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

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The objective of this study was to generate data that allow for estimation of the validity of oxygen saturation (S02) values in superficial cortical capillaries as calculated by a microreflectometric system (EMPHO /19). Capillary S02 and tissue oxygen pressure 002) were measured simultaneously in the cortex of n = 13 Wistar rats under normocapnic (PaCO2= 36 mmHg) arterial normoxia (PaO2= 92 mmHg), moderate (pa 02 = 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

Keywords: Capillary oxygen saturation; tissue oxygen pressure; rat cortex; hypoxia; reflection-spectrophotometry; multiwire-surface electrodes

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 (Pt02) 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 Pt02 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 PO^sup 2^, PCO^sup 2^, pH and temperature.5-7 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 14,15. Yet they are very useful for laboratory work, because they measure local organ surface PO^sub 2^ with a very high spatial resolution. Representative PtO^sub 2^-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 S02 (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 S02-distributions in superficial cortical capillaries. We and others have used a high resolution microreflectometric system (EMPHO Il, BGT, Uberlingen, Germany) for in vivo measurements of capillary S02 in the human cortex . It has been proven that the obtained S02 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 SO^sub 2^ values obtained by this method in the brain cortex under various arterial oxygenation levels in vivo.

MATERIALS AND METHODS

Measurements of capillary cortical oxygen saturation (SO^sub 2^)

Capillary SO^sub 2^ values were measured with the Erlangen Microlightguide Spectrophotometer (EMPHO)(R) II, BGT, Germany), which was introduced and described in 198939. It was designed for fast, diffuse remission spectrophotometry by flexible microl ightgu ides in small tissue volumes of moving organs in situ. Light in the visible domain illuminates tissue via the iluminating fiber and backscattered light is transmitted via six detecting fibers (0 70 (mu)um) 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 SO^sub 2^ value per spectrum is calculated by an algorithm described elsewhere 40,41 . The high temporal (100 spectra sec^sup -1^) and spatial (75*250 mum) 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 (Pt02) Cortical Pt02 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 Pt02 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 Pt02 values are recorded on a first-generation

PC system calculating frequency histograms and mean values. For a more detailed description of the complex calibration and measurement principles of this well known technique we refer to the existing literature.

Experimental protocol

Thirteen male Wistar rats weighing between 350 and 400 g were anesthetized by 0.015 mg kg-' Fentanyl and 0.8 mg kg-' 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 (PaC02: .:;36 mmHg) during three different stages of arterial oxygenation obtained by changing the inspired 02 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 380C.

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 Pa02, cortical capillary S02 and Pt02 was measured by scanning the exposed parietal cortex in each animal under (a) normoxia (Pa02: ,92 mmHg), (b) moderate hypoxia (PaO2: 53 mmHg) and severe hypoxia (Pa02: .31 mmHg).

Data were transferred to a commercially available computer software for graphical and statistical analysis (ANOVA, p

RESULTS

Physiological variables such as PaC02, MABP and rectal temperature were comparable during different stages of arterial hyoxia respectively normoxia (Table 1). A total number of n=9617 S02 respectively n=1118 Pt02 values was obtained during normoxia (Pa02=92.3 +2.2 mmHg) in all animals, n=7512 S02 respectively n=976 Pt02 values during moderate hypoxia (PaO2=53.51.2 mmHg) and n=6175 S02 respectively n=788 PtO2 values during severe hypoxia (Pa02=30.71.6 mmHg). The calculated mean values SD for pooled data of capillary oxygen saturation and tissue oxygen pressure in the parietal cortex were 45.6% + 14.6% SOZ respectively 26.8 + 8.2 mmHg PtO2 during arterial normoxia, 32.6%10.2% SOZ respectively 20.26.6 mmHg PtO2 during moderate arterial hypoxia and 12.3%11.1% S02 respectively 8.75.0 mmHg during severe arterial hypoxia (Table 2). Comparison of S02 and Pt02 frequency histograms correspondingly showed a parallel shift to the left with decreasing arterial oxygenation (Figure 1). During severe hypoxic hypoxia more than 40% of Pt02 values were below 10 mmHg and more than 80% of S02 values below 25% SO2. Distributions of oxygen saturation and oxygen pressure values became more homogeneous from normoxia to moderate hypoxia indicating a reactive increase in regional cortical blood flow. In a Hill plot an 'in vivo tissue oxygen dissociation curve' was accomplished with SOZ values ranging from 1 % to 65% SOZ and PtO2 values from 0.1 to 41 mmHg. Linear regression analysis showed an excellent correlation with a coefficient of determination of 12 of 0.88 (Figure 2). However, on closer examination of the plot it became obvious that the scatter of values around the line of regression is definitely increased below 10% SOz respectively 1.5 mmHg Pt02.

