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ON THE MECHANISM OF BARBITURATE ANAESTHESIA

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SUMMARY

1. The effects of pentobarbitone (0·05–0·6 mM in saline solution) on the evoked field potentials of *in vitro* preparations of guinea-pig olfactory cortex were studied.

2. The evoked field potentials comprised an initial diphasic wave – the lateral olfactory tract (l.o.t.) compound action potential – followed by a surface negative wave (e.p.s.p.) of 1–3 mV amplitude and about 10 msec duration. Superimposed on the negative wave were a number of positive peaks (population spikes).

3. Pentobarbitone depressed the e.p.s.p. but not the l.o.t. compound action potential. The number and size of the population spikes were progressively reduced as the e.p.s.p. became depressed, indicating a failure of transmission through the cortical relay. The e.p.s.p. depression increased with increasing concentrations of pentobarbitone.

4. Pentobarbitone had no effect on the threshold to electrical stimulation of the l.o.t. fibres or on that of the post-synaptic cells to synaptic excitation.

5. Post-tetanic potentiation and frequency potentiation were either of normal magnitude or were enhanced in the presence of 0·2–0·3 mM pentobarbitone.

6. It is concluded that pentobarbitone probably reduces the output of transmitter from the presynaptic nerve terminals of the olfactory cortex and that this mechanism could be the basis of the depressant action of the barbiturates.

INTRODUCTION

Barbiturates have been shown to depress synaptic potentials in the spinal cord without affecting the conduction of impulses along nerve fibres (Løyning, Oshima & Yakota, 1964; Somjen & Gill, 1963; Somjen, 1967). Similarly, pentobarbitone preferentially depressed synaptic potentials in sympathetic ganglia (Larrabee & Posternak, 1952). However, as Somjen (1967) has pointed out the use of the spinal cord as a model for the whole

of the central nervous system assumes that the synapses of the brain are affected in a similar way to those of the spinal cord.

In the work described in this paper an isolated preparation of the olfactory cortex of guinea-pigs has been used. The preparation has a simple synaptic organization which can be activated by stimulation of a well defined nerve tract. The preparation comprises a functionally intact sheet of nerve cells together with an afferent input, the lateral olfactory tract (l.o.t.). Like other *in vitro* preparations of excitable tissues it is possible to control the electrical activity of the nerve cells as well as the environment into which they are placed. The properties of the preparation have already been described and analysed in detail (Richards & Sercombe, 1968, 1970; Richards & ter Keurs, 1971; Richards, 1972) but are briefly reviewed here for the sake of clarity.

In vitro preparations from the guinea-pig brain usually have no spontaneous activity. This facilitates the interpretation of the results obtained from the neuronal population after it has been synchronously activated by an afferent volley. A further advantage is that the evoked potentials are very stable compared to those found *in vivo*. If the l.o.t. is stimulated at its anterior end, predominantly negative potentials can be recorded from all areas of the cortical surface (Yamamoto & McIlwain, 1966; Richards & Sercombe, 1968, 1970). The evoked potentials recorded adjacent to the l.o.t. on the surface of the prepiriform cortex comprise an initial diphasic wave, the l.o.t. compound action potential, followed by a negative wave (N-wave) upon which a number of positive peaks are superimposed. This negative wave has been identified as an extracellularly recorded monosynaptic excitatory post-synaptic potential (e.p.s.p.) and the positive peaks have been shown to be correlated with the synchronous discharge of the underlying cortical cells in response to the evoked e.p.s.p. (Richards & Sercombe, 1968, 1970; Richards & ter Keurs, 1971). In this paper the N-wave will be referred to as the e.p.s.p. field potential or e.p.s.p. and the positive peaks will be called population spikes. The evidence for this interpretation of the evoked potentials has been extensively reviewed in the discussion of the paper by Richards & Sercombe (1970) and by Richards & ter Keurs (1971). These potentials are summed potentials of many synchronously activated cortical synapses and cells in response to the l.o.t. volley. Typical responses of the olfactory cortex to l.o.t. stimulation can be seen in Fig. 1.

In all the experiments described in this paper, the doses of pentobarbitone applied to the *in vitro* preparations of olfactory cortex were comparable to those which would be found in the blood of an intact animal under barbiturate anaesthesia. This point is examined in detail in the Discussion.

The depressant effects of barbiturates on excitatory synaptic trans-

mission could result from interference with one or more of the following aspects of neuronal activity: (1) conduction of impulses along axons, (2) chemical transmission, (3) the propagation of excitatory potentials from the synapse to the axon hillock, (4) the threshold at which impulses are initiated. In this paper it will be shown that pentobarbitone depresses excitatory synaptic transmission in the olfactory cortex primarily by interfering with chemical transmission. A preliminary note has already appeared (Richards, 1971).

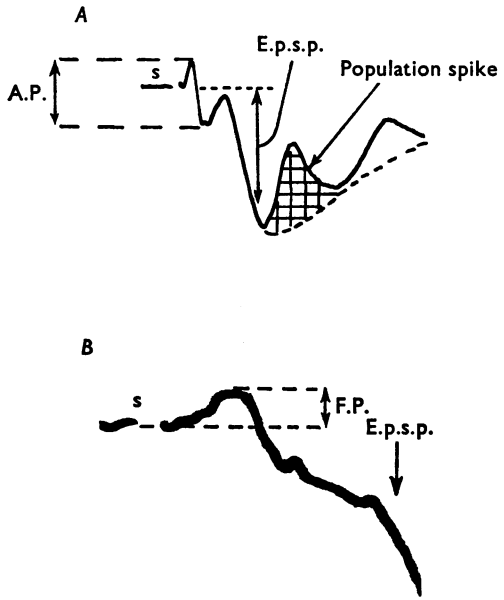

Fig. 1. Examples of the evoked potentials labelled to show the nomenclature and measurements used. *A*, recorded on the prepiriform cortex close to the olfactory tract. A.P., l.o.t. compound action potential; s, stimulus artifact; the e.p.s.p. was measured from the base line at a fixed latency from s. The area of the first population spike was measured as the shaded area under the peak and bordered by the projected return of the e.p.s.p. field potential (interrupted curve). *B*, response recorded on the prepiriform cortex away from the l.o.t. and shows the focal fibre field potential (F.P.) and the beginning of the e.p.s.p. (this response is shown on an expanded time base compared with *A* and at a higher amplification). Both *A* and *B* were recorded with negative potentials shown as downward deflexions. The same convention applies to all the other Figures shown.

