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REDUCTION BY GENERAL ANAESTHETICS OF GROUP Ia EXCITATORY POSTSYNAPTIC POTENTIALS AND CURRENTS IN THE CAT SPINAL CORD

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SUMMARY

1. The effects of thiopentone and halothane on excitatory synaptic transmission at group Ia afferent synapses on lumbosacral motoneurones were studied in the anaesthetized or decerebrate cat.

2. Thiopentone (10 mg kg^{-1}) infused on a background of light pentobarbitone anaesthesia caused a decrease in single-fibre monosynaptic group Ia excitatory postsynaptic potentials (EPSPs) of between 0 and 24%. A step increase in inspired halothane concentration in the range 0·7–0·9% produced a decrease in EPSP amplitude of between 0 and 31%. These effects were reversible when the anaesthetic level was reduced.

3. Fluctuation analysis of selected single-fibre group Ia EPSPs revealed that these effects could be accounted for by a decrease in the probability of occurrence of EPSPs of larger amplitude, and an increase in the probability of occurrence of EPSPs of smaller amplitude. The mean separation between discrete amplitudes was not altered by either anaesthetic agent.

4. EPSPs whose time course indicated a somatic site of origin were voltage clamped to study the effect of the anaesthetics on the time course of the synaptic currents. Neither thiopentone nor halothane produced a consistent effect on the time constant of decay of the current, although they both depressed its peak amplitude.

5. The results are interpreted as indicating a presynaptic site of action of both anaesthetics at the concentrations studied: the probability of release of neurotransmitter is reduced, without any detectable change in the mean duration of the postsynaptic conductance increase. These findings are discussed in relation to the mechanisms of action of anaesthetics on exocytosis and presynaptic inhibition.

INTRODUCTION

General anaesthetics have been shown to affect many of the events involved in excitatory synaptic transmission, including the release (Matthews & Quilliam, 1964; Collins, 1980) and re-uptake of neurotransmitters (Cutler, Markowitz & Dudzinski,

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1974), and postsynaptic receptor-mediated conductance changes (Galindo, 1969; Richards & Smaje, 1976). They also appear to reduce the release of excitatory transmitters by prolonging presynaptic inhibition (Eccles, Schmidt & Willis, 1963; Nicoll, 1975). It is still unclear, however, which of these actions predominates at concentrations which produce clinical anaesthesia (see Richards, 1983; Willow & Johnston, 1983, for reviews). Quantal analysis of single-fibre excitatory postsynaptic potentials (EPSPs) of spinal motoneurones, for instance, has suggested that barbiturates (Weakly, 1969), halothane (Zorychta, Esplin & Capek, 1975) and diethyl ether (Zorychta & Capek, 1978), all depress the quantal content without affecting the quantal size, implying a reduction in the probability of neurotransmitter release. Voltage clamp studies of hippocampal cells, on the other hand, have provided evidence that inhibitory currents are prolonged by pentobarbitone and halothane (Gage & Robertson, 1985), suggesting a postsynaptic site of action.

The present study attempts to address the issue by applying both quantal analysis and voltage clamping at group Ia synapses on motoneurones, in order to study the mode of action of thiopentone and halothane *in vivo*. The first part of the study reproduces some of the work of Weakly (1969) and of Zorychta *et al.* (1975). This was judged necessary because the conclusions drawn by these authors depended upon assuming a simple binomial model for the fluctuation in the amplitude of group Ia EPSPs, which has since been shown to be inappropriate for this synapse (Jack, Redman & Wong, 1981). Instead, a statistical analysis was applied to the fluctuation which made no assumptions about the underlying distribution of neurotransmitter release probabilities. This nevertheless gave a similar conclusion: anaesthetic depression was accompanied by a reduction in the probability of occurrence of larger EPSPs, and an increase in the probability of occurrence of smaller ones, without any appreciable effect on quantal size. In the second part of the study, voltage clamping was used to measure the time courses of synaptic currents: these were unaffected by concentrations of anaesthetics which reduced their peak amplitudes.

METHODS

Preparation

Experiments were performed on fifty-one cats weighing 1·5–2·2 kg. In studies involving thiopentone, anaesthesia was induced with 35–40 mg kg^{-1} sodium pentobarbitone (Nembutal, Ceva Chemicals, Australia) I.P.; supplementary anaesthesia was given by intravenous infusion of 2–8 mg kg^{-1} h^{-1} sodium thiopentone, sufficient to abolish the forelimb withdrawal reflex in response to pinching the forepaw. In experiments involving halothane the animal was initially anaesthetized with 5% halothane in an oxygen–air mixture (30% oxygen); the level was subsequently reduced to 1·5–2%, sufficient to maintain minimal jaw tone and prevent the withdrawal reflex. Some experiments using thiopentone were carried out in animals which were induced with halothane anaesthesia prior to decerebration (see below).

After induction of anaesthesia the trachea, common carotid artery and left and right cephalic veins were cannulated. Body temperature was maintained in the range 36–37 °C by a thermostatically controlled electric blanket and by radiant heat. End-tidal CO_2 was monitored and maintained at 4–5% except during end-tidal monitoring of halothane (see below). Left hindlimb nerves supplying posterior biceps, semitendinosus, medial gastrocnemius, lateral gastrocnemius, plantaris and the toe flexors were separated from surrounding tissues, cut distally, and mounted on bipolar stimulating electrodes in a pool filled with warm liquid paraffin.

In experiments involving thiopentone, the C5 branch of the right phrenic nerve was dissected out in the neck and mounted on a recording electrode in an oil pool. Its discharge was integrated

(integrator time constant 100 ms) and was used to monitor the respiratory motor output. Most animals used for studies of halothane, and some for studies of thiopentone, were decerebrated at intercollicular level immediately after dissection of the leg nerves. For these animals the halothane anaesthesia was then discontinued.

A laminectomy was performed to expose the lumbosacral spinal cord, which was then covered by warm paraffin oil. After retracting the dura, naturally occurring filaments from the L7 or S1 dorsal roots were placed on a bipolar recording electrode to determine the number of conducting fibres from each leg nerve. A filament was selected which contained at least one group I fibre from all or nearly all leg nerves. When there were more than one fibre from a given leg nerve, their recruitment thresholds were determined to ascertain that they could be reliably separated, or the leg nerves were further dissected into smaller subdivisions. After selection of the dorsal filament, the remaining L6 to S2 dorsal roots were cut. The ventral roots from these segments were cut and those from L7 and S1 mounted together on a bipolar stimulating electrode to permit antidromic identification of the motoneurones in these segments.

In order to minimize the effects of movement on the intracellular records, cats were paralysed with pancuronium bromide (Pavulon, Organon, 4 mg kg^{-1} h^{-1}), artificially ventilated and given a bilateral pneumothorax. In non-decerebrate animals, the anaesthesia during this period was always kept at a level which maintained pupillary constriction and which also maintained the integrated phrenic nerve discharge rate and peak amplitude at its pre-paralysis level. The paralysis was allowed to wear off at 2 hourly intervals to ensure that these monitors were reliable. Arterial blood gas measurements were made at 2 hourly intervals throughout most experiments. If the arterial pH fell below 7·3, in spite of satisfactory arterial blood gases ($P_{a.CO_2}$ 28–35 mmHg; $P_{a.O_2}$ 95–120 mmHg), it was corrected by intravenous infusion of 1 M-sodium bicarbonate (1–3 ml).

