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INTRACELLULAR RECORDING FROM CAT SPINAL MOTONEURONES DURING ACUTE ASPHYXIA

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SUMMARY

1. Changes in membrane and action potentials of cat spinal motoneurones during acute asphyxiation and re-oxygenation were recorded with an intracellular technique.

2. The asphyxial potential of the grey matter, which develops in the first 2–2·5 min of asphyxiation, can be expected to interfere with the membrane potential record. After correcting for this effect a gradual depolarization of the soma at a rate of 3–4 mV/min was found, commencing within a fraction of a minute after the start of asphyxiation.

3. The orthodromic responses of the motoneurones were the most vulnerable to O_2 lack. They failed earlier than the responses to antidromic and to direct excitation of the cell through the micro-electrode. After failure of the orthodromic spike an excitatory post-synaptic potential remained for a short time. Failure of antidromic excitation began by the dropping out of the soma dendritic potential, followed by the arrest of the initial segment response.

4. It was concluded that the early arrest of orthodromic excitation is caused by presynaptic failure.

5. All changes in membrane and action potentials were completely reversible by re-oxygenation after periods of asphyxia lasting from 4 to 6 min. The orthodromic response recovered markedly slower than the antidromic and direct ones.

INTRODUCTION

Profound changes occur in the central nervous system within a few minutes after oxygen deprivation. In addition to the arrest of the normal functions asphyxial potentials develop, the tissue impedance increases markedly, and swelling of neuronal (and glial) elements occurs by an inward movement of electrolytes and water (see Van Harreveld, 1962). The asphyxial potentials observed in cerebral cortex (Leão, 1947, 1951),

cerebellum (Van Harreveld, 1961) and spinal cord (Van Harreveld & Hawes, 1946, Van Harreveld & Biersteker, 1964) have been ascribed to differences in the rate of asphyxial depolarization of somata and axons or of dendrites and somata. Direct observations on the membrane potential of spinal neurones during asphyxia and anoxia were made with intracellular techniques by Kolmodin & Skoglund (1959), Nelson & Frank (1963) and R. M. Eccles, T. Oshima & Y. Løyning (personal communication) in cats and by Washizu-Yoshiaki (1960) in toads. These investigations have led to conflicting results. An investigation of the effect of asphyxia on the membrane and action potentials of spinal motoneurones recorded intracellularly in cats is presented in the present paper.

METHODS

Adult cats were used, fully anaesthetized with sodium-pentobarbital and immobilized with Flaxedil (Am. Cyanamid Co.) under artificial respiration. The femoral artery was cannulated for blood pressure recording and the femoral vein for injections. The left pleural cavity was opened. A string was placed around the descending aorta and threaded through a polyethylene tube. By pulling the aorta with the string against the tube the blood flow could be interrupted. The lumbosacral cord was exposed, and the dorsal and ventral roots of L_7 or S_1 were mounted on silver–silver chloride electrodes. The bodies of the underlying as well as of the cranial and caudal vertebrae were rigidly fixed. The cord was covered with a layer of 4% agar in Ringer solution, on top of which a paraffin pool was established. Conventional glass micropipettes, filled with 2·7 M-KCl and of rather low resistance (5–15 MΩ) were introduced with a micromanipulator either from the dorsal surface, or after rotation of the cord from the lateral side. With the latter approach the required depth of penetration was much reduced. Three Grass S4 stimulators were connected in such a way that they stimulated in succession the dorsal root, the ventral root and the impaled cell directly through the micro-electrode. A bridge circuit essentially similar to that of Araki & Otani (1955) was used for the latter stimuli. The interval between two stimuli was 1 sec; the whole cycle was repeated every 4 sec. Stimulus duration was 0·1 msec. The micro-electrode was connected through a chlorinated silver wire to a Medistor A-34 cathode follower. The output was fed into a Tektronix 502 A oscilloscope, and into a Grass polygraph for the recording of the membrane potentials. The reference electrode was a chlorinated silver wire buried in the agar. The electrocardiogram was monitored by recording it on the polygraph.

