Capillary oxygen saturation and tissue oxygen pressure in the rat cortex at different stages of hypoxic hypoxia

Neurological Research, Oct 2000 by Meyer, Bernard, Schultheib, Rolf, Schramm, Johannes

The objective of this study was to generate data that allow for estimation of the validity of oxygen saturation (SO2) values in superficial cortical capillaries as calculated by a microreflectometric system (EMPHO /19). Capillary SO2 and tissue oxygen pressure (PtO2) were measured simultaneously in the cortex of n = 13 Wistar rats under normocapnic (PaCO2 = 36 mmHg) arterial normoxia (PaO2 = 92 mmHg), moderate (PaO2 = 53 mmHg) and severe hypoxic hypoxia (PaO2 = 31 mmHg) with microreflectometry and multiwire surface electrodes. Values were pooled according to arterial oxygenation levels, displayed as frequency histograms and compared via ANOVA (p < 0.05). Hypoxic hypoxia was induced by lowering the inspired oxygen fraction (FiO2) to 0.2. The study was approved by the local ethics committee.

INTRODUCTION

Monitoring of tissue oxygen supply and metabolism has gained widespread interest in the neuroscience community, because methods which have been applied in animal experiments for a very long time were made amenable to clinical research by technological advancements within the last years. There are two basic principles for those methods in clinical application.

Methods based on polarographic principles' on one hand measure tissue oxygen partial pressure (PtO2) and are now mainly in use in the form of oxygen probes that are inserted in the brain tissue close to regions of interest. They are established monitoring devices for regional PtO2 in intensive care patients and during operative interventions2-4. Recently developed sensors incorporate combinations of electrochemical and fiberoptic elements allowing for simultaneous recording of regional tissue PO2 (PtO2), PCO2, pH and temperature5-7 Other polarographic techniques such as multiwiresurface electrodes8-13, have never reached such acceptance in clinical research due to their complex set-up, calibration procedure etc., which renders intro-operative use cumbersome and time-consuming. Yet they are very useful for laboratory work, because they measure local organ surface PO2 with a very high spatial resolution. Representative PtO2-distributions can be obtained reflecting adequately the heterogeneity of oxygenation in the superficial cortical layers of the brain.

Optical sensor techniques on the other hand have also been used for studies of tissue oxygen metabolism for a very long time providing information about oxygenation of hemoglobin or the redox state of intracellular enzymes'. Many methods have been developed using different algorithms and wavelengths (e.g. infrared vs. visible light), which also differ basically with respect to their catchment volume (e.g. macroscopic vs. microscopic reflectance spectroscopy)21-24. Spatially resolved near-infrared spectroscopy systems (NIRS) are well established methods nowadays, and are used for non-invasive transcranial monitoring of regional SO2 (TCCO) in the clinical setting25-31. Microreflectometric systems work mainly on the domain of visible light. They are less frequently used clinically than in laboratory experiments 32. However, they can provide clinically important information about local SO2-distributions in superficial cortical capillaries. We and others have used a high resolution microreflectometric system (EMPHO II, BGT, Uberlingen, Germany) for in vivo measurements of capillary SO2 in the human cortex. It has been proven that the obtained SO2 values reflect changes of nutritive capillary flow in the cortex of rats very accurately and with high sensitivity under constant conditions of arterial oxygen supply and consumption36. The major criticism of this technique lies in the fact that no exact validation procedure for brain tissue exists 36,37, although in vitro experiments have shown an excellent correlation with tissue oxygenation levels38. The aim of this study was to generate data, which allow for a better estimation of the validity of SO2-distributions obtained by this method in the brain cortex under various arterial oxygenation levels in vivo.

MATERIALS AND METHODS

Measurements of capillary cortical oxygen saturation (SO2)

