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The action of general anaesthetic agents on root responses of the frog isolated spinal cord

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1. The action of volatile and barbiturate general anaesthetic agents on synaptic transmission in the frog isolated spinal cord has been studied by recording ventral root synaptic potentials and spike discharges evoked by volleys in a dorsal root and in the lateral column fibres.

2. Some observations on the distribution of the lateral column fibres and the characteristics of the dorsal root potentials have been presented.

3. Volatile agents depressed and eventually abolished all components of the ventral root responses. Failure of motoneurone discharge was the result of two factors, a decrease in the slope of the synaptic potential and an elevation of the critical depolarization required to trigger propagated impulses.

4. Barbiturate compounds, in contrast, readily abolished polysynaptic components of the ventral root responses, but the short latency discharge produced by lateral column stimulation was potentiated, and was accompanied by a lowering of the firing threshold of motoneurones. The mechanism of this potentiation by barbiturate compounds is discussed.

5. It is concluded that volatile agents act predominantly on the initial segment and subsynaptic elements of the motoneurone, whereas barbiturate compounds depress the presynaptic or postsynaptic components of interneuronal synapses.

Although general anaesthetic agents are widely used in animal experiments, surprisingly few studies of their action on spinal reflexes have been undertaken, and much of the existing work is conflicting. Early work on monosynaptic reflexes in the cat (Eccles, 1946 ; Brooks & Eccles, 1947) suggested that pentobarbitone depressed reflex discharge by elevating the firing threshold of motoneurones. Although large doses of the drug decreased the amplitude of the synaptic potential, this was considered to be of lesser importance. This has recently been challenged by Sömjen & Gill (1963) who found that thiopentone depressed the synaptic potential in relatively small intravenous doses, and considered this effect to be of equal importance. In addition, they reported the effect of ether on motoneurone discharge, finding it indistinguishable from that of thiopentone.

Earlier studies of frog spinal reflexes are difficult to compare with those in the cat, for monosynaptic connexions in the frog are rarely able to produce threshold

depolarization of the motoneurones (Eccles, 1946). The earliest component of the ventral root discharge is produced by a pathway interrupted by at least one interneurone. Using the isolated preparation, Eccles (1946) showed that polysynaptic components of the ventral root synaptic potential were rapidly abolished by pentobarbitone, leaving only a subthreshold monosynaptic component. Bonnet & Bremer (1948 ; 1952) and Bremer & Bonnet (1948) studied a variety of anaesthetic agents in the spinal frog and concluded that depression of the synaptic potential was the main cause of the failure of reflex discharge. In agreement with Eccles (1946) they demonstrated that polysynaptic pathways were highly sensitive to barbiturate compounds. The recent description of descending fibres in the lateral column of the frog spinal cord (Brookhart, Machne & Fadiga, 1959) has provided a pathway for activation of motoneurones which these workers consider to be monosynaptic. Initial observations of pentobarbitone on this pathway (Brookhart & Fadiga, 1960) showed that it was resistant to concentrations of the drug which completely blocked polysynaptic segmental reflexes. No reports have appeared, however, on the susceptibility of this descending pathway to volatile anaesthetic agents. The experiments reported in this paper have therefore been designed to compare the effects of barbiturate and volatile general anaesthetic agents on ventral root responses evoked by lateral column and dorsal root volleys, and to attempt to elucidate the mode of action of these drugs on synaptic transmission.

Methods

The frogs used in these experiments were usually *Rana temporaria,* but occasionally *R. esculenta* or *R. pipiens* were employed. The frogs were concussed and decapitated, and the spinal cord was exposed by dorsal laminectomy. A block of tissue was then removed, including the vertebral column and lumbar plexuses, and placed in a dissecting dish containing frog Ringer solution which had been equilibrated with 95% oxygen and 5% carbon dioxide. The preparation was maintained at 10°–12° C. for the remainder of the experiment by employing a working stage which was cooled by the evaporator pipes of a small gas refrigerator. In most of the experiments one or more pairs of lumbar roots of one side were dissected free at the intervertebral foraminae and severed close to the dorsal root ganglion. In a few experiments the tibial and peroneal branches of the sciatic nerve were dissected out and left in continuity with the ninth ventral root. Occasionally the contralateral sciatic nerve and its branches were prepared in addition. This procedure allowed recordings to be made of motor activity in flexor and extensor muscle nerves. The whole spinal cord was removed and transferred to the recording bath, where it was positioned on its side in a groove cut into the top of a Perspex pillar (Katz & Miledi, 1963). It was secured by attaching threads to the rostral end of the cord, to the filum terminale and to a spare pair of spinal roots ; the threads were tucked under an elastic band around the top of the pillar. The spinal cord was continuously superfused with frog Ringer solution which had been cooled and equilibrated with O_2 and CO_2. The solution was stored in a reservoir and fed into the recording bath at a constant rate through a blood-giving set ; a duplicate set was used for administering drug solutions. The level of solution in the bath was kept constant by sucking out the excess from a side chamber through a fine pipette attached to a suction line. Although the recording bath had a volume of approximately 100 ml.,

the local environment of the spinal cord could be changed within 1 min as the inlet tube was directed at the surface of the cord.

Recordings were made from the seventh to ninth spinal roots by lifting them on to pairs of silver wire electrodes insulated by a layer of liquid paraffin (Fig. 1), or by using suction electrodes and no paraffin. The electrodes were chlorided for DC recording. Potentials were amplified and displayed on a Solatron CD1183 oscilloscope, and photographed. The electrode used for stimulating fibres in the lateral funiculus was a glass-insulated platinum wire with a concentric reference electrode. This was lowered on to the lateral surface of the cord with a micromanipulator; the optimum position was just dorsal to the point of exit of the fourth or fifth ventral root. Rectangular pulses of 100 μsec duration were used for dorsal root and lateral column stimulation.

The frog Ringer solution had the following composition: NaCl 114 mM, KCl 2 mM, $CaCl_2$ 1·8 mM, $NaHCO_3$ 2 mM, and glucose 1 g/l. in de-ionized water. Solutions of volatile anaesthetic agents were prepared by dissolving the required quantity in Ringer solution which had previously been equilibrated with 95% oxygen and 5% carbon dioxide. The low boiling point of ethyl chloride necessitated cooling the Ringer solution and anaesthetic agent below 12° C before mixing. Although the vapour pressure exerted by the volatile agent decreased the oxygen content of the solution, this did not modify the results, as occasional control periods with non-oxygenated Ringer solution were without effect on the responses. Barbiturate compounds elevated the pH of the solution, but as the results were not affected by correcting the pH with hydrochloric acid, this was not performed routinely.

The following compounds were used: diethyl ether (Duncan Flockhart), ethyl chloride (Bengue), ethyl alcohol (B.D.H.), chloroform (B.D.H.), halothane (Fluothane, I.C.I.), methoxyflurane (Penthrane, Abbott), trichloroethylene (Trilene, I.C.I.), methohexitone sodium (Brietal, Eli Lilly), and propanidid (Epontol, Bayer).