RESULTS

Since the impalement of primary afferent fibres, motor axons and interneurones was almost invariably lost during asphyxiation, the study was restricted to motoneurones. After identification of a motor cell by antidromic activation, the stimulus parameters were adjusted for optimal responses and the cell was observed for a few minutes. If the membrane potential and spike responses remained stable, asphyxiation was initiated by closing the aorta which caused the blood pressure recorded from the femoral artery to drop to zero. In the majority of penetrations this procedure resulted in an immediate loss of the spikes and of the membrane

potential, indicating that the electrode tip had moved out of the cell. This obviously was caused by the sudden drop in blood pressure, resulting in tissue movements. In other cases the electrode remained intracellular, but the membrane potential dropped sharply in the ensuing seconds while cell responses to stimulation stopped or changed to a pattern of injury discharges. This was interpreted as a smaller movement of the cell which enlarges the hole around the electrode tip and causes a serious leak in the membrane. This kind of recording was discarded. Finally, in a number of more fortunate impalements clamping of the aorta caused during the first seconds at most a small and transient disturbance of the membrane potential, while normal responses to stimulation continued. The effects of asphyxiation on such cells were studied.

When clamping of the aorta did not disturb the intracellular electrode position immediately, the electrode often remained in the cell for several minutes. Any abrupt change in membrane potential during this time was considered to be an artifact. If a cell was lost, it was occasionally possible to re-introduce the electrode into the cell by a small movement of the micrometer, in which case the recording was continued. After 4–10 min, when the effects of asphyxiation were evident, the electrode was either retracted in order to determine the extracellular potential, or recovery was attempted by opening the aorta clamp. This again resulted often in a loss of the intracellular electrode position, but in a small number of cases the recording could be continued during recovery. Owing to these difficulties the number of satisfactory recordings is limited (Table 1).

The membrane potential during asphyxiation and recovery

The mean membrane potential of the seventeen cells from which successful recordings during asphyxiation were obtained was 58 mV, the highest and lowest values were 76 and 40 mV. The standard error was 2·5 mV. These values have been corrected for the loss in potential caused by the bridge set up. Figures 1–3 show asphyxial changes in membrane potential, measured between the intracellular and the indifferent electrode, in three different cells. Generally, one can distinguish two phases in these records during asphyxiation. During the first phase, lasting 2–2·5 min, the measured potential either remained more or less stable, decreased (Fig. 3), or increased (Figs. 1 and 2). During the second phase, the potential declined steadily at a rate of 3–4 mV/min. During re-oxygenation the potential often showed an initial drop followed by a speedy recovery (Figs. 2 and 3). The small, relatively rapid potential changes usually present during the actual clamping and release of the aorta may be artifacts due to the necessary handling of the preparation.

For an evaluation of these findings the asphyxial potential developing between the spinal grey matter and an indifferent electrode has to be taken into consideration. The latency of this grey matter negativity is in the spinal cord not more than a few to 10 sec (Van Harreveld & Hawes, 1946) and this potential is fully developed after 2–2·5 min. The asphyxial potential then remains more or less constant for a few min, followed by a slow decline (Van Harreveld & Hawes, 1946; Van Harreveld & Biersteker, 1964). Upon re-oxygenation the asphyxial potential is quickly reversed. Its amplitude can be as large as 25 mV in the centre of the cord, and is smaller in more dorsal and ventral regions (Biersteker, Collewijn & Van Harreveld, 1966).