DISCUSSION

The microspectrophotometric system described in this article (EMPHO 10) will obviously never be applied as often as non-invasive NIRS-systems in clinical routine, because it requires surgical exposure of the cerebral cortex. From a scientific point of view, however, both methods should be regarded as complementary, because they differ basically in their catchment volumes. Opposed to NIRS-systems the EMPHO II is able to provide information on tissue oxygenation in superficial cortical areas alone with very high spatial resolution. Its excellent temporal resolution allows for easy and fast scanning procedures rendering it thus a feasible tool during neurosurgical intervention S33-35,37. Data acquisition is possible over any desired extent of the exposed cortex under direct visual control of normal anatomy and accompanying pathologies. The obtained S02 distributions therefore accurately reflect variations in and/or natural heterogeneity of tissue oxygenation in relation to underlying cortical structures. With these properties the system can offer new and valuable insights into the cortical microcirculation in animal experiments as well as in clinical research

However, a potential source of error exists and has been acknowledged previously. The lack of an exact validation for brain tissue might violate assumptions in the algorithm, which would be of interest for assessment of truly absolute cortical S02 values, although comparison of EMPHO III data with S02 values of other systems for intravital microreflectometry under comparable conditions in the rat cortex 32 suggests that this error is probably minor. Therefore experiments have been performed to compare these S02 data with methods regarded as the gold standard for tissue oxygenation, that is multiwire surface electrodes, which measure tissue oxygen partial pressure 002) with approximately the same spatial resolution as microreflectometric systems (e.g. EMPHO 110)38. In this experimental setup jejunal mucosal oxygenation was measured with both methods simultaneously, and it was demonstrated that S02 and PtO2 values varied within the expected range, following in parallel major oscillations induced by vasomotion. To test the validity of the EMPHO II in an additional in vitro experiment S02 and Pt02 values were obtained in a tissue homogenate under varying levels of arterial oxygenation. This produced a 'tissue oxygen dissociation curve', which showed an excellent correlation (r=0.95) of both values. However, values ranged between 20% and 80% S02 only, omitting the critical range below 20%. We thus reproduced the experiments in the rat cortex in vivo within a lower range of arterial oxygenation levels to create a more realistic environment for comparison of both methods under pathophysiological conditions. Our results regarding the Pt02 distributions in the rat cortex under arterial normoxia and different stages of hypoxia are in full accordance with those of prior animal

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 PtO2 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 a1.38 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 calcualted as 0.88, which indicates that Pt02 values per se contain approximately 90% of all information needed to predict intracapillary SO, 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 PtO2. 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 PtO2 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 PtO2 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 PtO2 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 way", 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

1 Clark L, Wolf R, Granger D, Taylor Z. Continuous recording of blood oxygen tensions by polarography. J Appl Physiol 1953; 6: 189-193

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

3 Meixensberger J, Baunach S, Amschler J, Dings J, Roosen K. Influence of body position on tissue-p02, cerebral perfusion pressure and intracranial pressure in patients with acute brain injury. Neurol Res 1997; 19: 249-253

4 Dings J, Meixensberger J, Jager A, Roosen K. Clinical experience with 118 brain tissue oxygen partial pressure catheter probes. Neurosurgery 1998; 43: 1082-1095

5 Hoffman WE, Charbel FT, Edelman G, Abood C. Brain tissue response to COZ in patients with arteriovenous malformation. J Cereb Blood Flow Metab 1996; 16: 1383-1386

6 Hoffman WE, Charbel FT, Edelman G, Ausman JI. Thiopental and desflurane treatment for brain protection. Neurosurgery 1998; 43: 1050-1053