METHODS

Details of the methods of preparation, incubation, stimulation and recording have already been described (Yamamoto & McIlwain, 1966; Richards & Sercombe, 1968, 1970). Guinea-pigs were stunned by a blow on the back of the neck, the brain was removed and slices of the olfactory cortex were cut with a razor strip and glass guide. The slices were incubated at 37° C in the chamber described by Richards &

Sercombe (1970). They were stimulated by means of a pair of silver ball electrodes which were placed across the anterior end of the olfactory tract. The responses of the tissue slices were recorded from the cortical surface by glass micropipettes (1–2 μm tip diameter) filled with an aqueous solution of 20% (w/v) NaCl. The recordings were monopolar with respect to the indifferent electrode which was placed in the saline bath. The micro-electrodes were connected to a modified Narth (1969) pre-amplifier (flat band-width 4 Hz to 2·5 kHz) and then to a Tektronix 502 A oscilloscope and Thermionic Products T3002 FM tape recorder (flat band-width d.c. to 2·5 kHz).

Saline solutions. The composition of the saline was NaCl, 134 mM; KCl, 5 mM; KH_2PO_4, 1·25 mM; $MgSO_4$, 2·0 mM; $CaCl_2$, 1·0 mM; $NaHCO_3$, 16 mM; glucose, 10 mM. The saline solutions were saturated with oxygen and carbon dioxide (95:5) before use. The pentobarbitone (as sodium salt) was dissolved directly in the saline solutions; no correction for pH changes was necessary as the concentration of the barbiturates was very low (0·1–0·6 mM) in relation to that of the bicarbonate buffer (16 mM).

Measurement of waveforms. The amplitude of the l.o.t. compound action potential was measured peak to peak (Fig. 1). The local short-latency field potentials generated by fibre activity in the cortex were measured as peak positivity to avoid the difficulty of deciding where the negative phase of the fibre potential ended and the e.p.s.p. began (Fig. 1). It was not possible to record both the l.o.t. compound action potential and the local fibre potential at the same time because the conditions necessary to record one exclude recording the other. The l.o.t. compound action potential tends to 'swamp' other early components of the evoked potentials. The amplitude of the negative wave (e.p.s.p.) was measured at an arbitrary fixed latency from the stimulus artifact on the falling phase of the e.p.s.p. and thus estimates the rate of growth of the synaptic potential. The latency chosen (usually 2–4 msec) was dependent on the conduction time of the l.o.t. fibres. Careful checks showed that pentobarbitone did not affect the conduction velocity of the l.o.t. fibres. The positive peaks (population spikes) were measured in two ways, (*a*) the latency from the stimulus artifact was measured and (*b*) the area under the peaks was estimated as the difference between the observed wave form and that which would have been expected if no positive peak were present (see Fig. 1). The accuracy of these estimates is greatest when the e.p.s.p.–population spike complex is smallest, as the time course of the e.p.s.p. decay is easily predicted from slightly weaker l.o.t. volleys that do not evoke population spikes. As the population spike decreases in latency and approaches the trough of the e.p.s.p. the errors of the area measurement will become appreciable as one cannot be sure that the observed e.p.s.p. peak does in fact represent the peak negativity that would be observed if no population spike were present. There is no way around this difficulty as there is no gross axon tract out of the olfactory cortex, precluding the possibility of antidromic block of the cortical cells. Paired shocks show marked facilitation of the e.p.s.p. as well as the population spike (see Richards, 1972). The only way the population spike can be selectively blocked is by action of tetrodotoxin (Richards & ter Keurs, 1971) but this is largely irreversible. However, despite these difficulties the accuracy of measurement is the same regardless of the presence or absence of pentobarbitone and is greatest at the foot and on the slope of the e.p.s.p.–population spike curve where the information is of greatest value.

RESULTS

Effects of pentobarbitone on the evoked field potentials

When pentobarbitone was added to the saline solution that bathed the preparation, the evoked potentials showed several changes (Figs. 2–4). The e.p.s.p. was reduced and there was a reduction in the number and size of the population spikes that were superimposed on the e.p.s.p. The latency of the population spikes was increased as the e.p.s.p. and population spike size became depressed by the added pentobarbitone. The effects on