It will be evident that such a major shift in the potential between spinal grey matter and an indifferent electrode will affect the potential differences measured between an intracellular electrode and the indifferent electrode. Nelson & Frank (1963) attempted to circumvent this difficulty by the use of concentric micro-electrodes. In the present series of experiments the following correction for the effects of the asphyxial potential was applied. As the appearance of the asphyxial potential is well known, one can estimate its course if its magnitude just outside the cell under investigation is known a few minutes after asphyxiation. In the cell shown in Fig. 1, this information was obtained by retracting the electrode after 6 min of asphyxia. The extracellular potential was at this moment 22 mV more negative than at the moment of penetration of the cell. This value was considered as that of the fully developed asphyxial potential at the locus of the cell under investigation. The probable course of the asphyxial potential has been indicated in Fig. 1. By subtracting this potential from the observed intracellularly recorded potential a voltage–time relation is obtained which may approximate the actual membrane potential changes and which seems to indicate that the membrane potential starts to drop shortly (within a fraction of a minute) after the beginning of asphyxiation. The rate of decline during this first phase is perhaps slightly higher than during the second phase in which the drop is 3–4 mV/min.

Figure 2 shows a cell in which the membrane fully recovered from 5 min asphyxiation after re-oxygenation. This demonstrates that the asphyxial depolarization is not an artifact, caused by the mechanical damage of the penetration, but is a reversible result of circulatory arrest. The recorded change in the intracellular potential of this cell during the first 3 min is very similar to that of the cell in Fig. 1. After 3 min the cell was lost spontaneously, but the intracellular position of the electrode could be restored by a small adjustment of the micrometer. This interruption showed that the extracellular potential had shifted by -18 mV after 3 min of

asphyxiation. By subtracting the probable asphyxial potential a similar course of the membrane potential during asphyxiation was obtained as shown in Fig. 1.

During the recovery period, which resulted in the complete restoration of the original membrane potential and response to stimulation, an initial drop in the potential was observed preceded by some rapid fluctuations, after which the membrane potential recovered. It is possible to estimate the course of the disappearance of the asphyxial potential after re-oxygenation of the cord, and to subtract this potential from the recorded potential during the recovery period, as has been performed in Fig. 2. This procedure eliminates the drop in membrane potential after re-oxygenation and suggests a gradual recovery of this potential.

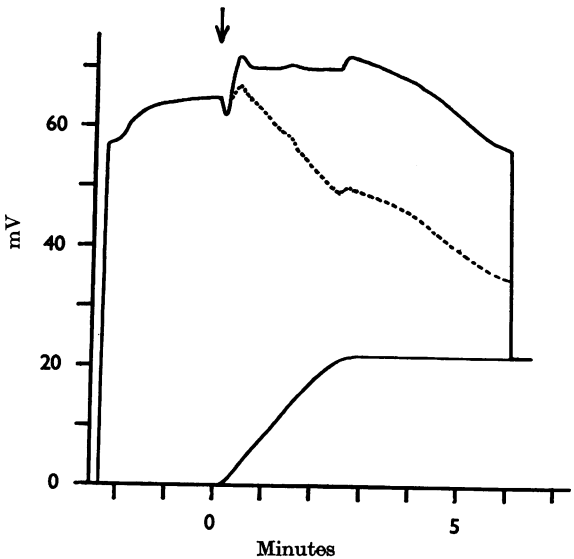

Fig. 1. Membrane potential of a motoneurone during asphyxia, starting at zero time (marked by arrow). Upper continuous line shows the potential difference between the intracellular and the indifferent electrode outside the cord. Lower continuous line is the estimated course of the asphyxial grey matter potential against the same indifferent electrode. The height of this potential is indicated by the base line shift in the micro-electrode recording upon retraction of the electrode after 6 min of asphyxia (−22 mV). Dotted line, obtained by subtraction of the lower from the upper continuous traces, gives an approximation of the true course of the asphyxial membrane potential changes. See text for additional explanation.

Figure 3 illustrates the de- and repolarization in another cell after an asphyxiation for 4 min. Since no value for the extracellular potential during the asphyxial period was determined in this instance, no correction for its effect can be made.

