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ASPHYXIAL POTENTIALS OF SPINAL GREY MATTER, AND OF VENTRAL AND DORSAL ROOTS

BY P. A. BIERSTEKER, H. COLLEWIJN AND A. VAN HARREVELD

From the Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena, California

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SUMMARY

1. Asphyxial potentials of short latency (from a few to about 10 sec) were recorded with mono- and bipolar electrodes from the cat's spinal cord. Monopolarly, a zone of maximum negativity was found somewhat dorsal of the central canal in the dorsal horn. With bipolar leads potentials of opposite polarity were observed in the dorsal and ventral horns. In the dorsal horn the more ventral electrode tip became negative with respect to the more dorsal one, in the ventral horn the more ventral tip became more positive. In the centre of the cord where the monopolar potential showed a maximum the bipolar potentials were small in either direction, or reversed during asphyxiation.

2. These observations can be explained by the development of two independent dipoles of opposite polarity, located in the dorsal and ventral horn respectively, oriented with their negative poles towards the centre of the cord.

3. In the ventral as well as in the dorsal root a negativity of a proximal electrode with respect to a more distal one developed during asphyxiation after the same latency as the asphyxial cord potentials. The asphyxial root potentials continued to grow during periods of asphyxiation as long as 30 min, and recovered promptly upon re-oxygenation.

4. Ventral and dorsal root potentials were abolished by asphyxiation of the cord for a period of 60 min, 2 weeks previously. This procedure destroys practically all the neurones, but not the dorsal root fibres. The dorsal root potential, but not the ventral one, was abolished by extradural sectioning of the roots, 2 weeks previously.

5. The asphyxial ventral and dorsal root potentials were interpreted as the result of depolarization of the intraspinal part of the motoneurones and primary afferent endings respectively, conducted electrotonically

along the roots. The short latency of these potentials suggests that an early depolarization of motoneurone, and of the primary afferent end knobs occurs. The latter, which may have some relation to presynaptic inhibition, explains the early failure of synaptic conduction during acute asphyxiation.

INTRODUCTION

Development of a surface negativity accompanied by a rise in impedance and a transport of electrolytes and water into cellular structures has been observed during asphyxiation of the cerebral and cerebellar cortex (see Van Harreveld, 1962). Similar observations have been made on the spinal cord. During asphyxiation by clamping the aorta an electrode placed in the spinal grey matter recorded a negativity with respect to an electrode on a root or in a pool of Ringer solution surrounding the cord (Van Harreveld & Hawes, 1946). A mean latency for the asphyxial potential of 8–9 sec. was found. Ventilation of the preparation with nitrogen caused the same potential changes with a somewhat longer latency (about 20 sec). The potentials produced by asphyxia and by anoxia were equal, except for the difference in latency which was explained by a difference in the oxygen store available to the cord after the start of the procedures used. It has therefore been concluded that the 'asphyxial' spinal grey potential is caused by oxygen lack. This potential was accompanied by an increase in impedance of the spinal grey matter and a transport of water and chloride into dendritic elements (Van Harreveld & Biersteker, 1964). On re-oxygenation of the cord the potential and impedance changes were readily reversible. In the present investigation the spinal asphyxial potential was subjected to a detailed investigation. In addition asphyxial potentials were led off from the ventral and dorsal roots.

METHODS

Cats were used, anaesthetized with pentobarbital and immobilized with Flaxedil (Am. Cyanamid Co.) under artificial respiration. The spinal cord was asphyxiated by clamping the thoracic aorta (Collewijn & Van Harreveld, 1966*a*). Potentials were led off with silver–silver chloride electrodes, placed with a micrometer device in the grey matter, against an indifferent electrode located in a pool of Ringer solution surrounding the cord. The electrodes introduced into the cord consisted of silver wires (0·15 mm in diameter) insulated up to the tip with epoxy resin. The tips of the wires were cut at an angle of about 30°. Such wires were used singly or fastened together with epoxy resin to form bipolar and multipolar electrodes. Potentials were led off from roots with the electrodes shown in the inset of Fig. 7. The root was pulled through cotton loops which had been fixed with 4% agar in Ringer solution in tubes filled with saline into which silver–silver chloride electrodes dipped. The potentials were recorded with a Grass polygraph, or after amplification with Kintel (114C) differential d.c. amplifiers with a Varian dual channel recorder.

After the experiment the cord was fixed in 95% alcohol and embedded in paraffin. The

electrode placement was studied in sections of this material stained with gallocyanin. The electrode positions were estimated from the distance over which the electrode had been advanced into the cord during the experiment, and the length of the electrode channel in the cord shrunken by fixation and embedding.

