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Microelectrode studies in the frog isolated spinal cord during depression by general anaesthetic agents

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1. Extracellular and intracellular potentials have been recorded from the isolated spinal cord of the frog during depression of synaptic transmission by volatile and barbiturate general anaesthetic agents.

2. Volatile agents did not impair conduction in presynaptic terminals in concentrations which completely blocked synaptic transmission.

3. Methohexitone consistently impaired conduction in presynaptic terminals long before transmission through polysynaptic pathways was blocked.

4. Volatile agents depressed the excitability of the motoneurone membrane, as evidenced by impaired antidromic invasion, reduced excitability to direct stimulation, depression of the synaptic potential and elevation of firing threshold. It is concluded that these actions are responsible for the depressant effect of volatile agents on spinal reflexes.

5. Methohexitone produced an increase in the excitability of the motoneurone membrane, as evidenced by enhanced antidromic invasion, increased excitability to direct stimulation and potentiation of short latency responses. Despite this excitatory action, the polysynaptic pathways through the cord were depressed by an action of the drug on conduction in presynaptic terminals.

6. It is suggested that the sensitivity of the motoneurone membrane to volatile agents may contribute to the good muscle relaxant properties of these drugs in clinical use.

Early observations of focal activity in the cat spinal cord using steel microelectrodes (Brooks & Eccles, 1947) suggested that the failure of reflex discharge during barbiturate anaesthesia was wholly a postsynaptic effect, and was largely the result of a progressive elevation of the firing threshold of the motoneurones. More recently, Sömjen & Gill (1963) have examined the responses of cat and rat motoneurones by intracellular recording during administration of ether or thiopentone, and concluded that depression of the synaptic potential was as important a cause of failure of discharge as was elevation of the firing threshold. They found that the actions of these two classes of compound were indistinguishable in these preparations. Although

the focal recording techniques of Brooks & Eccles (1947), Austin & Pask (1952) and Sömjen (1963) have not demonstrated any impairment of conduction in the terminals of primary afferent fibres during volatile or barbiturate anaesthesia, the more detailed investigations of Løyning, Oshima & Yokota (1964) revealed a significant impairment of conduction with small doses of thiamylal in the cat. This produced a reduction in the amplitude of the monosynaptic reflex in the absence of any direct effect upon the excitability of the motoneurone membrane.

Investigations of the action of anaesthetic agents on the ventral root responses of the frog isolated spinal cord (Richens, 1969) suggested that volatile agents act largely on the motoneurone membrane, whereas barbiturate compounds act mainly on the components of multisynaptic reflex pathways. This suggestion has been re-examined by recording focal extracellular activity and intracellular responses with glass microelectrodes during the administration of anaesthetic agents. This has enabled a more precise location of the site of action of these agents to be made (for a preliminary account see Richens, 1968).

Methods

The frogs used in these experiments were generally *Rana temporaria*, but occasionally *R. esculenta* or *R. pipiens* were used. A detailed description of the dissection and arrangement of the isolated cord and of the root electrodes are given in an earlier paper (Richens, 1969). Suction pipette electrodes were preferred for root recording and stimulation.

Microelectrodes were drawn from Pyrex glass on a Palmer horizontal electrode puller and filled with electrolyte solution by boiling under reduced pressure. Elec-

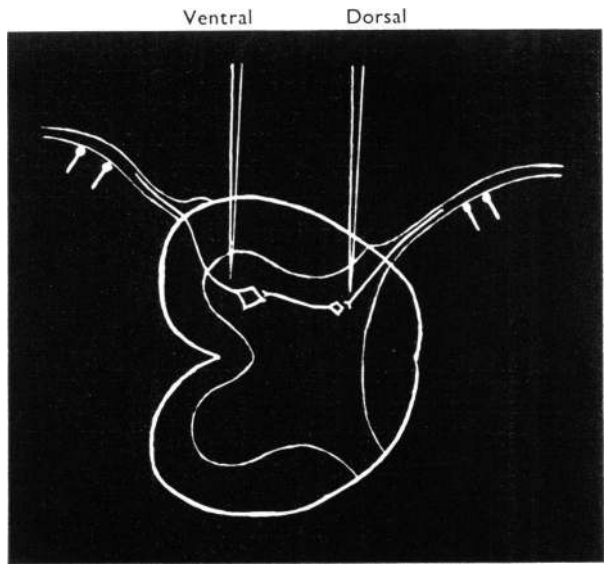

FIG. 1. Schematic diagram of the isolated spinal cord and the arrangement for microelectrode and root recording.

trodes filled with 4 M-NaCl and having a resistance of 1–2 MΩ were selected for recording focal potentials and for stimulating motoneurones directly, whereas 15–20 MΩ electrodes filled with 3 M-KCl were preferred for intracellular recording. The electrodes were held in a Palmer micromanipulator and inserted vertically into the lateral surface of the cord, as illustrated in Fig. 1. Connection to a cathode-follower was made with a chlorided silver wire or miniature silver/silver chloride/agar electrode, and the recording bath was earthed through a similar electrode. Microelectrode resistances were measured by introducing a rectangular pulse between the recording bath and earth while operating a microswitch which introduced a calibrating resistor across the cathode-follower input. Root responses and microelectrode potentials were displayed on two Solatron CD1183 oscilloscopes.

Recordings were made from the seventh to ninth segments of the spinal cord, as this provided roots of ample length for stimulating and recording, and allowed a concentric electrode to be applied to the lateral surface of the cord at the level of the fifth segment for stimulating the lateral columns. Before insertion of microelectrodes, the pia covering a small area of the lateral surface was carefully stripped off with a pair of fine watchmaker's forceps. Motoneurones were located by monitoring the focal potential produced by antidromic activation of the motoneurone pool. An abrupt drop in the potential with the appearance of a typical antidromic spike indicated penetration of a motoneurone. Only a small minority of cells were held for long enough to allow continuous observation of responses during the administration of an anaesthetic agent. In one case a cell was held for 75 min and it was possible to follow the depression and failure of the cell during anaesthesia, and its subsequent recovery. Focal potential recordings were made either from the dorsal horn, where primary afferent fibres synapse with internuncials or motoneurone dendrites, or from the ventral horn, where lateral column fibres synapse with the cell bodies of the motoneurones (Liu & Chambers, 1957; Fadiga & Brookhart, 1960; Kubota & Brookhart, 1963).