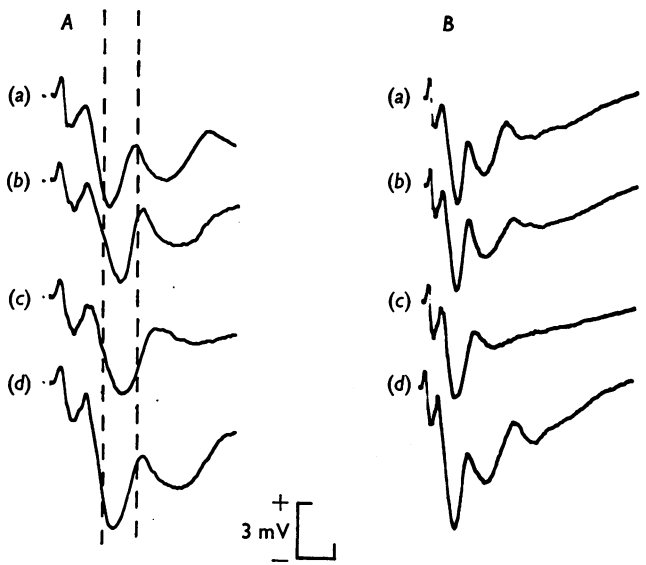

Fig. 2. The effects of 0·25 mM pentobarbitone on the evoked potentials of the guinea-pig prepiriform cortex. *A*: (*a*) control, (*b*) after 6 min exposure to 0·25 mM pentobarbitone, (*c*) after 18 min exposure to 0·25 mM pentobarbitone, (*d*) recovery 45 min after removing pentobarbitone from the incubation fluid. The first vertical interrupted line is drawn to indicate the (fixed) latency at which the e.p.s.p. was measured; the second is drawn 1 msec later and runs approximately through the peak of the first population spike of the control response *a*. The e.p.s.p. slope was reduced by pentobarbitone and the first population spike decreased in magnitude and increased in latency.

B, as for *A* but at a slower sweep speed to show the effects on the second population spike more clearly. Calibration: horizontal bar 1 msec in *A*, 2 msec in *B*.

the population spikes were more marked for the second and third population spikes than for the first (see Figs. 2–4). If the concentration of pentobarbitone was sufficiently high the population spikes were abolished.

The greater the latency of the population spike the greater was the effect of pentobarbitone upon it. The first population spike was the most resistant to the effects of pentobarbitone and showed smaller changes of latency and size for any given concentration of pentobarbitone than did the second or later population spikes. These changes in the e.p.s.p. and population spikes were not caused by a reduction in the size of the afferent l.o.t. volley as the l.o.t. compound action potential was unaffected by

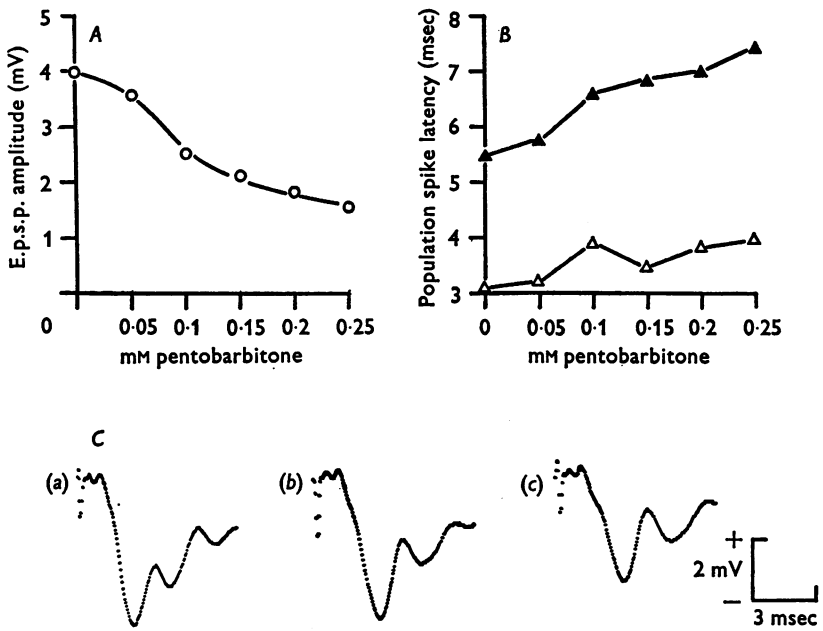

Fig. 3. The effects of various doses of pentobarbitone on the synaptic potentials. *A*, the e.p.s.p. amplitude plotted as a function of the saline pentobarbitone concentration. The e.p.s.p. was measured 3 msec after the stimulus artifact. The e.p.s.p. was first measured in control saline then in 0·05 mM pentobarbitone and successively in 0·25, 0·2, 0·15, and 0·1 mM pentobarbitone before returning to control. The effects were reversible. *B*, the latency of the first (△) and second (▲) population spikes plotted as a function of the saline pentobarbitone concentration. *C*, three of the averaged potentials used to compile *A* and *B*. (*a*) control, (*b*) 0·15 mM pentobarbitone, (*c*) 0·25 mM pentobarbitone. The potentials were averaged with a Biomac 200S signal averager.

pentobarbitone up to 0·6 mM (see below). The changes in the post-synaptic responses were reversible (Figs. 2, 4) and they were found in all fifty preparations that were examined.

The reduction in the e.p.s.p. amplitude, the decrease in the population spike size and the increase in the latency of the population spikes were

dose-dependent (Figs. 3 and 5). The sensitivity of preparations to pentobarbitone varied but the e.p.s.p. amplitude was halved by doses of pentobarbitone in the range 0·2–0·4 mM.

The time course of the e.p.s.p. depression caused by pentobarbitone can be seen from Figs. 4 and 5. Fig. 4 shows that as the e.p.s.p. became depressed, the latency of the population spikes increased. As the e.p.s.p. became maximally depressed the second population spike was abolished.