Capillary SO2 values were measured with the Erlangen Microlightguide Spectrophotometer (EMPHO) II, BGT, Germany), which was introduced and described in 198939. It was designed for fast, diffuse remission spectrophotometry by flexible microlightguides in small tissue volumes of moving organs in situ. Light in the visible domain illuminates tissue via the illuminating fiber and backscattered light is transmitted via six detecting fibers (0.7 mm) arranged in a hexagonal pattern around the illuminating fiber, to a rotating band pass interference filter disc. This serves as a monochromating unit in the spectral range of 502-628 nm in 2 nm steps. Spectra of 64 wavelengths per rotation are thus transmitted to a photo multiplier, an AD-converter and finally to a computer, in which one SO2 value per spectrum is calculated by an algorithm described elsewhere 40,41. The high temporal (100 spectra sec^-1^) and spatial (75*250 mm) resolution permits an easy scanning procedure of superficial cortical capillaries by moving the light-guide above the brain surface. Measurements of cortical tissue oxygen pressure (PtO2) Cortical PtO2 was measured with polarographic multiwire surface electrodes described by Kessler and Lubbers 30 years ago42,43 (MIT-system Dortmund, Germany) containing eight platinum wires allowing eight simultaneous PtO2 measurements in superficial cortical brain tissue based on the Clark principle. The electrode is counterbalanced on a lightweight arm, permitting it to follow brain pulsations without exerting pressure. It is further mounted on a micromanipulator to allow for scanning procedures by precise and free movement across the brain surface. The absolute PtO2 values are recorded on a first-generation
Thirteen male Wistar rats weighing between 350 and 400 g were anesthetized by 0.015 mg kg⁻¹ Fentanyl and 0.8 mg kg⁻¹ Droperidole i.m., maintained by 25% of the initial dose every 30 min throughout the experiments. The animals were intubated via tracheostomy. Ventilation was controlled by using a small animal respirator to maintain normocapnia (PaCO₂: 36 mmHg) during three different stages of arterial oxygenation obtained by changing the inspired O₂ fraction (21% -- 10% -- 4%) until a steady state was reached. The right femoral artery was cannulated for continuous blood pressure monitoring and intermittent blood gas analysis. Rectal temperature was controlled and maintained by a heating pad and lamp at 38°C.

The animals were mounted on a stereotactic head frame and a parietal craniotomy was carried out under the microscope using a diamond drill. Thereafter the dura mater was stripped off and the brain surface was continuously rinsed with warm saline solution. After reaching a steady state for the corresponding PaO₂, cortical capillary S0₂ and PtO₂ was measured by scanning the exposed parietal cortex in each animal under (a) normoxia (PaO₂: 92 mmHg), (b) moderate hypoxia (PaO₂: 53 mmHg) and severe hypoxia (PaO₂: 31 mmHg).

Data were transferred to a commercially available computer software for graphical and statistical analysis (ANOVA, p)
experiments using the same algorithm and methodology9-12,44. This holds true for mean values, shift of distributions and reaction to hypoxic-induced increase of local CBF 45,4s,as. The data derived from the multiwire surface electrode measurements can therefore be regarded as reliable reference values under these experimental conditions.

The stepwise decrease of arterial oxygenation produced an average desaturation from 46% to 33% to 12% S02 within the cortical capillaries, which paralleled a decrease of oxygen partial pressure in the same cortical areas from 27 to 20 to 9 mmHg Pt02. Thus a 30% drop of oxygen saturation in the vascular supply unit caused a 26% decrease of oxygen tension within the depending cortex, respectively a further reduction of S02 of 60% a subsequent decrease of Pt02 of 55%. The values calculated by the algorithm of the EMPHO II lie within the expected range of S02 values, when compared with those for calculated 02 dissociation curves in brain capillaries under the given arterial 02 partial pressures". Furthermore they correspond exactly to those described by Watanabe et al.32 in the brain of hypoxic Wistar rats measured by an analogous microspectrophotometric system. However, the seemingly linear relationship between S02 and Pt02 values in our experiment does not reflect the complicated interdependencies in reality and is most probably caused by the necessary averaging procedure for numerous values in multiple capillaries and cortical areas supplied by them. In reality the relation between 02 saturation and 02 partial pressure in brain capillaries is known to be sigmoid and the one between intracapillary 02 partial pressure and cortical Pt02 exponential in models regarding one isolated capillary segment47-49. To approximate these complicated relations in a descriptive linear manner usually a Hill-plot is used as done by Hasibeder et al.38 in their in vitro experiment. We have adopted this approach and have thus produced an 'in vivo tissue oxygen dissociation curve', which indeed demonstrated a significant linear dependency with an excellent correlation. The coefficient of determination r2 was calculated as 0.88, which indicates that Pt02 values per se contain approximately 90% of all information needed to predict intracapillary SO2, and vice versa.

