SUMMARY In rats with unilateral carotid artery ligation pyramidal tract responses were studied during hypoxia and during trimethaphan-induced hypotension. Observations on EEG activity during hypoxia suggest that unilateral carotid artery ligation produces a more severe perfusion defect in lateral portions of the hemisphere. During hypoxia and during trimethaphan-induced hypotension direct PTRs disappeared first from the hemisphere on the side of carotid artery ligation and next from the opposite hemisphere. This was followed by loss of direct PTRs in the same order. Animals could not be resuscitated once the direct PTR from the non-ligated hemisphere had disappeared. Hypotension appears to be a late contributing factor in impairing electrocerebral activity during hypoxia in this study.

Introduction

IT IS GENERALLY ACCEPTED that the human brain cannot survive anoxia for longer than approximately 5 to 10 minutes. During the last few years Hossmann and associates have demonstrated in the cat that some electrical activity of the brain returns even after one hour of anoxia. None of the cats which were made anoxic could be kept alive long enough to determine the extent of clinical recovery. Myers could not demonstrate residual impairment of neurological function in monkeys after circulatory arrest of less than 14 minutes. In some animals severe neurological deficits persisted for as long as 48 hours before complete recovery occurred. During hypoxia EEG activity is lost rapidly within 10 to 60 seconds. In the visual system hypoxia first produces loss of synaptic activity in the occipital cortex and only later is synaptic activity impaired in the lateral geniculate body. Hossmann and Sato have shown that pyramidal tract responses (PTRs) persist during anoxia in the absence of spontaneous EEG activity. Since the disappearance of EEG activity during hypoxia is not a reliable parameter to determine irreversible brain damage, we evaluated the disappearance of PTRs to determine if this was associated with irreparable brain damage. We chose the Levine anoxic-ischemic rat model because it has previously been examined pathologically, biochemically and electrophysiologically.

Pyramidal tract responses consist of direct early and indirect later components. The early response is the result of direct stimulation of pyramidal neurons. The later responses are smaller in amplitude and are due to activation of pyramidal neurons by cortical interneurons. We were thus able to study the effect of hypoxia on direct excitability of neurons and on synaptic activity. In the Levine model the hemisphere on the side of carotid ligation is impaired in its function earlier than the opposite hemisphere. The hemisphere with the patent blood supply therefore serves as control in each animal.

In earlier studies we were not certain if death of animals during hypoxia was related primarily to cardiovascular or cerebral dysfunction. Cardiovascular function was therefore assessed by continuous monitoring of EKG and blood pressure. In order to ascertain how much hypotension contributes to cerebral dysfunction during hypoxia we studied animals which were well oxygenated but were made hypotensive by injection of trimethaphan.

Methods

Sprague-Dawley rats weighing between 250 and 400 gm were anesthetized with 15 to 30 mg of pentobarbital intraperitoneally. Following the insertion of a tracheostomy tube the carotid system was dissected free on the left side of the neck. The internal and external carotid arteries were ligated separately. A PE 60 catheter was threaded into the left common carotid artery and advanced toward the aortic arch. Blood pressure was measured by connecting this catheter to a Statham 23 transducer.

The animal was then placed in a stereotactic headholder and the skull exposed. Part of the calvarium over each frontal lobe was removed for placement of bipolar cortical stimulating electrodes. Two gold-plated screws were inserted in the bone over both parietal areas, one located medially

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and the other laterally. EEG, EKG and blood pressure were simultaneously recorded on a polygraph. The motor cortex was electrically stimulated with single square wave pulses with a duration of 0.3 msec and an amplitude of 15 to 150 volts. Pyramidal tract responses in the medulla were recorded with pairs of electrodes placed in the pyramidal tract in a rostrocaudal manner. Coordinates from *The Rat Brain* were used but the final depth of the electrodes was determined by lowering the electrodes until optimal responses were obtained. Potentials were displayed on a Tektronix RM 565 oscilloscope and were photographed and later measured by projecting them on a screen. After all electrodes were placed the animal was curarized and placed on a small animal respirator. Rectal temperature was kept constant at 37°C by a heat lamp. Twenty animals were made hypoxic by mixing various amounts of nitrogen and oxygen. This mixture was adjusted to a level which abolished EEG activity without causing cardiac arrhythmias. Eleven animals were ventilated with oxygen but were made hypotensive with intravascular injection of 10 to 150 mg trimethaphan.