RESULTS

Asphyxial potentials of the spinal grey matter

The potential field in the cord during asphyxiation. The potential field developing in the cord during asphyxiation was investigated with bipolar electrodes. The tip of one wire was 0·5–0·8 mm more ventral than the other. These electrodes were advanced in steps from the dorsal side into the grey matter. After each step the cord was asphyxiated and the asphyxial potentials recorded. Since after each asphyxiation 10–15 min had to be allowed for recovery, only relatively few asphyxial potentials could be recorded from each preparation.

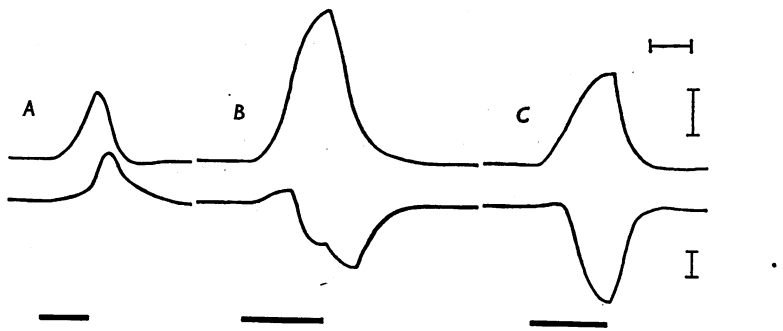

Fig. 1. Lower traces. Recordings of the asphyxial potential with a bipolar electrode located in the dorsal horn (*A*), in the central region of the cord (*B*) and in the ventral horn (*C*). An upward deflexion indicates relative negativity of the more ventral of the two electrode tips. The bars show the periods of asphyxiation. Calibration 1 mV.

Upper traces. Monopolar recordings obtained simultaneously by leading off from the dorsal tip of the bipolar electrode against an indifferent electrode outside the cord. Calibration 5 mV and 1 min.

The records led off in this way from the dorsal grey matter showed that in this region the more ventral tip of the bipolar electrode became negative with respect to the other. In the ventral grey matter the polarity of the potential was reversed as shown in Fig. 1*A* and *C* (lower records). In between these electrode positions there was an area where the potentials were either small in one direction or the other (Fig. 3*B*, middle record), or where the direction of the potential changed during asphyxiation (Fig. 1*B*, lower record). A transient increase of the asphyxial potential at the

start of re-oxygenation was sometimes observed in this region. The magnitude of the potentials led off with bipolar electrodes depended on the electrode distance and placement, the maximum observed was about 5 mV.

The bipolar potentials were correlated with monopolarly recorded potentials by leading off from the dorsal tip of the bipolar electrode, against an indifferent electrode in a pool of Ringer solution surrounding the cord (Fig. 1 *A*, *B* and *C*, upper records). Negativity of the grey matter was in this way recorded during asphyxiation from the ventral as well as from the dorsal horn. In the region where the reversal of the bipolarly recorded potential took place, the negativity of the grey matter with respect to an indifferent electrode showed a maximum which was sometimes very large (up to 25 mV). When the electrode was in the most ventral part of the ventral horn or in the adjoining white matter a small asphyxial positivity was occasionally observed (Fig. 2).

In Fig. 2 the magnitudes of the mono- and bipolarly recorded potentials after 2 min of asphyxiation (when the potential usually reached its maximum) are plotted against the position of the electrode in the cord estimated from the histological preparations (the zero point represents the dorsal surface). This figure demonstrates the reversal of the polarity of the asphyxial potential led off with bipolar electrodes and the maximum of the monopolar potential at the region of reversal of the bipolar potential. Histological study of the cord showed that this region was situated somewhat dorsal of the level of the central canal in the dorsal horn.

When the bipolar electrode was advanced in a transverse direction through the cord, entering a lateral column, then at first an asphyxial potential was recorded which indicated that the deeper electrode became negative with respect to the more superficial one. Then over some distance no consistent potentials were observed, and on reaching the other side of the cord an asphyxial potential was recorded of opposite polarity. In the centre of the grey matter where no consistent potentials were recorded with the bipolar electrode a large asphyxial negativity was led off, monopolarly.

The ventral surface of the cord showed usually a small asphyxial positivity with respect to the dorsal. To demonstrate these potentials it was necessary to reduce the shortening of surface potentials by bathing the cord in a pool of paraffin oil. Leading off from the surface of the dorsal and of the ventral column with respect to a region midway on the lateral column, a negativity of the latter spot with respect to both of the former was consistently observed. The magnitude of these potentials was 2–3 mV.

For the mapping of the asphyxial potential field repeated asphyxiations are necessary which could change the response of the cord to oxygen

deprivation. Figure 3 is a simultaneous recording from the dorsal and ventral horn and from the region in between using a multiple electrode. Potentials were led off from four locations 1 mm apart, providing the possibility of recording bipolarly from three regions at different depth in the grey matter. The simultaneous record (Fig. 3) is, but for the difference in time scale, similar to the consecutively recorded potentials of Fig. 1.