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. In a few experiments it was necessary to block synaptic transmission with magnesium ions (Katz & Miledi, 1963). This was done by mixing one part of isotonic (84 mM) $MgCl_2$ with five parts of the standard frog Ringer solution, giving a final concentration of 14 mM $MgCl_2$. The following anaesthetic agents were administered, dissolved in oxygenated Ringer solution: halothane (I.C.I.), chloroform (B.D.H.), ethyl chloride (Bengue), and methohexitone sodium (Eli Lilly).

Results

Before attempting to insert microelectrodes into a preparation, its reflex responses to dorsal root and lateral column stimulation were checked, and any preparation giving a poor ventral root discharge was rejected. This was commonly the result of damage inflicted during dissection of the pia.

Focal potentials

A low resistance micropipette positioned extracellularly among the primary afferent terminals in the dorsal horn records a potential with two main components on dorsal

root stimulation, an initial fast negative-positive sequence signifying the arrival of impulses in the afferent terminals, and a subsequent slow negative potential produced by activity in postsynaptic structures (Fig. 2D). The fibre degeneration studies of Liu & Chambers (1957) suggested that the synaptic contacts made by primary afferent fibres at a segmental level were restricted entirely to the dorsal regions of the cord. This has received ample support from the microelectrode studies of Brookhart & Fadiga (1960) and Fadiga & Brookhart (1960), who concluded that the post-synaptic structures activated by afferent terminals were interneurones and dorsal dendritic extensions of motoneurones. The monosynaptic connexion made by these fibres is only a weak influence on the motoneurone, for the synaptic potentials generated in these dendritic extensions have to conduct decrementally down the dendrites to the soma. This connexion is rarely adequate to fire the motoneurones. Because of this anatomical distribution of the primary afferents, a presynaptic focal potential can be recorded from only a restricted part of the dorsal horn, and an electrode placed more ventrally will record only an inverted image, as the structures in this region are acting as a current source. Records of this nature are illustrated in Fig. 2, D and E. Record F shows a ventral root potential taken at the same time as the focal potentials. The spikes produced by motoneurone discharge are reflected in the ventral horn focal potential (record E). In order to verify the presynaptic and postsynaptic nature of the two components of the focal potential, an experiment

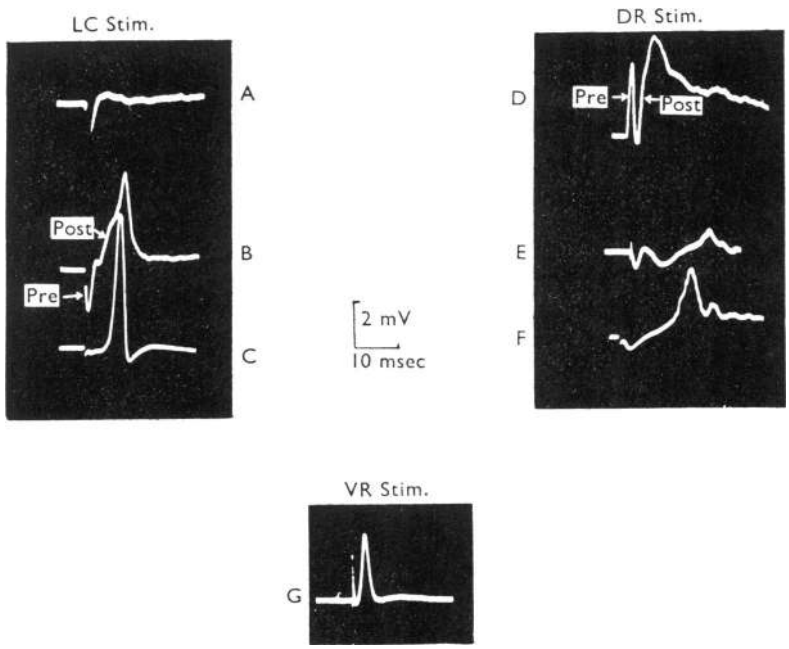

FIG. 2. Normal focal potentials. A and D: focal responses recorded with a microelectrode from the dorsal horn. B, E and G: focal responses recorded from the ventral horn. C and F: ventral root responses recorded simultaneously with the focal responses. A, B and C were recorded during stimulation of the lateral column, D, E and F during dorsal root stimulation and G during antidromic invasion of the motoneurone pool by a ventral root volley. Pre, Presynaptic component of the focal potential, Post, postsynaptic component. RC recording. Suction pipettes for root recording, and 1 MΩ NaCl pipettes for focal recording. Negativity is represented by an upward deflection.

was performed in which magnesium Ringer was substituted for the standard Ringer. Magnesium ions selectively block synaptic transmission in the spinal cord without impairing conduction of afferent impulses (Katz & Miledi, 1963) and would be expected to abolish postsynaptic components of the focal potential without altering those produced by afferent terminals. The records in Fig. 3 support this suggestion, and distinguish clearly between the two components.

The tract of descending fibres in the lateral column of the frog spinal cord is thought to activate motoneurones monosynaptically (Brookhart, Machne & Fadiga, 1959). These fibres appear to be distributed mainly to the ventral regions of the cord (Liu & Chambers, 1957 ; Brookhart & Fadiga, 1960) and synapse against the soma and proximal portions of the dendrites of the motoneurones (Kubota & Brookhart, 1963). They provide a powerful excitatory drive to the motoneurones, and evoke a synchronous ventral root discharge when stimulated (Fig. 2C). A microelectrode positioned in the ventral horn records the activity in the terminals of these fibres as well as the resultant synaptic potentials and spike discharges of the motoneurones in the vicinity (Fig. 2B). Magnesium Ringer abolishes the postsynaptic responses, leaving only the initial presynaptic component of the potential. An electrode positioned in the dorsal horn during lateral column stimulation records very little activity of any sort (Fig. 2A).

Activation of the motoneurone pool by antidromic impulses in the motor axons produces a negative focal potential in the ventral horn (Fig. 2G). This is used as a guide for manœuvering a microelectrode towards a motoneurone for intracellular recording. Its size is dependent upon the number of motoneurones whose somas are successfully invaded by the antidromic impulses, and a decrease in its size indicates failure of conduction at the initial segments of a greater proportion of the motoneurones in the pool. It has been used, therefore, to assess the action of anaesthetic agents on this part of the motoneurone membrane, while records of orthodromically-evoked focal potentials have been used to determine the effect of these drugs on presynaptic elements.

