Effect of Reduced Oxygen Availability upon Myogenic Depolarization and Contraction of Cat Middle Cerebral Artery

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SUMMARY. The goal of this study was to determine whether electrophysiological mechanisms contribute to the relaxation of cat middle cerebral artery in response to decreased ambient Po2 and whether decreased Po2 alters the myogenic depolarization and contraction of this vessel in response to elevations in transmural pressure. In one series of experiments, arterial segments (200–500 μm outer diameter) were isolated and mounted in an in vitro tension transducer to allow continuous measurement of active tension as bath Po2 was reduced. In these experiments, vessel relaxation occurred primarily between 150 mm Hg Po2 and 40 mm Hg Po2, suggesting that cerebral arteries are sensitive to alterations of Po2 in the physiological range. Relaxation did not result from the activation of dilator nerves in the vessel wall, since it was unaffected by tetrodotoxin. Arterial segments were also cannulated with micropipettes and subjected to elevations in transmural pressure during 300 mm Hg Po2 and 50 mm Hg Po2 superfusion. During 300 mm Hg Po2 superfusion, cannulated vessels exhibited myogenic depolarization and maintained their diameter as transmural pressure was increased; 50 mm Hg Po2 superfusion inhibited spontaneous spike activity, decreased the slope of the myogenic depolarization, and partially inhibited vessel contraction in response to elevated transmural pressure. These effects are independent of the parenchymal cell environment and appear to be mediated, at least in part, by electrophysiological mechanisms. (Circ Res 58: 565-569, 1986)

CEREBRAL arteries exhibit contraction, depolarization, and increased electrical spike activity in response to elevations of transmural pressure, suggesting that myogenic mechanisms play an important role in cerebral blood flow autoregulation (Harder, 1984). However, oxygen-dependent vascular control mechanisms also exist in some circulatory beds, and these may alter the effectiveness of myogenic mechanisms in regulating regional blood flow (Lombard and Duling 1981; Morff and Granger, 1982). Nonetheless, the exact nature of the interaction between myogenic and oxygen-dependent mechanisms remains unclear, especially at the level of the vascular smooth muscle (VSM) cell membrane. A number of studies (Carrier et al., 1964; Smith and Vane, 1966; Coburn et al., 1979; Detar, 1980; Ebeigbe et al., 1980; Jackson and Duling, 1983) have suggested that O2 can regulate active tone in blood vessels via a direct effect upon the VSM. Johansson and Somlyo (1980) have postulated that electrophysiological changes could occur in VSM as a direct result of the effect of altered Po2 upon some sensor located in the cell membrane, and that these in turn might control active tone in the vessel during alterations in local O2 availability. In addition, several studies have indicated that electrophysiological mechanisms regulate VSM contractile force in portal vein (Gurevich et al., 1977; Sigurdsson and Grampp, 1981) and ductus arteriosus (Noel et al., 1973; Roulet and Coburn, 1981) during alterations in local Po2. The goal of the present study was to determine whether electrophysiological mechanisms could mediate the responses of cerebral arteries to reduced O2 availability, and if reduced Po2 can affect the myogenic contraction and depolarization of cerebral vessels in response to increased transmural pressure. The results of these experiments indicate: (1) that isolated middle cerebral arteries relax in response to reductions of Po2 in the physiological range, (2) that reduced O2 availability inhibits action potentials in the middle cerebral artery, and (3) that decreased Po2 reduces but does not eliminate the myogenic depolarization and contraction of cerebral VSM in response to increases in transmural pressure.

Methods

Mongrel cats (3–5 kg) were premedicated with ketamine and anesthetized with 30 mg/kg pentobarbital, ip. The animals were then exsanguinated and decapitated. The brain was removed and placed in ice cold physiological salt solution (PSS), pH 7.35. The PSS had the following composition (in mm): Na+, 141; K+, 4.7; Ca++, 2.5; Mg++, 0.72; Cl-, 124; H2PO4-, 1.7; HCO3-, 25; glucose, 11.0; and HEPES, 5.0. Middle cerebral arteries were carefully removed, taking care not to stretch or damage the vessel.
The vessels were then transferred to an organ bath maintained at 37°C and containing PSS equilibrated with 95% O2-5% CO2.