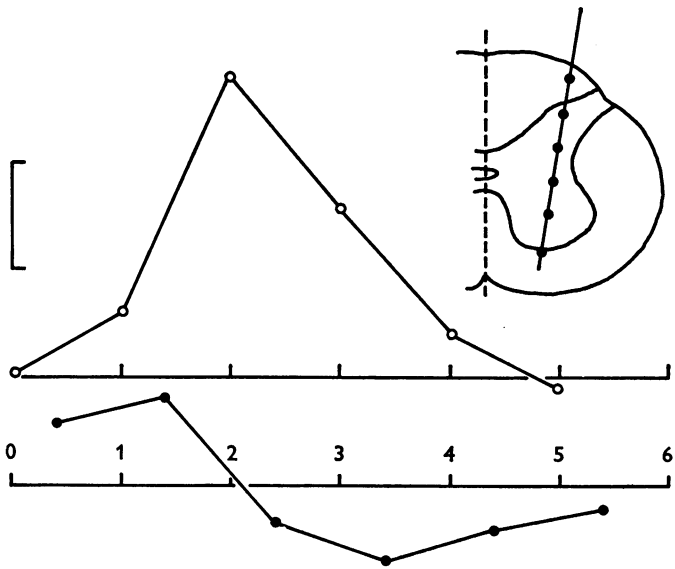

Fig. 2. The magnitude of the monopolar (upper graph) and bipolar (lower graph) potentials after 2 min of asphyxiation from six locations are plotted. The mid point of the bipolar electrode positions, reconstructed from the histological preparations, are indicated as points in the inset. The electrode was advanced in steps of 1 mm. The monopolar records were led off from the dorsal tip of the bipolar electrode. A deflexion above the base line in the lower graph (bipolar) indicates negativity of the ventral tip of the bipolar electrode with respect to the dorsal one; in the upper graph (monopolar) this indicates negativity of the electrode in the grey matter with respect to an indifferent electrode outside the cord. Note that the monopolar potential is maximal somewhat dorsal of the centre of the cord, and that the bipolarly recorded potential reverses at this site. Calibration 5 mV. Penetration of the electrode in mm is plotted on the abscissa.

The position of the electrode shown in the inset of Fig. 3 was estimated from the histological preparation.

The above observations could be explained by the assumption that during asphyxiation two dipoles of opposite polarity develop in the dorsal and ventral grey matter. In both dipoles the poles directed towards the cord surface are the positive ones. The negative poles are directed towards

the centre of the cord (Fig. 4). Differences in the rate of development of the dipoles could well cause a reversal of the bipolar potential in the central region of the cord during asphyxiation, and differences in the rate

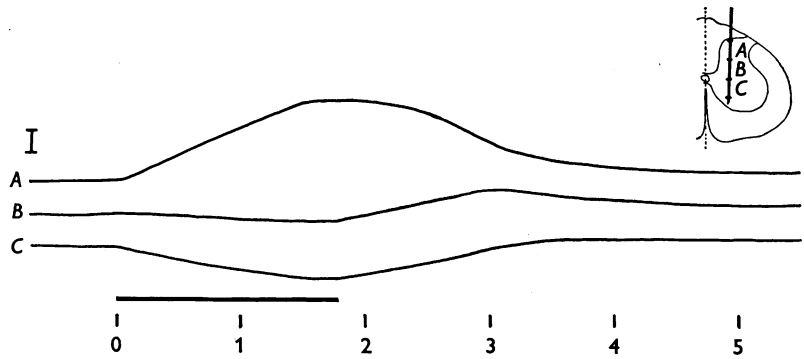

Fig. 3. Simultaneous recording of the asphyxial grey matter potential from three bipolar leads. An electrode was used leading off from the tissue at four points, the locations of which are indicated by the points in the inset. An upward deflexion represents a relative negativity of the more ventral of two electrode tips in each of the bipolar combinations. Calibration 1 mV, time in minutes. Horizontal bar indicates period of asphyxiation.

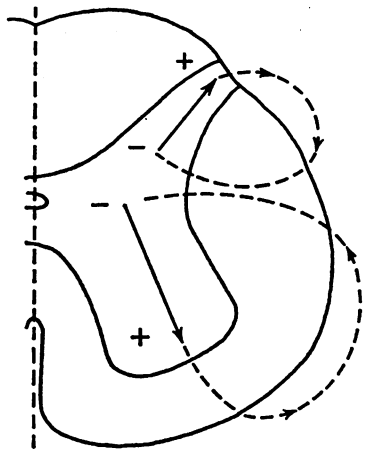

Fig. 4. A schematic representation of the two dipoles postulated to develop in the cord during asphyxiation.

of disappearance of the dipoles during re-oxygenation may explain the post-asphyxial enhancement of the potential (Fig. 1*B*, lower record). These observations support the concept that during asphyxiation two independent dipoles opposite in sign develop in the grey matter.