Results

Ventral root responses

The characteristics of the ventral root synaptic potential and spike discharge on dorsal root stimulation (DR-VRP) have been well documented (Barron & Matthews,

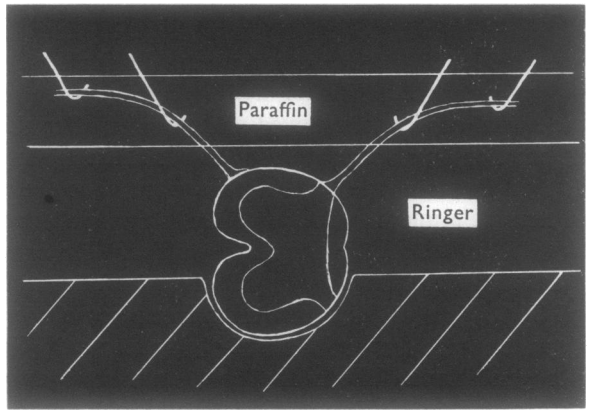

FIG. 1. Diagram of the isolated spinal cord and arrangement for recording with wire electrodes.

1938; Eccles, 1946; Bonnet & Bremer, 1952). Figure 2 illustrates the latency of the subthreshold monosynaptic component of the synaptic potential, the abrupt rise in amplitude signifying the arrival of impulses through polysynaptic pathways, the inflexion representing the firing threshold of the motoneurone pool, and the prolonged discharge produced by repetitive bombardment of the motoneurones. Figure 2 shows also, for comparison the ventral root response to stimulating the lateral column (LC-VRP), the characteristics of which have been analysed by Brookhart *et al.* (1959). The latency of the synaptic potential was the same as that on dorsal root stimulation in this preparation. In ten experiments performed at 12° C the average latency for the LC-evoked synaptic potential was 4·0 msec compared with 4·1 msec on dorsal root stimulation. The rate of rise, however, was much greater, and the firing threshold was rapidly reached producing a synchronous spike discharge with little polysynaptic activity. The contrasting properties of these two pathways have proved invaluable for identifying the pharmacological differences between barbiturate compounds and volatile agents.

During the course of this work a few observations were made on the distribution of the lateral column fibres. A single stimulus applied to the lateral column evoked a short latency synchronous discharge in the ipsilateral ventral roots, but produced only a small synaptic potential in the contralateral roots, which seldom reached threshold (Fig. 3 C and D). Recordings from the peroneal nerve (the physiological flexor branch) and tibial nerve (the physiological extensor branch) in several preparations revealed that most of the motor impulse traffic resulting from a lateral column

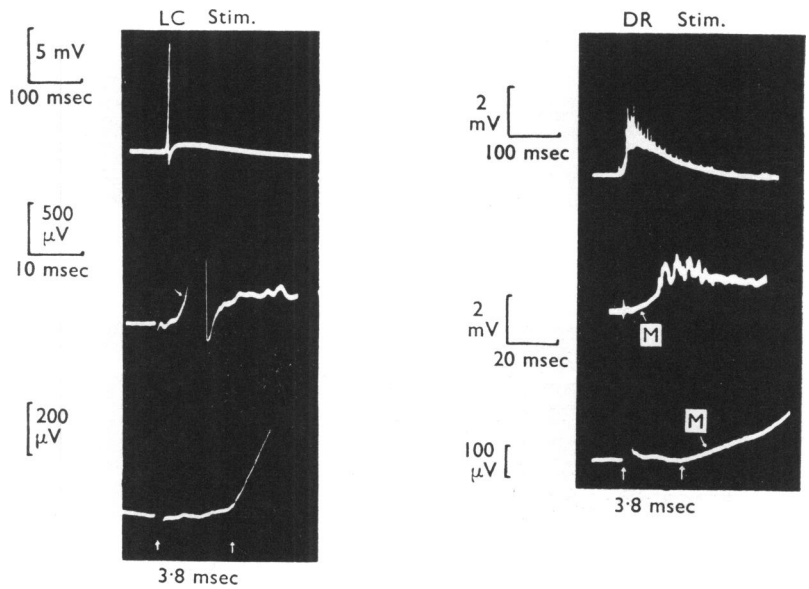

FIG. 2. Normal ventral root responses. The left hand block of records was produced by lateral column stimulation: the arrow in the centre record points to the inflexion representing the firing threshold for the motoneurone pool. The right hand block of records was produced by dorsal root stimulation: M, Monosynaptic component of the synaptic potential. The lowest record of each block was taken from the same preparation at 10° C. RC recording with time constant of 0·1 sec. An upward deflexion represents negativity at the proximal electrode.

volley was travelling to flexor muscles of the lower limbs (Fig. 3 E and F). Responses of this nature could be produced when the stimulating electrode was placed on the lateral column at the level of, or below, the brachial intumescence, but if placed at a higher level the responses were very variable and of longer latency. Repetitive stimulation of the lateral wall of the fourth ventricle evoked, after a short delay, bilateral ventral root discharges from the lumbar segments. In preparations where the spinal cord had been left *in situ* and the roots remained intact, stimulation of the wall of the fourth ventricle yielded co-ordinated alternating flexion and extension movements of the hind limbs.

Dorsal root responses

The depolarization of primary afferent fibres produced by a volley in a dorsal root or a ventral root was first extensively described by Barron & Matthews (1938).

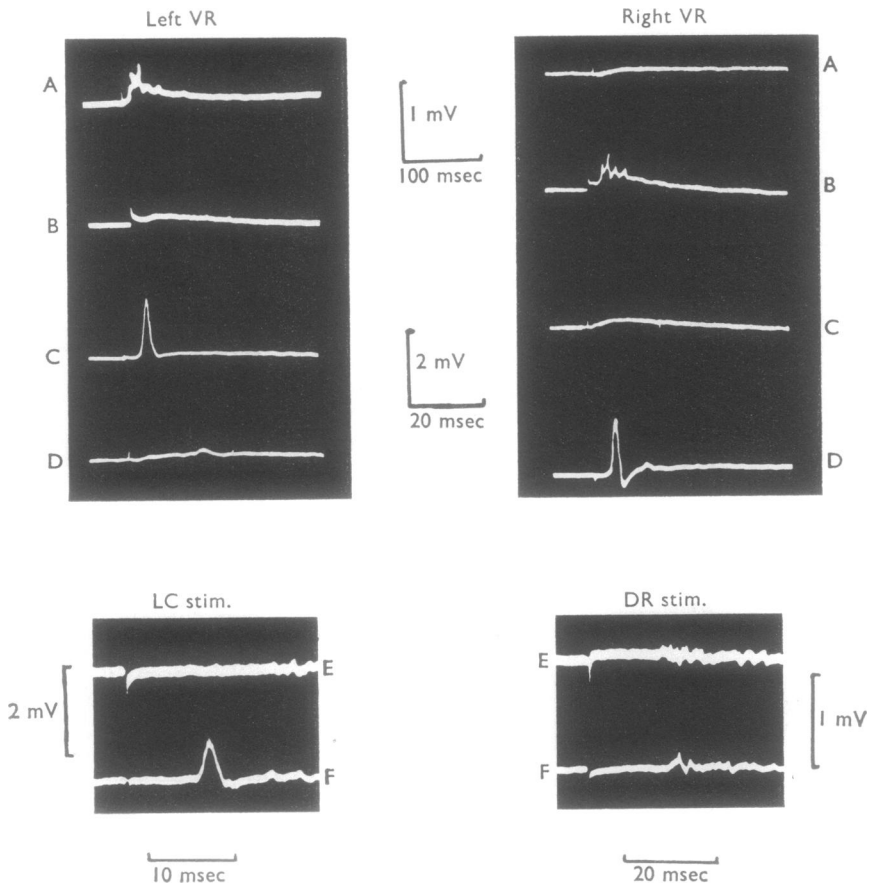

FIG. 3. Distribution of lateral column fibres. The sciatic nerves of both sides, and their peroneal and tibial branches, were dissected out and left in continuity with VR9. Upper blocks of records: recordings from the ninth ventral roots on stimulating the left DR9 (A), the right DR9 (B), the left lateral column (C) and the right lateral column (D). Lower blocks of records: recordings from the tibial branch (E) and the peroneal branch (F) of the left sciatic nerve on stimulating the ipsilateral lateral column and DR9. RC recording.

