Effects of Breathing O₂ or O₂ + CO₂ and of the Injection of Neurohumors on the PO₂ of Cat Cerebral Cortex

BY WILLIAM J. WHALEN, PH.D., ROGER GANFIELD, PH.D., AND PANKAJAM NAIR, PH.D.*

Abstract:

Tissue PO₂ was measured in the outer 1.7 mm of the brain of the lightly anesthetized cat by means of a micro O₂ electrode introduced through a small hole in the skull. There was considerable variation from one location to another and from animal to animal. The interindividual variation was not related to the level of the arterial pressure or to rectal temperature. Individual values for the PO₂ in 569 locations in the 11 cats ranged from 0 to 90 mm Hg with a mean of 25. There was a significant downward trend in PO₂ from the surface layers inward. When the electrode was left in one location, the PO₂ usually fluctuated, though not often rhythmically as seen in skeletal muscle. Breathing pure O₂ (N=41) or 95% O₂ - 5% CO₂ (N = 33) caused an equal (50%) increase in PO₂. The intravenous injection of epinephrine or norepinephrine caused a primary rise in PO₂ usually with a secondary decrease. Acetylcholine caused a primary decrease and usually a secondary increase.

ADDITIONAL KEY WORDS tissue PO₂ epinephrine norepinephrine hypercapnia acetylcholine hypocapnia brain PO₂

A number of investigators have measured oxygen tension on the brain surface or oxygen availability deep in the cerebral cortex since the original studies of Davies and Brink in 1942.1 The polarographic electrodes used have been of three types: (1) a recessed cathode which gives absolute values for PO₂ but can only be used intermittently and is too large for intratissue measurements;1 (2) a bare-ended cathode which can be made small enough for intratissue use, but whose calibration in tissues is uncertain;2 (3) Clark-type electrode3 in which both cathode and anode are covered with a membrane. The latter electrode gives absolute values for PO₂, but only Silver4 has made one with a tip (5μ) small enough for intratissue use. Using this electrode Silver has made some measurements of the PO₂ in the brain of the rat and rabbit. Our purpose was to extend and amplify Silver's observations as well as to test the response to the breathing of pure oxygen or 95% oxygen-5% carbon dioxide and the injection of the neurohumors epinephrine, norepinephrine and acetylcholine.

Methods

The electrode we have devised combines some of the advantages of the separate cathode and the membrane-covered electrode. The details of its construction and testing have been published.5 Briefly, it consists of a glass capillary tube drawn out to a tip of 1 to 2μ. The capillary is filled with an alloy to within about 20μ of the tip. A few microns of gold are plated on the alloy, and the balance of the recess is filled with collodion. An external reference anode is used. Tests of the electrode indicate that it is capable of measuring oxygen tension in tissues.6 Occasionally a larger-tipped electrode (30μ) was used for comparison. This electrode had a slower response time, but otherwise gave similar results (fig. 1A). Since there is good evidence that O₂ consumption of the

*St. Vincent Charity Hospital, Cleveland, Ohio, 44115.

This work was supported in part by PHS Grants # HE 11906 and FR 05631.
PO$_2$ IN CEREBRAL CORTEX

FIGURE 1

PO$_2$ profiles in cat cerebral cortex. Downward-pointing arrows in A and B indicate the moment of insertion of the electrode tip into the tissue surface. In C the record begins with the electrode tip in the brain surface. Further penetration is indicated by the dashed lines—depth scale at right. Withdrawal in A is indicated by upward-pointing arrow. Each profile is from a different animal.

Brain is essentially independent of PO$_2$, variations in cortical PO$_2$ must reflect variations in local blood flow.