The nature of the asphyxial spinal grey potential. The spinal asphyxial potential was originally considered as the result of depolarization of somata and dendrites which would form the sink, whereas the more slowly depolarizing motor nerve fibres would form the source of this potential (Van Harreveld & Hawes, 1946). Subsequent experiments made it more likely that the dendrites would form the sink, and the somata and axons the source of the potential (Van Harreveld & Biersteker, 1964). However, other explanations can be considered. Tschirgi & Taylor (1958) recorded potential changes between the blood in a jugular vein and central nervous tissue by changing the CO_2 concentration in the blood. These potentials were ascribed to changes in the hydrogen ion concentration across the blood–brain barrier. It seems unlikely, however, that this mechanism is involved in the asphyxial potentials since the latter were the same (but for a small difference in latency) during asphyxiation by clamping the aorta which can be expected to result in a CO_2 accumulation, and during anoxia by ventilating the preparation with N_2 which, as long as the circulation remains intact, will prevent an increase of the CO_2 concentration in the blood.

There is histochemical and electron microscopic evidence that during asphyxiation of the cerebral and cerebellar cortex a shift of chloride and water into dendritic elements occurs (Van Harreveld, 1957, 1961; Van Harreveld & Schadé, 1959; Van Harreveld, Crowell & Malhotra, 1965). Also in the spinal grey matter such a shift seems to occur (Van Harreveld & Biersteker, 1964). The silver–silver chloride electrodes used are chloride sensitive and the electrolyte shift could perhaps result in a change in the chloride concentration of the tissue debris surrounding the electrodes. The asphyxial potentials were therefore led off with an electrode system which is insensitive to changes in chloride concentration (Adrian, 1956). Coarse glass pipettes (0·1–0·2 mm in diameter), the tips of which had been filled with 4 % agar in Ringer solution, were used. The rest of the electrodes was filled with a NaCl solution into which silver-chloride plated silver wires dipped. One of the electrodes was placed in the spinal grey matter, the other was situated in a pool of Ringer solution surrounding the cord. With these electrodes asphyxial potentials of the usual magnitude and time course were recorded, indicating that the asphyxial potentials are not caused by changes in chloride concentration.

The original concept based on differential depolarization of neurones is supported by experiments in which the nerve cells in the cord had been destroyed by 60 min asphyxiation 2 weeks before the experiment. These cords failed to show asphyxial potentials (Van Harreveld & Hawes, 1946). This observation has been extended in the present series of experiments to preparations asphyxiated for 30–35 min. The majority of the inter-

neurones, but also 65–80 % of the motoneurones are destroyed in this way (Van Harreveld & Schadé, 1962; Biersteker & Van Harreveld, 1963). In some of these preparations no asphyxial potential, in others small and atypical potentials were recorded. These observations support the neuronal origin of the asphyxial potentials, although the possibility cannot be excluded that glia, which in a favourable object (molecular layer of the cerebellum, Van Harreveld, 1961) exhibits a similar asphyxial chloride and water uptake as dendrites, also contributes to asphyxial potentials.

Asphyxial potentials led off from spinal roots

Ventral roots. If the asphyxial potential is caused by differential depolarization of neurones including motoneurones, then electrotonically conducted potential changes can be expected in the ventral roots during oxygen deprivation. Such potentials were led off with the electrodes shown in the inset of Fig. 7. The root and cotton loops were submerged in a pool of paraffin oil. This electrode arrangement minimizes disturbing effects

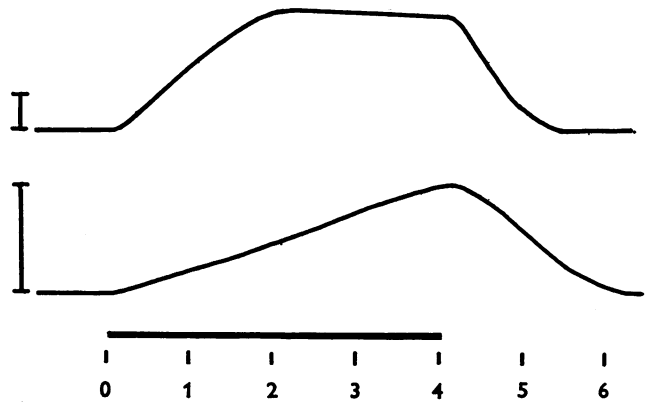

Fig. 5. Upper trace. Asphyxial cord potential recorded with a bipolar electrode from the ventral horn. Upward deflexion indicates negativity of the dorsal electrode tip. Calibration 1 mV.