There is now considerable evidence to support the hypothesis that this depolarization is generated by a chemical transmitter, and that it produces presynaptic inhibition (Eccles, 1961). The dorsal root potential following a conditioning dorsal root volley (DR-DRP) is probably generated through a short internuncial chain (Schmidt, 1963), while that following a ventral root volley (VR-DRP) is generated by a longer pathway whose first synapse is cholinergic (Kiraly & Phillis, 1961). The dorsal root potential which follows a conditioning volley in the lateral column (LC-DRP) has been the subject of only a brief description (Katz & Miledi, 1962). Figure 4 illustrates these three potentials. At 12° C the time to peak of the LC-DRP was 100–120 msec, presenting a slower rate of rise than the DR-DRP. In addition, the rising phase usually displayed a prominent "notch" occurring 20–25 msec after the stimulus (Fig. 4B and C). The amplitude was often greater than that of the DR-DRP, whereas the half decay time was comparable (250–350 msec). When only a few fibres in the lateral column were activated (using a microelectrode as the stimulating electrode) the "notch" became more obvious, the main component having a latency close to that of the VR-DRP (Fig. 4C and H). This long latency suggested that the potential may, in part, be produced by activity in recurrent collaterals from the synchronously discharging motoneurones, in much the same way as the VR-DRP. As this latter potential is completely abolished by anticholinergic drugs, it was of interest to determine the action of these drugs on the LC-DRP. Four experiments in which either tubocurarine or dihydro-β-erythroidine were used showed that the LC-DRP was only slightly depressed by concentrations (10^{-4} g/ml.) which completely abolished the VR-DRP (Fig. 5). Cholinergic collaterals must, therefore, contribute little to the LC-DRP. During these experiments it was noticed also that the synchronous ventral root spike evoked by a lateral column volley was not affected by these drugs (Fig. 5D). This is evidence against the suggestion of Crepax & Brookhart (1966) that acetylcholine might be produced by lateral column terminals.

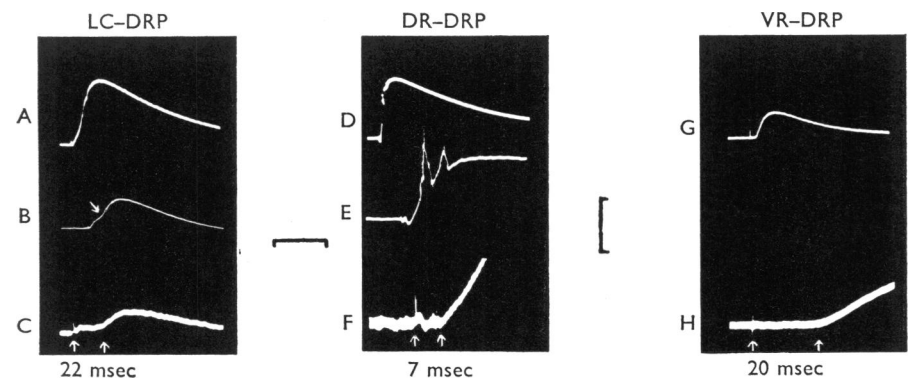

FIG. 4. Dorsal root potentials recorded from DR8 on stimulation of the lateral column (A, B and C), DR9 (D, E and F), and VR8 (G and H). Records A, D, E, F, G and H were taken from the same preparation with DC amplification. The time and voltage calibration bars are as follows: records A, D and G—200 msec and 2 mV; record B—100 msec and 2 mV; record E—40 msec and 2 mV; records C, F and H—500 μV. The arrow in record B points to the "notch" on the rising phase of the LC-DRP. Record C was produced by activation of a few lateral column fibres with a stimulating microelectrode. All records were taken at 12° C. Negativity at the proximal electrode is represented by an upward deflexion.

Although no attempt has yet been made in the frog to elucidate the physiological significance of primary afferent depolarization, the powerful depression of orthodromic responses (Fig. 6B) produced by the smallest of these potentials, the VR-DRP, suggests that they have an important influence on afferent input. The transmission from lateral column fibres to motoneurones, however, was not affected by a conditioning ventral root volley (Fig. 6D). In preparations in which the sciatic nerve and its branches remained attached to a ventral root, an antidromic volley in

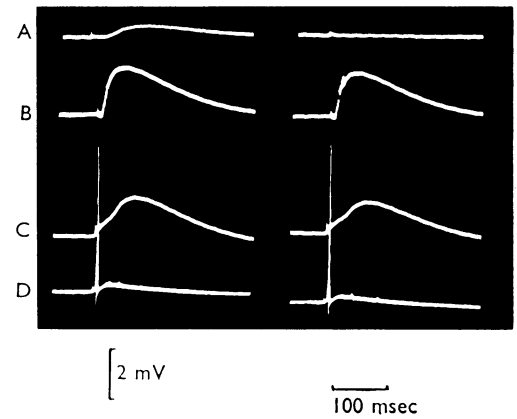

FIG. 5. Action of tubocurarine on dorsal and ventral root potentials. A: VR-DRP; B: DR-DRP; C: LC-DRP; D: LC-VRP. The left hand records are control records, while those on the right were taken 12 min after exposing the preparation to tubocurarine (10^{-4} g/ml.). RC recording.

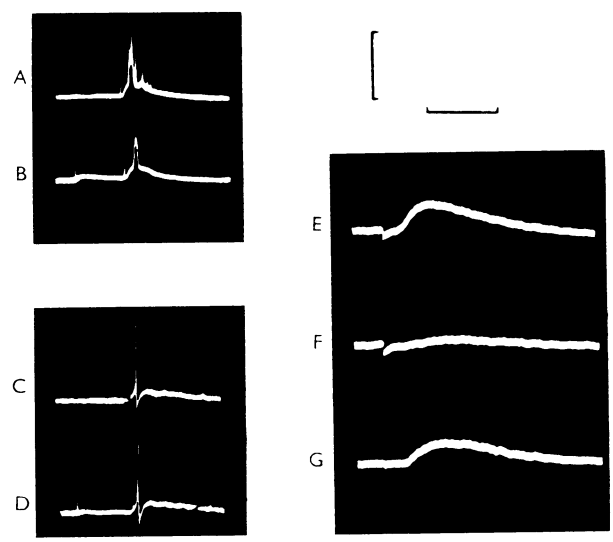

FIG. 6. Inhibition of ventral root responses and origin of VR-DRP. A and C: the ventral root responses to a dorsal root and lateral column volley respectively. B and D: the same responses 65 msec after a conditioning volley in an adjacent ventral root. E, F and G: another preparation in which the DRP was recorded from root 8 on stimulating the peroneal and tibial nerves simultaneously (E), the peroneal nerve alone (F) and the tibial nerve alone (G). DR8 had been sectioned close to the dorsal root ganglion. Time calibration: 100 msec. Amplitude calibration: 2 mV for A and B, 5 mV for C and D, 500 μV for E, F and G. RC recording.

the extensor branch (tibial nerve) produced a dorsal root potential (Fig. 6G) which was many times larger than that (F) produced by a volley in the flexor branch (peroneal nerve). The combined response (E) was little greater than that to an extensor nerve volley alone. The physiological significance of this observation is uncertain.