Cats were anesthetized with sodium pentobarbital (30 to 40 mg/kg) given intraperitoneally. In one of the cats urethane anesthesia (1 g/kg) was used and was supplemented with sodium pentobarbital (10 mg/kg). Since the results from this latter cat fell within the range of the others the data were lumped. A tracheal cannula was inserted, and the femoral artery was cannulated to monitor arterial pressure. The femoral vein was cannulated to permit injections. Rectal temperature was also monitored and usually held between 36 to 39°C. A dental drill was used to make a small hole in the skull (0.5 to 1.0 cm diameter) and a slit was made in the dura for the insertion of the electrode. The brain surface was continually bathed in warmed physiological solution (pH 7.2 to 7.3) usually equilibrated with 25% O$_2$-2% CO$_2$. Occasionally oxygen-free solutions were used, mainly as a calibration check. A slow flow rate was used, except during calibration checks, in order to permit near equilibration with the brain surface.

The anode was placed in the bathing solution, and under a stereomicroscope the PO$_2$ electrode was inserted by means of a micro manipulator into the brain surface. Measurements of PO$_2$ were made at the surface, and then at each 100 microns into the cortex. The micro-manipulator knob was connected to a potentiometer so that the depth (down to 1000 µ) into the brain could be recorded simultaneously with the PO$_2$. (In some experiments deeper penetrations were made but with far less accurate localization.) At every third or fourth step the electrode was left in place while the animal was given pure O$_2$ or 95% O$_2$-5% CO$_2$ to breathe for three to seven minutes. A planimeter was used to calculate the PO$_2$ from the area under the PO$_2$ trace.

After three to six penetrations in each animal injections of epinephrine (5 µg), norepinephrine (5 µg) or acetylcholine (10 µg) were given intravenously, in random order, while the electrode remained in one location. The agents were washed into the circulation with 1 to 2 ml of saline warmed to body temperature.

Results

It is difficult to describe a typical oxygen profile, for the variability was extreme. Figure 1 depicts three different profiles which illus-
Plot of mean PO$_2$ ± S. E. versus depth into the brain. Each point down to 1000μ represents at least 39 locations and a minimum of nine beyond that depth—in all, 569 locations in 11 cats.

The values found for PO$_2$ in 569 locations in 11 cats ranged from 0 to 90 mm Hg. The highest value was found near the surface of the brain when oxygen-free solution was bathing the surface. The data from the 569 locations have been plotted and illustrated in figure 2. A highly significant downward trend from the surface inward is evident. When the values from the brain surface to the 1000μ depth, inclusive, were averaged, a mean of 25 mm Hg was obtained. The values from the deeper locations were not included since (1) there was less certainty about the depth, and (2) the electrode tip may have been in white matter.

The average values for tissue PO$_2$ for each animal ranged from 5 to 44 mm Hg. There was no correlation with arterial pressure. The only animal with a mean arterial pressure of less than 75 mm Hg had a mean value for tissue PO$_2$ of 35 mm Hg. In this animal the mean arterial pressure was about 60 mm Hg throughout the experiments. Likewise, rectal temperature was not correlated with tissue PO$_2$. As stated earlier, the rectal temperature of most animals remained between 36 and 39° C. One animal, however, was inadvertently heated to 41.2° C, and the PO$_2$ values showed no change. In another animal the temperature fell to 35° C without noticeably affecting the PO$_2$ readings.

The effect of breathing pure O$_2$ or 95% O$_2$-5% CO$_2$ varied considerably. Responses from three cats are illustrated in figure 3. Commonly the PO$_2$ rose for the first two to three minutes and then levelled off or declined. When compared to the first air-breathing control period, breathing pure O$_2$ caused an increase in tissue PO$_2$ in 35 trials, no change in one, and a decrease in five. Breathing 95% O$_2$-5% CO$_2$ caused an increase in 21 trials, no change in one, and a decrease in 12. The mean percent of increase was 50% for both gasses. These results are graphed in figure 4.