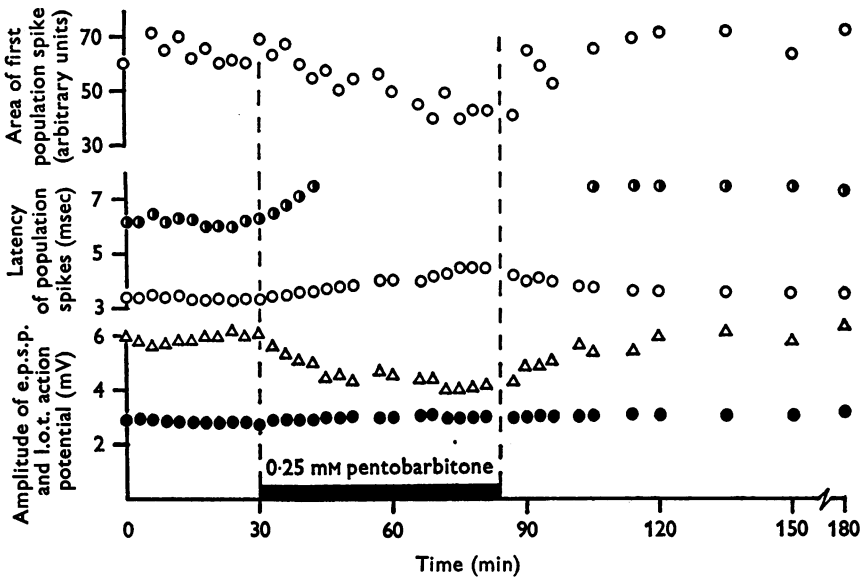

Fig. 4. The quantitative changes found in the evoked potentials of the prepiriform cortex during exposure to a saline solution containing 0·25 mM pentobarbitone. The three ordinates are: top, the area of the first population spike (○) in arbitrary units; middle, the latency of the first (○) and second (◑) population spikes; bottom, the amplitude of the e.p.s.p. (△) and the l.o.t. compound action potential (●). In all cases the abscissa is time in min. The exposure to pentobarbitone (0·25 mM) is indicated by the black bar along the abscissa. Pentobarbitone (0·25 mM) abolished the second population spike after about 12 min exposure.

Conversely, as the e.p.s.p. increased after the pentobarbitone had been withdrawn from the incubation fluid the population spikes also recovered (Fig. 4).

The compound action potential of the l.o.t. fibres was unaffected by doses of pentobarbitone of up to 0·6 mM (Figs. 2 and 4). The field potentials of active fibres in the cortex were also unaffected by pentobarbitone even at doses (0·6 mM) that virtually abolished the synaptic potentials (Fig. 5). In one case (out of nine examined) a reduction in the amplitude of the presynaptic fibre potentials was seen in 0·4 mM pentobarbitone.

Absence of effect on threshold

Pentobarbitone in concentrations of 0·1–0·4 mM did not significantly alter the stimulation voltage at which the l.o.t. compound action potential could first be recorded (Fig. 6). The inference drawn from these results is that pentobarbitone did not alter the threshold of the afferent fibres.

Fig. 7 shows the relation between the e.p.s.p. and the area of the first population spike in the presence and absence of 0·2 mM pentobarbitone. The area of the population spike may be considered a measure of the

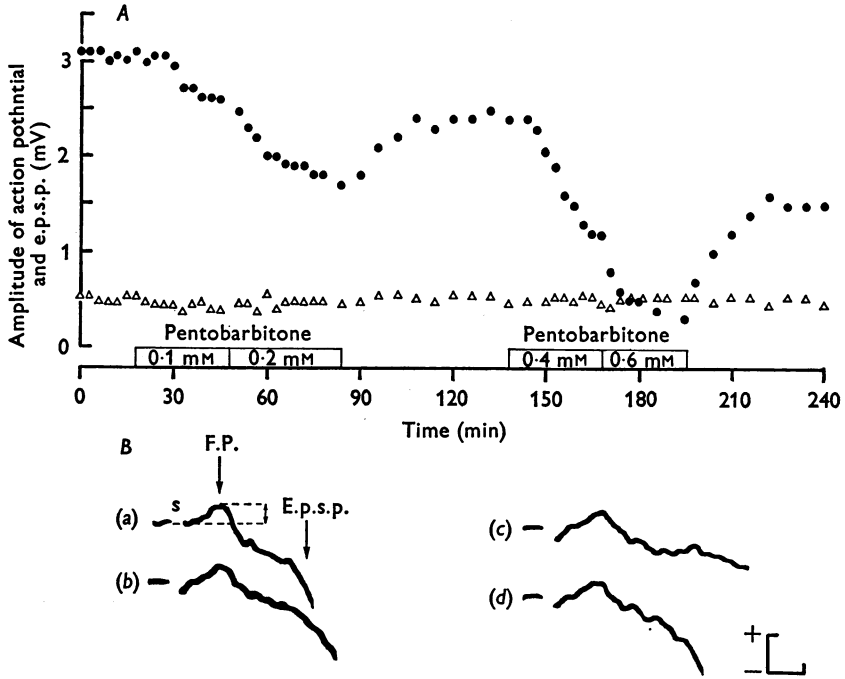

Fig. 5. The effects of pentobarbitone on the evoked e.p.s.p.s and fibre potentials recorded locally in the cortex. *A*, the amplitude of the e.p.s.p. (●) and fibre potential (△) plotted against time in the presence of differing concentrations of pentobarbitone. In this experiment the e.p.s.p. showed a slow, progressive decline which did not seem to be correlated with exposure to pentobarbitone. *B*, four of the potentials from which *A* was compiled (*a*) control (F.P. = fibre potentials); S is stimulus artefact (*b*) 0·2 mM pentobarbitone, (*c*) 0·6 mM pentobarbitone, (*d*) recovery after 0·6 mM pentobarbitone. Calibrations: horizontal bar 1 msec, vertical bar 1 mV.

number of cells firing (Andersen, Bliss & Skrede, 1971; Richards & Sercombe, 1968; Richards & ter Keurs, 1971) (this is equivalent to the fractional pool discharge of motoneurones). The relationship between the e.p.s.p. and the area of the first population spike is, therefore, a measure

of the effective coupling between the e.p.s.p. and the discharge of the cortical cells. This relationship was determined by variation of the intensity of the l.o.t. volley. If pentobarbitone had impaired the coupling between the e.p.s.p. and the discharge of cortical cells, for example by raising the threshold for action potential generation, a large re.p.s.p. would be required to evoke the same number of cortical cells. If this were the case, the plot of e.p.s.p. amplitude against the area of the first population spike should be displaced to the right. However, as Fig. 7 shows, no such displacement