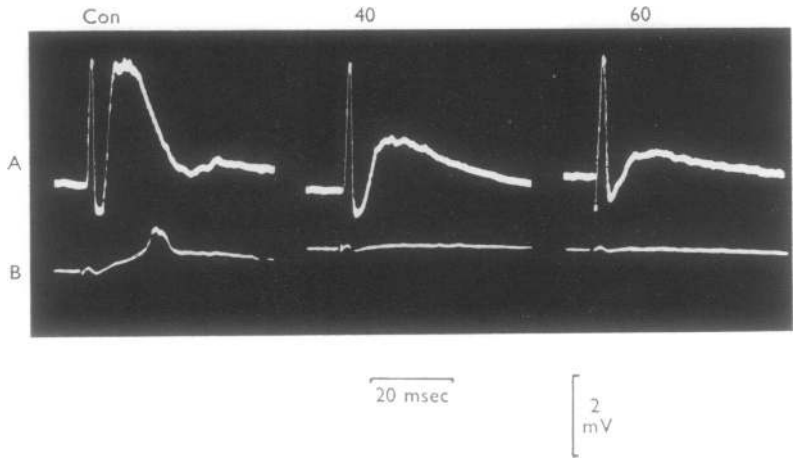

FIG. 3. Action of magnesium on the dorsal horn focal potential. A: focal response in the dorsal horn on supramaximal dorsal root stimulation, B: ventral root responses recorded simultaneously. Following the control records (Con) the bathing solution was changed to magnesium Ringer (14 mM) and further records taken at 40 and 60 min. RC recording.

Intracellular potentials

Resting membrane potentials were usually low, but occasionally values of 70–80 mV were recorded, which is in agreement with Katz & Miledi (1963). Figure 4 illustrates typical responses to volleys in the two orthodromic pathways and on antidromic activation. Lateral column volleys evoked a synaptic potential of about 4 msec latency (at 10–12° C), which suggests a monosynaptic connection, although there may be time for a single interneuronal synaptic delay. Dorsal root volleys usually evoked a small subthreshold potential of similar latency, with further build-up 8–9 msec after the stimulus from bombardment through polysynaptic pathways. The initial component produced by dorsal root volleys was the result of monosynaptic connexions between primary afferent fibres and dorsal dendritic extensions of the motoneurones (Kubota & Brookhart, 1963). A wide variation in threshold was seen from one cell to another, being from 4 mV to 22 mV. The threshold on dorsal root excitation was usually 1–2 mV higher than that on lateral column excitation, most of the difference resulting from extracellular field currents (Machne, Fadiga & Brookhart, 1959). In one case, however, the difference was unusually great (Fig. 11A).

Antidromic invasion of a motoneurone (Fig. 4) produced a spike with an inflexion on its rising phase, which represented the transition from the initial segment to the soma-dendritic component of the response. The spike was followed by a negative dip in the after-hypopolarization, sometimes amounting to a transient hyperpolariza-

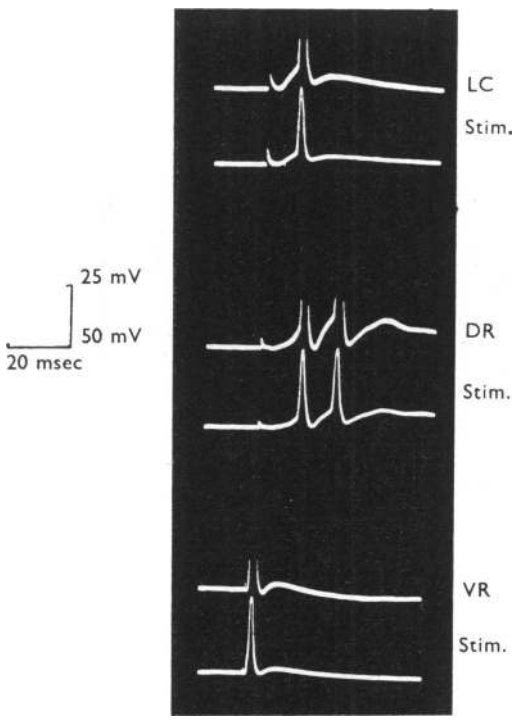

FIG. 4. Normal intracellular responses from a motoneurone recorded with a 20 MΩ KCl pipette. The responses to a lateral column volley (upper pair), a dorsal root volley (centre pair) and to antidromic activation (lower pair) are displayed at two different gains, the upper record of each pair being at twice the gain of the lower. RC recording in upper traces, DC in lower traces.

tion. This is a feature which is not seen in cat motoneurones, and may be the result of a delayed rise in the potassium permeability (Machne *et al.*, 1959). Occasionally a cell was impaled which produced only an initial segment spike on antidromic invasion, and this was a common finding in cells which were dying as a result of penetration by the electrode.

Action of anaesthetic agents on presynaptic conduction

Because of the anatomical arrangement of the frog spinal cord it has been possible to assess independently the effects of drugs on the terminals of two fibre systems: the dorsal root afferents by recording from the dorsal horn, and the lateral column terminals by recording from the ventral horn. Both types of fibre responded in a similar fashion to anaesthetic drugs. A low concentration of a volatile agent—for example chloroform 0·5 mg/ml. or ethyl chloride 1·5 mg/ml.—did not impair conduction in presynaptic terminals despite a severe depression of the reflex dis-