The reliability of the subtraction method depends on the measurement of the difference in the potential directly outside the cell under investigation before and after impalement. Possible changes in electrode potential owing to material being picked up by the tip during penetration and to differences in the ratio of Na and K ion concentrations (Adrian, 1956; Kostyuk, 1965) in the medium outside the cell before and after impalement and asphyxiation have to be considered. The measurements of Ito & Oshima (1964*a*) seem to indicate that the effect of the latter variable is not great with concentrations to be expected in the tissues. Figure 4, in which base line shifts observed by retracting the electrode or losing the

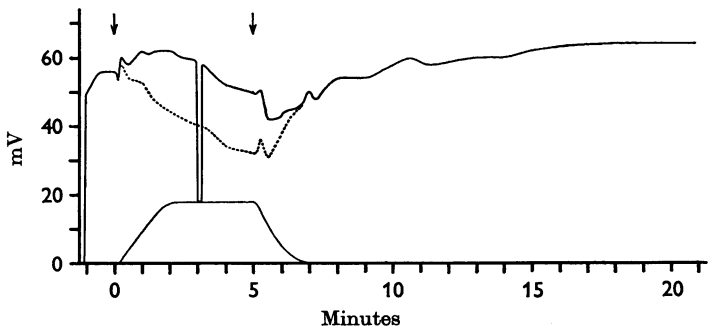

Fig. 2. Membrane potential of a motoneurone during asphyxia starting at zero time and recovery after 5 min asphyxiation (marked by arrows). After 3 min asphyxiation the intracellular electrode position was lost, but could be regained by a slight micrometer adjustment. Explanation as in Fig. 1.

cell are plotted against the period of asphyxiation, suggest that these factors are not of major importance for the changes in base line potentials observed. The shifts are all negative (except one), which agrees with the direction of the asphyxial spinal grey potential. Furthermore, the largest potentials are found after the first 2 min of asphyxiation when the spinal grey potential is fully developed. As mentioned above the magnitude of this asphyxial potential depends not only on the duration of asphyxiation but also on the location of the tip in the ventral horn (Biersteker *et al.* 1966) which accounts for the spread in base line shifts. During the first minute when the asphyxial potential is developing the base line shift is much smaller. Although the base line shift seems to be an expression of the developing asphyxial potential there is an element of uncertainty in the estimate of the magnitude and course of the extracellular asphyxial potential shift which makes the details of the corrected membrane potential during the first minutes of asphyxiation unreliable. However, the overall course will probably be correct. The effect of the developing asphyxial

potential seems to explain in a satisfactory way the increase in the recorded membrane potential often observed during the first minutes of asphyxiation (Figs. 1 and 2) and the sometimes marked decrease of the potential at the start of re-oxygenation (Figs. 2 and 3).

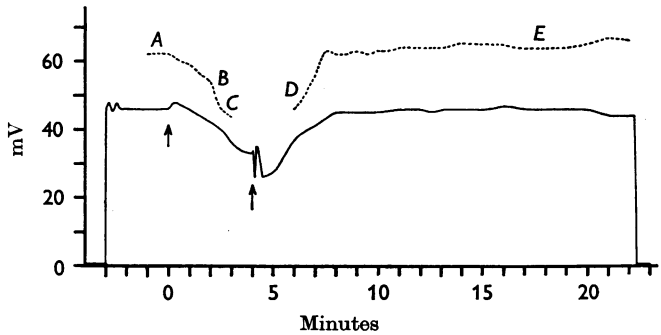

Fig. 3. Membrane potential of a motoneurone during asphyxia starting at zero time and recovery after 4 min asphyxiation (marked by arrows). Continuous line shows the potential differences between the intracellular and indifferent electrodes. Dotted line indicates the height of the antidromic SD spikes. The letters *A–E* indicate the moments at which action potentials shown in Fig. 5 were recorded. Due to the use of a bridge circuit for direct stimulation the recorded membrane and spike potentials are lower than the actual potentials.

