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Cerebral Blood Flow Autoregulation During Hypobaric Hypotension Assessed by Laser Doppler Scanning

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Summary: Hypobaric hypotension was used to reduce systemic blood pressure in rats below the lower threshold of CBF autoregulation to evaluate a new laser Doppler (LD) "scanning" technique. Spontaneously breathing male Wistar Kyoto rats (n = 8) were anesthetized with chloral hydrate and the head fixed in a stereotaxic head holder. A cranial window with intact dura mater was introduced to assess local CBF (lCBF) by LD. One stationary probe served to detect rapid flow changes, whereas the second probe was used to sample lCBF recordings from many cortical locations by means of a stepping motor-controlled micromanipulator to obtain lCBF frequency histograms. Advantages are an improved spatial resolution together with the easy detection of low-flow areas and a better comparison of data from individual experiments. Arterial blood pressure was stepwise reduced by exposing the lower body portions to subatmospheric pressures (hypobaric hypotension), thus avoiding the use of drugs or heparinization. The lower threshold of CBF autoregulation was detected by "scanning" at arterial pressures between 50 and 46 mm Hg, with low-flow spots occurring immediately. The data suggest LD scanning as a method suited particularly for studies where lCBF inhomogeneities are expected, e.g., the ischemic penumbra or sinus vein thrombosis. **Key Words:** Blood flow autoregulation—Hypobaric hypotension—Laser Doppler flowmetry—Local cerebral blood flow.

Laser Doppler flowmetry (LDF) represents a rather new technology that permits the assessment of changes in cortical blood flow with an excellent temporal resolution. The sampling volume of conventional LD probes is ~1 mm^3. Therefore, LDF can yield CBF data for only small, circumscribed areas, i.e., local CBF (lCBF). Although lCBF changes detected by LDF have been shown to correlate with results obtained with other techniques (DiResta et al., 1987; Skarphedinsson et al., 1988; Haberl et al., 1989; Lindsberg et al., 1989), the currently available LDs are not calibrated to provide conventional CBF units, i.e., ml $100\,g^{-1}\,min^{-1}$, but rather machine-specific flow units. This drawback notwithstanding, LDF is used with increasing frequency to monitor lCBF alterations and to control the success of vessel ligations to induce global or focal ischemia (Dirnagl and Pulsinelli, 1990). lCBF, however, varies considerably depending on the site of measurement, which has led us to develop a "scanning" procedure by moving a second Doppler probe to many different locations (Ulrich et al., 1993; Ungersböck et al., 1993*a*,*b*). Thereby, typical lCBF histograms are obtained for given anatomical sites. Shifts of the median and the mode of the histogram indicate flow changes. With this scanning technique, errors due to dislocations of the Doppler probe are avoided. The second LD is used to maintain the high temporal resolution afforded by continuous measurements.

The scanning technique is now used to study CBF autoregulation in a model of hypobaric hypotension recently described for the induction of forebrain ischemia in rats by reducing systemic arterial pressure after carotid occlusion (Dirnagl et al., 1993). With this model, hypotension is caused by venous blood pooling in the lower body portions, which are exposed to a reduced atmospheric pressure of −10 to −20 cm H_2O. The use of heparin or

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Abbreviations used: lCBF, local CBF; LDF, laser Doppler flowmetry.

other drugs is avoided. Moreover, the technique has already been used to study regional CBF autoregulation (Thorén et al., 1989). These advantages suggest the use of hypobaric hypotension together with the LD scanning technique as well as with continuous LDF to study microcirculatory changes at the lower threshold of blood flow autoregulation for a detailed analysis of the temporal and spatial variations of lCBF.