Lower trace. Simultaneous record of the asphyxial ventral root (S_1) potential using the electrodes shown in the inset of Fig. 7. Upward deflexion indicates relative negativity of the electrode nearest to the cord. Calibration 1 mV, time in minutes. Horizontal bar indicates period of asphyxiation.

of changes in temperature and ion concentration owing to the arrest of the circulation. Figure 5 shows an asphyxial potential obtained in this way from the ventral root (S_1) and a bipolarly recorded asphyxial potential from the ventral horn. A potential develops in the root (proximal electrode negative with respect to the distal one) which has the same short latency

as the spinal grey potential. The latency was often unusually short (not more than a few sec) in these experiments which may have been due to an impairment of the circulation in the cord caused by the severing of roots. The spinal grey potential reaches a maximum after about 2 min of asphyxiation but the ventral root potential keeps on rising during the entire period of O_2 lack reaching a maximum of about 1 mV in 4 min. Such a period of asphyxiation has been found to cause a soma depolarization of 12–15 mV (Collewijn & Van Harreveld, 1966*a*). With a length constant for the ventral root of 4·5–6·5 mm (Eccles, 1946) such a soma depolarization would seem to be sufficient to cause the asphyxial ventral root potential observed, especially since all motoneurones can be expected to depolarize in this way.

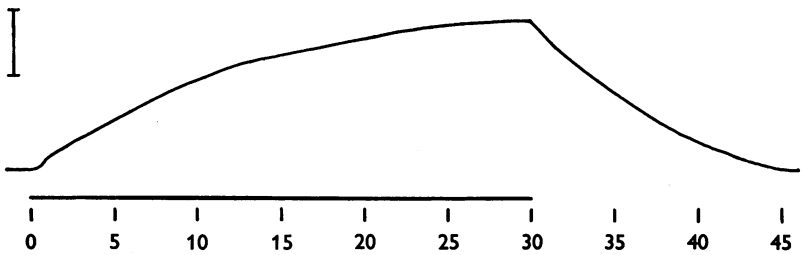

Fig. 6. Recording from a ventral root (S_1), during and after a period of asphyxiation of 30 min duration. Upward deflexion indicates relative negativity of the electrode nearest the cord. Drift has been eliminated from the record by assuming that the beginning and the end of the graph represent the same root potential. Calibration 1 mV, time in min. Bar indicates period of asphyxiation.

The difference in the course of the asphyxial potentials led off from the spinal grey matter and from the ventral root becomes more pronounced with longer asphyxiations. Figure 6 shows a 30 min asphyxiation. The ventral root potential keeps on rising during the entire period of asphyxiation although at a diminishing rate. The maximum ventral root potential recorded in this experiment was 2·5 mV. Potential recordings of this duration invariably show base line shifts. The curve in Fig. 6 was constructed on the assumption that the position of the recording pen before asphyxiation and after recovery therefrom indicated the same tissue potential. The course of this ventral root potential is quite different from the asphyxial potential of the spinal grey matter which slowly declines after reaching a maximum in 2–2·5 min (Van Harreveld & Biersteker, 1964). Release of the aorta clamp after 4 min of asphyxiation caused a reversal of the potential in a few minutes (Fig. 5). Even after 30 min of asphyxiation the ventral root potential reversed (Fig. 6). As could be expected asphyxial ventral root potentials were not present in prepara-

tions in which the nerve cells had been destroyed by asphyxiation for 60 min, 2 weeks before the experiment.

Dorsal roots. Asphyxial potentials similar to the ventral root potential could be led off from the dorsal roots. Figure 7 shows such a potential and a monopolarly recorded spinal grey potential during a cord asphyxiation of 3 min duration. The course of the dorsal root potential is again different from the grey matter potential. The former grows for the entire duration of the asphyxial period, whereas the spinal grey potential approaches its maximum in about 2 min. Re-oxygenation of the cord reverses both potentials in a few minutes.

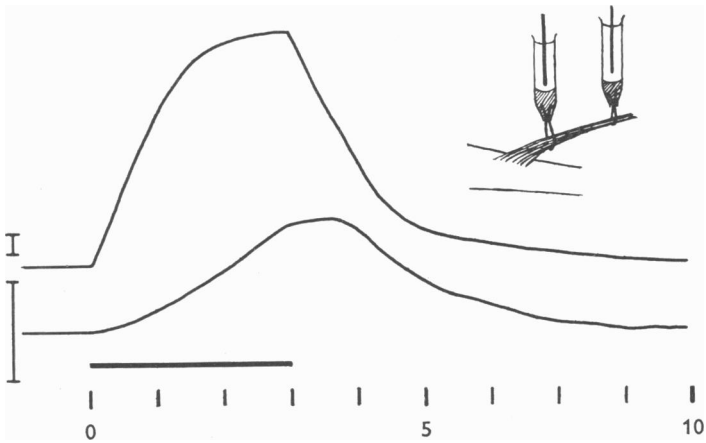

Fig. 7. Upper trace. Asphyxial cord potential recorded with a monopolar electrode. Upward deflexion indicates grey matter negativity. Calibration 1 mV.