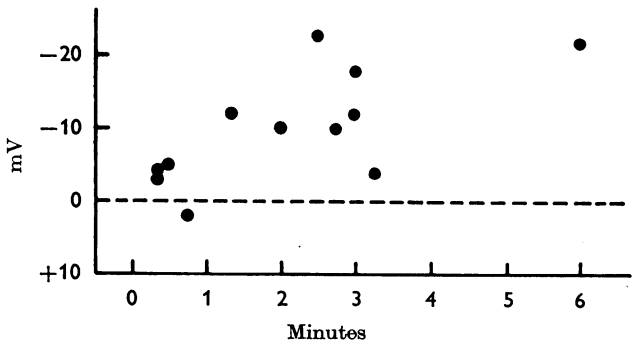

Fig. 4. Shifts in the base line of the micro-electrode recording after various periods of asphyxia, as found by accidental or intentional removal of the electrode tip from the cell. The shifts are all negative except for one. The largest shifts occur after about 2·5 min asphyxiation. They can be considered as a representation of the asphyxial grey matter potential.

Response to stimulation during asphyxiation and recovery

Orthodromic excitation. In many cells stimulation of the dorsal root evoked a monosynaptic soma-dendritic (SD) spike; in some only an excitatory post-synaptic potential (EPSP) was elicited. The orthodromic spike was the first response of the cell to fail during asphyxiation. Table 1

shows that this occurred after 0·2–2·5 min. After clamping of the aorta the rate of rise of the SD spike, but especially of the initial segment (IS) response, decreased gradually resulting in a more pronounced inflection between these potentials (Fig. 5*A* and *B*). Also the rate of decline was reduced. These changes resulted in a longer duration of the orthodromic response. Furthermore, the SD spike became reduced in size. There was a gradual increase in the latency of the spike, as the threshold level for firing of the IS was reached by the EPSP at a later and later moment

TABLE 1. Asphyxial survival times (min) of various responses to stimulation

Cell	Orthodromic spike	EPSP	Antidromic spike	Initial segment response	Direct excitation
1	1·0	3·0	4·7	5·0	—
2	—	—	7·0	7·3	—
3	1·0	3·0	0·9	—	—
4	2·5	3·0	4·5	5·0	—
5	—	—	6·6	7·5	—
6	—	—	7·9	8·0	—
7	1·9	2·3	4·0	4·1	—
8	2·0	2·5	1·6	2·3	—
9	—	2·5	3·0	5·0	5·0
10	2·5	3·0	3·0	3·1	3·1
11	0·2	3·0	3·2	—	4·5
12	—	2·5	3·4	3·5	—
13	—	—	2·3	2·4	—
14	—	—	1·8	2·0	—
15	2·1	—	2·2	2·4	—
16	0·6	1·5	2·2	2·4	—
17	0·7	2·0	1·4	1·5	—
Means	1·5	2·6	3·5	4·1	4·2

(Fig. 5*B*). After failure of the spike, an EPSP was still present which decreased gradually in amplitude and could not be recognized about 3 min after the beginning of asphyxiation (amplification 5 mV/cm). The mean survival time of the spikes in ten cells was 1·5 min, that of the EPSP 2·6 min. Figure 3 shows the changes in membrane potential during asphyxiation and recovery of the cell of which the action potentials are shown in Fig. 5.

The ventral root responses to dorsal root stimulation often grow markedly during the first minute after clamping the aorta indicative of an increase in the number of motor cells activated by the stimulus. With intracellular recording it was observed a few times that a cell, which before arrest of the blood supply responded only with an EPSP, produced a SD spike shortly after asphyxiation. These responses stopped at the moment which was usual for the failure of orthodromic conduction. The increased excitability may be a result of a slight depolarization of the motoneurone.