Yet, apart from pure statistical calculations, it is obvious that the scatter of values around the line of regression is not homogeneous (Figure 2). A definitive increase can be observed at its lower end, that is at values below 10% SOz, respectively 1.5 mmHg Pt02. The reason for this divergence at very low levels of oxygenation cannot be pinpointed exactly. The easiest explanation would be that the algorithm for calculation of S02 data is not valid at very low oxygen levels despite the fact that the EMPHO II works on the basis of multiple wavelengths. This would be true under the assumption that Pt02 values by multiwire surface electrodes are the gold standard for reference. It is, however, a known fact that multiwire surface electrodes are neither sensitive nor stable enough to measure very low Pt02 values with absolute precision. 50. Exact quantification of values in this range remains somewhat arbitrary since even the best of electrodes will show drift artefacts. Furthermore it is not clear whether the logarithmic transformation of the Hill plot is still valid to approximate the relation between S02 and Pt02 in a severely hypoxic situation, because intracellular and intravascular changes in pH, PaCO2 and lactate levels etc. will influence 02 capacity, 02 affinity, 02 diffusion and local CBF in an uncontrolled way56, z,sz.

We could, therefore, show that S02 values in superficial cortical capillaries as calculated by the algorithm of the EMPHO III are highly accurate over a wide range of oxygenation levels. Only with respect to extremely low values does it remain questionable whether they reflect truly absolutes of capillary 02 saturation and one should therefore be cautious in interpretation of single values in the range below 10% SO2. This shortcoming, however, is not unique to the method described here, but probably inherent to every method applied for data sampling in cerebrovascular microvolumes, including laser Doppler flowmetry. In microreflectometric measurements single values in general, irrespective of 02 saturation level, should never be regarded as representative. We and others have shown previously36,37, that to reach reliable and reproducible results scanning procedures are mandatory with these methods. Thus we consider only accumulations of low S02 values, that significantly exceed those found due to natural heterogeneity of local CBF unequivocal indicators for cortical hypoxia or ischemia.

We conclude that the EMPHO II is a highly reliable and feasible instrument for experimental and clinical cerebrovascular research, if information about cortical capillary 02 saturation with high spatial and temporal resolution is desired.

REFERENCES
2 van Santbrink H, Maas Al, Avezaat CJ. Continuous monitoring of partial pressure of brain tissue oxygen in patients with severe head injury. Neurosurgery 1996; 38: 21-31
7 Hoffman WE, Charbel FT, Portillo GG, Edelman G, Ausman JI. Regional tissue $P_{O_2}$, $P_{CO_2}$, pH and temperature measurement. Neurol Res 1998; 20: S81-S84
11 Leniger-Follert E, Lubbers DW, Wrabetz W. Regulation of local tissue $P_{O_2}$ of the brain cortex at different arterial 02 pressure. Pfuiigers Arch 1975; 359: 81-95
12 Leniger-Follert E, Lubbers DW. Behavior of microflow and local $P_{O_2}$ of the brain cortex during and after direct electrical stimulation. A contribution to the problem of metabolic regulation of microcirculation in the brain. Pfuiigers Arch 1976; 366: 39-44
16 Chance B. Spectrophotometry of intracellular respiratory pigments. Science 1954; 120: 767-774
34 Hoper J, Gaab MR. Effect of arterial $P_{CO_2}$ on local $HbO_2$ and relative $Hb$ concentration in the human brain - a study with the Erlangen micro-lightguide spectrophotometer (EMPHO). Physiol Meas 1994; 15: 107-113
40 Kubelka P, Munk F. Ein Beitrag zur Optik der Farbanstriche. Z Technische Physik 1931; 11: 76-77
48 Krogh A. The rate of diffusion of gases through animal tissue with some remarks on the coefficient of invasion. J Physiol (Lond) 1918/19; 52: 391
Bernhard Meyer, Rolf Schulteibeta and Johannes Schramm
Department of Neurosurgery, University of Bonn, Germany
Correspondence and reprint requests to: Bernhard Meyer, MD, Department of Neurosurgery, University of Bonn, Sigmund Freud Str. 25, 53127 Bonn, Germany. Accepted for publication June 2000.
Copyright Forefront Publishing Group Oct 2000
Provided by ProQuest Information and Learning Company. All rights Reserved