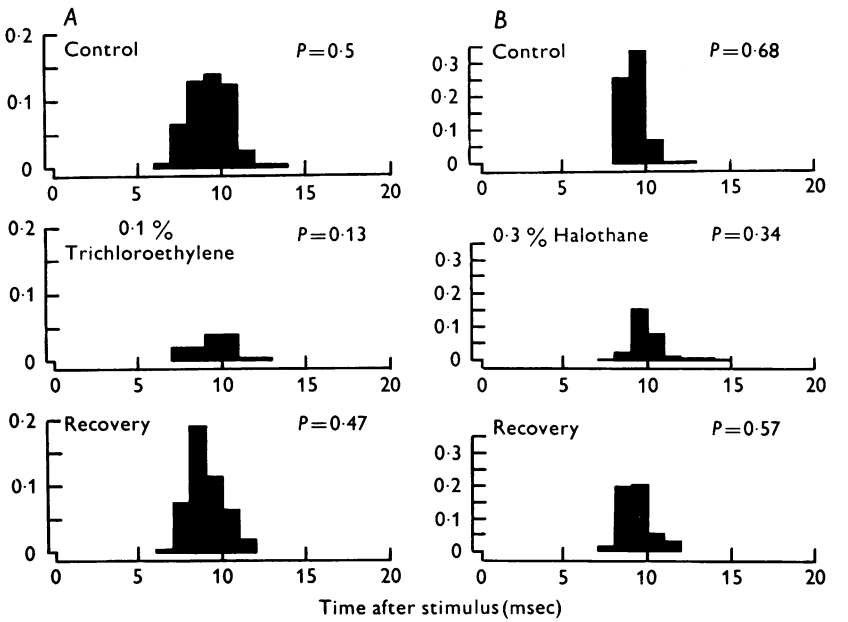

Fig. 5. The action of trichloroethylene and halothane on the stimulus-evoked spike discharges of two granule cells. *A*, the action of trichloroethylene. *B*, the action of halothane. The results are expressed as post-stimulus time histograms, each being compiled from the responses to 200 stimuli given at 1/sec. The ordinates show the probability of cell discharge following stimulation of the perforant path. Both anaesthetics depressed the evoked activity of the cells. The number to the right of each histogram indicate the average probability, *P*, of cell discharge during the sampling period.

No effect of anaesthetics on granule cell threshold

One of the most striking features of these experiments was the sensitivity of the population spike to low concentrations of anaesthetics. Small changes in the population e.p.s.p. of the order of 10% caused by low concentrations of anaesthetic depressed the population spike by up to 60%