Anaesthetic administration

Thiopentone. When ready to study the effects of sodium thiopentone (Ceva Chemicals, Australia) on synaptic transmission a dose of 10 or 15 mg kg^{-1} was infused in 2 ml of 0·9 % saline over 2 min. Slow infusion of anaesthetic was suspended until the peak amplitude of the integrated phrenic nerve discharge had returned to control levels; it was then restarted at the same rate as before. In six experiments (including one on a decerebrate cat) the unbound blood concentrations of thiopentone before and after the dose were determined as follows. 0·5 ml of arterial blood was sampled through a wide-bore needle, allowed to clot and centrifuged at 13000 r.p.m. for 4 min. The serum was decanted into a microconcentrator tube (Amicon, Centricon 30) and spun at 3500 r.p.m. for 50 min to separate blood proteins of molecular weight greater than 30000 Da (to which thiopentone and pentobarbitone are mostly bound). An aliquot of filtrate (70–200 μl depending upon the total volume of filtrate) was measured and added to an equal volume of acetonitrile containing 25 μg of sodium thiamylal (Surital) as the internal standard. Any proteins which had precipitated were then removed by centrifugtion at 13000 r.p.m. for 4 min, and the supernatant was subjected to liquid chromatographic analysis (Varian Liquid Chromatograph, Model 5000, fitted with a 5 μm C_{18} column 25 cm in length and internal diameter 4·6 mm). The mobile phase was an acetonitrile–water gradient (100–0 %) and the flow rate was constant at 2 ml min^{-1}. The UV detector was set at 226 nm for determination of peaks corresponding to sodium pentobarbitone, sodium thiopentone and sodium thiamylal. The area under each relevant peak was calculated using a digitizing tablet and the concentration of pentobarbitone and thiopentone calculated in relation to the concentration of the internal standard.

Halothane. To study the effects of halothane (ICI) on synaptic transmission, the inspired concentration was abruptly increased from 0–0·3 to 0·8–1·2 % (decerebrate animals) or from 0·8–1·0 to 1·5–1·75 % (animals with intact neuraxis). After data collection the inspired halothane concentration was returned to the control level. In some experiments on decerebrate animals a background level of 0·1–0·3 % halothane was maintained before and after the higher concentration to reduce the intracellular synaptic noise. Arterial halothane levels during the control and experimental periods were approximated using an end-tidal halothane monitor (Datex Normac AA-102 Anaesthetic Agent Monitor). An end-tidal plateau was achieved by artificially ventilating at 22 breaths min^{-1}, and variations in ventilation to maintain end-tidal CO_2 at 4–5 % were made entirely by adjustments to tidal volume.

Recording

Intracellular recordings for fluctuation analysis were made using glass microelectrodes filled with 2 M-potassium methyl sulphate (resistances 8–12 MΩ). After obtaining a stable penetration of an antidromically activated motoneurone (membrane potential more negative than −60 mV) a single-fibre EPSP was evoked by stimulating a muscle nerve at 6 Hz. The dorsal root filament was monitored to ensure that the EPSP was elicited by impulses in only one axon, activated without any latency variation. Between 1000 and 6000 records of the EPSP (5–10 ms in duration) were digitied (20 kHz) and stored on computer disc. The level of anaesthesia was then changed as described above and successive averages of 500 records were monitored (but not always stored) until the EPSP had decreased in amplitude. A further group of 1000–6000 records was then stored as before. Under favourable conditions (notably, when the membrane potential changed by less than 5 mV) the peak amplitude of the EPSP was followed until full recovery, and another large group of single records was collected. Most EPSPs studied with fluctuation analysis had a rise time of less than 1 ms and were between 200 and 400 μV in peak amplitude. If the time course or peak amplitude of the EPSP changed during either of the data collection periods, the results were rejected from the analysis.

For voltage clamp studies intracellular recordings were made using either a single-electrode voltage clamp (SEVC) or a two-electrode voltage clamp (TEVC). Electrodes were constructed as described by Finkel & Redman (1983*a*) and Clements, Nelson & Redman (1986). Only EPSPs which could be assigned to a somatic site of origin were clamped (Finkel & Redman, 1983*b*). This was determined by normalizing their rise time and half-width by the membrane time constant, and referring to a nomogram prepared by Jack, Miller, Porter & Redman (1971). If an EPSP satisfied this condition, its average amplitude (500–1000 trials) was first recorded unclamped. The amplifier (Axoclamp 2A) was then set at a switching frequency between 17·5 and 23·2 kHz (SEVC), and the current and residual voltage of the clamped EPSP were digitized at 50 kHz and averaged over 1000–2000 trials. The anaesthetic level was then switched as before and further unclamped and clamped records were taken.

Data analysis

Fluctuation analysis. A histogram of the peak amplitude of the EPSP on successive trials was constructed by measuring the mean difference in the intracellular voltage between a short (0·5–1·0 ms) period just before the start of the transient and a similar region coinciding with its peak. A histogram was correspondingly constructed for the background noise by measuring the difference in voltage over similar intervals before the EPSP. (The methods used in these calculations are similar to those described in Jack *et al.* 1981.) The histogram of peak amplitudes was then treated as a finite mixture of several distributions (Everitt & Hand, 1981) each associated with a different discrete amplitude of the EPSP. Each distribution was sampled from the corresponding peak amplitude with additive background noise in proportion to its probability of occurrence. The shape of the background noise distribution was estimated by treating the histogram of measured noise amplitudes as a finite sample taken from it.

The statistical task was to obtain the maximum likelihood estimates of the mixing proportions and voltages of the different discrete amplitudes making up the fluctuation of the EPSP amplitude. This was achieved using a different error criterion from that used in previous analyses of this kind (e.g. Jack *et al.* 1981; Clements, Forsythe & Redman, 1987). The maximum likelihood estimator (MLE), a variant of the Expectation–Maximization (E–M) algorithm (Dempster, Laird & Rubin, 1977) first described by Hasselblad (1966), was applied in two stages. Briefly, the noise histogram was approximated by a continuous monotonic distribution made up from the sum of two Gaussian curves, using the MLE to find their optimal probabilities, standard deviations and positions relative to zero on the voltage axis. The mixture problem was then solved by applying the MLE to the histogram of the evoked EPSP, this time treating the noise fit as the contaminating distribution, and allowing only the probabilities and positions of the underlying discrete amplitudes of the EPSP to vary.

An important difficulty which arises in interpreting MLE solutions is that confidence intervals cannot easily be obtained. If the number of discrete amplitudes is not known *a priori*, the maximum likelihood solution can on occasion be very different from the 'true' distribution underlying the data sample. Ling & Tolhurst (1983) have given some results of Monte Carlo simulations using a similar implementation of the E–M algorithm, in which the solution was

constrained to have the correct number of discrete amplitudes. These indicated that errors were likely to arise if the separation between these amplitudes was too small relative to the standard deviation of the noise. For the purpose of the present study, further simulations have been carried out where the number of discrete amplitudes was not known *a priori*. These indicate that reliable solutions can be obtained as long as the following conditions are met:

(i) it is assumed that the underlying distribution is made up of discrete amplitudes separated by roughly equal increments (that is, quantal variability is negligible compared with the noise standard deviation);

(ii) the sample size is greater than 1000, with a smaller confidence interval associated with larger samples;

(iii) the noise is well fitted by a unimodal sum of two Gaussian distributions;

(iv) the separation between discrete amplitudes is greater than 1·5 noise standard deviations (σ_N) for a mixture of two amplitudes with roughly equal probability, with greater separation required if the number of amplitudes is greater or their probabilities are unequal.

If these conditions are not met, it is possible to find distributions which give incorrect solutions when run through a Monte Carlo simulation. An example of some simulation results with three discrete amplitudes is shown in Fig. 1. This was carried out using 2000 random samples of real intracellular noise records, 50% with a superimposed voltage increment corresponding to one 'quantum' and another 25% with an increment corresponding to two quanta. 2000 other samples were used to make up a histogram of noise amplitudes. For each quantal size, the simulation was repeated 30 times. When the MLE solution contained pairs of discrete amplitudes separated by less than 10% of the noise standard deviation, these were routinely fused.