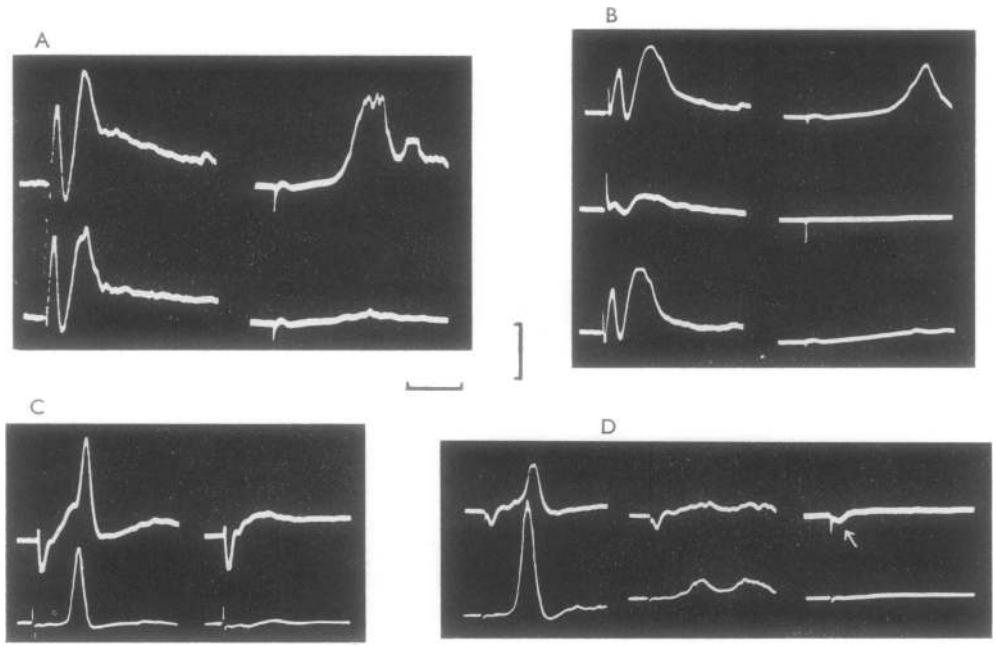

FIG. 5. Action of chloroform and ethyl chloride on presynaptic conduction. A & B: focal potentials recorded from the dorsal horn (left-hand records of each block) and ventral root potentials recorded simultaneously (right-hand records), produced by a supramaximal dorsal root stimulus. A: upper records are control responses, and lower records were taken after a 14 min exposure to chloroform 0·5 mg/ml. B: Another preparation in which the concentration of chloroform was trebled to 1·5 mg/ml. The centre records were taken after a 6 min exposure, and the lower records 90 min after washing. C and D: focal potentials recorded from the ventral horn (upper records) and ventral root potentials recorded simultaneously (lower records), produced by a lateral column volley. C: Control responses on the left. The right-hand records were taken after a 20 min exposure to ethyl chloride 1·5 mg/ml. D: Control responses on the left. Two concentrations of chloroform have been used: 0·5 mg/ml. (centre records, 14 min exposure) and 1·5 mg/ml. (right-hand records, 10 min exposure). The presynaptic response is depressed by the higher concentration (arrow). Low resistance NaCl pipettes for focal potentials. Time calibration: 10 msec. Amplitude calibration: 1 mV in A, 2 mV in B and D, 4 mV in C.

charge (Fig. 5A and C). If, however, the concentration of anaesthetic agent was approximately trebled, a reversible conduction block rapidly ensued (Fig. 5B and D). In recordings from the dorsal horn the second negative wave, representing postsynaptic activity, was depressed by volatile agents, although it was never unduly sensitive, suggesting that the activity of interneurones is not severely curtailed by these compounds (Hughes & Gasser, 1934). In the ventral horn the postsynaptic components, representing activity in motoneurones, were highly sensitive. The measurement of firing threshold of the motoneurone pool—the inflexion between the focally recorded synaptic potential and spike—did not yield consistent results, contrary to the findings of Brooks & Eccles (1947) in the cat.

The action of the barbiturate compound, methohexitone, on presynaptic conduction was always one of depression, although the degree of depression varied from one experiment to another. Figure 6 illustrates the two extremes of depression of the focal spike generated by primary afferents in the dorsal horn. In Fig. 6A the spike was reduced to 78% of the control value before the ventral root reflex discharge (B) was abolished, whereas in Fig. 6C the reduction was only to 92% of the control value despite complete abolition of the polysynaptic ventral root discharge (D). The average reduction in amplitude of the presynaptic spike for six experiments was 13% at the point at which all polysynaptic ventral root discharge had

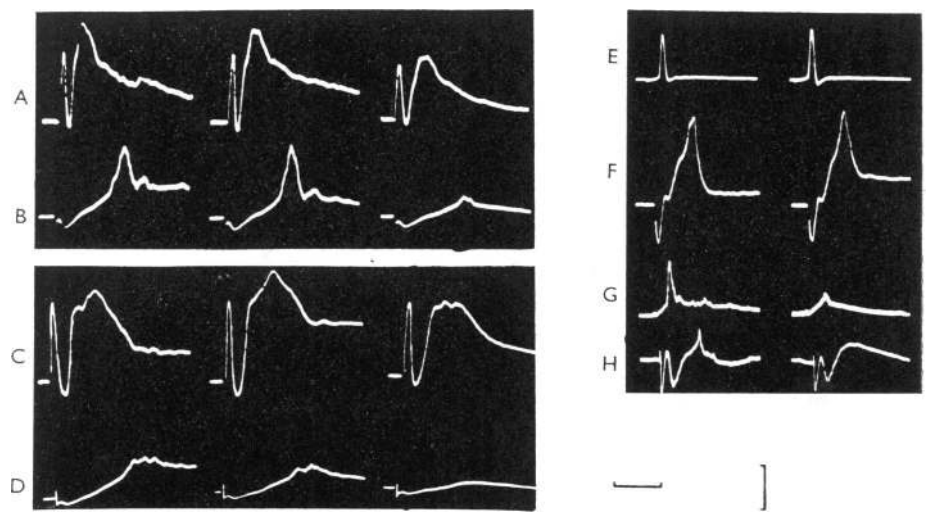

FIG. 6. Action of methohexitone on presynaptic conduction. A and C: focal responses from the dorsal horn produced by a supramaximal dorsal root volley. B and D: ventral root responses recorded simultaneously. A and B: Control responses (left) and responses recorded after a 20 min and 40 min exposure to methohexitone 100 μg/ml. C and D: Control records from another preparation, and the same responses after a 20 min and a 40 min exposure to methohexitone 100 μg/ml. E, F, G and H: Control responses (left) from another preparation and the same responses after a 25 min exposure to methohexitone 100 μg/ml. E: Ventral root response to a lateral column volley. F: focal potential from the ventral horn recorded simultaneously with E. G: Ventral root response to a dorsal root volley. H: focal potential from the ventral horn recorded simultaneously with G. Time calibration: 10 msec in A, B, C, D, F and H, 20 msec in E, and 40 msec in G. Amplitude calibration: 1 mV in B and G, 2 mV in A, C, D, F and H, 5 mV in E. DC recording in C.

been abolished. If prolonged exposure to the drug was allowed, such that the monosynaptic reflexes were irreversibly depressed, the conduction in presynaptic terminals became severely impaired. During barbiturate depression an alteration in the contour of the postsynaptic wave was often seen, its amplitude being transiently increased and its duration prolonged (Fig. 6C). The significance of this is not clear, although it could result from changes in field currents which occur during depression of the activity of interneurone pools. As depression deepened the decay of the postsynaptic response became smoothed out into an exponential time course, suggesting that only the first synapses activated by primary afferents were still in operation. At this stage slow potentials of elemental time course could be recorded from the ventral root on stimulating a dorsal root (Brookhart & Fadiga, 1960).