Action of anaesthetic agents on ventral root responses

Initial observations on the DR-VRP alone, drew attention to features distinguishing the depression produced by volatile agents from that produced by barbiturate compounds. With chloroform the loss of reflex discharge was always a generalised one, involving both early and late spike discharge. Sometimes the early discharge was more sensitive than the later polysynaptic spikes, as in Fig. 7G. In contrast, thiopentone rapidly abolished the late discharge while potentiating the earliest

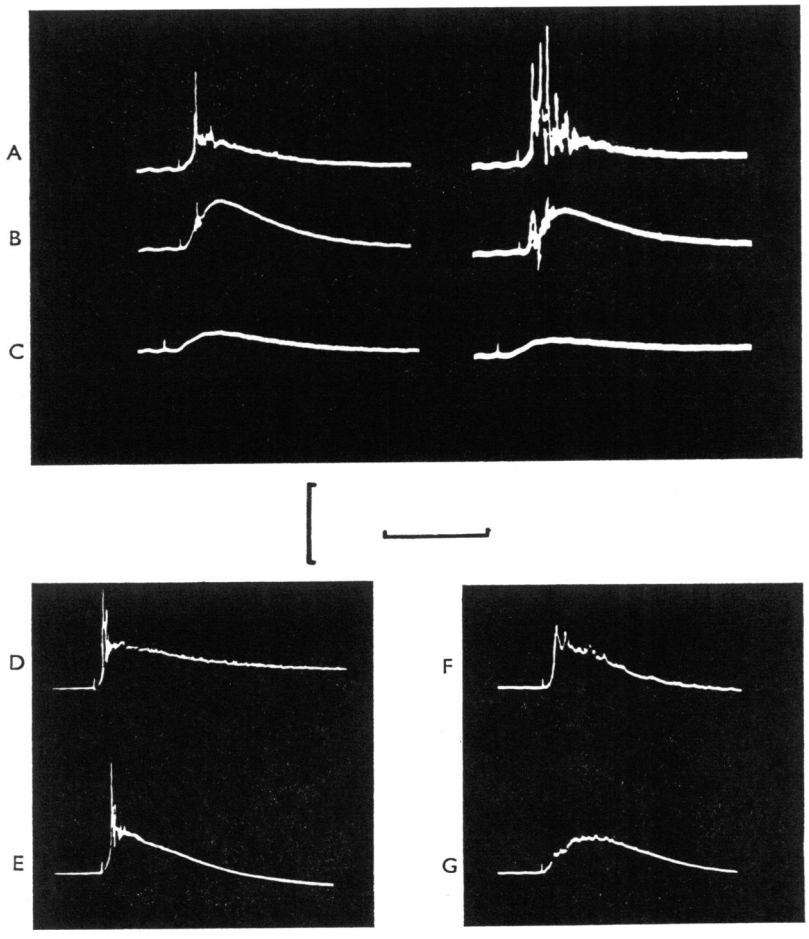

FIG. 7. Action of anaesthetic agents on the DR-VRP. Upper block: A: DR-VRP, B: DR-DRP, C: VR-DRP. Control records are shown on the left, while those on the right were taken after a 40 min exposure to trichloroethylene 0·5 mg/ml. Note the excitatory action. Lower blocks: D and F: control records from two preparations. E and G: the same potentials after a 20 min exposure to thiopentone 100 μg/ml. (E) and after a 7 min exposure to chloroform 1 mg/ml. (G). Time calibration: 100 msec for A, B and C; 150 msec for D, E, F and G. Amplitude calibration: 1 mV for C; 2 mV for the remaining records. RC recording (time constant 0·5 sec in D and E, 0·1 sec in other records).

spikes (E). The suppression of polysynaptic discharge was accompanied by a loss of the later components of the synaptic potential, suggesting that the failure of discharge resulted from a loss of synaptic bombardment of the motoneurones through multisynaptic chains, or from a curtailment of the after-discharge of interneurones. With chloroform the spike discharge was often lost without much diminution in the synaptic potential, suggesting a failure of the spike-generating mechanism of the motoneurone. These observations prompted a more detailed study of several volatile agents and another barbiturate compound to determine whether this differing pattern of activity held for other related compounds. The actions of diethyl ether, ethyl chloride, halothane, methoxyflurane, and trichloroethylene were found to be indistinguishable from chloroform except in the degree to which they produced initial excitation in low concentration. This was greatest with trichloroethylene, which sometimes failed to depress the reflexes even after prolonged exposure (see Fig. 7A). The other barbiturate compound used was methohexitone, and this was found to have an identical action to thiopentone. As it is a more soluble compound than thiopentone it was chosen for all subsequent experiments.

Actions on monosynaptic and polysynaptic responses

Studies of the action of anaesthetic agents on the DR-VRP suggested that the responses evoked by lateral column volleys should be highly sensitive to volatile agents, but resistant to, or even potentiated by, barbiturate compounds. To test this hypothesis both types of response were recorded in the same preparation during exposure to anaesthetic agents. Figure 8 illustrates the contrasting actions of thiopentone and halothane on these responses. Halothane (1 mg/ml.) depressed the LC-VRP and DR-VRP to an equal extent (Fig. 8C and D). Initially the discharge of motoneurones was suppressed, followed by a progresive reduction in the amplitude, but not the time course, of the synaptic potential. This type of activity was found with other volatile agents tested: chloroform (0·5 mg/ml.), ether (3 mg/ml.), ethyl chloride (1·5 mg/ml.) and methoxyflurane (0·75 mg/ml.). In addition, ethyl alcohol (4 mg/ml.) produced a weak depression which resembled that of volatile agents. When a preparation was exposed to methohexitone 100 μg/ml. the early spike discharge of the DR-VRP was initially potentiated (Fig. 8B), whereas the later synaptic potential and discharge were quickly abolished (see also Fig. 12E). Suppression of motoneurone discharge by methohexitone was always accompanied by depression of the synaptic potential, in contrast to volatile agents, which abolished the discharge before depressing the synaptic potential to any extent. The LC-evoked spike (Fig. 8A) showed marked potentiation, which persisted until the DR-VRP was profoundly depressed. If exposure to methohexitone was continued to a stage at which the DR-VRP was abolished, the LC-evoked spike dropped below the control value and eventually became considerably depressed (see Fig. 11). This, however, always signified irreversible depression of the preparation despite several hours' washing in Ringer solution. This is in agreement with the findings of Brookhart & Fadiga (1960), who used pentobarbitone. Usually it was possible to reduce the DR-VRP to a small synaptic potential of short latency and exponential time course before obvious depression of the LC-evoked spike had occurred (see Fig. 12G). This synaptic potential was the electrotonic manifestation of a monosynaptic EPSP which had been revealed by removal of polysynaptic bombardment of the motoneurones (Eccles, 1946) and was produced by the synaptic activity of primary

afferent fibres on dendritic terminals in the dorsal horn (Brookhart & Fadiga, 1960; Fadiga & Brookhart, 1962).