MATERIAL AND METHODS

Animal preparation and hypobaric hypotension

Male Wistar Kyoto rats (300 ± 20 g, n = 8) were premedicated with 0.5 mg atropine. Anesthesia was introduced with ether and continued by intraperitoneal injection of chloral hydrate (36 mg/100 g body wt). During the experiment, spontaneous ventilation was maintained, and body temperature was controlled by a feedback-controlled homeothermic blanket control unit (Harvard, South Natick, MA, U.S.A.). Blood pressure in the right carotid artery was continuously monitored via an intraarterial catheter connected to a pressure transducer (Gould 134615-50). Arterial blood gases were determined during the initial control period using an ABL3 blood gas analyzer (Radiometer, Copenhagen, Denmark). The head was fixed in a stereotaxic frame (Stoelting, Wood Dale, IL, U.S.A.). Access to the brain surface was gained via a 5 × 2.8–mm large cranial window, drilled 2 mm lateral and 1 mm caudal to the bregma, and a 1 × 2–mm hole over the contralateral hemisphere. On both sides the dura was left intact (OP-microscope; Zeiss, Wetzlar, Germany). During the craniectomy, the drill tip was continuously cooled with physiologic saline. The lower body portion of the animals was placed in a sealable steel chamber, connected to an electronically controlled vacuum pump for later induction of hypobaric hypotension. To do so, the barometric pressure within the steel chamber could be reduced down to −30 cm H_2O, thereby causing a pooling of venous blood in the lower body portion of the rat. Care was taken not to impede the spontaneous breathing of the animals.

lCBF measurement

Local cortical blood flow was measured by two TSI laser flow blood perfusion monitors (model BPM 403a; TSI, St. Paul, MN, U.S.A.) using two identical 0.8-mm needle probes. lCBF is expressed in LD units since the calibration of LDs remains controversial. The needle probes were fixed to micromanipulators and were placed over the intact dura under microscopic control. The gap between dura and LD probes was always kept as small as possible, never to exceed 100 μm. One LD probe was positioned over the right parietal cortex at a location without visible, i.e., larger vessels. This LD was defined as "stationary" (LD_{stat}), since the probe was not moved during the experiment. The other LD probe was translocated by the stepping motor-driven micromanipulator (0.1-μm resolution; Märzhäuser, Germany) to multiple positions in 100-μm steps within the cranial window and for this reason is called the "scanning" LD (LD_{scan}). Thus, the random registration of 25 individual measurements results in one scanning procedure with information from 25 different locations over a total distance of 2.5 mm. To avoid artifacts caused by measurements recorded with a moving probe, a delay of 2 s was allowed before each measurement. A fast A/D-conversion board with onboard signal-processing capacity (DAP; Microstar Laboratories, Redmont, WA, U.S.A.) permitted sampling of data with a running average for 8 s (i.e., over approximately two breathing cycles) for each point of measurement. Therefore, one scan took ~4 min; scanning would be much faster if no averaging of respiration-dependent flow changes were performed. Data thus collected were used to calculate observation frequency histograms. Measurements were assigned to flow classes with a class width of 5 LD units and a range between 0 and 140 LD units, before they were mathematically normalized to 100% and plotted as frequency histograms. The technique permits repeated scans from a given set of locations. The accuracy of repeated scans has been found to be excellent (Ungersböck et al., 1993*b*) and has since been further improved by the use of the stepping motor-driven micromanipulator.

Experimental protocol

After a 30-min control phase, MABP was lowered by hypobaric hypotension in 5-mm Hg steps down to 30 mm Hg. In <1 min, the MABP was reduced to the intended level and was then maintained constant for the ensuing 10 min. During this plateau phase, MABP and CBF_{stat} were continuously recorded. A scanning procedure was performed also. At the end of the experiment, the animals were killed by an overdose of anesthesia. Then the biological zero was determined for both LDF techniques.

Statistical analysis

Data from blood gas analysis (Table 1) and MABP (Table 2) are reported as means ± SD together with medians and 95% confidence intervals. lCBF data from LD_{stat} were averaged for each MABP class of each animal. Data thus generated were included in box plots to allow for comparison with the data from LD_{scan} (see Fig. 2b), which are not normally distributed. LD_{scan} box plots were obtained by using median values of the lCBF frequency histograms calculated for each pressure level. lCBF changes after MABP reduction were evaluated for statistical significance using an analysis-of-variance test on ranks for repeated measures according to Friedman (SIGMASTAT; Jandel Scientific, Erkrath, Germany). Differences were assumed statistically significant at $p < 0.05$.

RESULTS

MABP and hypobaric hypotension

Means, SDs, and 95% confidence intervals of the blood pressure classes are shown in Table 2. MABP

TABLE 1. *Data from arterial blood gas analyses, sampled during initial control phase of experiments*

	Po_2 (mm Hg)	Saturation (%)	Pco_2 (mm Hg)	HCO_3^- (m*M*)	pH
Mean	79.15	94.7	41.97	23.27	7.358
Median	78.3	94.6	42.2	23.45	7.36
SD	5.28	1.07	1.66	2.32	0.026
95% CI	7.33	1.49	2.31	3.22	0.036

All parameters were normally distributed. CI, confidence interval.