The asphyxial survival time of reflex activity in the segment of the cord under investigation was determined in most preparations by recording the

monosynaptic ventral root responses to dorsal root stimulation. The mean survival time was 2·7 min. The mean time after which the amplitude of the ventral root response was reduced to half its size was 1·1 min. The latter value can be considered as an estimate of the time after which half of the motoneurones lost their excitability. Table 1 shows that out of ten cells

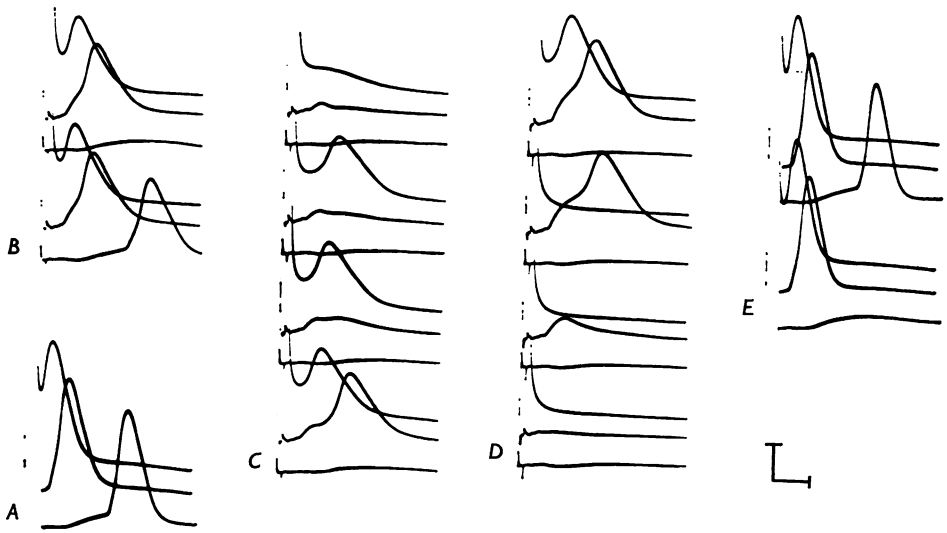

Fig. 5. Failure and recovery of excitation of a spinal motoneurone during and after asphyxia. Groups of three traces are shown, representing the results of orthodromic (lower trace), antidromic (middle trace) and direct (upper trace) excitation. The sequence in time is upward. Calibration bars indicate 20 mV and 1 msec. Figure 3 shows the membrane potential change in this motoneurone. *A*: Normal spikes before asphyxiation. *B*: 2·5 min after asphyxiation. Last orthodromic spike, after which only an EPSP remains. *C*: 3 min after asphyxiation. Failure of IS–SD transmission of antidromically elicited action potential, followed by block or direct excitation. *D*: 2 min recovery. Re-appearance of IS spike, followed by SD spike elicited by antidromic and direct excitation. *E*: 12·5 min recovery. Re-appearance of orthodromic spike. Virtually complete recovery.

five had stopped firing after 1·1 min. The longest observed firing in a cell was 2·5 min. The survival times found with micro-electrodes agree thus well with those of the ventral root response, indicating that impalement does not seriously interfere with the asphyxial survival.

In the cells which showed a good recovery from asphyxiation (up to 6 min in duration) a small EPSP became visible within minutes after re-oxygenation and grew gradually to threshold for the orthodromic spike (Fig. 5*E*). The cell shown in Fig. 5 was fired orthodromically 12·5 min after the end of a 4 min asphyxiation. In the cell shown in Fig. 2 an

orthodromic spike was elicited after recovering for 6 min from 5 min asphyxia. No spontaneous activity was observed during asphyxiation or the recovery thereof.