When the quantal size was 1·5 σ_N (Fig. 1*B*), the estimated discrete amplitudes and associated probabilities were grouped around the correct values. As quantal size increased the scatter became smaller (Fig. 1*D*). If, on the other hand, quantal size was much less than 1·5 σ_N, then the number, positions and probabilities of the discrete amplitudes could be incorrectly identified (Fig. 1*C*). The resolution of the MLE was, however, improved by increasing the number of samples used to form the histograms.

The experimental data were compared to the simulation results as follows. For every experimental MLE solution, simulation results were found which were of a similar form. That is, they were made up of the same number of discrete amplitudes, with a similar ratio for their separation to σ_N, and a similar number of measurements in the histograms. If the simulation results reflected the known underlying distribution with no contamination from other non-homologous distributions, the experimental MLE result was accepted. Otherwise, it was rejected and formed no further part of the analysis. It was inevitably necessary to reject the majority of the results on these criteria, and even for those results which were accepted, confidence intervals could only be estimated indirectly, because of the complexity of the solution space from which they were drawn. It was impossible, for instance, to get confidence intervals by obtaining a range of MLE solutions on repeated data samples. An indicator, however, that the confidence intervals were small as a result of applying these restrictive criteria for acceptance, comes from examples where the data sample could be divided by half and still be of sufficient size to treat with the MLE: in those cases which had been accepted by the criteria outlined above, the MLE solutions for half samples and for the whole data sample generally coincided within less than 0·2 σ_N on the abscissa and 0·05 on the ordinate.

The results accepted for analysis are perforce selective, and therefore biased towards those EPSPs which had a small number of discrete amplitudes, and for which the average separation of discrete amplitudes relative to σ_N was large. This is an inevitable consequence of applying stringent criteria for reliability without constraining the results to conform to *a priori* assumptions about the form of the solution.

Voltage clamp experiments. Voltage clamp results were rejected if the EPSP was inadequately clamped (residual peak voltage deflection greater than 20% of the unclamped peak amplitude) or if the steady-state current required to hold the membrane potential at the reference value exceeded $\pm$8 nA. An exponential curve was fitted to the early part of the decay of the synaptic current (0·5–1·0 ms duration, immediately after the current maximum) by minimizing the squared error. The time constant of this exponential decay was compared with the time constant obtained in the same region after the anaesthetic was given.

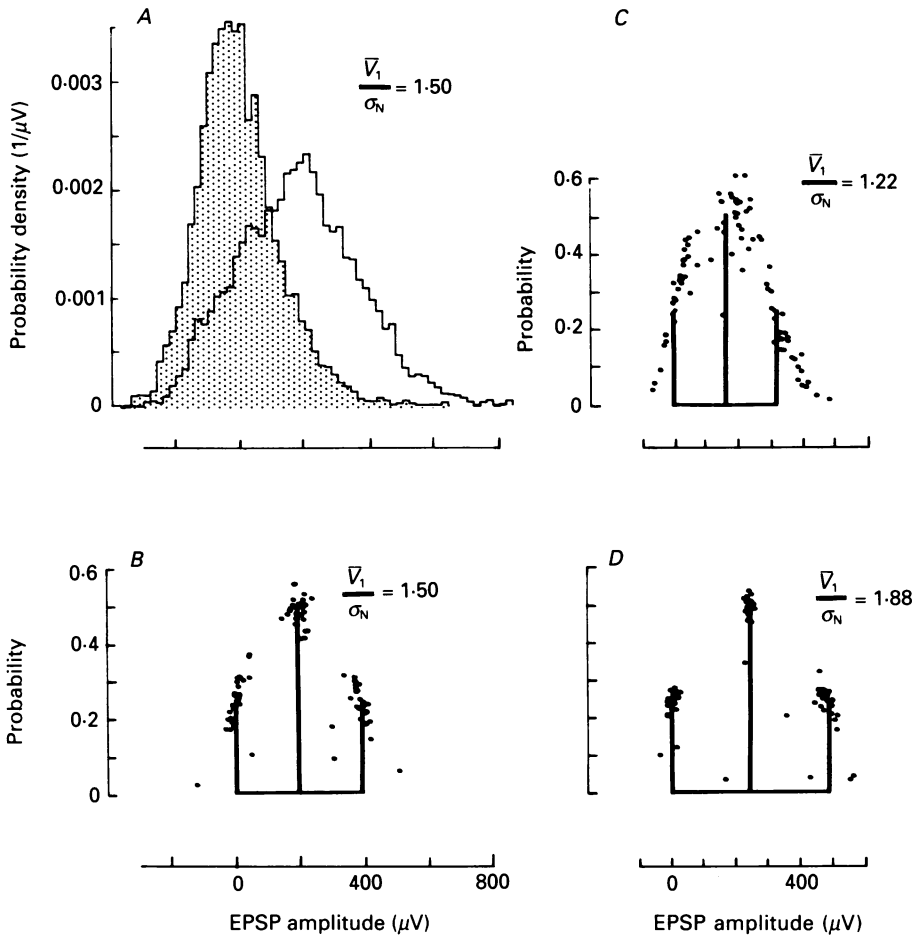

Fig. 1. *A* contains two histograms: the shaded histogram was made up from 2000 measurements of intracellular noise voltage with zero mean and $\sigma_N = 130\ \mu V$. The unshaded histogram was made up from 2000 samples of a mixture of three distributions. Fifty per cent of the samples were obtained by adding 195 μV to further measurements of intracellular noise; 25% were obtained by adding 390 μV to noise measurements and the remaining 25% were simply drawn from the noise distribution. This made the ratio $\bar{V}_1/\sigma_N = 1{\cdot}50$, where $\bar{V}_1 = 195\ \mu V$ and represents the 'quantal' increment. Thirty pairs of histograms were constructed in this manner, and the maximum likelihood estimates of the noise and mixture parameters were obtained in each case. The correct result for the mixture parameters (assuming perfect sampling) is shown by the filled bars in *B*, where the height of each bar indicates its probability of occurrence, and its position on the abscissa indicates its amplitude. The maximum likelihood estimates for each pair of histograms have been superimposed, with each filled circle corresponding to a probability and an amplitude. The results in *C* and *D* were obtained similarly, except that in *C* the quantal amplitude was reduced to make $\bar{V}_1/\sigma_N = 1{\cdot}22$, and in *D* it was increased to make $\bar{V}_1/\sigma_N = 1{\cdot}88$.

RESULTS

Effects of anaesthetics on EPSP amplitudes

Thiopentone

The peak amplitudes of EPSPs were reduced to various extents by the standard dose of 10 mg kg^{-1} sodium thiopentone injected in 2 ml 0·9% saline over 2 min. The maximal reduction in amplitude ranged from 0 to 100 μV, corresponding to decreases from the control amplitudes by 0–24% ($n = 47$), mean $11{\cdot}0 \pm 0{\cdot}9$% (S.E.M.) (Fig. 2*A*). Fifteen per cent of EPSPs were not decreased in amplitude, or were reduced by less than 2·5%, and have not been included in the remainder of the analysis. The major fall in EPSP amplitude was generally complete by 5 min, and was accompanied by a fall in blood pressure and in the peak integrated phrenic nerve discharge. The time course of the decrease in mean peak amplitude is shown in Fig. 2*B*. There was no difference between intact and decerebrate animals in the rate of decrease or maximal decrease of the EPSP. Recovery of the EPSP amplitude occurred at widely varying rates, sometimes returning to control values between 15 and 60 min after thiopentone injection, but was often incomplete when the membrane potential fell and recording was discontinued. Arterial unbound thiopentone concentrations ranged from 0·3–29·9 μg ml^{-1} before a dose of thiopentone to 5·1–45·5 μg ml^{-1} after (mean change $7{\cdot}1 \pm 0{\cdot}8$ μg ml^{-1}; 10 μg ml^{-1} = 37·8 μM). No obvious relation was detected between the EPSP reduction and either the change in or the absolute level of the arterial unbound thiopentone concentrations.