Conduction in the terminals of the lateral column fibres was similarly impaired

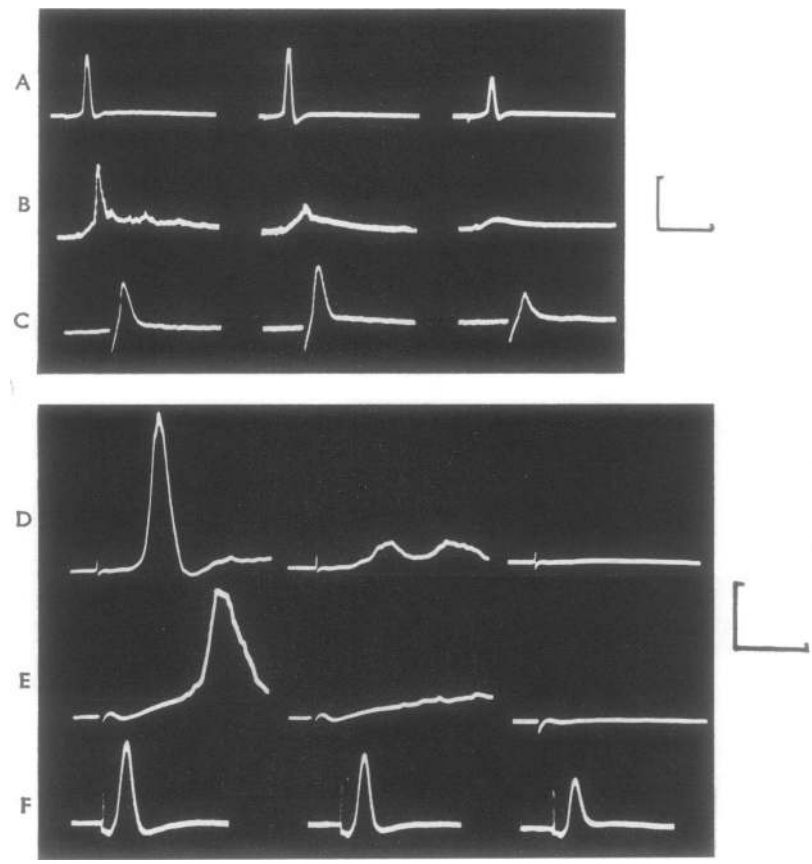

FIG. 7. Action of methohexitone and chloroform on antidromic invasion of the motoneurone pool. A and D: Ventral root responses to a lateral column volley. B and E: Ventral root response to a dorsal root volley. C and F: Focal response on antidromic invasion of the motoneurone pool. Control responses on the left. A, B and C: The preparation was exposed to methohexitone 100 μg/ml. and records taken at 25 min (centre records) and 50 min. D, E and F: Another preparation, which was exposed to two concentrations of chloroform, 0·5 mg/ml. (centre records, 14 min exposure) and 1·5 mg/ml. (right hand records, 10 min exposure). Calibration of upper block : A, 5 mV and 20 msec. B, 1 mV and 40 msec. C, 2 mV and 10 msec. Calibration of lower block: D and F, 2 mV and 10 msec. E, 1 mV and 10 msec. RC recording.

by methohexitone. In Fig. 6F a 10% reduction in the presynaptic component of the focal potential is seen, whereas the postsynaptic spike recorded either from the motoneurone pool (F) or from the ventral root (E) is potentiated. This potentiation must reflect a great increase in the excitability of the motoneurone membrane in the face of an impaired bombardment by afferent fibres. The polysynaptic discharge evoked by dorsal root stimulation (G) is depressed rapidly by methohexitone, the earliest components being the last to succumb. Often an initial potentiation of the early components is seen (Richens, 1969), resembling the behaviour of the synaptic response to lateral column stimulation. Figure 6H illustrates the inverted image of the dorsal horn response which can be recorded from the ventral horn on the arrival of a volley in a dorsal root. The presynaptic component is depressed as in record F.

Action of anaesthetic agents on motoneurone excitability

The preceding observations suggested that the site of action of barbiturate compounds on frog spinal reflexes was different from that of volatile agents. While the latter in low concentration depressed the responses of motoneurones without affecting presynaptic conduction, a small dose of methohexitone increased the excitability of the motoneurones while impairing conduction in presynaptic terminals. It was of interest to test the action of these drugs on antidromic invasion of the motoneurone pool, as the amplitude of the focal response is related to the number of motoneurones successfully invaded, and hence the excitability of their soma membranes. Chloroform, in concentrations which effectively depressed synaptic transmission, reduced the amplitude of the focal potential (Fig. 7F). In seven experiments, at the point of total synaptic block, the focal potential was reduced to 70–90% of the control level, but returned to normal on washing the preparation. Much higher

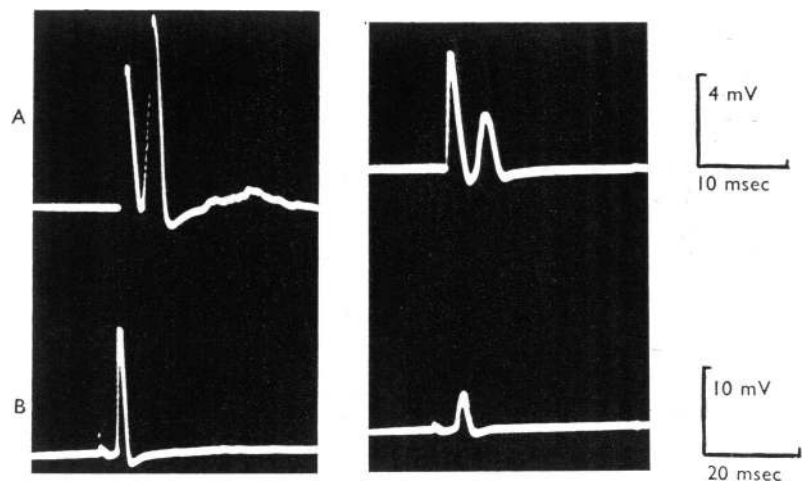

FIG. 8. Action of chloroform on the response of motoneurones to direct stimulation. A stimulating current pulse was applied through a low resistance NaCl pipette which had been inserted into the motoneurone pool. A: response to direct stimulation recorded from the ventral root. The initial spike is the direct response of the motoneurones to the current pulse, while the second spike is the result of synaptic activation from nerve terminals in the motoneurone pool. B: Ventral root response to a lateral column volley. Control responses on the left, and responses after 17 min exposure to chloroform 0·5 mg/ml. on the right. The depression of the synaptic responses (A and B) are comparable, but is accompanied by a 20% reduction in the height of the direct responses of the motoneurones.