During recovery from the actions of volatile agents (or sometimes during incomplete recovery from excessive concentrations of methohexitone) the synaptic potential often returned to, or became even larger than, the control value before any spike discharge reappeared, suggesting that the mechanism responsible for spike generation was impaired by the drug and recovered more slowly than that responsible for the production of the synaptic potential.

The recent introduction of an entirely new class of intravenous anaesthetic agent prompted examination of the action of the eugenol derivative propanidid (Epontol, Bayer) on the ventral root responses of the spinal cord. Figure 9 illustrates the

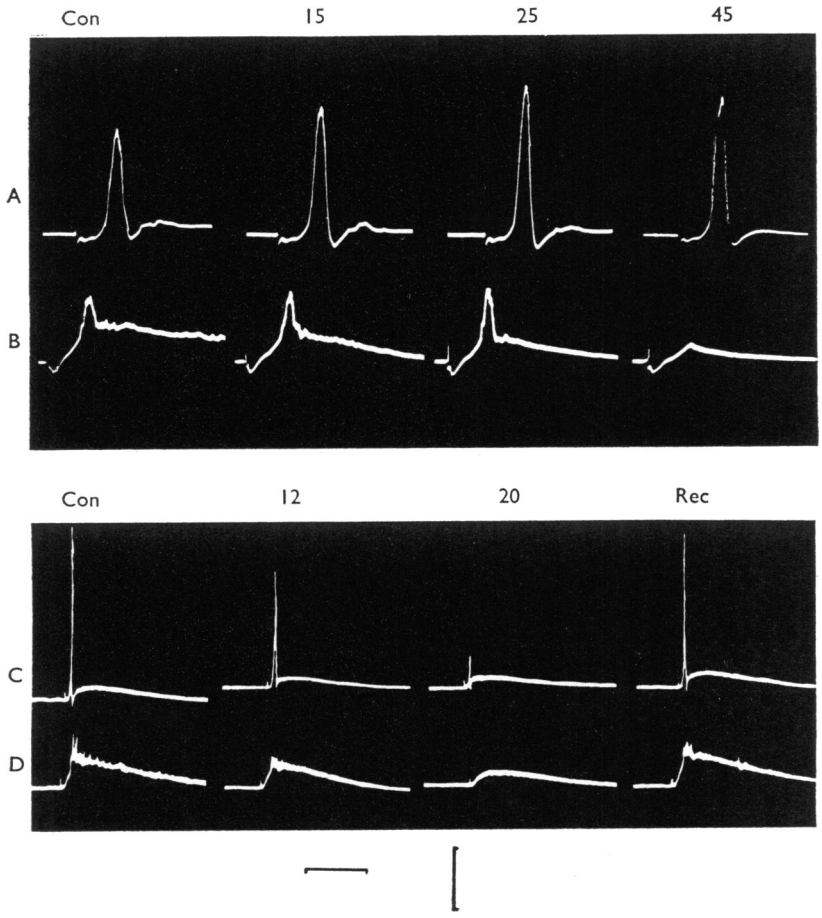

FIG. 8. Action of methohexitone and halothane on ventral root responses. Upper block of records: A: LC-VRP; B: DR-VRP. Following the control records (Con) the preparation was exposed to methohexitone 100 μg/ml. and further records taken at 15 min, 25 min and 45 min. Lower block of records: C: LC-VRP; D: DR-VRP. Another preparation, which was exposed to halothane 1 mg/ml. and further records taken at 12 and 20 min. One hour after washing, the recovery of the preparation was almost complete (Rec). RC recording. Time calibration: 10 msec for A, 20 msec for B, 100 msec for C and D. Amplitude calibration: 1 mV for B and D, 2 mV for A and C.

responses during exposure to propanidid 350 μg/ml. The early depression of polysynaptic reflex activity (C) and resistance of the LC-evoked response (D) indicated an action resembling barbiturate compounds rather than volatile agents.

Actions on firing threshold

The inflexion between the synaptic potential and spike on records of the LC-VRP have been used as a measure of the firing threshold of the motoneurone pool. Measurements from these records are much more reliable (see **Discussion**) than those made from the DR-VRP by Bonnet & Bremer (1952), and similar measurements have been successfully used by Sömjen & Gill (1963) from monosynaptic responses in the cat. Figure 10 shows the effect of ethyl chloride on the threshold and amplitude of the spike. A decrease in amplitude (B) was accompanied by a progressive rise in threshold (A); the relationship between these two changes has been plotted in the graph illustrated in Fig. 10D. The increase in threshold remained fairly small as long as the spike was only moderately depressed, but during severer depression the elevation became progressively greater. The semi-logarithmic plot (Fig. 10E) shows a straight line relationship between the two, suggesting that a given increment of threshold corresponds to the dropping out of a constant fraction of the motor pool. This confirms the findings of Sömjen & Gill (1963) using ether in the cat. Further analysis of these records (Fig. 10C) shows, in addition, a small reduction in the rate of rise of the synaptic potential. Although this pattern was seen in several experiments, the relative changes in firing threshold and slope of the synaptic potential varied from one preparation to another. Table 1 summarizes the results of five experiments with volatile agents.

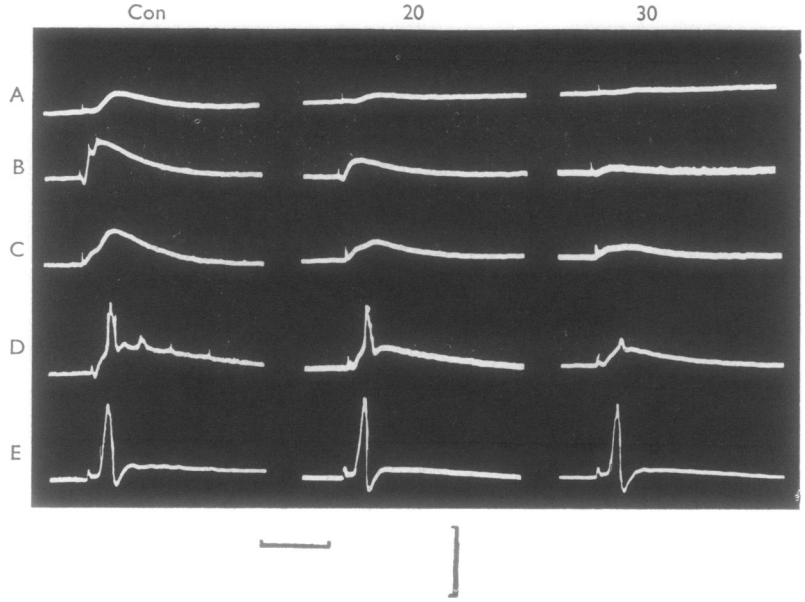

FIG. 9. Action of propanidid on dorsal and ventral root potentials. A: VR-DRP; B: DR-DRP; C: LC-DRP; D: DR-VRP; E: LC-VRP. After taking the control records (Con) the preparation was exposed to propanidid 350 μg/ml. and further records taken at 20 min and 30 min. Amplitude calibration: 2 mV for A, B, C and D; 4 mV for E. Time calibration: 100 msec for A, B and C; 50 msec for D; 20 msec for E. RC recording.