Halothane

Changes in end-tidal halothane concentration from 0–0·3 to 0·8–1·2% or from 0·8–1·0 to 1·5–1·75% caused reductions in EPSP amplitudes ranging from 0 to 224 μV ($n = 28$), corresponding to 0–31%, mean $16{\cdot}5 \pm 1{\cdot}4$% (Fig. 2*A*). The time course of the decrease in EPSP, specifically followed in only a small number of cases, was apparently slower in decerebrate animals ($n = 5$), when the end-tidal level was increased from 0–0·3 to 0·8–1·2%, than in intact animals ($n = 8$) when the end-tidal halothane was increased from 0·8–1·0 to 1·5–1·75% ($P < 0{\cdot}08$, analysis of variance). The maximal reduction in the EPSP was similar in both preparations (Fig. 2*B*). After reducing the halothane level the recovery of the EPSP took place over a similar interval. Again, there was no obvious correlation between the decrease in EPSP amplitude and the end-tidal halothane measurements.

Neither thiopentone nor halothane was observed to cause a consistent change in membrane potential.

EPSP latency

A small latency increase of 30–50 μs was seen in approximately 70% of both thiopentone- and halothane-treated EPSPs, and did not generally revert with a reduction in the depth of anaesthesia. When visible in the intracellular average records, the field potential associated with impulse invasion of the preterminal arborization showed an identical latency increase, suggesting that there was no appreciable increase in synaptic delay. Recordings of the arrival time of the afferent

impulse in the dorsal root filament were not made and a 50 μs or smaller latency increase would not have been detected on the monitor oscilloscope. It was thus impossible to determine from the available data whether the latency shift occurred centrally or peripherally, or in both regions of the group Ia afferent pathway.

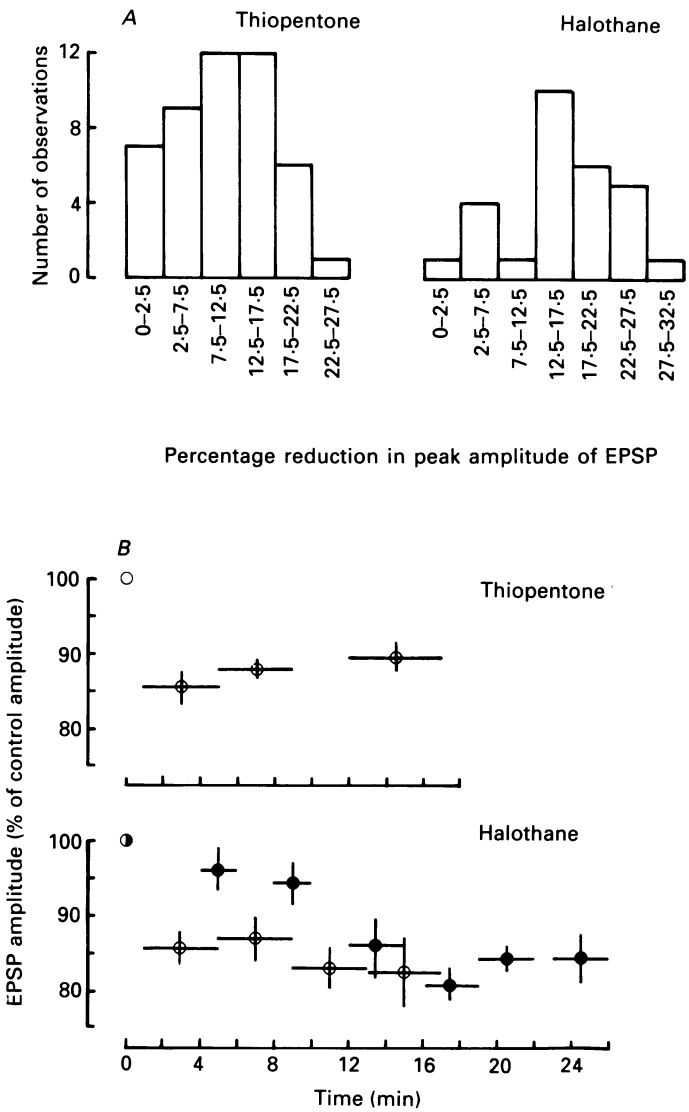

Fig. 2. *A* shows frequency histograms of the maximal percentage reduction in EPSP peak amplitudes after administration of 10 mg kg^{-1} thiopentone (left histogram) or after increasing the end-tidal halothane concentration by 0·7–0·9 % (right histogram). In *B*, the mean time course of the reduction in EPSP amplitudes is shown, in the top panel, after thiopentone administration (○) and, in the bottom panel, separately for intact (○) and decerebrate (●) animals after halothane administration. The horizontal bars represent the range of times included for the calculation of the mean and the vertical bars S.E.M. The difference between intact ($n = 5$) and decerebrate cats ($n = 8$) in the time course of the reduction of the EPSP amplitudes was significant at $P < 0·08$ (analysis of variance).

Fluctuation analysis

Thiopentone

Only four out of twenty-one EPSPs studied using fluctuation analysis were accepted as having been resolved into their underlying discrete amplitudes with a high degree of confidence both before and after thiopentone administration.