concentrations of volatile agent (Fig. 7F, right-hand records) severely depressed the focal potential, but this may have resulted partly from a block of axonal conduction. Conversely during administration of methohexitone (Fig. 7C), the focal potential was increased in amplitude, indicating that motoneurones which had previously failed to invade were able to do so under the influence of the barbiturate compound. This increase coincided with the potentiation of the synaptic response to lateral column stimulation (A) and with suppression of the polysynaptic discharge to dorsal root stimulation (B). When the monosynaptic response became depressed by prolonged exposure to methohexitone (Fig. 7, 50 min records) the focal potential was likewise reduced in amplitude. This pattern was observed in each of five experiments.

An alternative method of testing motoneurone excitability is by passing a current through an extracellular pipette to stimulate the motoneurones in the vicinity of the tip, while recording the evoked action potentials from the ventral root. It is not certain, however, whether this technique is testing the excitability of the motoneurone soma, the initial segment (whose threshold is normally low) or even the axon itself. Of four experiments of this type with chloroform, all showed a reduction in the direct excitability of the motoneurones, ranging from 9% to 26% at the point when synaptic conduction was almost blocked. Figure 8 illustrates typical results. With methohexitone, measurements of the size of the volley were made when the monosynaptic response to lateral column stimulation was maximally potentiated. The results were more variable than with chloroform, ranging from no change to a 12·5% increase in four experiments. The greatest changes were seen when the potentiation of the monosynaptic response was greatest.

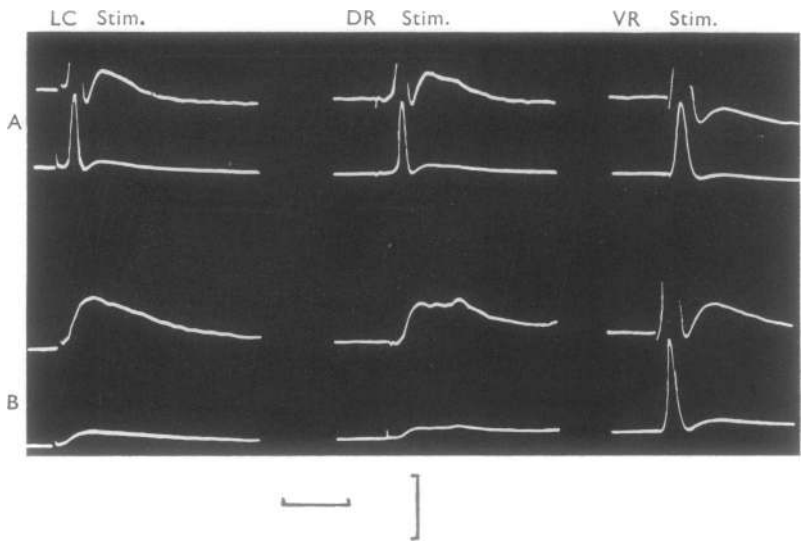

FIG. 9. Action of chloroform on intracellular responses of a motoneurone. A: Responses from a cell in an untreated preparation to lateral column, dorsal root and ventral root stimulation. Upper records at high gain RC and lower records at low gain DC. B: Responses from another cell in the same preparation after a 12 min treatment with chloroform 0·5 mg/ml. The cell fails to generate a spike potential on orthodromic stimulation, but invades normally antidromically. Time calibration: 20 msec for responses to orthodromic activation, 10 msec for responses to antidromic activation. Amplitude calibration: 12·5 mV for high gain records, 50 mV for low gain.

Action of anaesthetic agents on intracellular potentials

Most recordings were made from cells in a control preparation, and then from different cells in the same preparation during the administration of an anaesthetic agent. Several cells were impaled at various stages of depression by chloroform. Those cells whose synaptic drive was not sufficiently depressed to prevent spike