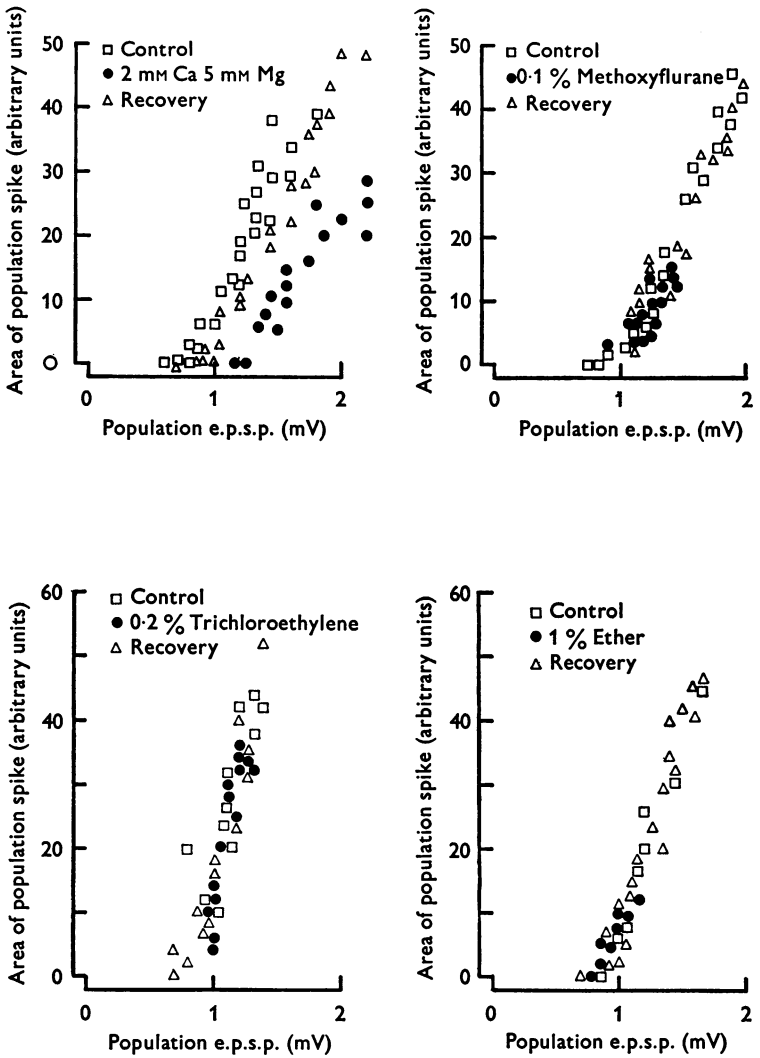

Fig. 6. The relationship between the population e.p.s.p. and the population spike and the action of various substances on it. *A*, the effect of increasing saline Ca^{2+} and Mg^{2+} from 1 mM-Ca^{2+}, 2 mM-Mg^{2+} to 2 mM-Ca^{2+}, 5 mM-Mg^{2+}. *B*, the action of 0·1 % methoxyflurane. *C*, the action of 0·2 % trichloroethylene. *D*, the action of 1 % ether.

Note that elevating Ca^{2+} and Mg^{2+} shifted the relationship to the right indicating an increase in the threshold of the granule cells. The anaesthetics had no effect on the relationship.

(see Fig. 2). Clearly, either there was a steep relationship between the amplitude of the population e.p.s.p. and the size of the population spike, or the anaesthetics had altered the relationship between the synaptic depolarization and the discharge of the granule cells, so that a larger depolarization of the synapses would be required to discharge the granule cells. To see which of these alternatives was correct we examined the relationship between the amplitude of the population e.p.s.p. and the area of the population spike by varying the intensity of the perforant path volley.

If the safety factor for the discharge of the granule cells had been decreased by anaesthetics, for example by raising their threshold for spike discharge or by decreasing the electrotonic propagation of the synaptic potential to the axon hillock, then a larger population e.p.s.p. would be required to elicit a population spike of a given size. Such a change is seen if the concentrations of Ca^{2+} and Mg^{2+} in the artificial c.s.f. is increased (Fig. 6*A*) and would be expected from the known actions of these ions on the electrical threshold of peripheral nerve (Frankenhaeuser & Hodgkin, 1957) and other cortical neurones (Richards & Sercombe, 1970).

Similar experiments with the volatile anaesthetics experiments on thirty preparations showed no significant change in the relationship between the population e.p.s.p. and the population spike (Fig. 6*B–D*). Thus there was no evidence that the volatile anaesthetics increased the threshold of the granule cells.

It is also apparent from Fig. 6 that there is indeed a very steep relationship between the population e.p.s.p. and the population spike, sufficient to account for the great sensitivity of the population spike to the action of general anaesthetics.

Repetitive stimulation

It is likely that transmission between the perforant path synapses and the granule cells is mediated by a transmitter substance. Therefore, the depression of the population e.p.s.p. caused by anaesthetics may have resulted from a decrease in transmitter synthesis or mobilization, which in turn would lead to a decrease in the amount of transmitter available for release by nerve impulses. Such a hypothesis predicts that, as the presynaptic stores of transmitter become depleted by successive nerve impulses, the evoked e.p.s.p.s should also decrease. Therefore, if we were to accelerate the rate of transmitter depletion by repetitive afferent stimulation at high frequencies in the presence of anaesthetic, the e.p.s.p.s evoked after such stimulation should be more depressed than before. This effect can be seen in cholinergic synapses when the re-uptake of choline is blocked by hemicholinium-3 (Birks & McIntosh, 1961; Elmqvist & Quastel, 1965). In our experiments we found no persistent increase in the

depression of the population e.p.s.p. after high frequency stimulation in ten experiments. One example can be seen in Fig. 7. Experiments were conducted with all four anaesthetics using either 0·6–0·7 % halothane, or

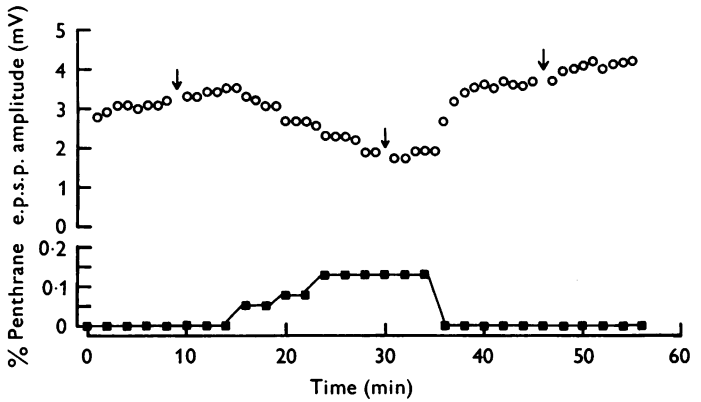

Fig. 7. The effect of high frequency stimulation on the evoked population e.p.s.p. before, during, and after exposure to methoxyflurane (Penthrane). At each arrow, stimuli were delivered at 20 Hz for 72 sec which is equivalent to 2 hr stimulation at the normal rate of 0·2 Hz. No increase in the depression of the e.p.s.p. was seen following this test when the preparation was exposed to the anaesthetic.