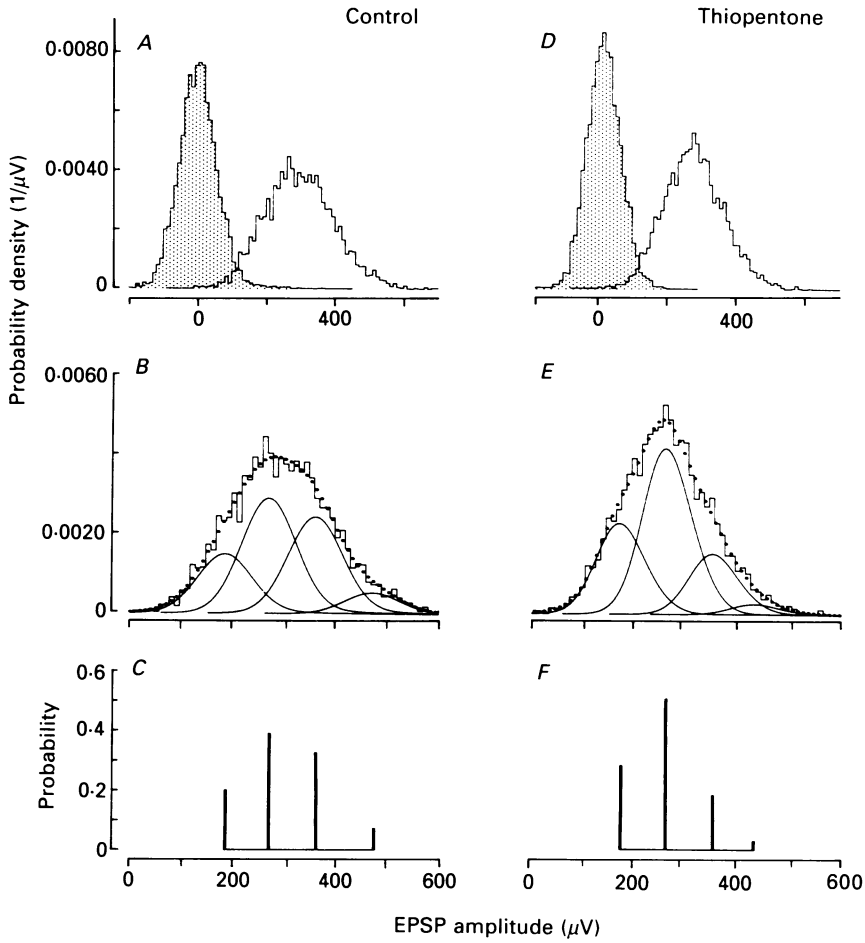

Fig. 3. The histograms in *A* were made from 3900 records of intracellular noise (shaded) and of the peak amplitude of an evoked EPSP (unshaded). Thiopentone (10 mg kg^{-1}) was infused over 2 min, and 5 min later the collection of another 4000 records began. These records were used to obtain the noise and EPSP histograms in *D*. The maximum likelihood estimates of the underlying peak amplitudes, and their probabilities of occurrence, are shown in *C* and *F*. The continuous lines in *B* and *E* represent the noise distributions convolved with each discrete amplitude, such that the area under each of these curves is the probability associated with the corresponding peak amplitude. The sum of these distributions is shown as dots (·), and this can be compared with the original histograms of the evoked EPSPs, also shown in *B* and *E*. Note that the amplitude scale in *B*, *C*, *E* and *F* has been expanded from that used in *A* and *D*. Amplitudes with probabilities less than 0·03 have not been illustrated. Further details are given in Table 1 (EPSP *C*).

The analysis of one EPSP (*C* in Table 1 and Fig. 4) is illustrated in Fig. 3. Histograms describing the fluctuation of the EPSP before and 5 min after 10 mg kg^{-1} thiopentone are shown in Fig. 3*A* and *D*, together with the corresponding histograms for the noise amplitude. After applying the MLE, the fluctuation of the synaptic potential was resolved into four principal discrete amplitudes as shown by the continuous lines in Fig. 3*C* and *F*. The distributions associated with these discrete amplitudes are shown by continuous lines in Fig. 3*B* and *E*. These sum to form

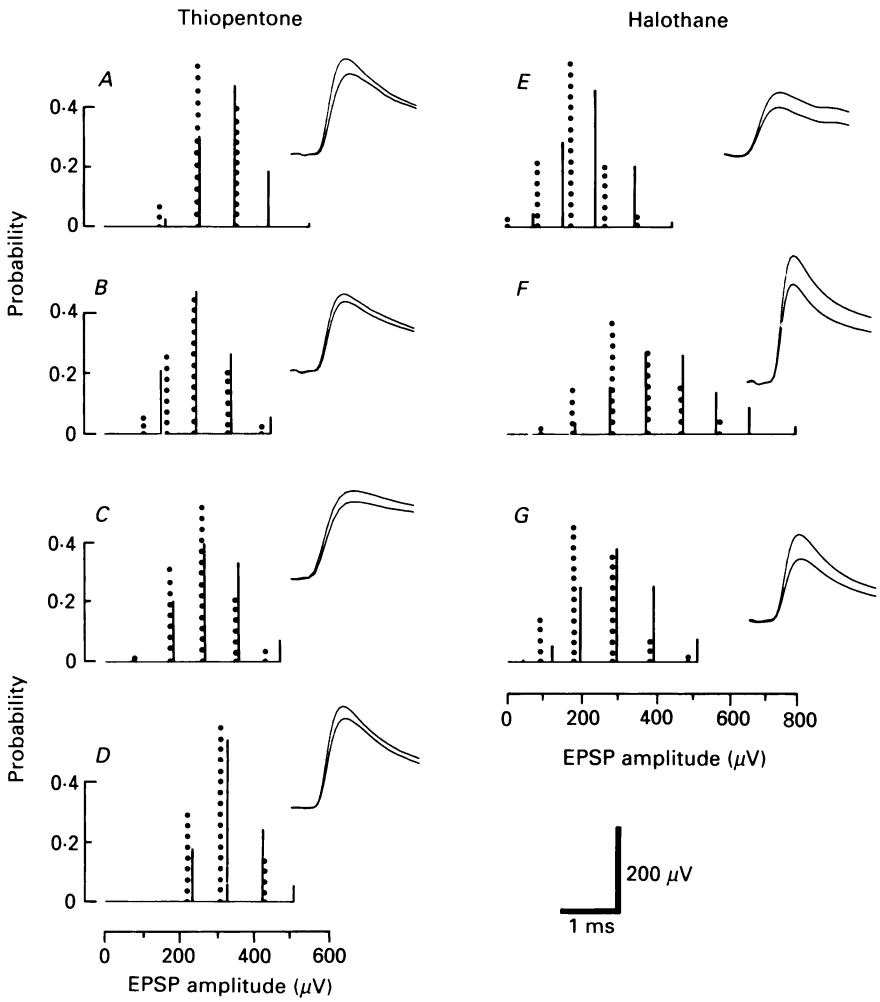

Fig. 4. This is a summary of the results obtained from amplitude fluctuation measurements, where both the control EPSP and the EPSP in the presence of anaesthetic were resolved into their discrete amplitudes. The filled bars indicate peak amplitudes and probabilities for the control EPSPs, while the interrupted bars apply for EPSPs reduced by anaesthetics. When the two bars superimposed (identical peak amplitudes, as in *A* and *C*) the interrupted bar has been offset. Each result is accompanied by an inset showing the average EPSP before and during anaesthesia. The results shown in *C* is the same as that fully illustrated in Fig. 3. The same probability, voltage and time calibrations apply to all sections of the figure. Amplitudes with probabilities less than or equal to 0·01 have not been illustrated. Further details for each EPSP are given in Table 1.

continuous distributions shown as dotted lines, which represent maximum likelihood fits to the original histograms. The results are summarized in Fig. 3*C* and *F*: the EPSP both before and after thiopentone fluctuated between discrete amplitudes lying at approximately 180, 270, 360 and 470 μV, and most of the 12% reduction in its peak size was accounted for by a reduction in the probabilities of occurrence of the 360 and 470 μV EPSPs and an increase in the probabilities of occurrence of the

TABLE 1. Summary of fluctuation analysis results. EPSPs designated *A*–*G* correspond to those shown in Fig. 4. EPSP mean amplitude (V, μV), number of sweeps (N), noise standard deviation (σ_N, μV) and quantal size ($\bar{V}_1$, μV) are shown on the left and the voltages and probabilities of the underlying discrete amplitudes are given on the right. Lines marked by * refer to results obtained after increasing the depth of anaesthesia. Line marked by † refers to results obtained on recovery of the EPSP to control amplitude. Amplitudes with probabilities of less than 0·006 have not been listed

THIOPENTONE

EPSP	V	N	σ_N	$\bar{V}_1$	Voltages and probabilities				
A	330	200	46	93	160·1/0·025	250·5/0·303	344·1/0·471	435·6/0·187	545·0/0·014
	*281	1000	57	99	143·6/0·067	247·1/0·531	346·3/0·395	513·2/0·006	
B	268	1500	49	95	157·0/0·209	252·6/0·470	346·0/0·264	451·2/0·056	
	*243	1500	50	80	109·5/0·052	171·2/0·251	247·1/0·441	334·2/0·200	425·6/0·021
	†264	1500	49	92	82·2/0·015	165·2/0·201	253·6/0·482	348·1/0·238	441·8/0·047
C	305	3100	61	90	73·1/0·007	186·7/0·201	270·8/0·390	360·8/0·328	472·1/0·072
	*261	4000	47	89	79·9/0·010	176·9/0·273	265·4/0·499	354·5/0·182	432·6/0·031
D	347	3700	62	93	235·8/0·173	331·3/0·530	423·6/0·237	507·4/0·058	
	*301	3100	61	98	221·8/0·285	311·3/0·577	426·7/0·137		

HALOTHANE

EPSP	V	N	σ_N	$\bar{V}_1$	Voltages and probabilities				
E	229	3400	57	94	67·9/0·042	148·6/0·283	235·5/0·454	341·0/0·204	442·1/0·016
	*169	3500	47	90	0·5/0·023	79·3/0·211	168·7/0·539	260·9/0·197	346·7/0·029
F	435	3900	52	96	69·2/0·007	181·7/0·037	273·8/0·158	369·2/0·270	470·1/0·260
					559·1/0·139	647·5/0·090	770·2/0·028	865·9/0·006	
	*328	2800	47	95	88·3/0·020	178·6/0·145	279·4/0·364	374·3/0·264	464·3/0·151
					568·1/0·042	659·5/0·010			
G	298	2800	59	98	40·2/0·006	117·7/0·050	194·6/0·243	291·4/0·372	389·2/0·247
					505·7/0·076				
	*213	4000	60	100	84·8/0·135	174·4/0·442	278·2/0·344	379·0/0·063	481·8/0·012

180 and 270 μV EPSPs. Minor changes in the discrete amplitudes were seen, but these could only account for a 3% reduction in peak EPSP amplitude.