**Results**

Resting systolic blood pressure varied between 120 and 180 mm Hg and diastolic values ranged from 90 to 120 mm Hg. During most of the experiments the mean blood pressure was kept between 50 and 80 mm Hg. At these levels the EKG remained unchanged. When the nitrogen content of the inhaled nitrogen-oxygen mixture was increased to lower blood pressure further, the QRS complex widened and increased in amplitude and S-T segment elevation occurred. Furthermore, numerous PVCs appeared and many animals had second-degree heart block. Even after blood pressure had fallen to zero the EKG continued often at rates of about 200 per minute for several minutes.

A few animals were noted to have an asymmetric EEG during the control period prior to induction of hypoxia. This asymmetry consisted of lower voltage EEG activity over the hemisphere on the side of carotid ligation. The decrease in amplitude was more striking over the lateral electrode compared with the medial electrode. Animals with pre-hypoxic EEG asymmetries were not included in this study. Following induction of hypoxia initial changes of the EEG consisted of loss of activity in the 20 to 50 Hz range and appearance of irregular 1 to 2 Hz waves. Amplitude of the remaining activity gradually decreased until it completely disappeared. In the majority of animals burst activity with a frequency of 14 to 16 Hz occurred just before all EEG activity disappeared. The sequential EEG changes during hypoxia were similar in the two hemispheres but always occurred later over the hemisphere with the patent carotid system.

The time required for total suppression of EEG activity varied between two and eight minutes after adding nitrogen to the inspired air. The shorter time was always found in those animals whose blood pressure fell rapidly. Changes in EEG activity were usually seen over the ligated hemisphere when the mean blood pressure decreased below 100 mm Hg, while they were not apparent over the non-ligated hemisphere until the mean blood pressure fell below 75 mm Hg. When nitrogen administration was stopped blood pressure promptly returned to resting levels. EEG activity returned to normal first over the hemisphere with the patent carotid system followed by normalization of EEG activity over the opposite hemisphere. Intermittent burst activity with a frequency of 14 to 16 Hz was usually the earliest indication of return of EEG activity. In one animal EEG activity returned when nitrogen administration was stopped even though the EEG had been absent for one hour. In all other animals EEG activity did not return after it had been absent for 25 to 30 minutes even though recording was continued for several hours following termination of hypoxia.

Neither the direct nor the indirect pyramidal tract responses changed shortly after the EEG was completely abolished. As hypoxia progressed the multiple indirect responses disappeared first. Before they were abolished their amplitude decreased. The indirect responses first disappeared with stimulation of the hemisphere on which side the carotid system was ligated. The direct responses gradually decreased in amplitude but did not disappear until after the indirect responses had disappeared. While the amplitude decreased, latency and duration of direct responses increased. Similar changes and the ultimate disappearance of the direct response were temporally delayed with stimulation of the hemisphere whose carotid was patent (see figs. 1 and 2). The order of loss of pyramidal tract responses was as follows: (1) multiple indirect responses from the ligated side; (2) multiple indirect responses from the non-ligated side; (3) first indirect response from the ligated side; (4) first indirect response from the non-ligated side; (5) direct response from the ligated side; and (6) direct response from the non-ligated side. Re-oxygenation of the animals at various stages of hypoxia produced return of pyramidal tract responses in reverse order of their disappearance. There were two exceptions. When the animal was kept hypoxic until the direct response was lost from both hemispheres, hypotension or ventricular arrhythmias caused death of the animal. However, it was possible to abolish the direct response from the ligated hemisphere and have it return following re-oxygenation of the animal. The second exception related to the multiple secondary responses

![Figure 1. Sequential changes of pyramidal tract responses during hypoxia. Left carotid artery ligated. A: Control, B: early hypoxia, C: two minutes later than B, and D: one minute after C.](http://stroke.ahajournals.org/)

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which often did not return following interruption of hypoxia.