In one series of experiments, an in vitro tension transducer was utilized to measure the change in vessel contractile force continuously as bath PO2 was reduced. In these experiments, 2- to 3-mm-long segments of the middle cerebral artery were mounted on wires attached to a pair of jaws, which were then given a passive load of 0.5 g and allowed to equilibrate. The vessels were allowed to equilibrate for 1 hour in PSS equilibrated with 95% O2-5% CO2. After the equilibration period, the arteries were activated by addition of KCl to the bath to achieve a final concentration of 50 mm K+. The osmotic effect of the added KCl was compensated by removal of an equivalent amount of NaCl. When a steady level of contraction was attained, the gas equilibration mixture was changed to 0% O2-5% CO2-95% N2, and tension changes were recorded while the decrease in PO2 was simultaneously measured in the vessel chamber with the oxygen electrode from a Radiometer blood gas analyzer. In one set of arteries, the response to reduced PO2 was tested in an identical manner in the presence of 10−6 g/ml tetrodotoxin (TTX) to ensure that the relaxation of the vessel did not result from the activation of dilator nerve fibers in the vessel wall.

In another series of experiments, 3- to 5-mm-long vessel segments (200–500 μm, outer diameter) were cannulated with a glass micropipette attached to a volume reservoir filled with physiological salt solution (Halpern et al., 1984). This system could be pressurized with a sphygmomanometer bulb while the pressure was monitored with a Statham transducer in line. One end of the vessel was stretched over the micropipette and tied in place with a 25-μm silk suture, while the opposite end was tied off and secured in a pair of plastic jaws which could be adjusted to maintain the vessel at in situ length. All side branches were tied off and the vessel was pressurized to 100 mm Hg and allowed to equilibrate at this pressure for at least 1 hour. Preparations which did not hold the equilibration pressure because of leakage, and vessels which exhibited large passive dilations when transmural pressure was increased during 95% O2-5% CO2 superfusion, were discarded.

In the cannulated vessel studies, the arteries were observed through a Zeiss model DRC stereomicroscope coupled to a closed circuit television system. Vessel diameters were measured from videotape recordings with an IPM model 907 image measuring monitor. Since it was often difficult to measure inner diameter and wall thickness because the lumen of the artery was not always clearly visible, outer diameters were measured in the present experiments. Vessels which exhibited blowout, i.e., a very large and irreversible increase in diameter at any point in the experiment, were excluded from analysis.

In the electrophysiological studies, glass microelectrodes filled with 3 M KCl were used to measure VSM transmembrane potentials (Em) in the cannulated vessels. The electrodes utilized for these studies had tip impedances of 50–80 MΩ and tip potentials of less than 3 mV. The methods, apparatus, and criteria for impalements utilized in the present studies were similar to those which have been described earlier (Harder, 1984).

In the cannulated vessel experiments, the PO2 of the PSS in the vessel chamber was also measured with the oxygen electrode from a Radiometer blood gas analyzer. With the apparatus used for these experiments, equilibration of the supply reservoir with the 95% O2-5% CO2 mixture consistently produced a PO2 of approximately 300 mm Hg in the chamber. After the initial equilibration period at a transmural pressure of 100 mm Hg and a bath PO2 of 300 mm Hg, the response of the vessel to increases in transmural pressure was tested by releasing the pressure in the artery and then reinflating it to a transmural pressure of 40 mm Hg, where there was no passive collapse of the vessel segment. After 1–2 minutes at 40 mm Hg, transmural pressure was increased in various increments to a final value of 160 mm Hg. Each pressure step was maintained for 1–2 minutes. After myogenic contractile responses were tested at 300 mm Hg PO2, the vessel was returned to the equilibration pressure (100 mm Hg), and bath PO2 was lowered by equilibrating the PSS in the reservoir with 5% O2-5% CO2-90% N2. This gas equilibration procedure consistently produced a PO2 of about 50 mm Hg in the vessel chamber. After a 30-minute equilibration period at 50 mm Hg PO2, the response of the vessel to elevations of transmural pressure was tested again.

Active tone in the vessel was then blocked by the addition of verapamil to the bath to achieve a final concentration of 10−6 M. After 20 minutes, the pressure-diameter curve was repeated in the presence of the verapamil.