Similar results were obtained for the other EPSPs which were accepted: the probability of occurrence of larger discrete amplitudes was reduced, and that of smaller ones increased, with only small and inconsistent changes in their position on the abscissa. The thiopentone-treated EPSPs are illustrated in Fig. 4*A*, *B*, *C* and *D*, together with the superimposed averages of the early parts of their time course to indicate the degree of reduction. Details are given in Table 1. Of the four EPSPs, one (EPSP *B*) returned to control amplitude some 15 min after the standard dose and in this case the result of the fluctuation analysis could be accepted with a high degree

of confidence. The probabilities of occurrence of larger discrete amplitudes increased and the probabilities of occurrence of smaller discrete amplitudes decreased such that the probabilities associated with the various components were near control values (Table 1). The amplitudes of the remaining three EPSPs did not return to control within 22–57 min.

Where the discrete amplitudes failed to coincide before and after anaesthetic, the discrepancies fell within the scatter of solutions for the corresponding Monte Carlo simulations. This implies that the minor differences seen in the discrete amplitudes could be accounted for by finite sampling of the data. Assuming that the underlying fluctuation of the EPSP reflects the intermittent release of quanta of transmitter from presynaptic sites (Jack *et al.* 1981), the depolarization produced by an individual quantum ($\bar{V}_1$) can be estimated from the mean separation between the discrete amplitudes in the MLE solution. A decrease in the postsynaptic depolarization produced by a quantum should then be detected as a reduction in $\bar{V}_1$. No such trend can be seen in the results illustrated in Fig. 4 (computed values of $\bar{V}_1$ before and after anaesthetic are listed in Table 1). For most of the accepted EPSPs, the discrete amplitudes do not align perfectly before and after anaesthetic. This misalignment can also occur if two sets of data, recorded under identical conditions, are resolved into their fluctuation patterns. Finite sampling of the noisy EPSP results in small differences in each EPSP amplitude histogram. If the quantal sizes recorded at the soma differ slightly for different release sites, this will also cause small misalignments of discrete amplitudes before and after anaesthetic.

A guide to the sensitivity of the MLE solutions to genuine changes in $\bar{V}_1$ and in the probabilities of occurrence of discrete amplitudes can be obtained from simulations such as those illustrated in Fig. 1. For any given underlying distribution the scatter of $\bar{V}_1$ around the correct value is roughly normally distribution. A standard error can therefore be estimated for $\bar{V}_1$ obtained from an experimental MLE solution by referring to the appropriate set of simulation results. For $\bar{V}_1 = 1{\cdot}5\ \sigma_N$ and 2000 trials, a 25% change in $\bar{V}_1$ would be detected by rejecting the null hypothesis at the 5% level. The changes in $\bar{V}_1$ measured from the MLE solutions (Table 1) were well within this margin of error. While we cannot reject the null hypothesis for any one EPSP, the possibility that quantal size did change by a factor up to the size of the change in the EPSP cannot be ruled out for any one example. The conclusion that no change occurred in quantal size is based on acceptance of the null hypothesis for all seven EPSPs. Conversely, the null hypothesis can be rejected at the 5% level if the probability of occurrence of a discrete amplitude alters by more than about 0·08. This applies for all but one EPSP in Table 1. In the exception (EPSP *B*), the largest change in probability for any one of the discrete amplitudes is a decrease from 0·264 to 0·200, giving a change of 0·064 which falls just short of failing the null hypothesis.

Seventeen out of twenty-one EPSPs studied using fluctuation analysis were rejected because the MLE solutions for either the control or the thiopentone-treated periods, or both, were considered unreliable. Their form was such that they could have arisen from several different fluctuation patterns, because the discrete amplitudes were either too numerous or too closely spaced in relation to σ_N for the sample size obtained. If the criteria for acceptance were relaxed the additional results generally revealed the same effect of thiopentone on the MLE solutions, but

in some cases changes in σ_N were also accompanied by changes in the separation between discrete amplitudes. Since this tendency was observed in simulations when the ratio $\bar{V}_1/\sigma_N$ was reduced, it was interpreted as a bias built into the optimization if appropriate safeguards were not observed.

Halothane

Fluctuation analysis of twenty-one EPSPs gave three results before and after administration of halothane which were accepted with a high degree of confidence. Again, the reduction of the peak amplitude was brought about by increases in the probability of occurrence of the smaller discrete amplitudes and decreases in the probability of occurrence of the larger discrete amplitudes (Fig. 4*E*, *F* and *G*; Table 1). Amplitudes of the EPSPs did not recover to control values within 30 min.

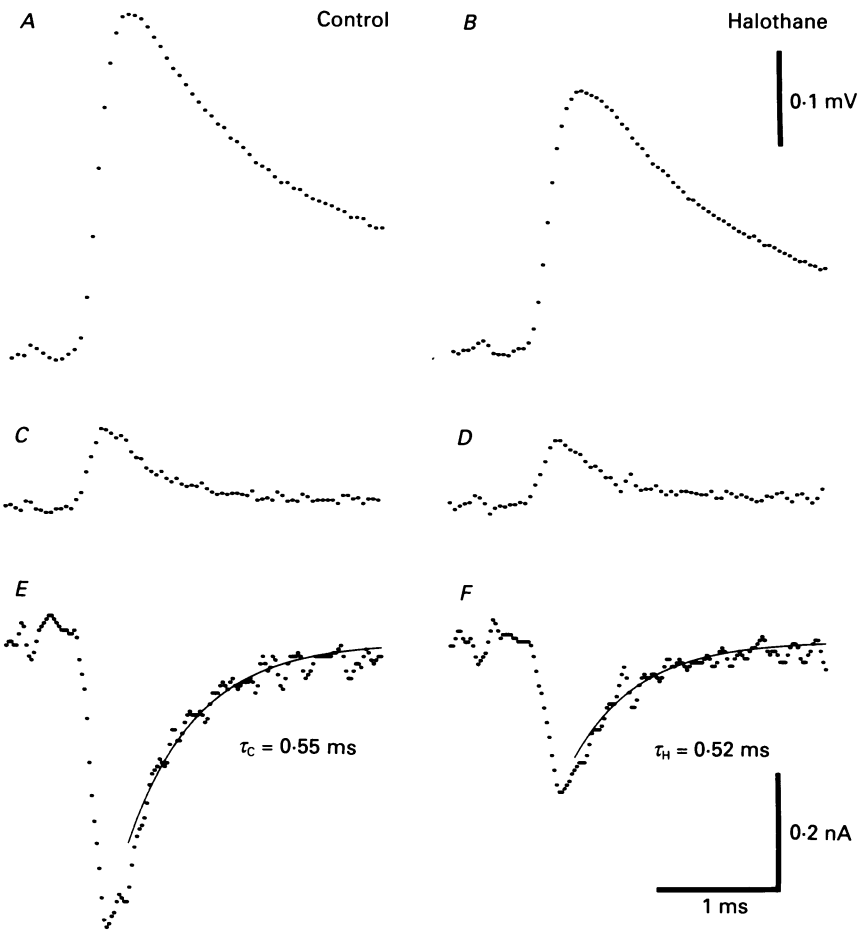

Fig. 5. Voltage clamp of a somatic EPSP before and after halothane. The top records are averages of the unclamped EPSP. The middle records indicate the residual EPSP during clamp, and the bottom records are averages of the clamp current. The continuous lines are single exponentials which best fitted the decay of the current in the period 1·0–2·0 ms from the start of the trace, and time constants are indicated.