7 Hoffman WE, Charbel FT, Portillo GG, Edelman G, Ausman JI. Regional tissue P02, PCO^sub 2^, pH and temperature measurement. Neurol Res 1998; 20: S81-S84
8 Crockard HA, Symon L, Branston NM, Juhasz J, Wahid A. Measurements of oxygen tension in the cerebral cortex of baboons. J Neurol Sci 1976; 27: 17-28
9 Grote J, Zimmer K, Schubert R. Effects of severe arterial hypocapnia on regional blood flow regulation, tissue POz and metabolism in the brain cortex of cats. Pflugers Arch 1981; 391: 195-199
10 Grote J, Zimmer K, Schubert R. Tissue oxygenation in normal and edematous brain cortex during arterial hypocapnia. Adv Exp Med Biol 1984; 180: 179-184
11 Leniger-Follert E, Lubbers DW, Wrabetz W. Regulation of local tissue POz of the brain cortex at different arterial 02 pressure. Pflugers Arch 1975; 359: 81-95
12 Leniger-Follert E, Lubbers DW. Behavior of microflow and local P02 of the brain cortex during and after direct electrical stimulation. A contribution to the problem of metabolic regulation of microcirculation in the brain. Pflugers Arch 1976; 366: 39-44
13 Leniger-Follert E. Direct determination of local oxygen consumption of the brain cortex in vivo. Pflugers Arch 1977; 372: 175-179
14 Assad F, Schultheibeta R, Leniger-Follert E, WOllenweber R. Measurements of local oxygen partial pressure (P02) of the brain cortex in cases of brain tumors. Adv Neurosurg 1984; 12: 263-270
15 Schultheiss R, Leuwer R, Leniger-Follert E, Wassmann H, Wullenwebert R. Tissue P02 of human brain cortex-method, basic results and effects of pentoxifylline. Angiology 1987; 38: 221-225
16 Chance B. Spectrophotometry of intracellular respiratory pigments. Science 1954; 120: 767-774
17 Jobsis FF. Noninvasive, infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters. Science 1977; 198:1264-1267
18 Jobsis FF, Keizer JH, LaManna JC, Rosenthal M. Reflectance spectrophotometry of cytochrome aa3 in vivo. J Appl Physiol 1977; 43:858-872
19 Kariman K, Burkhart DS. Non-invasive in vivo spectrophotometric monitoring of brain cytochrome aa3 revisited. Brain Res 1985; 360: 203-213
20 Bashford CL, Barlow CH, Chance B, Haselgrove J, Sorge J. Optical measurements of oxygen delivery and consumption in gerbil cerebral cortex. Am J Physiol 1982; 242: C265-C271
21 Kinuta Y, Kikuchi H, Ishikawa M, Hirai 0, Imataka K, Kobayashi S. Reflectance spectrophotometric measurement of in vivo local oxygen consumption in the cerebral cortex. J Cereb Blood Flow Metab 1987; 7: 592-598
22 Narita N, Tominaga T, Koshu K, Miozoi K, Yoshimoto T. Monitoring of brain tissue hemoglobin concentration and oxygen saturation using a three wavelength spectrophotometric method. Neurol Res 1994; 16: 428-432
23 Newton DJ, Harrison DK, Delaney CJ, Beck JS, McCollum PT. Comparison of macro- and micro-lightguide spectrophotometric measurements of microvascular haemoglobin oxygenation in the tuberculin reaction in normal human skin. Physiol Meas 1994; 15: 115-128
24 Tateishi N, Maeda N, Shiga T. A method for measuring the rate of oxygen release from single microvessels. Circ Res 1992; 70: 812-819
25 Ausman JI, McCormick PW, Stewart M, et al. Cerebral oxygen metabolism during hypothermic circulatory arrest in humans. J Neurosurg 1993; 79: 810-815
26 Dujovny M, Ausman JI, Stoddart H, Slavin KV, Lewis GD, Widman R. Somanetics INVOS 3100 cerebral oximeter [letter, comment]. Neurosurgery 1995; 37: 160
27 Hock C, Muller-Spahn F, Schuh-Hofer S, Hofmann M, Dirnagl U, Villringer A. Age dependency of changes in cerebral hemoglobin oxygenation during brain activation: A near-infrared spectrosocpy study. J Cereb Blood Flow Metab 1995; 15: 1103-1108
28 Kirkpatrick PJ, Smielewski P, Czosnyka M, Menon DK, Pickard JD. Near-infrared spectroscopy use in patients with head injury. I Neurosurg 1995; 83: 963-970
29 McCormick PW, Stewart M, Goetting MG, Balakrishnan G. Regional cerebrovascular oxygen saturation measured by optical spectroscopy in humans. Stroke 1991; 22: 596-602
30 Samra SK, Dorje P, Zelenock GB, Stanley JC. Cerebral oximetry in patients undergoing carotid endarterectomy under regional anesthesia. Stroke 1996; 27: 49-55
31 Smielewski P, Kirkpatrick P, Minhas P, Pickard JD, Czosnyka M. Can cerebrovascular reactivity be measured with near-infrared spectroscopy? Stroke 1995; 26: 2285-2292
32 Watanabe M, Harada N, Kosaka H, Shiga T. Intravital microreflectometry of individual pial vessels and capillary region of rat. J Cereb Blood Flow Metab 1994; 14: 75-84
33 Hoper J, Gaab MR, Batz M, Feyerherd F. Local oxygen supply to the cerebral cortex during thiopental and propofol anesthesia. First results. Anaesthetist 1994; 43: 534-538
34 Hoper J, Gaab MR. Effect of arterial PCO2 on local HbO2 and relative Hb concentration in the human brain - a study with the Erlangen micro-lightguide spectrophotometer (EMPHO). Physiol Meas 1994; 15: 107-113
35 Meyer B, Schaller C, Frenkel C, Schramm J. Physiological steal around AVMs of the brain is not equivalent to cortical ischemia. Neurol Res 1998; 20: SI 3-S1 7