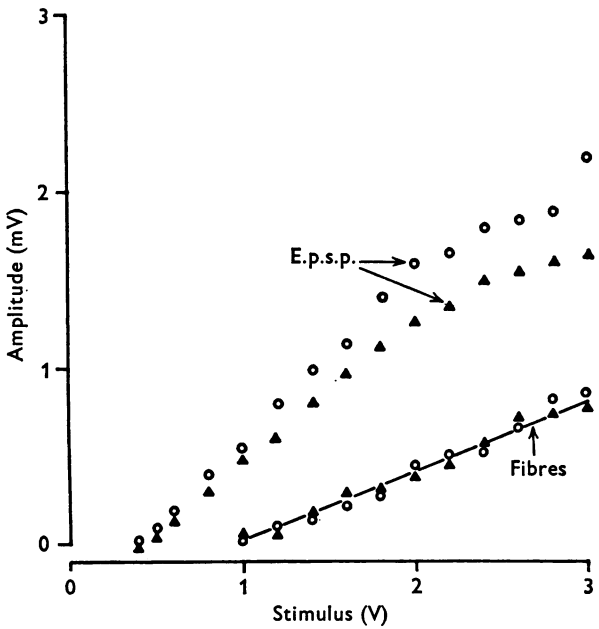

Fig. 6. The absence of effect of pentobarbitone on the threshold of fibre potentials or of e.p.s.p.s in the cortex. ○, control; ▲, 0·2 mM pentobarbitone. The abscissa shows the voltage applied to the l.o.t. stimulating electrodes.

occurred. Therefore, pentobarbitone (0·2–0·3 mM) did not affect the coupling between the e.p.s.p. and the discharge of cortical cells. This result was consistent in all seven preparations examined. It was not possible to test the effects of higher doses of pentobarbitone on the e.p.s.p.–population spike relationship because higher doses of pentobarbitone (0·4 mM or more) depress the e.p.s.p. to such an extent that the discharge of the postsynaptic cells is effectively blocked.

Frequently the latency of the population spikes was increased by 0·5 to 1 msec after treatment with pentobarbitone (Fig. 4) even though the e.p.s.p. was the same size as a submaximally stimulated control. However,

this effect was not reversible nor was it always observed and in some preparations a steady increase in the latency of the population spikes with time was observed even in the absence of any treatment with drugs. Thus this effect may reflect the gradual failure of a preparation rather than a specific effect of pentobarbitone.

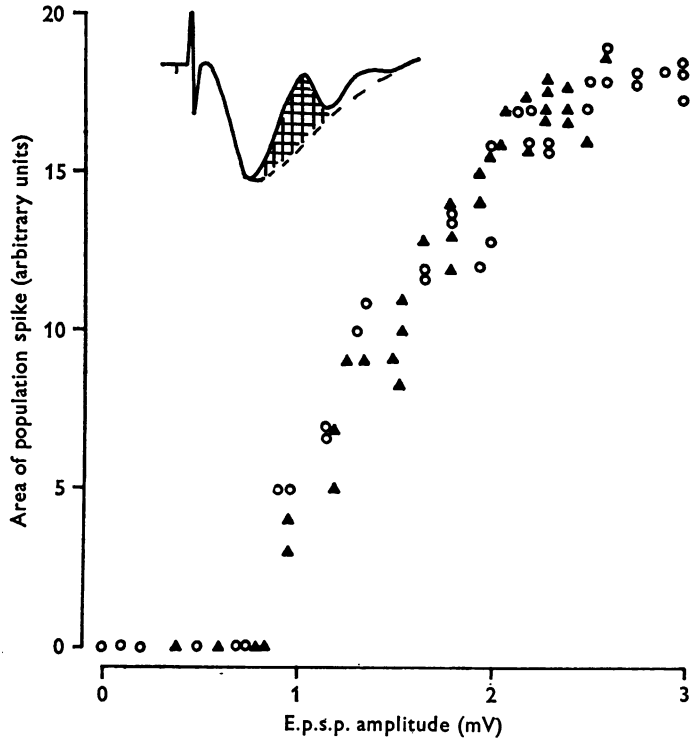

Fig. 7. The absence of any effect of pentobarbitone on the relation between the population spike (measured by its area) and the e.p.s.p. (measured at a fixed latency on the falling phase, see Fig. 1*A*). ○, control, ▲, 0·2 mM pentobarbitone. The e.p.s.p. evoked by maximal stimulation was reduced to 80% of control by 0·2 mM pentobarbitone. The area of the population spike that was measured is indicated by the cross-hatching in the schematic drawing of the potential.

Pentobarbitone and repetitive synaptic activity

The amplitude of the evoked e.p.s.p. varied with the frequency of stimulation (see Richards, 1972). At low frequencies of stimulation (0·5–2/sec) the final, steady amplitude of the e.p.s.p.s was slightly depressed compared to an unconditioned control e.p.s.p. For frequencies between 5 and 20/sec, the steady e.p.s.p. amplitude was greater than the control (i.e. the responses showed frequency potentiation) and for frequencies above 20/sec

the steady amplitude of the e.p.s.p. declined with increasing frequency of stimulation (see Fig. 8). These changes in the relative steady e.p.s.p. amplitude were qualitatively similar when pentobarbitone (0·2–0·3 mM) was added to the bathing medium. Frequency potentiation was often enhanced in the presence of pentobarbitone (see Fig. 8) but this was not a constant finding. However, in no case was frequency potentiation reduced or abolished.