Antidromic excitation. Table 1 shows that with a few exceptions antidromic excitation went on for a considerably longer time than orthodromic activity. The mean survival time of the antidromic SD response was 3·5 min, the longest time was about 8 min. Failure of the response developed as in the case of the orthodromic spike after a reduction in the rate of rise of the SD spike but especially of the IS response, resulting in a marked inflexion on the rising phase of the potential (Fig. 5*A* and *B*). Also the rate of decline was decreased, and the duration of the antidromic response increased. The spikes decreased furthermore markedly in height. The heights of the antidromic spikes of the cell giving the responses shown in Fig. 5 are plotted in Fig. 3. It should be remembered that, although the spikes are plotted in the same direction as the membrane potential, their directions are really opposite. Taking this into consideration, Fig. 3 shows that an overshoot remains as long as the spike is elicited by the antidromic stimulus. It is, furthermore, of interest that the spikes start to decline after a latency comparable with that of the decrease in membrane potential. A similar early decline of the orthodromic spikes was observed. The last antidromic stimulus to elicit the full response in Fig. 5*C* showed a long delay between the IS and SD spike, indicating that threshold for the excitation of the soma membrane by the IS spike had been reached. At the next response the SD component dropped out, while the IS spike still could be observed for some time. After the arrest of the IS potential only a very small response, which may be attributed to the medullated axon, was sometimes observed. The mean survival time of the IS response was 0·6 min longer than that of the SD spike. In a few cases the antidromic response disappeared abruptly, without a transitional IS–SD block. In these cases the block may have been situated at the axon–initial segment junction, which was considered by Lloyd (1953) to be the usual locus of an asphyxial block of the antidromic impulse.

During recovery from 4 to 6 min asphyxiation, the IS spike returned first, shortly after re-oxygenation. It increased gradually in amplitude until it triggered the SD spike again (Fig. 5*D*).

Direct excitation. In instances in which the electrode resistance was sufficiently low, the motor cells could be stimulated by passing currents through the electrode in a bridge circuit. The stimulus artifact was usually large, causing distortion of the spike, especially of its rising phase. The latency of the spike increased markedly during asphyxiation (Fig. 5*A*, *B* and *C*). The rates of rise and decline decreased and the spikes became smaller and of longer duration. Asphyxial failure of directly elicited spikes

occurred usually very shortly after the development of an IS–ED block during antidromic excitation (Fig. 5*C*). After this a small deflexion was still present, which resembled an IS potential, suggesting that the soma was invaded from the initial segment and not stimulated directly. It should then have been possible to evoke an SD spike by increasing the stimulus strength sufficiently to reach threshold for the soma membrane, which is considerably larger than that of the initial segment. Though this could apparently be achieved in one instance, in general the large stimulus artifact made the recording of such responses impossible.

DISCUSSION

Asphyxiation of the spinal cord seems to result in a depolarization of the soma membrane of the motoneurone which starts after a short (fraction of a minute) latency. The initial enhancement of the membrane potential sometimes recorded was ascribed to the development of the asphyxial grey matter potential which interferes with the recording. This view is supported by recordings from rigid preparations in which the cord had been asphyxiated 2 weeks previously for 30–35 min. In such cords, which show little or no asphyxial grey matter potential, a clear cut asphyxial depolarization of the motoneurone with a short latency was observed (Collewijn & Van Harreveld, 1966). Furthermore, electrotonically conducted potentials in the ventral root observed during asphyxiation which have a similar short latency and are indicative of depolarization of the intraspinal portion of the motoneurone (Biersteker *et al.* 1966), are in agreement with an early soma depolarization. Finally the early decline of the ortho- and antidromic SD spikes could well be due to a drop in membrane potentials.

An early asphyxial depolarization was observed by Kolmodin & Skoglund (1959). These authors considered the possibility that an asphyxial increase in interneuronal bombardment of the motoneurone is partly responsible for the observed depolarization. In the anaesthetized preparations used in the present investigation there is little evidence for such an occurrence. R. M. Eccles, T. Oshima & Y. Løyning (personal communication) observed no change in the membrane potential during a 15 min period of hypoxia by ventilation with 5 % O_2 in N_2 when the membrane potential was high (75 to 80 mV), but in cells with lower potential (60 to 70 mV) a gradual depolarization of 1–2 mV/min was observed. Nelson & Frank (1963) observed during ventilation of the preparation with N_2 or arrest of the artificial respiration a decrease of the membrane potential which usually could not be reversed by re-oxygenation and was ascribed to electrode movements. During 30–40 sec periods of