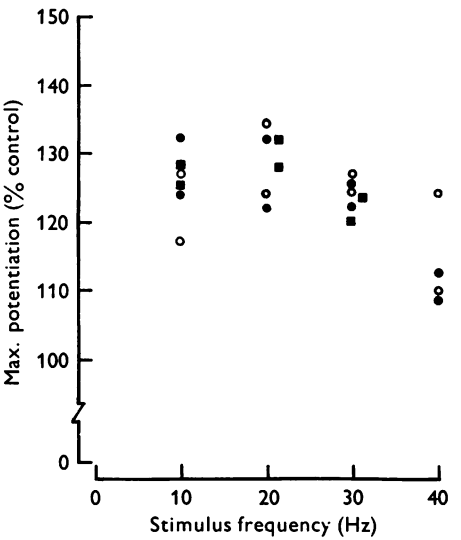

Fig. 8. Action of halothane on the potentiation of the population e.p.s.p. by repetitive stimulation. Halothane (0·3 %) depressed the unconditioned population e.p.s.p. by 35 % in this preparation. Key to symbols: ○, control; ●, 0·3% halothane; ■, recovery. Two tests were made at each frequency.

2·5–3 % ether, or 0·15–0·2 % methoxyflurane, or 0·1–0·4 % trichloroethylene. One preparation did, however, show an increase in the depression of the population e.p.s.p. after the high frequency stimulation in 3 % ether but the e.p.s.p. reverted to its earlier amplitude while the ether continued to superfuse the slice.

None of the anaesthetics studied prevented the potentiation of the population e.p.s.p.s elicited by stimulation at 10–40 Hz (Fig. 8). Neither set of experiments are compatible with the idea that anaesthetics depress synaptic transmission by impairing transmitter synthesis or mobilization.

DISCUSSION

Our aims in the experiments reported here were twofold, to see whether general anaesthetics depressed excitatory synaptic transmission in the dentate gyrus and to define the processes involved in any depression of synaptic transmission.

A detailed consideration of the relationship between the concentrations of anaesthetic administered to *in vitro* preparation and intact animals can be found in the papers by Richards (1973*a*) and Richards *et al.* (1975). However, the depression of synaptic transmission in the dentate gyrus occurs at concentrations of anaesthetic much lower than those required to maintain anaesthesia (defined by the minimum alveolar concentration, see Results and Table 1). Therefore, it is probable that the depression of synaptic transmission in the dentate gyrus is among the central actions of these anaesthetics during anaesthesia.

It will be clear from the Results that the four anaesthetics investigated all depressed transmission between the perforant path and granule cells. Furthermore, our results show that this was neither the result of a failure of impulse conduction in the perforant path nor the result of a generalized increase in the threshold depolarization required for granule cell discharge. The depression of synaptic transmission can be accounted for by a single effect, the depression of the population e.p.s.p.s.

Cause of e.p.s.p. depression

Since the evoked e.p.s.p.s of the dentate gyrus show frequency potentiation, post-tetanic potentiation and are dependent upon the levels of Ca^{2+} and Mg^{2+} in the extracellular fluid (see Bliss & Lømo, 1973; Bliss & Richards, 1971; Lømo, 1971) it is probable that transmission between the perforant path and granule cells is mediated by an unknown transmitter substance. There are, therefore, four possibilities that could account for the depression of the population e.p.s.p.s by anaesthetics: (*a*) presynaptic failure; (*b*) a decrease in transmitter synthesis and mobilization; (*c*) a

decrease in transmitter output for a given presynaptic volley; (*d*) a decrease in the sensitivity of the post-synaptic membrane to released transmitter substance. It is clear from Figs. 2 and 3 that the depression of the population e.p.s.p. was not the result of failure of conduction in the perforant path fibres as the e.p.s.p. was profoundly depressed by concentrations of anaesthetic that had no effect on the perforant path compound action potential. A decrease in transmitter synthesis or mobilization caused by anaesthetics is similarly unlikely, because anaesthetics did not abolish frequency potentiation (Fig. 8) nor did they cause an increase in the depression of the population e.p.s.p. following intense stimulation for long periods (Fig. 7). Thus, it is probable that all four anaesthetics (ether, halothane, methoxyflurane and trichloroethylene) depress synaptic transmission in the dentate gyrus either by decreasing the output of transmitter from the presynaptic terminals or by decreasing the sensitivity of the post-synaptic membrane to the released transmitter or by both of these effects together.