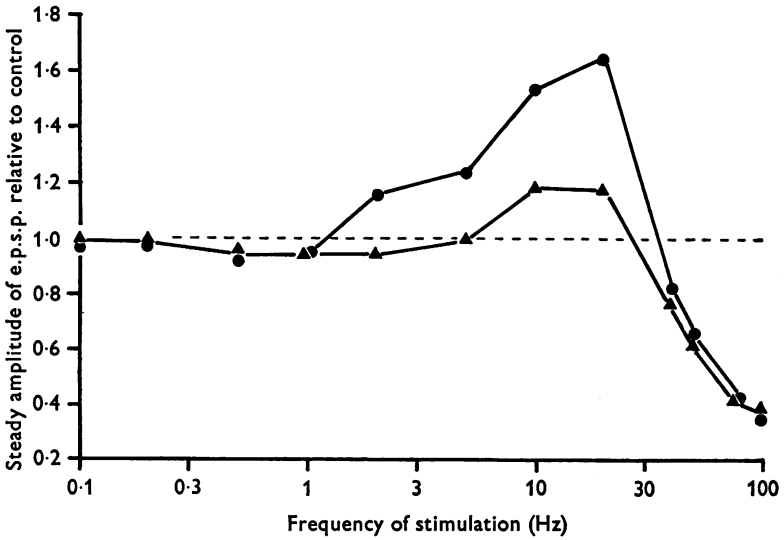

Fig. 8. The effect of pentobarbitone on the size of e.p.s.p.s evoked at different frequencies of stimulation. 0·25 mM pentobarbitone (●), control (▲). The frequencies are shown on a logarithmic abscissa and the ordinate shows the steady amplitude of the e.p.s.p. after many stimuli relative to a single unconditioned e.p.s.p. (the first in the train of impulses). The unconditioned e.p.s.p.s were depressed by 35% by the pentobarbitone.

Four preparations were specifically examined for any changes in the pattern of post-tetanic potentiation (PTP) that could be brought about by pentobarbitone (0·2–0·3 mM). E.p.s.p.s were evoked by l.o.t. stimulation every 5 sec for 1 min before a 30 sec burst at 50/sec. After the tetanic stimulation the e.p.s.p.s were evoked again once every 5 sec and the amplitude of those e.p.s.p.s that were evoked after tetanic stimulation were compared with the mean unconditioned e.p.s.p. amplitude. In no case did pentobarbitone cause a significant reduction in PTP compared to the controls. Indeed, in some preparations there was an increase in the magnitude of PTP in the presence of pentobarbitone. The peak potentiation and the duration of the potentiation were dependent on the frequency and duration of the conditioning train and this was independent of the presence of pentobarbitone. The results of one experiment are shown in Fig. 9.

One preparation showed considerable drift in the amount of PTP recorded at different times. In this case, the PTP observed in the presence of pentobarbitone always lay between the initial control level and the eventual recovery level. Several tests were made and the PTP drifted both up and down during the testing.

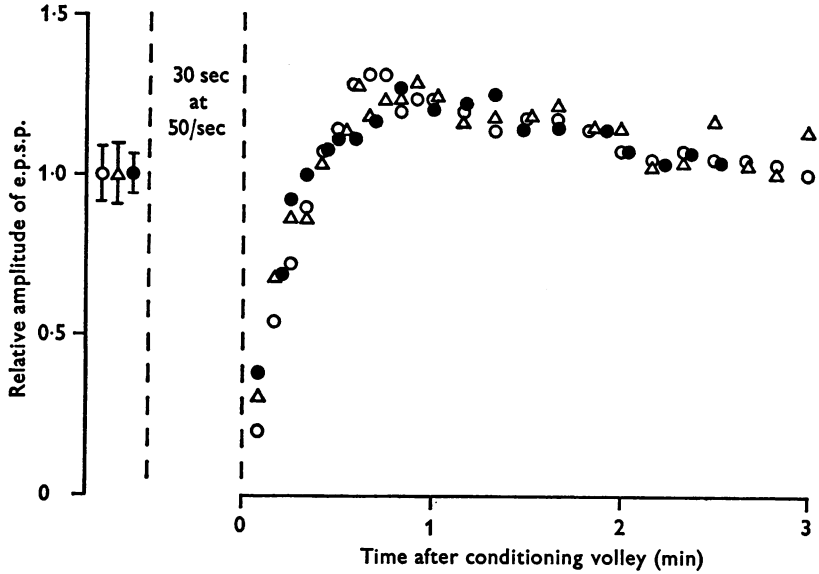

Fig. 9. The results of one experiment showing post-tetanic potentiation of e.p.s.p.s before (○), during (△) and after (●) exposure to 0·2 mM pentobarbitone. E.p.s.p.s were evoked by stimulation of the l.o.t. once every 5 sec for 1 min before a 30 sec burst at 50/sec (indicated by the interrupted vertical lines). The control values (left) are shown together with their 95% confidence limits. After tetanic stimulation the e.p.s.p.s were evoked once every 5 sec for 3 min and the amplitude of each successive e.p.s.p. relative to the mean unconditioned e.p.s.p. amplitude is plotted against the time after the conditioning volley. Pentobarbitone (0·2 mM) halved the amplitude of the unconditioned evoked e.p.s.p.s.

Effects on unit discharge

The effects of pentobarbitone on the spikes recorded from cells in the cortex was not exhaustively examined. For technical reasons it is difficult to record continuously from a single cell when solutions are changed. In three cases this was successfully achieved and no significant difference in the amplitude of the spikes was found after addition of pentobarbitone (0·2 and 0·3 mM) to the bathing fluid. However, evoked unit activity was greatly depressed after the administration of pentobarbitone.

DISCUSSION

Ideally, any study of the mechanisms of anaesthesia should be made on preparations that can be studied in the unanaesthetized state as a control, and in such a study (whether *in vivo* or *in vitro*) the levels of the anaesthetic in the extracellular fluid must be known. With the barbiturates this is especially important as these agents are not rapidly eliminated from the body but become bound to serum proteins or accumulated in the tissues of the body (Brodie, Mark, Papper, Leif, Bernstein & Rovenstine, 1950; Goldstein & Aronow, 1960). The work described in this paper was concerned with elucidating how barbiturates (pentobarbitone in particular) affect synaptic transmission in doses that might be expected to be found during anaesthesia and, by extension, how the barbiturates might cause anaesthesia in the intact animal. Accordingly, the doses of pentobarbitone applied to the *in vitro* preparations were comparable to those which might be found during anaesthesia in an intact animal.