Changes in cortical PO$_2$ following injection of neurohumors were followed in eight experiments performed on five cats. In one experiment there was no response to any of the agents. Since the control PO$_2$ in this instance
was 0, it is possible that the electrode tip was in an avascular location. In the remaining seven experiments epinephrine caused an initial increase in PO$_2$ (sometimes slight) in all trials, and a delayed decrease in five. Norepinephrine also caused an increase in all trials, but a secondary decrease in only two. Acetylcholine caused a decrease in six, an increase in one, and a secondary increase above the control level in four trials. In these seven trials the control PO$_2$ ranged from 5 to 30 mm Hg. Typical responses are shown in figure 5. Note in this illustration that breathing 100% O$_2$ did not increase the PO$_2$. This lack of response was also found in three other of the eight locations. Although the small number of trials makes the evaluation uncertain, there was no obvious relationship between the response to O$_2$ and that to the neurohumors.

**Discussion**

The range of values we report here for PO$_2$ in cat brain cortex are similar to those found by Silver$^4$ in rat and rabbit brain cortex. The highest value he found was 97 and the lowest 2 mm Hg. Silver noted, as others had before him, that the high values were found near blood vessels and the low values approximately midway between vessels. Silver did not report a mean value or a plot of the PO$_2$ versus depth. However, the PO$_2$ profile he showed was not dissimilar from several we saw in the cat brain. He made no mention of a downward trend in PO$_2$ from the surface inward, and we infer that there was none. Likewise, in another similar study, but with a bare-ended platinum electrode, no such trend was reported.$^5$ It is possible that the PO$_2$ in the outer 100 microns of the surface was affected by the PO$_2$ of the solution bathing the brain surface but not deeper layers, for we have shown that when exposed to a PO$_2$ of 380 mm Hg at the surface the critical depth in an isolated slice of the cat cortex is only about 110 microns (to be published). In the present study the PO$_2$ of the bathing solution was never more than 190 mm Hg. Thus we are inclined to believe that the downward trend is real and may reflect loss of oxygen in the vessels leading down from the pia. In line with this view, we previously noted that even large arteries leak oxygen,$^6$ and Duling has found a substantial longitudinal O$_2$...
gradient along arterioles (personal communication). Furthermore, Staub\textsuperscript{10} has shown that in the lung oxygen exchange occurs in vessels much larger than the capillaries.

Our study indicates that cortical PO\textsubscript{2} is considerably above that in white skeletal muscle, where we found mean values of about 4 and 6 mm Hg for the guinea pig\textsuperscript{6} and cat\textsuperscript{11} respectively. In the studies on skeletal muscle we were quite certain that the electrode tip was seldom if ever in a blood vessel and, therefore, the values for PO\textsubscript{2} represented tissue PO\textsubscript{2}. In the present study the electrode tip may at times have been in a blood vessel so that the mean value is higher than the true mean for tissue PO\textsubscript{2}. However, it was nearly always possible to return to a particular location and obtain the original value—an unlikely possibility if the electrode had damaged the blood vessel. In addition, there was no significant change with time in any one location as might be expected if the electrode interfered with the blood supply. Finally, Silver\textsuperscript{1} reported that even with the larger-tipped electrode he used spontaneous electrical activity of neurones recorded by his electrode continued for hours. Thus, it is likely that the mean values are representative of mean tissue PO\textsubscript{2}. Whether all cells are at times exposed to the mean PO\textsubscript{2} is problematical. The relative stability of the values at one particular location would suggest that they are not, but rather that cells near blood vessels are always exposed to a higher oxygen tension, and one population of cells remote from blood vessels may always exist on the brink of hypoxia. More work is necessary to resolve this question.