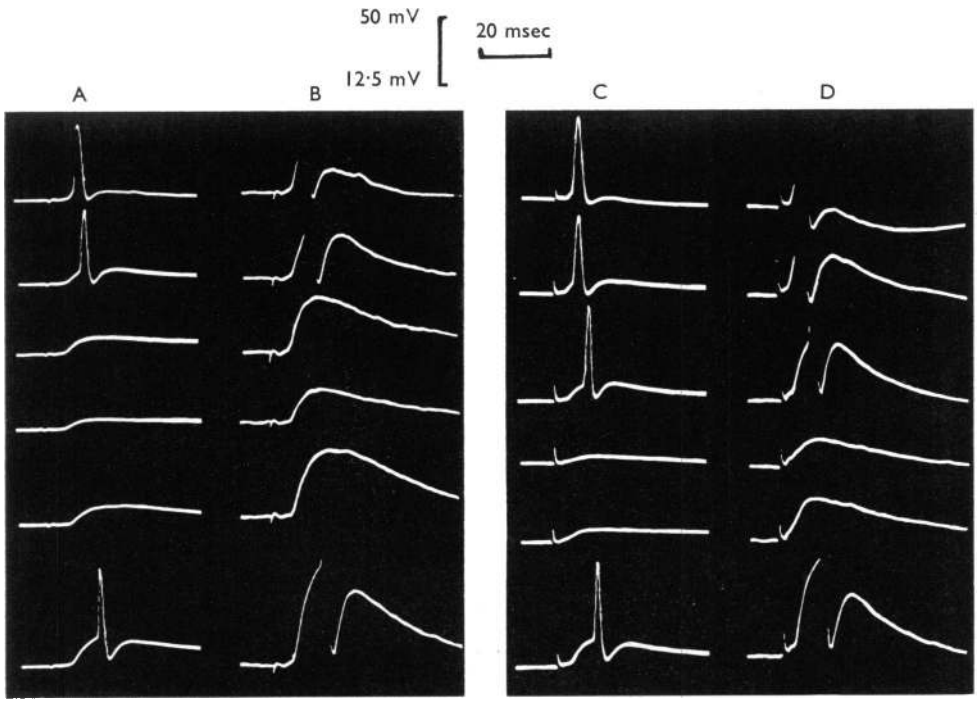

FIG. 10. Action of chloroform on intracellular responses of a motoneurone. Responses of a single cell to supramaximal dorsal root stimulation (A and B) and to a lateral column volley (C and D). A and C were recorded at low gain DC (calibration 50 mV) while B and D were recorded at high gain RC (calibration 12·5 mV). The sequence of responses from above downwards is as follows: A and B, control responses, responses after 20, 25 and 30 min exposure to chloroform 0·5 mg/ml., then 20 and 40 min after changing to normal Ringer solution. C and D, Control responses, responses after 20, 25 and 35 min exposure, then 15 and 40 min after washing. 25 MΩ KCl electrode.

TABLE 1. *Summary of the results of the experiment illustrated in Fig.* 10

Route of stimulation	Control threshold (mV)	Threshold before failure (mV)	Smallest synaptic potential (mV)	Threshold at recovery of discharge (mV)
LC	4·8	12·0	5·4	16·0
DR	6·0	9·5	4·6	18·2

Measurements of threshold were made before administration of chloroform, just before failure of spike generation, and on recovery of transmission. The smallest synaptic potential recorded after 35 min exposure to chloroform has been measured.

generation usually produced a spike to both lateral column and dorsal root volleys. The safety factor of the latter, however, seemed to be rather less than the former, probably due to the weaker synaptic connexions of the segmental pathway and also to damage to inter-neurones during dissection of the pia. It was not possible to decide whether the threshold of these cells was elevated, for the threshold of normal cells varies over a wide range and insufficient records are available for a quantitative analysis of this nature. A few cells from which recordings were made showed only a subthreshold synaptic potential to orthodromic stimulation by both routes, despite normal antidromic invasion (Fig. 9B). Occasionally a cell was held for a few minutes after penetration and during deepening anaesthetic depression, and showed progressive diminution in the size of the synaptic potentials despite a stable resting membrane potential.

In one experiment a motoneurone was successfully held under stable conditions for 75 min, during which the suppression of its discharge by chloroform and its subsequent recovery was followed. The results are illustrated in Fig. 10 and are summarized in Table 1. Small fluctuations in membrane potential and spike height were observed, but appeared not to be related to the administration of the anaesthetic agent and were probably due to variation in the seal of the membrane around the electrode. The control threshold to dorsal root excitation was slightly higher than that to lateral column excitation, owing to extracellular field currents (Machne *et al.*, 1959). Administration of chloroform caused a progressive rise in threshold until it exceeded the height of the synaptic potential, and spike generation failed.

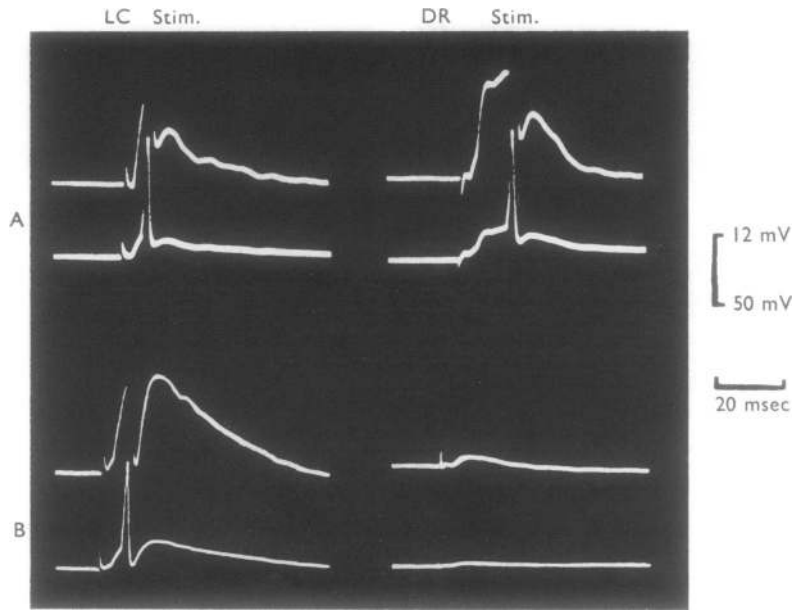

FIG. 11. Action of methohexitone on intracellular responses of a motoneurone. A: Control responses to lateral column and dorsal root stimulation in the untreated preparation. Upper records at high gain RC and lower records at low gain DC. Note the unusually large difference between the threshold to LC stimulation and that to DR stimulation. B: The responses of another cell in the same preparation 35 min after commencing treatment with methohexitone 100 μg/ml. 15 MΩ KCl electrode.

Threshold reached a much higher level on lateral column excitation before failure occurred, because the lateral column fibres produced a more powerful synaptic drive. The slope of the synaptic potential was only slightly reduced during the early stages of depression. Once transmission ceased, however, the slope and amplitude of the synaptic potentials were progressively decreased. The synaptic potential produced by the lateral column impulses was as sensitive to chloroform as that produced by dorsal root impulses. In addition, the monosynaptic component of the synaptic potential (B) on dorsal root stimulation appeared to be as susceptible to depression as the polysynaptic components. During recovery the potentials reached amplitudes of just over three times the control level before spike discharge recommenced. It was not possible to follow complete recovery of the cell. No impairment of antidromic invasion was found in this cell, even when orthodromic excitation was severely depressed.

Recordings of ventral root potentials during depression of reflex activity by methohexitone (Richens, 1969) demonstrated a selective block of polysynaptic responses with sparing of two-neurone reflex arcs. Intracellular recording from five motoneurones confirmed this observation. In every case the responses to lateral column and ventral root stimulation were normal, whereas a maximal dorsal root volley produced only a subthreshold synaptic potential which was largely monosynaptic (Fig. 11). Methohexitone had effectively blocked the polysynaptic components, upon which the reflex discharge to segmental afferent stimulation largely depends. It was not possible, from the results available, to decide whether a change in threshold of the motoneurone membrane had been produced by drug action.