Choice of dose

The choice of dose to apply to an *in vitro* system can be calculated as follows. As the anaesthetic must reach the extravascular space before it can produce any effect, the anaesthetic can be considered diluted by the total extracellular water. Estimates for the extracellular water vary but range from 20 to 30 % of the total body weight (Spector, 1956). Thus a dose of 25 mg/kg of pentobarbitone (mol.wt. 226) should have a *maximum* concentration in the extracellular fluid in the range of 0·3–0·5 mM and this induction dose will progressively decrease as the drug distributes itself throughout the total body water. The concentration of pentobarbitone will progressively decrease to the 'steady' levels found during surgical anaesthesia (about 0·2 mM: Fisher, Walker & Plummer, 1948) as the drug is taken up by the various tissues of the body. These calculations do not take into account the possible differential distribution of the drug but available evidence (Goldstein & Aronow, 1960) suggests that pentobarbitone is uniformly distributed between plasma and the brain substance. The *minimum* concentration of pentobarbitone present in the extracellular fluid during anaesthesia *must* exceed the blood level of free pentobarbitone at the time an animal awakens from barbiturate-induced sleep. The levels found range from 1·0 mg/100 ml. plasma in the rabbit, mouse and in man to 2·0 mg/100 ml. plasma in the rat (Spector, 1956). About a half of this will be bound to plasma protein. The unbound plasma pentobarbitone will be in equilibrium with the extracellular fluid and so this would imply a concentration range of 0·02–0·04 mM pentobarbitone in the extracellular water. The anaesthetic dose of pentobarbitone for many species lies

between 20 and 30 mg/kg (Barnes & Eltherington, 1966). For these reasons doses of pentobarbitone in the range 0·05–0·6 mM were chosen for this study.

Site of action of barbiturates

The following cellular mechanisms are those that could be affected by barbiturates.

1. Conduction of impulses along either the incoming axons or the outgoing axons.
2. Chemical transmission.
3. The propagation of excitatory potentials from the synapse to the axon hillock.
4. The threshold at which impulses are initiated.

These possibilities will be considered in turn.

Conduction of impulses. No evidence was found to support the notion that pentobarbitone (up to 0·6 mM) interfered with impulse transmission in the afferent axons. The field potentials of the l.o.t. fibres were unaffected by the drug as were the fibre field potentials recorded in the cortex (Figs. 2–5). The latency of the e.p.s.p. was unchanged with moderate doses (0·4 mM) of pentobarbitone. These observations taken together support the idea that pentobarbitone does not significantly interfere with conduction in the presynaptic axons.

Chemical transmission. The e.p.s.p. field potential was progressively depressed by increases in the concentration of pentobarbitone in the bathing fluid. Similar reductions in e.p.s.p.s have been found in motoneurones following administration of barbiturates (Somjen & Gill, 1963; Somjen, 1963; Løyning *et al.* 1964; Shapovalov, 1964; Weakly, 1969). Apart from direct interference by barbiturates with presynaptic conduction the reduction in e.p.s.p. field potential amplitude could be caused by one or more of the following mechanisms: (*a*) a reduced synthesis or mobilization of transmitter in the presynaptic nerve terminal; (*b*) a reduced amount of transmitter liberated by a given presynaptic volley; (*c*) a diminished sensitivity of the post-synaptic membrane to the released transmitter substance; (*d*) a reduction in the resting membrane potentials of the post-synaptic cells; (*e*) a decrease in the number of active nerve terminals.

(*a*). As post-tetanic potentiation and frequency potentiation were clearly observed in the presence of pentobarbitone and as the e.p.s.p. amplitude during prolonged repetitive stimulation was maintained in the presence of pentobarbitone (Figs. 8, 9), a reduction in the synthesis or mobilization of transmitter does not seem to be the cause of the reduction in e.p.s.p. amplitude to a single volley.

(*b*) and (*c*). As the identity of the transmitter substance is unknown, direct

determination of the amounts of transmitter released by nerve stimulation in the presence and absence of pentobarbitone is not yet practicable. For the same reason it is not yet possible to see whether the sensitivity of the post-synaptic membrane to the released transmitter substance is reduced by pentobarbitone.

(*d*) Another possible explanation for the reduction in the amplitude of the e.p.s.p.s is that the resting membrane potential could have been reduced by pentobarbitone. This possibility has only been indirectly investigated in this paper. (For technical reasons it has not proved possible to determine the membrane potential of these small cortical cells before, during and after exposure to pentobarbitone.) No change was found in the amplitude of extracellularly recorded spike potentials following administration of pentobarbitone. Thus it is unlikely that a significant reduction in the resting membrane potential of the cortical cells was the cause of the reduction in the amplitude of the evoked e.p.s.p.s. More direct evidence has come from Somjen & Gill (1963) and Weakly (1969). With intracellular micro-electrodes these workers found no consistent effects of thiopentone or pentobarbitone on the resting membrane potential of motoneurones.

(*e*) Pentobarbitone could have reduced the e.p.s.p. by a block of nerve impulse conduction in a proportion of the fine terminal branches of the afferent axons. This would result in a reduction of the number of active synapses. This mechanism cannot be excluded but seems to be unlikely for the following reasons. (1) The focal recordings of fibre activity in the cortex show no reduction in the presence of pentobarbitone. It is, however, possible that these focal potentials reflect the activity of the diverging l.o.t. axons rather than their terminal branches. (2) A failure of conduction in the terminal branches of the l.o.t. axons should be more evident after a tetanic train of stimuli because the fine terminal branches of the afferent axons are hyperpolarized after a tetanic train and their excitability is depressed (Eccles & Krnjević, 1959; Wall & Johnson, 1958). Thus one might expect a reduction in the magnitude of PTP when the synapses are exposed to pentobarbitone. In fact the PTP observed during exposure to pentobarbitone was either of normal magnitude or increased (see also Somjen, 1963, 1967).