Although the results of the experiments reported here show that general anaesthetics depress synaptic transmission in the olfactory cortex and the dentate gyrus by essentially similar mechanisms, two differences were apparent. Synaptic transmission was more readily depressed by anaesthetics in the dentate gyrus than in the olfactory cortex; and ether and trichloroethylene did not cause repetitive discharge of the granule cells in the dentate gyrus although both did so in the olfactory cortex (see Richards, 1973*b*, 1974 and Richards *et al.* 1975).

The greater sensitivity of the perforant path synapses to depression by general anaesthetics reflects a lower safety factor for these synapses than for those between the lateral olfactory tract and the pyramidal cells of the prepiriform cortex. In the dentate, a 25% reduction in the population e.p.s.p. results in a depression of the population spike of 60% or more, whereas a similar reduction in the size of the population e.p.s.p. in the olfactory cortex results in a depression of the population spike there of only 20–40%. (Compare Fig. 6 with Fig. 7 of Richards, 1973*b* or Fig. 5 of Richards *et al.* 1975.) Such a difference can be partly explained by the differences of stimulation in the two preparations, always maximal in the olfactory cortex but submaximal in the dentate gyrus.

However, this does not explain why the population e.p.s.p. in the dentate gyrus is more readily depressed than that of the olfactory cortex. It has been suggested (Seeman, 1972; Staiman & Seeman, 1974) that general anaesthesia results from a blockage of impulse conduction in fine axons of central neurones. It is known that the perforant path consists of small myelinated fibres of 0·6–0·8 μm diameter (Nafstad, 1967) whereas the lateral olfactory tract consists of larger myelinated fibres of approximately

1·0 μm diameter (L. W. Duchen & C. D. Richards, unpublished observations). The apparent relationship between the ease with which anaesthetics cause depression of synaptic transmission and the size of the afferent fibres in the two regions tempts one to assume that this does indeed explain the greater sensitivity of the dentate synapses. However, there are difficulties. In both cortical areas the general anaesthetics depress the population e.p.s.p. *before* they begin to depress the presynaptic fibre potential. Furthermore, so far as we can tell, there is no difference in the concentration of anaesthetic required to impair conduction in the perforant path or the lateral olfactory tract. For example, ether (the least 'selective' of the general anaesthetics studied) begins to impair conduction in both the lateral olfactory tract and the perforant path above 4% and methoxyflurane begins to impair conduction in both fibre tracts above 0·5–0·6%. Therefore, some other explanation must be sought.

One possibility is that the amount of transmitter released in response to a nerve impulse is less for the nerve terminals of the perforant path than for those of the lateral olfactory tract. If this were so, our results can be simply explained. As increments in transmitter release do not produce a proportional increase in synaptic current (see Martin, 1955), the smaller the initial e.p.s.p. the greater will be the decreasein the e.p.s.p. caused by a given reduction in the amount of transmitter release (or that caused by a given reduction in the sensitivity of the post-synaptic membrane to released transmitter.)

Unlike the pyramidal cells of the olfactory cortex, the granule cells of the dentate gyrus failed to show repetitive discharge in response to afferent stimulation when they were exposed to trichloroethylene or ether. This failure probably reflects the rapid depression of synaptic transmission rather than a fundamental difference between the two preparations, as repetitive discharge of granule cells during exposure to either anaesthetic can be shown if they are stimulated antidromically using transverse slices of the hippocampus (A. E. White, unpublished observations). In these experiments it has been shown that multiple population spikes can be generated during exposure of the preparations to 3% ether or 0·3–0·5% trichloroethylene.

The great susceptibility of synaptic transmission in the dentate gyrus to general anaesthetics is intriguing, as the hippocampus is thought to be one of the cortical structures concerned with memory and its recall (Milner & Penfield, 1955; Olds, 1972; Penfield & Mathieson 1974). Furthermore, the perforant path synapses with the granule cells show changes in synaptic conductivity following brief trains of impulses which is one characteristic likely to be required of synapses involved in the establishment of memory (Bliss & Lømo, 1973; Bliss & Gardner-Medwin, 1973). It is, therefore,

possible that our results could help to explain the amnesia that is commonly associated with the early stages of general anaesthesia (see Artusio, 1954; Guedel, 1937; Robson, Burns & Welt, 1960).

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