Lower trace. Simultaneous recording from the dorsal root of S_1. Upward deflexion represents relative negativity of the electrode nearest to the cord. Calibration 1 mV, time in minutes. Period of asphyxiation is indicated by the bar.

The inset shows the electrode arrangement used for the recording of root potentials. A cotton loop was fixed with 4 % agar in Ringer solution in a glass tube filled with saline in which a silver chloride plated silver electrode dipped. One electrode was placed as close to the cord as feasible, the other near the cut end. Root and cotton loops were covered with mineral oil. Special care was taken to avoid contact of the root with the preparation at any other place than at the origin of the root.

The asphyxial dorsal root potential could be explained by the assumption that during asphyxiation depolarization of the intraspinal section of the primary sensory fibres occurs which is electrotonically conducted over the dorsal root. This concept is supported by experiments in which the dorsal and ventral roots had been severed extradurally 1 week to 10 days previously resulting in degeneration of the dorsal root. Asphyxial dorsal root potentials could not be demonstrated in these preparations, although

the usual asphyxial spinal grey potentials were present. Figure 8 shows a record led off from the ventral and dorsal root of such a preparation during aorta clamping. The ventral root potential is present but the potential in the dorsal root remains stationary. It would seem therefore that also the endings of the primary sensory fibres depolarize during asphyxiation. No asphyxial potentials could be led off from the dorsal roots in preparations in which the cord had been asphyxiated for 60 min 2 weeks previously. This may seem surprising since such cord asphyxiations (by increasing the pressure in the dural cavity above the blood pressure) do not affect the primary sensory fibres (Van Harreveld, 1940).

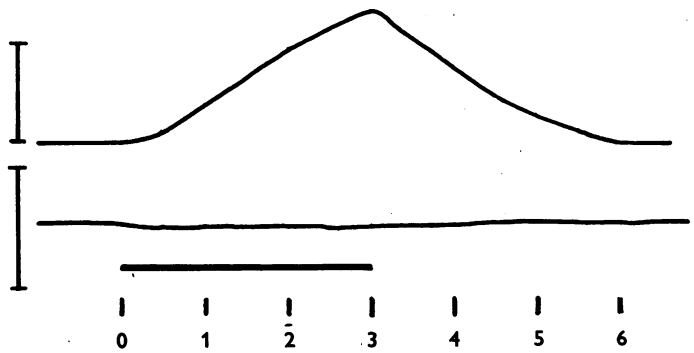

Fig. 8. Recordings from the ventral (upper trace) and dorsal root (lower trace) of a preparation in which these roots (S_1) had been sectioned extradurally 2 weeks previously. Horizontal bar indicates period of asphyxia. Note the absence of the asphyxial potential in the dorsal root. Calibration 1 mV, time in minutes.

DISCUSSION

The reduction or absence of the asphyxial potential in the spinal grey matter of cats in which a large percentage of the nerve cells had been destroyed by a previous asphyxiation suggested that this potential is produced by neurones, probably by the differential depolarization of certain parts of the nerve cell. The original suggestion (Van Harreveld & Hawes, 1946) that the somata and dendrites would depolarize, and that the axons would form the source of the asphyxial potential is unlikely in view of the slight soma depolarization during the period (2–2·5 min) in which the asphyxial potential grows to its maximum (Collewijn & Van Harreveld, 1965*a*). An asphyxial water and chloride uptake by cerebral cortical (Van Harreveld, 1957; Van Harreveld & Schadé, 1959), cerebellar (Van Harreveld, 1961) and spinal dendrites (Van Harreveld & Biersteker, 1964) has been found whereas little or no chloride uptake by the somata was seen. The observations are in agreement with the concept that the

dendrites depolarize during the development of the asphyxial potential and the somata and axons form the main source of these potentials.

Two mechanisms, asphyxial arrest of ion pumps and increase in Na permeability of the cell membrane, have been proposed which can both explain the depolarization as well as the electrolyte and water transport into dendrites (Van Harreveld, 1966). The arrest of the ion pumps, preventing the removal of the Na which continually diffuses into the cell, could cause a more rapid depolarization of dendrites than of somata because of the larger surface to volume ratio of the former (Collewijn & Van Harreveld, 1965*a*). In the second mechanism it was assumed that an asphyxial increase in sodium permeability of the dendritic membrane results in Donnan forces which move extracellular NaCl accompanied by water into the dendrites (Van Harreveld & Ochs, 1956). The release of a compound like glutamic acid could cause the postulated increase in membrane permeability for Na (Van Harreveld, 1959). For the asphyxial potential of the cerebral cortex the second explanation seems more likely since this potential develops suddenly after a relatively long latency (2–3 min in rabbits, Leão, 1947, 1951; up to 6 min in cats, Van Harreveld, 1957). Furthermore, potential changes, an increase in tissue impedance and a chloride and water transport into apical dendrites in all respects comparable with the asphyxial potential and its concomitants, have been observed in the cerebral cortex during spreading depression (Van Harreveld & Ochs, 1957; Van Harreveld, 1958; Van Harreveld & Schadé, 1959) which is the result of cortical stimulation, and not caused by oxygen deprivation of the tissue (Van Harreveld & Ochs, 1957; Marshall, 1959). For the spinal asphyxial potential both mechanisms proposed seem possible, although in view of the above mentioned features of cortical asphyxiation and spreading depression and of the similarity of spinal and cortical asphyxial potentials the assumption of a change in membrane permeability is perhaps more likely.