Absence of effect on propagation of excitatory potentials and cell threshold. Pentobarbitone could reduce synaptic transmission if it reduced the effectiveness of the coupling between the e.p.s.p. and the discharge of the post-synaptic cell. This could be brought about either by a reduction in electrotonic conduction of the e.p.s.p.s along the post-synaptic cell dendrites, or by preventing the conduction of dendritic spikes, or by an increase in the threshold depolarization of the post-synaptic cell necessary to cause the cell to discharge. If the e.p.s.p.-cell discharge coupling was

impaired in any of the ways suggested (or in any combination) then the relationship between the population e.p.s.p. and the discharge of the cortical cell population would reflect this, so that a larger e.p.s.p. would be required to discharge a given population of cells. This point was specifically examined (Fig. 7) and no increase in the e.p.s.p. necessary to evoke the same number of cortical cells (measured by the size of the population spike) was found. This result thus excludes the possibility that pentobarbitone reduced the effective coupling between the e.p.s.p. and the discharge of the cortical cells.

Conclusions about barbiturate action on cortex

The synaptic current generated by the cells of the olfactory cortex in response to an l.o.t. volley is reduced in the presence of pentobarbitone. This could be a result of either a reduction in the amount of transmitter released in response to the afferent volley, or of a reduction in the sensitivity of the post-synaptic membrane to the released transmitter substance. As the results of this investigation are entirely compatible with those of Weakly (1969) (see below) for the effects of barbiturates on synaptic transmission in motoneurones and as he provided evidence which suggested that barbiturates reduce the output of transmitter from the presynaptic nerve terminals in the spinal cord, this seems to be the most likely explanation for the depressant effects of barbiturates on synaptic transmission in the cortex. Whatever the precise mechanism, the reduction in synaptic current caused by pentobarbitone could contribute to the anaesthetic action of this substance in the intact animal.

Barbiturate effects on other mammalian synapses

Brooks & Eccles (1947) and Løyning *et al.* (1964) believe that interference with conduction in the presynaptic axons is the mechanism whereby barbiturates impair synaptic transmission in the spinal cord. However, both Brooks & Eccles (1947) and Løyning *et al.* used barbiturate anaesthetized animals with repeated injections of barbiturate and the exact cumulative dose of barbiturate that was given is uncertain. The serum barbiturate levels were not monitored and the final level of barbiturate may well have been substantially in excess of that required for surgical anaesthesia. Furthermore, Løyning *et al.* rest their case on the evidence that the negative phase of the presynaptic fibre potential recorded from the motor nucleus was reduced in amplitude. The preceding positive phase, however, was not reduced. Since the negative phase is immediately followed by, and is partly superimposed on, the negative extracellular e.p.s.p. and since the e.p.s.p. is reduced by the barbiturate a reduction of this phase of the compound potential might be expected no matter what the cause of the e.p.s.p. reduction. Finally, in the example they show (their Fig. 9), the

recovery of the e.p.s.p. is not clearly paralleled by a recovery in the negative presynaptic fibre potential as would be expected if the e.p.s.p. reduction was caused by a failure of presynaptic fibre conduction. Thus there is no clear evidence to suggest that conduction in the afferent axons of any part of the C.N.S. is adversely affected by pentobarbitone at least in concentrations likely to be found during anaesthesia.

Brooks & Eccles (1947) and Somjen & Gill (1963) both found that the threshold of motoneurones was increased by barbiturates but the doses of barbiturate administered were higher than those normally required to produce anaesthesia. At lower dose levels the threshold of motoneurones was not increased (Løyning *et al.* 1964; Weakly, 1969). Thus, an increase in the threshold of nerve cells does not seem to be an important factor in the depression of neuronal activity produced by barbiturates.

The effects of barbiturates on the monosynaptic e.p.s.p.s of triceps surae motoneurones have been studied by Weakly (1969). He found that the quantal content of the e.p.s.p.s was reduced by barbiturates and that the unit e.p.s.p.s were unchanged in amplitude. These results were obtained with single fibre activation of motoneurones and strongly suggest that barbiturates depress excitatory synaptic transmission by reducing the output of transmitter from the presynaptic nerve terminals. This idea is further supported by the experiments of Matthews & Quilliam (1964) who found that amylobarbitone reduced the output of acetylcholine from stimulated ganglia. It would, however, be wrong to conclude from these results that all synaptic transmission is reduced by barbiturates. Nicoll (1972) has found that the unusual dendro-dendritic excitatory synapses between the mitral cells and the granule cells of the olfactory bulb are not especially sensitive to anaesthetic agents. He also found that barbiturates increase the duration of the inhibition of the mitral cells caused by the granule cells. Larson & Major (1970) found the recurrent inhibition of motoneurones to be potentiated by hexobarbitone. The potentiation of presynaptic inhibition by barbiturates is well established (Eccles, Schmidt & Willis, 1963). In contrast, Weakly, Esplin & Zablocka (1968) were unable to detect a specific increase in the duration of direct inhibition after administration of barbiturates and Frank & Ohta (1971) found that the reticulospinal inhibitory pathway was readily blocked by pentobarbitone. Thus, different synapses appear to be affected in different ways by barbiturates (and by other anaesthetic agents). None the less, the various effects of barbiturates: depression of excitatory synaptic transmission, potentiation of presynaptic inhibition and potentiation of at least some post-synaptic inhibitory pathways – will all combine to reduce the over-all level of neural activity. These mechanisms are, therefore, presumably the basis of barbiturate anaesthesia.

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