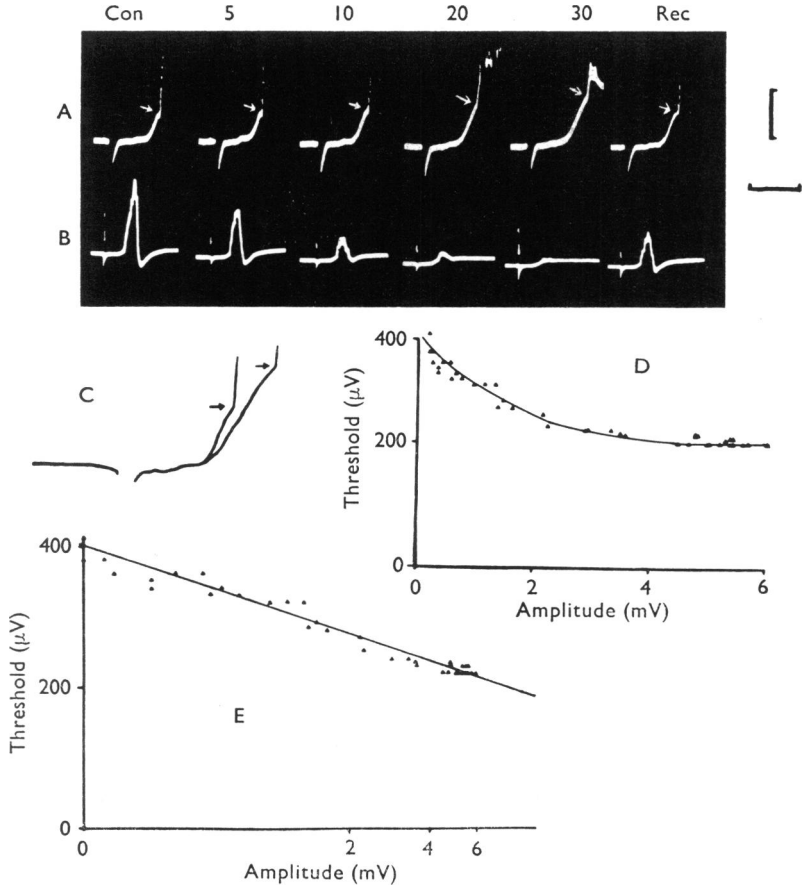

FIG. 10. Action of ethyl chloride on firing threshold of the motoneurone pool. Records of the synaptic potential and spike discharge evoked by LC stimulation are shown at high gain (A) and low gain (B). Following the control records (Con) the preparation was exposed to ethyl chloride 1 mg/ml., further records being taken at 5, 10, 20 and 30 min. The recovery records (Rec) were taken 45 min after washing in Ringer solution. Calibrations: 500 μV and 5 msec for A; 5 mV and 10 msec for B. RC recording. In C, tracings have been made of the control and 30 min records to show the increase in threshold (arrow) and decrease in slope of the synaptic potential. Graph D has been plotted to show the relationship between threshold and amplitude of the responses in A and B, while graph E shows the same results plotted logarithmically.

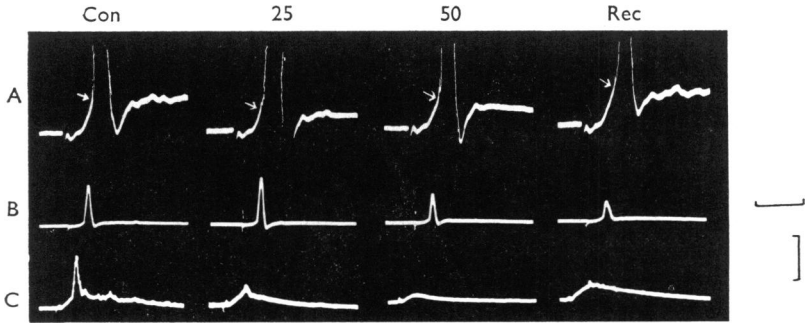

FIG. 11. Action of methohexitone on firing threshold. A: LC-VRP at high gain; B: LC-VRP at low gain; C: DR-VRP. The preparation was exposed to methohexitone 100 μg/ml. following the control records (Con), and further records were taken at 25 and 50 min. The exposure to methohexitone was prolonged, and despite washing the preparation for 2 hr in normal Ringer solution the spike discharge was irreversibly depressed (Rec). This depression was accompanied by an increase in firing threshold above the control level. Calibrations: 500 μV and 10 msec for A; 4 mV and 20 msec for B; 1 mV and 50 msec for C. RC recording.

Because the LC-VRP was not depressed by an exposure to methohexitone that did not produce irreversible effects in the preparation, no alteration in threshold was expected. Four experiments with methohexitone, however, showed that potentiation of the spike discharge was accompanied by a *decrease* in threshold (Fig. 11).

TABLE 1. *Actions on threshold and slope of synaptic potential.*

Experiment Number	Anaesthetic agent	Control			Anaesthetized		
		A	B	C	A	B	C
1	Ethyl chloride	230	0·23	7·7	480	0·16	0·3
2	Ethyl chloride	230	0·17	5·5	270	0·13	1·5
3	Chloroform	210	0·11	2·9	200	0·08	0·2
4	Chloroform	190	0·22	10·2	300	0·22	4·0
5	Halothane	200	0·14	7·5	240	0·11	0·9
6	Methohexitone	620	0·47	9 3	520	0·47	17·3
7	Methohexitone	400	0·31	4·5	330	0·31	6·7
8	Methohexitone	600	0·49	10·7	430	0·49	11·3
9	Methohexitone	270	0·12	4·0	170	0·12	5·4

The wide range of values for threshold and slope of synaptic potential is the result of differences in electrode positioning. The closer the electrodes were to the emergence of the root from the spinal cord, and the greater the inter-electrode distance, the larger was the synaptic potential in relation to the height of the monosynaptic spike. A, Threshold of monosynaptic spike in μV. B, Slope of synaptic potential in mV/msec. C, Amplitude of monosynaptic spike in mV. The anaesthetic agents were used in the following concentrations: ethyl chloride 1·5 mg/ml., chloroform 0·5 mg/ml., halothane 1·0 mg/ml., methohexitone 100 μg/ml.