36 Nakase H, Heimann A, Kempski 0. Alterations of regional cerebral blood flow and oxygen saturation in a rat sinus-vein thrombosis model. Stroke 1996; 27: 720-727
37 Meyer 13, Schaller C, Frenkel C, Ebeling B, Schramm J. Distributions of local oxygen saturation and its response to changes of mean arterial blood pressure in the cerebral cortex adjacent to arteriovenous malformations. Stroke 1999; 30: 2623-2630
38 Hasibeder W, Germann R, Sparr H, et al. Vasomotion induces regular major oscillations in jejunal mucosal tissue oxygenation. Am J Physiol 1994; 266: 6978-6986
39 Frank KH, Kessler M, Appelbaum K, Dummler W. The Erlangen micro-lightguide spectrophotometer EMPHO I. Phys Med Biol 1989; 34: 1883-1900
40 Kubelka P, Munk F. Ein Beitrag zur Optik der Farbanstriche. Z Technische Physik 1931; 11: 76-77
41 Frank KH, Kessler M, Appelbaum K, Albrecht HP, Mauch ED. Measurements of angular distributions of Rayleigh and Mie scattering events in biological models. Phys Med Biol 1989; 34: 1901-1916
42 Kessler M, Grunwald W. Possibilities of measuring oxygen pressure fields in tissue by multiwire surface electrodes. Progr Resp Res 1969; 3: 147-152
43 Lubbers D, Baumgartl H, Fabel H, et al. Principle and construction of various platinum electrodes. Progr Resp Res 1969; 3: 136-146 44 Grote J, Reulen JJ, Schubert R. Increased tissue water in the brain:
Influence on regional cerebral blood flow and oxygen supply. Pathology of cerebrospinal microcirculation. Adv Neurol 1978; 20: 333-339
45 Bewrecski D, Wei L, Otsuka T, et al. Hypoxia increases velocity of blood flow through parenchymal microvascular systems in rat brain. J Cereb Blood Flow Metab 1993; 13: 475-486
46 Kozniewska E, Weller L, Hoper J, Harrison DK, Kessler M. Cerebrocortical microcirculation in different stages of hypoxic hypoxia. J Cereb Blood Flow Metab 1987; 7: 464-470
47 Astrup P, Engel K, Severinghaus J, Munson E. The influence of temperature and pH on the dissociation curve of oxyhemoglobin in human blood. Scand Clin Lab Invest 1965; 17: 515-523
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
49 Grote J, Siegel G, Zimmer K, Adler A. The interaction between oxygen and vascular wall. Adv Exp Med Biol 1989; 248: 575-581 50 Lubbers DW, Baumgartl H, Zimelka W. Heterogeneity and stability
of local POz distribution within the brain tissue. Adv Exp Med Biol 1994; 345:567-574
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