The depolarization of spinal neurones after circulatory arrest could be conceived as follows. Dendritic depolarization would start after a short latency (from a few to 10 sec) and would be near completion after 2–2·5 min, resulting in the development of the asphyxial grey matter potential. Soma depolarization proceeds much slower, at a rate of 3–4 mV/min. (Collewijn & Van Harreveld, 1966*a*), suggesting that the soma potential reaches a low value 15–20 min after the start of O_2 deprivation. Depolarization of the intraspinal portion of the motoneurone may take considerably longer. The ventral root was found to remain excitable for 1 hr under the conditions of the present experiments, from which it can be surmised that the root did not depolarize markedly during this time. Such a sequence not only accounts for the development of the asphyxial grey

matter potential but also for its slow decline (Van Harreveld & Biersteker, 1964) since the sources of this potential (soma and intraspinal part of the motor axons) depolarize slowly. The depolarization of motoneurones starting with the dendrites and ending with the intraspinal part of the motoneurones is furthermore in agreement with the potentials led off from the ventral root which started with a short latency and slowly increased during long (30 min) asphyxiations.

Repeated probing of the spinal cord with micro-electrodes showed potential jumps indicative of the penetration of cellular elements, which slowly declined in the course of 60–90 min asphyxiation (Van Harreveld & Biersteker, 1963; Van Harreveld, 1964). It was assumed that the impaled structures were somata. However, in view of the relatively rapid drop in the soma potential (15–20 min) observed with indwelling micro-electrodes (Collewijn & Van Harreveld, 1966*a*) this conclusion has to be revised and it has to be surmised that during probing with the micro-electrode not only the membrane potentials of somas but also those of axons were recorded.

The mechanism proposed for the asphyxial grey matter potential requires not only a differential depolarization of neurones, but also a more or less uniform orientation of the nerve cells in the spinal cord. Such an arrangement exists in the cerebral cortex where the somata of pyramidal cells and their apical dendrites are all located in the same direction with respect to the cortical surface. In the spinal cord such an orientation is much less obvious although a measure of orientation in a dorso–ventral direction of the dendrites in the dorsal horn accumulating Cl during asphyxiation was observed (Van Harreveld & Biersteker, 1964). To account for the different direction of the dipoles developing in the cord during asphyxiation the orientation of the majority of the somata and their dendrites would have to be opposite in the dorsal and ventral horns. The large values of the asphyxial potential recorded somewhat dorsal of the spinal canal may be caused by the summation of the potentials of serially placed neurones. Furthermore, the increase in tissue resistance observed during asphyxiation will enhance the potentials which can be led off by limiting the shunt on the potentials of individual elements.

Potential changes led off from the dorsal root indicate that during asphyxiation depolarization of the intraspinal part of the primary sensory neurones takes place. Depolarization of the endings of these neurones could explain the failure of the presynaptic terminals to produce an EPSP after 2–3 min asphyxiation (Collewijn & Van Harreveld, 1965*a*). The depolarization and functional arrest of the endings of the sensory neurones is reminiscent of the mechanism of presynaptic inhibition proposed by Eccles, Kostyuk & Schmidt (1962). It would seem possible that the

asphyxial depolarization of primary sensory neurones is caused by a release of the transmitter substance from the interneurones normally involved in presynaptic inhibition. This possibility is supported by the observation that asphyxiation of the cord for 60 min 2 weeks previously abolished the asphyxial potential in the dorsal root. Such long asphyxiations do not seem to affect the primary sensory fibre (Van Harreveld, 1940), but cause extensive destruction of interneurones probably including those responsible for presynaptic inhibition. This would result in the loss of the mechanism postulated to cause asphyxial depolarization of the primary sensory endings. The observation that previous asphyxiation for 30–35 min makes the EPSP (and orthodromic conduction) quite insensitive to renewed asphyxiation (Collewijn & Van Harreveld, 1966*b*) could be explained in the same way by the severe interneuronal destruction found in such cords. Indeed, recovery cycles recorded from the latter preparations often showed a less severe and shorter lasting post-activation depression than found in normal controls, which was explained, at least in part, by damage to the presynaptic inhibitory mechanism (Van Harreveld & Spinelli, 1965).