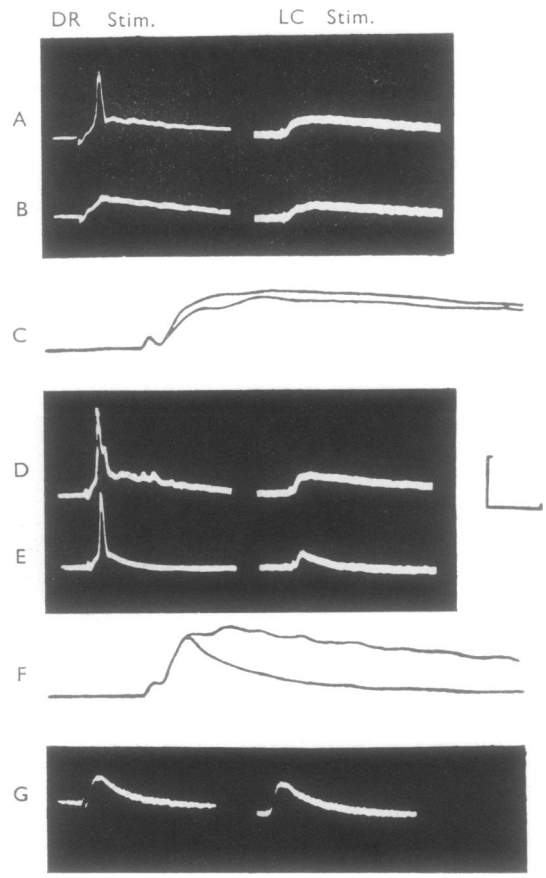

FIG. 12. Action of chloroform and methohexitone on pure synaptic potentials. A: control response to supramaximal dorsal root stimulation (left) and to a small lateral column volley subthreshold for motoneurone discharge (right). B: the same responses after 10 min exposure to chloroform 0·5 mg/ml. C: superimposed tracings of the LC responses in A and B. D and E: recordings from another preparation, but arranged in the same manner as A and B. The responses in E were taken after 18 min exposure to methohexitone 100 μg/ml. F: superimposed tracings of the LC responses in D and E. G: ventral root responses to supramaximal dorsal root stimulation (left) and to a lateral column volley the size of which was adjusted to produce a synaptic potential comparable in size to the dorsal root-evoked response. The records were taken after 40 min exposure to methohexitone. Amplitude calibration: A, B, D and E: 1 mV for DR responses, 500 μV for LC responses. G: 200 μV. Time calibration: 40 msec. DC recording.

No alteration in the slope of the synaptic potential could be detected in these experiments, the results of which are summarized in Table 1. If exposure was prolonged sufficiently to cause irreversible depression, the threshold began to rise and the slope of the synaptic potential lessened, resembling the changes produced by volatile agents.

Actions on a pure synaptic potential

In order to assess the sensitivity of the synaptic potential to anaesthetic agents, subthreshold synaptic potentials were produced by small volleys in the lateral column fibres. In theory, an anaesthetic agent which acts either on the subsynaptic membrane of the motoneurone or on the terminals of the lateral column fibres should depress this potential. Figure 12 illustrates records from two typical experiments, one with chloroform (A, B and C) and the other with methohexitone (D, E and F). It was expected that the subthreshold potential would be purely monosynaptic, but the radical change in its contour produced by methohexitone proved that this was not the case. Although the slope and amplitude of the initial component of the response was not changed by methohexitone, the duration was severely curtailed and the decay became exponential (E and F). Thus the resulting potential was a pure monosynaptic response which had been revealed by suppression of activity in polysynaptic chains. Its amplitude was maintained as long as the spike discharge produced by a large lateral column volley remained unimpaired, but

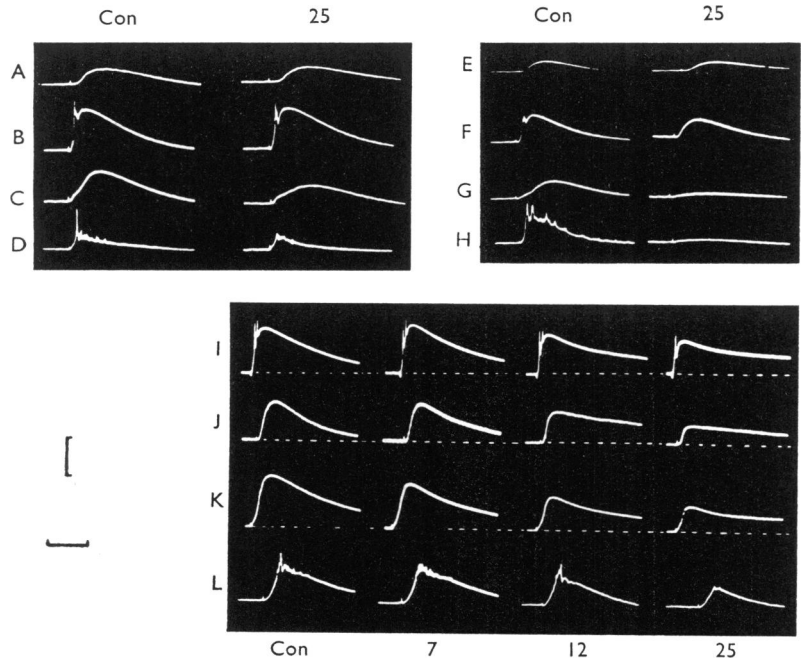

FIG. 13. Action of anaesthetic agents on dorsal root potentials. A, E and J: VR-DRP. B, F and I: DR-DRP. C, G and K: LC-DRP. D, H and L: DR-VRP. Three different preparations. A, B, C and D: records taken before (Con) and after 25 min exposure to diethyl ether 3 mg/ml. E, F, G and H: records taken before and after a 20 min exposure to chloroform 1 mg/ml. (a high concentration). I, J, K and L: records taken before (Con) and after a 7, 12 and 25 min exposure to methohexitone 100 μg/ml. Amplitude calibration: 1 mV for J; 2 mV for all other records. Time calibration: 40 msec for L, 100 msec for all other records. DC recording in I, J and K.

attenuation of the spike, signifying irreversible depression of the preparation, was accompanied by a progressive fall in the amplitude of the pure synaptic potential. In most preparations it was possible to produce responses of this nature (Fig. 12G) to dorsal root as well as to lateral column stimulation (Brookhart & Fadiga, 1960).

During exposure to chloroform the synaptic potential was affected in a very different manner. During early depression the potential was little altered although motoneurone discharge to a dorsal root volley diminished, resulting presumably from an elevation of firing threshold. As depression deepened, however, the amplitude of the synaptic potential gradually decreased, but its time course remained unaltered (Fig. 12B and C). Several attempts were made to obtain a pure monosynaptic response to dorsal root volleys, as with methohexitone, but these failed. The monosynaptic component disappeared into the noise level long before polysynaptic bombardment was suppressed. This drug was therefore affecting both monosynaptic and polysynaptic components indiscriminately, an action which can be adequately explained only by postulating a stabilizing action on the motoneurone membrane.

Actions on dorsal root potentials

Schmidt (1963) has studied the action of several anaesthetic agents on the dorsal root potentials of the toad. In the present experiments the effects of some additional drugs have been studied: chloroform, halothane, ethyl chloride, methoxyflurane, trichloroethylene, methohexitone and propanidid. These experiments confirm, in general, those of Schmidt. The dorsal root potentials evoked by a volley in an adjacent root (DR-DRP) or in a ventral root (VR-DRP) were usually little affected by concentrations of volatile agents which severely depressed the ventral root responses (Fig. 13A, B, E and F). The dorsal root potential evoked by a lateral column volley (LC-DRP) was, however, usually depressed much more readily than the other dorsal root potentials (C and G). The amplitude of the three dorsal root potentials was often transiently increased during the initial excitatory phase produced by many volatile agents, but thereafter the amplitude decreased and the time to half-decay shortened.

An exposure to methohexitone which blocked the ventral root spike discharge always produced a considerable reduction in the amplitude of the dorsal root potentials (Fig. 13I, J and K). For a given degree of depression of ventral root discharge, the dorsal root potentials were more sensitive to methohexitone than to volatile agents. The VR-DRP and LC-DRP were slightly more sensitive to this barbiturate compound than was the DR-DRP. Reduction in the amplitude of these potentials was accompanied by a lengthening of the half-decay times (25 min records), as described by Schmidt (1963). Recovery from the effects of this drug was usually delayed for several hours. Although the action of propanidid on the ventral root potentials resembled that of methohexitone, the dorsal root potentials were highly sensitive to propanidid (Fig. 9A, B and C) and did not show the lengthening of decay time which characterizes the action of barbiturate compounds.