anoxia by N_2 ventilation of the preparation no changes in membrane potential recorded with concentric electrodes were observed. However, taking the asphyxial potential of the grey matter as an indicator, depolarization of spinal cord elements starts with a latency of about 20 sec with this procedure (Van Harreveld & Hawes, 1946), and at a rate of depolarization of 3–4 mV/min very minor potential changes could be expected during the periods of anoxia employed by Nelson & Frank (1963).

The membrane potentials are indirectly dependent on the cell metabolism since the ion concentration gradients which are generally believed to generate the potentials are maintained by energy requiring ion pumps. It is therefore not surprising that Ito & Oshima (1964*b*) observed a decrease of membrane potential after the intracellular injection of azide which affects the cytochrome system and uncouples oxidative phosphorylation. This depolarization was ascribed to the arrest of the sodium pump and accumulation of sodium in the cell. The short latency of the depolarization (less than 1 min) would indicate that the store of high energy phosphates available to the ion pumps is small. The depolarization after oxygen deprivation could be ascribed to the same mechanism. It has been suggested (Van Harreveld & Biersteker, 1964; Biersteker *et al.* 1966) that the development of the asphyxial grey matter potential is due to a difference in the rate of depolarization of dendrites and somata. It would seem possible that owing to the much greater surface–volume ratio the asphyxial depolarization of dendrites due to arrest of the ion pumps proceeds at a much higher rate than in the somata. An alternate possibility is that the dendrites depolarize because of a greatly enhanced increase in Na permeability of the membrane caused by the release of compounds like glutamic acid (Biersteker *et al.* 1966). The initial phases of the soma depolarization could then be due, at least in part, to electrotonic conduction of an early dendritic depolarization. It would seem very likely, however, that the later phases of soma depolarization are due to the arrest of the ion pumps in the soma.

The affect of asphyxiation on ortho- or antidromically elicited action potentials of motoneurones are in agreement with the findings of Kolmodin & Skoglund (1959). Antidromic-survived orthodromic excitation sometimes for a considerable period. The antidromically elicited SD spike was observed up to about 8 min after the start of asphyxiation and was usually survived by the IS response for a short period. A long (6–14 min) survival of the SD potential elicited by antidromic activation was observed by Brooks & Eccles (1947) by focal recording from the spinal cord. These values are a measure for the survival time of the most resistant motoneurones. The arrest of the orthodromic spike which occurred from 0·2 to 2·5 min after circulatory arrest can therefore not be ascribed to a

failure of the initial segment, but is due to the gradual decline of the EPSP which becomes subthreshold for that part of the neurone. After arrest of the orthodromic spike the EPSP continued to decline and could not be distinguished about 3 min after the start of O_2 deprivation. The small depolarization of the soma during this period cannot account for the failure of the EPSP. The decline of the EPSP could be ascribed to an insensitivity of the post-synaptic membrane for the transmitter compound developing during asphyxiation, or to the inability of the presynaptic terminals to produce the transmitter. The former mechanism is highly unlikely in view of observations on preparations made rigid by a previous asphyxiation in which the EPSP was the phenomenon most resistant to O_2 deprivation (Collewijn & Van Harreveld, 1966). The finding of an electrotonically conducted potential in the dorsal roots of normal cats during asphyxiation which would be indicative of depolarization of primary sensory endings (Biersteker *et al.* 1966) is in agreement with the latter concept. Depolarization of end knobs would, as in the mechanism proposed for presynaptic inhibition (Eccles, Kostyuk & Smith, 1962) prevent the release of the transmitter compound. Asphyxial arrest of spinal monosynaptic reflex activity thus would be due to presynaptic failure as was already suggested by Brooks & Eccles in 1947.

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