The results of the experiments in which 100\% O\textsubscript{2} was breathed were not unexpected. Cross and Silver\textsuperscript{9} reported that they occasionally found areas where breathing pure O\textsubscript{2} did not increase the cortical tissue PO\textsubscript{2}. However, they interpreted this finding as an indication either that the electrode was not working properly or that the tip (1-10\(\mu\) bare-ended) was in unhealthy brain tissue. We prefer to interpret our results to mean that in some areas constriction of blood vessels reduced blood flow sufficiently to maintain tissue PO\textsubscript{2} constant or even to decrease it. The constrictor effect of oxygen on brain blood vessels is well known.\textsuperscript{12} Furthermore, Cooper et al.\textsuperscript{13} sometimes saw evidence of a fall in blood flow in the area around their O\textsubscript{2} electrode when their patients breathed 100\% O\textsubscript{2}. We considered but rejected the possibility that the locations which showed no change or decrease in PO\textsubscript{2} in our studies were those too far from open blood vessels to be affected—presumably those areas having low PO\textsubscript{2} values. One of the locations which showed a decrease (slight) in PO\textsubscript{2} when 100\% O\textsubscript{2} was breathed had a control value of 52. The largest decrease of PO\textsubscript{2} of 8 mm Hg occurred in a location having a control value of 27. On the other hand, locations with control values as low as 0 mm Hg responded to O\textsubscript{2} with an increase in PO\textsubscript{2}, although with a delay of about one minute.

The surprising finding that breathing 95\% O\textsubscript{2}-5\% CO\textsubscript{2} caused only the same increase in tissue PO\textsubscript{2} and, in fact, no change or decrease
Effect of the injection (I.V.) of 5 μg epinephrine (E), 5 μg norepinephrine (NE), or 10 μg acetylcholine (Ach); or breathing 100% O₂ (100) on cortical tissue PO₂ (lower trace left-hand scale) and arterial pressure (right-hand scale). Record begins with insertion of the electrode at arrow. Subsequent depth indicated by dashed lines (right-hand scale).

in more locations, is difficult to explain in view of the dilator action of carbon dioxide on cerebral blood vessels.\textsuperscript{12} In other studies we found that administering the gas to cats in the same manner caused a rise in arterial PO₂ to 480 mm Hg and a drop in arterial pH of about 0.1 (to be published). Assuming this pH change occurred in these experiments, it should have been adequate to increase cerebral blood flow\textsuperscript{12, 14} and tissue PO₂. As with pure O₂ breathing the control level of tissue PO₂ was not correlated with the direction or magnitude of the response. In 12 of 28 locations, where paired comparison was possible, the response to the two gasses was in opposite directions. In explanation, we can only suggest that some blood vessels respond to carbon dioxide by dilating, others by constricting.

The results of the injections of the neurohumors resemble those obtained by others\textsuperscript{4, 9, 15, 16} using a bare-ended electrode in the cerebral cortex. The injections of the catecholamines consistently caused a rise in cortical PO₂. The rise was greatest and more prolonged with norepinephrine, in association with the prolonged arterial pressure response. However, the delayed decrease in PO₂ which occurred most often with epinephrine often took place in advance of the return of the arterial pressure to the control level. This response may be due to a stimulating effect of catecholamines on cerebral metabolism,\textsuperscript{17} or it may simply be a manifestation of autoregulation. Further studies in which arterial pressure or blood flow are controlled and blood gasses monitored will be required to reveal the mechanism. The same comment applies to the overshoot in PO₂ which we commonly saw after acetylcholine injection; also, before the arterial pressure reached the control level.

References

8. Purves MJ: Fluctuations of arterial oxygen tension which have the same period as respiration. Res Physiol 1: 281-296, 1966
11. Whalen WJ, Nair P: Skeletal muscle PO\textsubscript{2}: Effect of inhaled and topically applied O\textsubscript{2} and CO\textsubscript{2}. Am J Physiol (to be published)
Effects of Breathing O₂ or O₂ + CO₂ and of the Injection of Neurohumors on the PO₂ of Cat Cerebral Cortex

WILLIAM J. WHALEN, ROGER GANFIELD and PANKAJAM NAIR

Stroke. 1970;1:194-200
doi: 10.1161/01.STR.1.3.194

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1970 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/1/3/194