Discussion

Recording focal potentials from dorsal and ventral horns has shown that the ability of primary afferent and lateral column fibres to conduct impulses was not impaired by exposure to concentrations of volatile agents which powerfully depressed synaptic transmission. If, however, the dose was doubled or trebled a marked conduction block developed. This is wholly in agreement with the findings of Larrabee & Posternak (1952), who used the more simple synaptic system of the superior cervical ganglion of the cat. Sömjen (1963) also demonstrated no impairment of conduction in afferent fibres in the cat spinal cord with concentrations of ether which were sufficient to block reflex discharge, but he pointed out that this does not eliminate the possibility of a conduction block in fine terminals, for a microelectrode may be recording activity mainly from the parent axons. His observation that ether does not affect post-tetanic potentiation, however, suggests that fine terminals are unaffected. It has not been possible to use this technique in the isolated spinal cord, for deterioration of the preparation rapidly occurs on high frequency stimulation.

Although no evidence can be offered to eliminate the possibility that volatile agents act by interfering with the release of transmitter, it seems probable that the depressant effect of these drugs is exerted mainly on the motoneurone membrane, for failure of synaptic transmission was always accompanied by evidence of a decrease in the excitability of the membrane. Low concentrations of volatile agents reduced the excitability of motoneurones to direct stimulation and caused a failure

of antidromic invasion in a proportion of the cells of the motoneurone population, leading to an attenuation of the focal potential. This failure occurred probably at the initial segment, as was demonstrated for cat motoneurones by Sömjen & Gill (1963). A reduction in the size of the initial segment spike or a stabilization of the soma membrane would lead to conduction failure, especially in cells where the critical value for invasion was only just being reached before exposure to the anaesthetic agent. An action at this site, which is considered to be the trigger zone of the motoneurone (Eccles, 1957), would also explain the rise in firing threshold to orthodromic excitation which was seen in recordings of ventral root potentials (Richens, 1969) and confirmed in this present study by intracellular recording. In the cell illustrated in Fig. 10, failure of orthodromic excitation appeared to be the result of two factors: (i) elevation of threshold and (ii) depression of the synaptic potential. Although the slope of the synaptic potential showed only small changes during the early stage of depression, the threefold elevation of the threshold on recovery of transmission suggests that considerable reduction in the amplitude of the synaptic potential must have occurred. These two changes, therefore, gradually reduced the safety factor for transmission until eventually the falling synaptic potential dropped below the rising threshold. Recovery of the mechanisms responsible for the synaptic potential appeared to occur more quickly in this cell than those responsible for firing threshold. This had often been observed for the motoneurone pool on ventral root recording (Richens, 1969). In view of the other evidence for a direct action of volatile agents on the motoneurone membrane, the depression of the synaptic potential was probably the result of stabilization of the subsynaptic regions of the soma-dendritic membrane. This depression involved all components of the synaptic potential equally, so that the contour of the compound synaptic potentials was little altered, in spite of a reduction in amplitude. The responses to lateral column volleys were as sensitive to depression as those to dorsal root volleys. If suppression of reflex activity was the result of an unselective action at all synapses the polysynaptic connexions would be disproportionately affected (Barany, 1947). This was not a characteristic of the action of volatile agents on the cat spinal cord (Austin & Pask, 1952) or on the frog spinal cord, and this fact is emphasized by comparison with the action of barbiturate compounds. Although it is unnecessary to postulate any site of action for volatile agents other than the motoneurone membrane to account for their action on reflex motor discharge, other elements in the spinal cord are also affected, but to a much smaller extent. The dorsal root potentials of the anuran spinal cord are generated by synaptic depolarization of primary afferent fibres by small interneurones, activated by dorsal or ventral root fibres. These pathways are depressed by volatile agents, although their sensitivity is much less than the sensitivity of the pathways producing ventral root discharge (Schmidt, 1963 ; Richens, 1969).

With methohexitone, focal recording has shown consistent impairment of presynaptic conduction long before the reflex discharge to a dorsal root volley was abolished, and usually during potentiation of the monosynaptic responses to a lateral column volley. Although it is uncertain whether an extracellular electrode records activity from the finest of terminals, if the presynaptic component is produced largely by parent axons it is probable that changes of conduction in their terminals may be even more severe than is indicated by changes in the focal potential. Although these findings do not agree with those of Sömjen (1963) using focal recording and post-tetanic potentiation techniques in the cat, they accord with

the more recent studies of Løyning *et al.* (1964). These workers, using small injections of thiamylal in lightly anaesthetized cats, found that depression of monosynaptic responses was accompanied by an impairment of conduction in the afferent terminals in the absence of any change in the excitability of the motoneurones themselves. They postulated that the drug had a local anaesthetic action on the nerve terminals, thus reducing the output of excitatory transmitter, and considered that the postsynaptic action observed by Brooks & Eccles (1947) and Sömjen & Gill (1963) resulted from their use of much larger doses of barbiturates. The present experiments on the frog spinal cord have always shown an excitatory action on the motoneurone membrane, which more than offset any reduction in synaptic drive from impaired conduction in the lateral column terminals, and often allowed an initial potentiation of the earliest discharges to dorsal root stimulation. Depression of the lateral column response was accompanied by evidence of depression of the excitability of the motoneurones, but always occurred after a prolonged exposure to the drug and was irreversible. The increased excitability has been demonstrated by a reduction in firing threshold of the motoneurone pool to orthodromic excitation (Richens, 1969), facilitation of antidromic invasion, and by an increased excitability to direct stimulation through a micropipette. Possible explanations for this effect have been considered in the preceding paper (Richens, 1969).

Intracellular recording has confirmed the sensitivity of polysynaptic pathways to methohexitone. The synaptic potential produced by a dorsal root volley was gradually reduced in duration and amplitude until a small potential of exponential time course remained. The response to lateral column volleys was usually unaffected unless prolonged exposure was allowed, in agreement with Fadiga & Brookhart (1960). Allowing for the unusual excitatory action of methohexitone on the motoneurone membrane, it would appear justified to ascribe to this drug an unselective action on synaptic transmission in the spinal cord, probably by a local anaesthetic effect on nerve terminals. This would be supported by the observation that the dorsal root potentials are more sensitive to methohexitone than to volatile agents (Richens, 1969).

It is well known that in clinical use volatile anaesthetic agents are more efficient muscle relaxants than barbiturate compounds. The selective action of volatile agents on the final common pathway to skeletal muscle—the alpha motoneurone—may, at least in part, account for this property.

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