Voltage clamp studies

Single- or two-electrode voltage clamp techniques were used to measure the time constants of the currents underlying single-fibre somatic EPSPs. Five EPSPs recorded in experiments involving thiopentone, and four with halothane, were successfully clamped before and after increasing the depth of anaesthesia. Figure 5 illustrates results obtained in one example from an experiment in a decerebrate cat. The unclamped EPSP (average of 500 records) is shown before and after halothane, demonstrating a reduction of 24% in its peak amplitude. Also shown are the clamp current and residual EPSP records. An exponential curve was fitted to the early part of the current records, and its time constant compared before and after anaesthetic. In this example the peak synaptic current decreased by 45%, but the time constants before and after were almost unchanged, from 0·55 to 0·52 ms. The discrepancy between the relative change in current and EPSP peak amplitude is probably explained by the fact that the current record was obtained 11 min after the EPSP record.

The clamp currents obtained from a further eight EPSPs, together with the fitted time constants, are shown in Fig. 6. No consistent trend emerged for the time course of the synaptic current to be altered by either anaesthetic.

Control experiments: hypotension and hypercapnia

For the seven EPSPs for which the fluctuation analysis was considered reliable, mean systolic and diastolic blood pressures were 98/67 mmHg (range 117/93–72/54 mmHg) in the control period and 71/42 mmHg (range 98/73–49/29 mmHg) after increasing the depth of anaesthesia. To verify whether hypotension could have an indirect effect on synaptic transmission, the mean peak amplitude of the single-fibre EPSP was measured in one experiment before and after removal of whole blood to maintain systolic blood pressure 25 mmHg below control values (of 77 mmHg in this case) for 25 min. The EPSP was decreased by 2·6% during the hypotension, and subsequently recovered to control amplitude when the blood was returned and the blood pressure elevated to its original value.

In another animal the arterial P_{CO_2} was changed while the EPSP mean peak amplitude was monitored. An increase of P_{a,CO_2} by 8·5 mmHg (from 29 to 37·5 mmHg) did not alter the peak amplitude of the EPSP. The changes in P_{a,CO_2} observed after anaesthetic administration were always smaller (0–7 mmHg).

DISCUSSION

The concentrations of anaesthetics used in the present study were comparable to those achieved in human clinical practice. Total plasma thiopentone concentrations following repeated bolus doses during surgery can reach 70 μg ml^{-1} (Frank, Savege, Leigh, Greenwood & Holly, 1982), although during continuous infusion levels have been measured at 4–16 μg ml^{-1} (Morgan, Crankshaw, Prideaux, Chan & Boyd, 1986). Since the unbound fraction in these patients represents 15–30% of the total (Morgan *et al.* 1986), the unbound concentrations obtained in the present study (5–45 μg ml^{-1}) are in the upper half of this range. Arterial halothane levels were not determined but

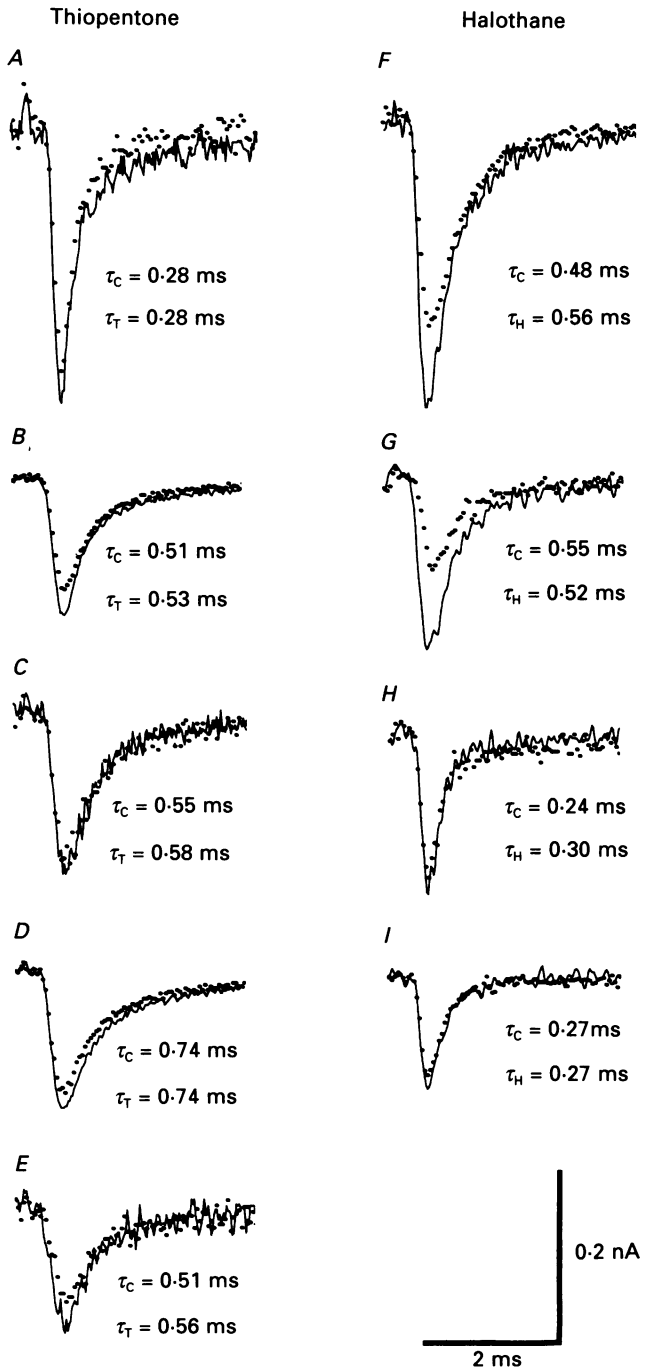

Fig. 6. Averaged records of synaptic currents before (continuous line) and after (·) thiopentone or halothane. The decay time constants are indicated.

the concentration of this anaesthetic can be inferred from the end-tidal levels. These were well within the range used for maintenance of light anaesthesia (0·7–1·5% end-tidal). In spite of these differences EPSP peak amplitudes were only decreased by small amounts by either anaesthetic, and no obvious relation could be established between the depression of EPSPs and the concentration over the ranges used in either decerebrate animals or in animals with maintained anaesthesia. This does not, however, detract from the relevance of the present study to the mechanisms of general anaesthesia, since a relatively small effect on EPSPs can be amplified by passage of signals through a chain of neurones lying near their firing thresholds.

Reduction of EPSP peak amplitude

Both thiopentone and halothane decreased the peak amplitude of single-fibre EPSPs by between 0 and approximately 30%. The time course of this effect, however, was quite different: the major effect of thiopentone occurred about 5 min after the intravenous infusion, whereas that of halothane reached a plateau 15–20 min after a change in inspired concentration. Among pharmacokinetic mechanisms which could account for the time course of thiopentone action are redistribution between binding sites, and secondary effects mediated by cardiovascular and metabolic disturbances. As a weak acid, its binding to lipids and proteins is potentiated by a fall in pH, such as that accompanying hypotension or hypercapnia (Brodie, Mark, Papper, Lief, Bernstein & Rovenstine, 1950). A possible sequence of events to account for the effects of injected thiopentone in the present experiments is as follows: an initial effect on vasomotor centres induces a fall in blood pressure, which, through a secondary action on the distribution of thiopentone in the spinal cord, causes a more profound delayed depression of EPSPs. This would be a quite different sequence of events from that seen with bolus injections, where the short duration but very rapid onset of anaesthesia are explained by redistribution of thiopentone from well-perfused sites in the brain to poorly perfused sites in fat and muscle (Brodie, Bernstein & Mark, 1952). Thiopentone does not appear to be converted to active metabolites (Brodie *et al.* 1950).