Results

Relaxation of Cerebral Vessels in Response to PO2 Reduction

Figure 1 illustrates the relaxation of the wire-mounted arteries as a function of bath PO2 in the presence and absence of tetrodotoxin (TTX). Data are expressed as percent of maximum relaxation of the 50 mm K+-induced contraction, i.e., total relaxation of the tension developed in response to 50 mm K+. Approximately 70% of the total relaxation of the K+-induced contraction occurred between 150 mm Hg and 40 mm Hg PO2. Relaxation of the vessel segments was identical in the presence and absence of TTX.

Effect of Reduced O2 Availability Upon Em Changes at Various Transmural Pressures

Reduction of superfusion PO2 from 300 mm Hg to 50 mm Hg had two major effects upon the Em of cannulated cerebral arteries: (1) inhibition of spontaneous action potentials, and (2) reduction of the slope of the myogenic depolarization of the vessels in response to increases in transmural pressure. Figure 2 illustrates a record of an Em measurement in a cannulated vessel segment pressurized to 120 mm Hg. This vessel exhibited spontaneous spike activity at a bath PO2 of 300 mm Hg, but the action potentials were inhibited about 15 seconds after bath PO2 was reduced to 50 mm Hg. Spontaneous action potentials were observed at 300 mm Hg PO2 in two of six preparations. In both cases, these were inhibited when bath PO2 was reduced to 50 mm Hg. Although most preparations appear to have areas of cells which exhibit action potentials, it is quite difficult to maintain cell impalements in these areas. Therefore, most of our Em measurements were obtained from quiescent cells. However, the resting Em of the spontaneously active cells which we studied was not significantly different from that of the quiescent cells.
Reduction of superfusion PO₂ to 50 mm Hg reduced the slope of the myogenic depolarization of the arteries whether or not action potentials were present. However, 50 mm Hg PO₂ superfusion did not completely eliminate the pressure-dependent depolarization of the vessels. Figure 3 summarizes the Em changes which occurred as transmural pressure was elevated during 300 mm Hg PO₂ and 50 mm Hg PO₂ superfusion. In both cases, the vessels exhibited myogenic depolarization as transmural pressure was increased. However, the slope of the Em vs. transmural pressure relationship at 50 mm Hg PO₂ was significantly less than that at 300 mm Hg PO₂.

**Effects of Reduced PO₂ and Verapamil on Arterial Diameters during Increases in Transmural Pressure**

The effect of decreased superfusion solution PO₂ upon the diameter of cannulated vessels during increases in transmural pressure is summarized in Figure 4. During 300 mm Hg PO₂ superfusion, there was no significant change in vessel diameter as transmural pressure was increased in 40 mm Hg increments through the entire pressure range from the control value of 40 mm Hg. During 50 mm Hg PO₂ superfusion, the vessels dilated in response to the initial elevations in transmural pressure and then tended to maintain the larger diameter through the upper part of the pressure range. In contrast, vessel diameters increased passively with each step increase in transmural pressure during verapamil superfusion.

**Discussion**

Many studies have suggested that altered O₂ availability can regulate active tone in blood vessels by directly affecting VSM contractile force (Carrier et al., 1964; Smith and Vane, 1966; Coburn et al., 1979; Detar, 1980; Ebeigbe et al., 1980). Detar (1980) proposed that VSM relaxation in response to reduced PO₂ has two components. One occurs at low PO₂ levels and high levels of VSM activation, and results from anoxia of the VSM cells. The other ('physiological hypoxia-induced depression of VSM contraction') is observed at low-to-moderate levels of VSM activation, occurs over the physiological range of PO₂ values, and does not appear to involve restricted energy metabolism.