Discussion

The results of the initial experiments on the distribution of the lateral column fibres suggested that the fibres which activate lumbar motoneurones arise no higher

than the brachial intumescence, and descend mainly on the ipsilateral side to activate chiefly the flexor motoneurones. In support of this suggestion the histological sections produced by C. N. Liu & W. W. Chambers (personal communication, 1968) show an absence of degeneration in the lumbar region following a hemisection at the level of the first segment, but show profuse degeneration (mainly on the ipsilateral side) following a hemisection at the fifth spinal segment. The fibre tract in the lateral column which has been used in the experiments reported in this paper probably corresponds with the reticulo-bulbo-spinal tract of Abbie & Adey (1950). Although Brookhart *et al.* (1959) consider the short latency discharge on lateral column stimulation to be monosynaptic, no direct connexion between fibres in this column and the motoneurones of the lumbar region was demonstrated by Abbie & Adey (1950).

In theory, a drug depressing spinal reflexes by acting solely on the motoneurone membrane would be expected to increase the firing threshold of the motoneurone by an action on the initial segment, to depress synaptic potentials by stabilizing the subsynaptic membrane, and as a result of these two effects, to suppress all reflex discharge whether produced monosynaptically or polysynaptically. All the volatile agents used satisfied these criteria. The ventral root responses to lateral column and dorsal root stimulation were equally affected, and the contour of the synaptic potentials on which the spike discharges were superimposed was little altered despite a reduction in amplitude. It was never possible to abolish polysynaptic components of the slow wave without also abolishing the monosynaptic. This supports the observation of Austin & Pask (1952) that ether depressed polysynaptic reflexes in the cat no more readily than the monosynaptic spike produced by muscle afferent stimulation, indeed they were often more resistant. If volatile agents were acting presynaptically by reducing transmitter output or by lowering the safety factor for conduction in fine terminals, their depressant effect would involve multisynaptic chains to a much greater extent than two neurone arcs (Barany, 1947). This was never the case.

A reduction in the rate of rise of the synaptic potential of the motoneurones was evident in most preparations during the early stages of depression, and together with the elevation of threshold was responsible for the failure of discharge. This confirms the results of Sömjen & Gill (1963) using extracellular and intracellular recording techniques in the cat and rat. Although a decrease in the slope of the synaptic potential could result from a presynaptic effect, taken in conjunction with other evidence, it is probably the result of stabilization of the subsynaptic membrane to the depolarizing action of the excitatory transmitter. As pointed out by Sömjen & Gill (1963), caution must be used in the interpretation of measurements of firing threshold from ventral root recordings, for the amplitude of the synaptic potential is determined by the sum of all the electrotonically conducted activity in each motoneurone whose axon is contained in the ventral root, regardless of its synaptic delay and whether or not it fires in response to the afferent volley. The initial upstroke of the reflex spike, however, is produced by the first impulse to arrive at the site of the recording electrode, and is not an average for the motoneurone pool. This argument applies even more strongly to measurements made from ventral root responses produced by dorsal root stimulation, for here the first impulse to emerge is a multisynaptic one, and is preceded by a slower development of the synaptic potential. This may account for the failure of Bonnet & Bremer (1952) to demonstrate a rise in threshold with non-barbiturate anaesthetic agents. Bearing these

limitations in mind, the consistent elevation of threshold that has been demonstrated in the present experiments with several volatile agents confirms the findings of Sömjen & Gill (1963) and almost certainly reflects a genuine rise in the critical depolarization required to trigger individual motoneurones. As the trigger zone of the motoneurone is considered to be the initial segment of the axon (Eccles, 1957) the elevation of threshold must be produced by an action of the drug at this part of the membrane.

The results with thiopentone and methohexitone indicate that these compounds act on spinal reflex mechanisms in a manner quite different from volatile agents. Polysynaptic elements of the ventral root response were highly sensitive to the barbiturate compounds, whereas short-latency spikes were depressed only by prolonged exposure. This finding confirms the results of Eccles (1946) and Brookhart & Fadiga (1960) in the isolated cord, and of Bonnet & Bremer (1952) in the spinal frog. Bonnet & Bremer found no alteration in threshold (measured from responses evoked by dorsal root stimulation) and concluded that depression was the result of a decrease in the amplitude of the synaptic potential. As this was polysynaptic in nature this effect can be explained by an action of the drug on interneuronal synapses. Such an action was suggested by their finding of an exaggerated "post-reaction subnormality" on double stimulation of a dorsal root, which they ascribed to an elective action on after-discharge mechanism of the neurones.

Apart from a brief mention by Bonnet & Bremer (1952) of an increased amplitude of the initial spikes of the reflex discharge during barbiturate action, there appear to be no reports of potentiation of motoneurone discharge in the frog with these compounds. This, however, was a consistent finding on activation of the motoneurones by lateral column volleys, and was accompanied by a reduction in firing threshold. It is possible that this potentiated response was the result of a greater degree of synchronization of the impulses in the ventral root, although this would not explain the observed decrease in firing threshold. The latency of the spike was not altered by barbiturate compounds. Studies of cat spinal reflexes (Eccles, 1946 ; Sömjen & Gill, 1963 ; Løyning, Oshima & Yokota, 1964) have shown only a reduction in monosynaptic discharge by barbiturate compounds, except when the body temperature of the experimental animal was lowered. At 34° C thiamylal in small doses produced enhancement of the monosynaptic reflex in the cat, instead of depression (Løyning *et al.*, 1964). Temperature effects, therefore, may account for the invariable potentiation by barbiturates of the lateral column response in the present experiments, in which the frog cord was kept at 10°–12° C. A species difference, however, may also be important, for frog motoneurones are known to differ slightly in their potassium permeability from cat motoneurones (Araki & Otani, 1955). If a barbiturate compound selectively depressed potassium permeability, the motoneurone membrane would become more, rather than less, excitable to synaptic bombardment. There is evidence that barbiturates may act in this way on muscle fibres (Thesleff, 1956). An alternative explanation is that background inhibitory bombardment of the motoneurones might be suppressed by drug action, resulting in a decrease in their resting membrane potential and an increase in their excitability. Intracellular studies, however, have revealed little evidence of such a bombardment (Katz & Miledi, 1963). Finally, an anti-enzyme effect at the synapses of the lateral column fibres could produce a potentiated response, and Eccles, Schmidt & Willis (1963) have postulated an action of this type

to account for the prolongation of the dorsal root potentials by barbiturate compounds.

Løyning *et al.* (1964) have produced convincing evidence that the short-acting barbiturate, thiamylal, produces depression of monosynaptic reflexes in the cat by an action on presynaptic terminals, having little effect on the responses of the motoneurone membrane in small doses. Earlier studies (Eccles, 1946; Sömjen & Gill, 1963) had shown an elevation of threshold and reduction in the slope of the synaptic potential, but this postsynaptic effect is probably produced only by larger doses of anaesthetic agent. A presynaptic effect of barbiturates would adequately explain the sensitivity of polysynaptic chains to these compounds (Barany, 1947).

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