In the case of halothane by contrast, the slow onset of action may reflect the time taken for blood levels to rise (Chenoweth, Robertson, Erley & Golhke, 1962). *In vitro*, depression of EPSPs by halothane varies from an almost immediate response (Richards, 1973; cortical slices) to one with at least a 15 min delay (Takenoshita & Takahashi, 1987; spinal cord). Our data suggest that the initial concentration could be important since there was some evidence that the rate of decrease of the EPSP was slower in decerebrate than intact cats.

Neurotransmitter release

The important result derived from fluctuation analysis of single-fibre EPSPs is that both thiopentone and halothane decreased the frequency of occurrence of large discrete amplitudes and increased that of small amplitudes. What change was seen in quantal size was smaller than could be reliably resolved by the analysis technique. This statement must be qualified by stressing that the results are biased towards those EPSPs for which the MLE solutions could be accepted with high confidence. Approximately 80% of the EPSPs were rejected because of uncertainty about the

accuracy of resolution into discrete amplitudes either before or after anaesthetic, or both. This is an inevitable consequence of applying stringent criteria for reliability in results obtained for the group Ia afferent–motoneurone synapse in the cat. The bias in the selection of EPSPs will be towards those for which quantal size relative to σ_N is high. As σ_N varied for different motoneurones, we are unable to assess whether the analysis is biased towards those EPSPs with a large quantal size. The average quantal size for the seven EPSPs analysed in this study was 95 μV. It was 96 μV in Clements *et al.* (1987) and 92 μV in Jack *et al.* (1981), but again, similar selection criteria were in force in these earlier analyses.

It has previously been suggested that postsynaptic receptors are saturated by neurotransmitter released from Ia terminals, to account for the striking lack of variability in the quantal EPSP evoked at a given synapse (Jack *et al.* 1981). If so, a reduction by anaesthetics of the amount of transmitter released per quantum, or a reduction in receptor affinity, could be missed by quantal analysis. However, a reduction in the conductance of the postsynaptic channels or in the number of channels activated by the neurotransmitter would be reflected in a decrease in $\bar{V}_1$. Such an effect was not observed in the results which were accepted, but it is possible that the rejected data contained evidence for this.

Even with the different strategy for statistical analysis, our results are in agreement with those of Weakly (1969) and Zorychta *et al.* (1975). The changes in fluctuation were most simply explained by a reduction in the probability of transmitter release, without any consistent effect on quantal amplitude. Direct *in vitro* measurements of spontaneous inhibitory postsynaptic potentials with halothane (Takenoshita & Takahashi, 1987) also confirm that quantal size is unaltered. The reduction in probability of transmitter release could be accounted for either by a direct effect of thiopentone and halothane on the presynaptic cell, or by potentiation of presynaptic inhibition of transmitter release at the afferent terminal. Among the possible direct actions of thiopentone on the presynaptic cell, impairment of action potential conduction has been discounted by Somjen & Gill (1963), on the grounds that no delay was observed in the onset of ventral root potentials or EPSPs elicited by muscle afferent stimulation. A 30–50 μs delay, as seen in some of the present experiments, would have been beyond the temporal resolution of latency measurements made by these authors, and could reflect a lower safety factor for action potential conduction in the fine terminal arborization. That this reflects intermittent failure of invasion of presynaptic terminals is less likely since action potential block has only been demonstrated at much higher equivalent concentrations of pentobarbitone (Staiman & Seeman, 1974).

Other actions of general anaesthetics have been demonstrated at concentrations of barbiturates and halothane equivalent to those achieved in the present study. Potentiation of γ-aminobutyric acid (GABA) release occurs at similar barbiturate dose and concentration ranges as depression of glutamate and aspartate release (Collins, 1980). Phenobarbitone, pentobarbitone and halothane have also been shown to potentiate the postsynaptic effects of GABA by prolonging the duration of inhibitory synaptic currents (Barker & McBurney, 1979; Gage & Robertson, 1985). Taken together, these effects allow the possibility that the depression of group Ia afferent EPSPs results from a potentiation and/or a mimicking (Nicoll, 1975) of

presynaptic inhibition. Indeed, presynaptic inhibition at this synapse has been shown to cause a decrease in quantal content as measured by fluctuation analysis (Clements *et al.* 1987). Furthermore, pentobarbitone and halothane have been shown to affect stimulus–secretion coupling by inhibiting calcium influx into adrenal chromaffin cells (Yashima, Wada & Izumi, 1986; Pocock & Richards, 1987), and a similar mechanism could underlie the depression of neurotransmitter release.

The present results cannot distinguish between these different mechanisms but a similar approach at a different synapse may resolve the issue. The excitability of vestibulospinal terminals in the spinal cord, for instance, is not affected by GABA (Curtis, Wilson & Malik, 1984), and since EPSPs in spinal motoneurones elicited by descending fibre activity can be resolved by fluctuation analysis (Harrison, Jack & Kullmann, 1989), this pathway could be examined to study effects of anaesthetics in the absence of presynaptic inhibition.

Synaptic currents

The results obtained with the voltage clamp did not reveal an effect of either thiopentone or halothane on the duration of the excitatory synaptic current (EPSC). The present findings therefore failed to reveal any effect of the anaesthetics on the kinetics of channel closure. This contrasts with the prolongation by phenobarbitone, pentobarbitone and halothane of GABA-activated inhibitory synaptic currents *in vitro* (Barker & McBurney, 1979; Gage & Robertson, 1985), and may be explained by different effects on receptors at excitatory and GABA-mediated inhibitory synapses (Lodge & Curtis, 1978). Barbiturates and halothane have notably been shown to potentiate binding of GABA to its receptor (Willow & Johnston, 1980), and barbiturates appear to prolong the mean GABA-induced channel lifetime (Barker & McBurney, 1979). Their effects on excitatory amino acid receptors, in contrast, are less well understood. While barbiturates depress the sensitivity of many central neurones, including spinal motoneurones, to glutamate (Crawford & Curtis, 1966; MacDonald & Barker, 1979), it is not clear whether this reflects a receptor antagonism or an effect on the ionophore.

In contrast to barbiturates, halothane does not appear to depress the response of central neurones to glutamate (Galindo, 1969; Richards & Smaje, 1976). Its major effect at excitatory synapses on motoneurones must therefore be mediated by depression of neurotransmitter release. This may, furthermore, be a direct effect on the presynaptic terminal since dorsal root reflexes measured in the rat spinal cord *in vitro* are suppressed by halothane (Takenoshita & Takahashi, 1987). The opposite would be expected if halothane exerted its action through potentiation of background presynaptic inhibition (Eccles *et al.* 1963).

Action at other synapses

The conclusions drawn from the present work may not hold at other sites in the central nervous system. If barbiturates do exert their action on Ia afferent EPSPs predominantly by potentiating presynaptic inhibition, other excitatory synapses without this specialization may be relatively immune to their effects. Moreover, the sensitivity of glutamate receptors to pentobarbitone differs considerably between

subclasses (Sawada & Yamamoto, 1985). Until this has been investigated more thoroughly, the present results cannot be extended to other excitatory synapses without consideration of the receptor subtypes acting at each site.

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