In the present experiments, we employed small segments of cat middle cerebral artery to investigate the direct effect of reduced PO₂ upon cerebral VSM. The in vitro tension measurements in this study demonstrate that most of the relaxation of K⁺-stimulated cerebral arteries in response to reduced O₂ availability occurs at bath PO₂ levels between 150 mm Hg and 40 mm Hg, which presumably includes the range of PO₂ levels encountered by the vessel in vivo. The average wall thickness of the vessels used in our experiments was about 30 μm, which is much less than that of the smallest strips which Pittman and Duling (1973) used to provide evidence that
relaxation of isolated arteries in response to reduced Po2 could result from an "anoxic core" due to diffusion limitation of O2 to the center of the vessel. In that study, the critical solution Po2 levels, i.e., the bath Po2 levels at which norepinephrine-induced contractions of 170- to 200-μm thick sections of hog carotid artery just began to decrease (presumably, due to diffusion limitation of O2 to the center of the vessel) were between 15 and 35 mm Hg. These values are less than the Po2 at which complete relaxation of K+-stimulated contractions occurred in our experiments. Therefore, we feel that the relaxation of middle cerebral artery which we observed does not occur because of anoxia of VSM cells in the center of the vessel wall. Finally, the response of the arteries to decreased Po2 does not involve the activation of dilator nerves in the vessel wall, since vessel relaxation was unaffected by tetrodotoxin.

A likely explanation for the direct relaxation of VSM by reduced Po2 is that a decrease in Po2 reduces the availability of the activator Ca++ which is necessary for the generation of contractile force by the muscle. This could occur via inhibition of transmembrane Ca++ influx, via inhibition of the release of membrane bound Ca++ stores, or (less likely) via stimulation of Ca++ uptake into membrane stores or extrusion of Ca++ from the cell.

Several studies have suggested that membrane electrical events may regulate VSM responses to O2. For example, decreased Po2 inhibits the spontaneous action potentials and rhythmic contractions of rat portal vein (Gurevich et al., 1976; Hellstrand et al., 1977; Sigurdsson and Grampp, 1981). In addition, Roulet and Cobum (1981) reported that action potentials occur during the development of O2-induced contractions of the guinea pig ductus arteriosus, whereas tonic contractions of this vessel in response to O2 are associated with a sustained depolarization of the VSM.

In our experiments, it appears as if the inhibition of active VSM tone in middle cerebral artery by reduced Po2 may be partially mediated by an inhibition of action potentials similar to that occurring in rat portal vein (Gurevich et al., 1976; Hellstrand et al., 1977; Sigurdsson and Grampp, 1981). However, the slope of the Em vs. transmural pressure...
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fusion, it does not appear to eliminate completely the myogenic contraction of cerebral arteries in response to elevated transmural pressure (Harder, 1984) both appear to be Ca++-dependent (since the concentration gradient for Ca++ across the muscle membrane is high, even a small change in Ca++ permeability might be expected to depolarize the muscle membrane either directly or via its action on other ion conductance systems (Sperelakis, 1979), the present results support the hypothesis of Ebeigbe et al. (1980, 1982) that reduced Po2 inhibits active VSM tone by interfering with transmembrane Ca++ influx.

The reduced slope of the E_m vs. transmural pressure relationship may result from a hyperpolarization of the VSM by reduced Po2 at a given transmural pressure or a reduction of the depolarizing response of the VSM to the increase in transmural pressure. Either of these events could result from stimulation or inhibition of various membrane ionic conductances whose net effect would be to reduce the amount of depolarization at each level of transmural pressure. Since we did not reduce the Po2 during each transmural pressure step, we are unable to determine which of these mechanisms is operating in the present study. Nonetheless, our results suggest that the effect of reduced Po2 upon E_m may involve alterations in membrane ionic conductances other than those involved in the production of action potentials.

Although 50 mm Hg Po2 superfusion reduces active VSM tone relative to 300 mm Hg Po2 superfusion, it does not appear to eliminate completely the myogenic contraction of cerebral arteries in response to elevated transmural pressure, since the vessels still depolarize as transmural pressure is increased and do not exhibit the marked passive dilation which was seen during verapamil superfusion. However, since the slope of the E_m vs. transmural pressure relationship is significantly reduced during 50 mm Hg Po2 superfusion relative to 300 mm Hg Po2 superfusion, it appears as if reduced O2 availability may shift the operating point of the myogenic response so that it maintains a larger diameter as transmural pressure is increased. This would have the effect of allowing blood flow to increase in the face of reduced O2 availability while preserving the myogenic responses of the vessel to large and sudden increases in transmural pressure.

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