The postulated asphyxial depolarization of primary sensory endings suggests an additional mechanism for the asphyxial grey matter potential in which the endings would form the sinks, the primary sensory fibres the sources of the potential.

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REFERENCES

ADRIAN, R. H. (1956). The effect of internal and external potassium concentration on the membrane potential of frog muscle. *J. Physiol.* **133**, 631–658.

BIERSTEKER, P. A. & VAN HARREVELD, A. (1963). The nature of the rigidity caused by spinal cord asphyxiation. *J. Physiol.* **166**, 382–394.

COLLEWIJN, H. & VAN HARREVELD, A. (1966*a*). Intracellular recording from cat spinal motoneurones during acute asphyxia. *J. Physiol.* **185**, 1–14.

COLLEWIJN, H. & VAN HARREVELD, A. (1966*b*). Intracellular recording from spinal motoneurones in cats with post-asphyxial rigidity. *J. Physiol.* **185**, 30–41 .

ECCLES, J. C. (1946). Synaptic potentials of motoneurones. *J. Neurophysiol.* **9**, 87–120.

ECCLES, J. C., KOSTYUK, P. G. & SCHMIDT, R. F. (1962). Central pathways for depolarization of primary afferent cells. *J. Physiol.* **161**, 237–257.

LEÃO, A. A. P. (1947). Further observations on the spreading depression of activity in the cerebral cortex. *J. Neurophysiol.* **10**, 409–414.

LEÃO, A. A. P. (1951). The slow voltage variation of cortical spreading depression of activity. *Electroenceph. clin. Neurophysiol.* **3**, 315–321.

MARSHALL, W. H. (1959). Spreading cortical depression of Leão. *Physiol. Rev.* **39**, 239–279.

TSCHIRGI, R. D. & TAYLOR, J. L. (1958). Slowly changing biolectric potentials associated with the blood–brain barrier. *Am. J. Physiol.* **195**, 7–22.

VAN HARREVELD, A. (1940). The effect of asphyxiation of the spinal cord on pain sensibility. *Am. J. Physiol.* **131**, 1–9.

VAN HARREVELD, A. (1957). Changes in volume of cortical neuronal elements during asphyxiation. *Am. J. Physiol.* **191**, 233–242.

VAN HARREVELD, A. (1958). Changes in the diameter of apical dendrites during spreading depression. *Am. J. Physiol.* **192**, 457–463.

VAN HARREVELD, A. (1959). Compounds in brain extracts causing spreading depression of cerebral cortical activity and contraction of crustacean muscle. *J. Neurochem.* **3**, 300–315.

VAN HARREVELD, A. (1961). Asphyxial changes in the cerebellar cortex. *J. cell. comp. Physiol.* **57**, 101–110.

VAN HARREVELD, A. (1962). Water and electrolyte distribution in central nervous tissue. *Fedn Proc.* **21**, 659–664.

VAN HARREVELD, A. (1964). Effects of spinal cord asphyxiation. *Prog. Brain Res.* **12**, 280–307.

VAN HARREVELD, A. (1966). *Water and Electrolyte Distribution in Central Nervous Tissue.* Washington: Butterworth Inc. (In the Press.)

VAN HARREVELD, A. & BIERSTEKER, P. A. (1963). Asphyxial depolarization of spinal and cortical cells. *Fedn Proc.* **22**, 280.

VAN HARREVELD, A. & BIERSTEKER, P. A. (1964). Acute asphyxiation of the spinal cord and of other sections of the nervous system. *Am. J. Physiol.* **206**, 8–14.

VAN HARREVELD, A., CROWELL, J. & MALHOTRA, S. K. (1965). A study of extracellular space in central nervous tissue by freeze-substitution. *J. cell Biol.* **25**, 117–137.

VAN HARREVELD, A. & HAWES, R. C. (1946). Asphyxial depolarization in the spinal cord. *Am. J. Physiol.* **147**, 669–684.

VAN HARREVELD, A. & OCHS, S. (1956). Cerebral impedance changes after circulatory arrest. *Am. J. Physiol.* **187**, 180–192.

VAN HARREVELD, A. & OCHS, S. (1957). Electrical and vascular concomitants of spreading depression. *Am. J. Physiol.* **189**, 159–166.

VAN HARREVELD, A. & SCHADÉ, J. P. (1959). Chloride movements in cerebral cortex after circulatory arrest and during spreading depression. *J. cell. comp. Physiol.* **54**, 65–77.

VAN HARREVELD, A. & SCHADÉ, J. P. (1962). Nerve cell destruction by asphyxiation of the spinal cord. *J. Neuropath. exp. Neurol.* **21**, 410–423.

VAN HARREVELD, A. & SPINELLI, D. (1965). Reflex activity in spinal cats with post-asphyxial rigidity. *Archs int. Physiol. Biochim.* **73**, 209–230.