Since nitrogen hypoxia produced hypotension we wanted to assess the effect of lowering the blood pressure on pyramidal tract responses and induced hypotension with trimethaphan. The amount of trimethaphan required to lower blood pressure to similar levels as during hypoxia was quite variable. When blood pressure was lowered below 100 mm Hg the EEG from the ligated side was lost within two minutes and within two and one-half minutes from the non-ligated side. EEG activity rapidly returned when the blood pressure was raised to normal levels at this time. Pyramidal tract responses were attenuated and disappeared in the same order as in the hypoxic animals (see figs. 3 and 4). First to be lost were the multiple indirect responses from the ligated side followed by loss of multiple indirect responses from the non-ligated side. All indirect responses on the ligated side were lost within six to seven minutes and within seven to eight minutes on the non-ligated hemisphere. The direct response on the ligated side persisted as long as 15 minutes and as long as 18 minutes on the non-ligated side. Direct and indirect responses returned when the blood pressure was raised to control values immediately after their disappearance.

Discussion

In previous experiments with spontaneously breathing animals, hypoxia was complicated by apnea requiring frequent resuscitations. This was eliminated in the present
study by mechanically ventilating curarized animals. Nitrogen-induced hypoxia eventually produced hypotension, cardiac arrhythmias and EKG changes in all animals. The changes in EKG morphology were similar to those reported in the monkey by Massopust et al. The time course of development of hypotension and cardiac abnormalities was quite variable from animal to animal. Cardiac rates below three per second and/or mean arterial pressures below 30 mm Hg were incompatible with successful resuscitation.

Nitrogen administration per se does not produce narcosis when administered with less than three atmospheres of pressure. The electrophysiological changes observed in the present experiments therefore are secondary to hypoxia. When mean arterial blood pressure was maintained well above 50 mm Hg during hypoxia EGG activity disappeared and a progressive decrease of PTRs occurred, particularly with stimulation of the hemisphere on the side of carotid ligation. PTRs on both sides did not disappear until systemic blood pressure had fallen well below 50 mm Hg. These observations are in agreement with findings by Etholm et al. that nitrogen hypoxia depressed neuronal activity beyond that which could be expected from hypotension alone. In our experiments trimethaphan-induced hypotension produced similar sequential changes of the PTRs as observed during hypoxia. This does not imply that in hypoxia impairment of neuronal function is solely due to hypotension because, as discussed above, impairment of function precedes significant lowering of systemic blood pressure. Furthermore, trimethaphan may have a direct effect on cortical function separate from its effect on blood pressure. As illustrated in figure 4 secondary PTRs do not recover completely or not at all when adequate perfusion pressure is re-established with neosynephrine after trimethaphan-induced hypotension.

As reported by many investigators the EGG disappears early during hypoxia and the time from onset of hypoxia to electrocortical silence is not a reliable parameter to predict ultimate outcome of cerebral function. On the other hand, when EGG activity remained absent in our experiments for longer than 30 minutes almost all animals eventually died. This occurred even when normal perfusion pressure could be re-established after hypoxia of 30 minutes' duration.

EEG asymmetries when present before or during hypoxia were always more prominent when recording from the lateral cortical electrode on the side of carotid artery ligation. This observation suggests that medial parts of the hemisphere on the ligated side are better perfused than more lateral parts. This interpretation is supported by histological findings that medial cortical areas are frequently spared while lateral ones are infarcted.

Sensory-evoked potentials, cortical steady potentials, strychnine-induced spikes and pyramidal tract responses persist for some time after EEG activity is abolished during hypoxia. In agreement with these observations we also found persistence of PTRs after disappearance of EEG activity. The most sensitive of these responses were the late indirect PTRs which depend upon multisynaptic pathways. The next response to be abolished was the first secondary potential followed by disappearance of the direct response. Patton and Amassian reported that blood loss, cooling, mechanical injury, drying of the cortex and deep barbiturate anesthesia had a much earlier and more severe effect on indirect responses than on direct responses of the PTR. In our studies indirect and direct responses were always decreased and eventually abolished on the side of carotid ligation before similar changes took place in the opposite hemisphere. This indicates that carotid ligation effectively reduces cerebral blood flow on the same side, enhancing the effect of hypoxia.

Whenever hypoxia was terminated prior to loss of the direct PTR on the non-ligated side, most animals could be resuscitated with restoration of direct and indirect PTRs and EGG activity. All animals died when the direct response on the ligated side was allowed to disappear. Kayama observed in cats that EGG and visual evoked potentials recovered almost completely when animals were resuscitated before presynaptic activity of visual cortex disappeared during anoxia. These findings are very similar to ours since the direct response of the PTR is due to direct excitation of pyramidal neurons.

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