TABLE 2. *Intended and actually measured arterial blood pressures (mm Hg) before and during hypobaric hypotension (n = 8)*

	MABP classes								
	>70	66–70	61–65	56–60	51–55	46–50	41–45	36–40	30–35
MABP mean	76.37	67.02	62.70	57.09	53.32	47.77	42.38	37.59	32.61
SD	4.87	0.78	1.79	1.66	1.42	1.28	0.72	1.57	1.99
95% CI	4.86	1.09	1.79	1.65	1.27	1.04	0.59	1.58	2.00

CI, confidence interval.

ranged between 71 and 85 mm Hg under control conditions. Hypobaric hypotension permitted a gradual decrease of MABP in 5-mm Hg steps and the maintenance of that level for the intended duration. During hypobaric hypotension, SDs within the MABP classes never exceeded 2 mm Hg. The inset of Fig. 1 demonstrates the time course of a typical experiment, illustrating the stepwise reduction of MABP.

lCBF changes

The reaction of lCBF to MABP variation as registered by LDF is illustrated by Figs. 1–3. The time course of an individual experiment is seen in Fig. 1, as an example of LD data from the stationary probe (LD_{stat}): (a) During the plateau phase of the pressure/flow relationship (MABP 60–85 mm Hg), lCBF remained stable as expected (autoregulation); (b) a subthreshold MABP was accompanied by a decrease in lCBF, in proportion to MABP; (c) the individual LD_{stat} recordings typically varied considerably over time, although registered with an LD probe not moved during the experiment. These fluctuations were independent of the MABP level and were found in all animals.

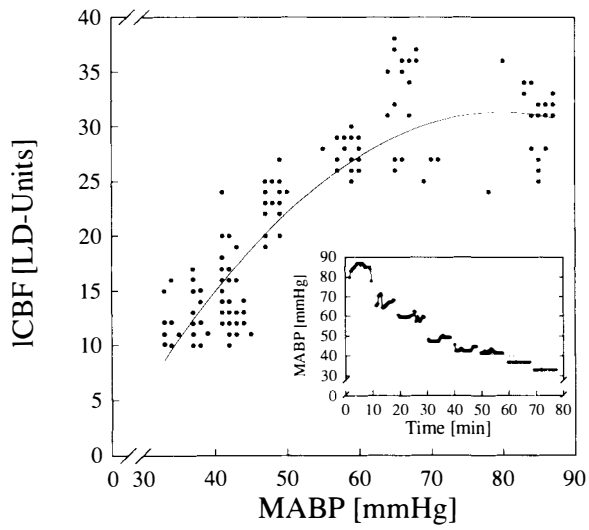

FIG. 1. Time course of local CBF (lCBF) of an individual experiment measured by the stationary laser Doppler (LD). Lowering MABP by hypobaric hypotension below a threshold of 60 mm Hg induced a proportional reduction of lCBF ($y = -34 + 1.65x - 0.01x^2$; $r = 0.915$). There was, however, a considerable flow variation at each pressure level in individual animals. **Inset:** Time course of MABP for the pressure classes chosen.

Figure 2a shows the medians of the average lCBF registered by LD_{stat}. During the control phase, the median flow remained constant at MABP of >55 mm Hg. Lowering MABP by hypobaric hypotension below 50 mm Hg induced a 20% reduction of lCBF. The first statistically significant lCBF decrease, however, was found only at MABP of <45 mm Hg, when lCBF was decreased by 48% ($p < 0.01$). The LD_{stat} signal continued to decrease with further progressing hypobaric hypotension. Figure 2b gives the median lCBF as registered by the mobile LD used for scanning (LD_{scan}). At MABP levels of >55 mm Hg, LD_{scan}—just like LD_{stat}—detected a plateau phase that did not change significantly at MABP variations between 56 and 85 mm Hg. MABP below 50 mm Hg was followed by reduced flow in the investigated area, with lCBF reduced to 69% as compared with the plateau phase ($p < 0.001$). Any further MABP reduction caused further decreases of lCBF.

In another step, the individual LD_{scan} data were assigned to flow classes (see Material and Methods), and observation frequencies were calculated (Fig. 3). Histograms thus obtained during the plateau phase describe the typical distribution of lCBF. The normal lCBF pattern is reflected by the first four histograms in Fig. 3 (MABP classes >55 mm Hg) with an asymmetric, left-shifted histogram distribution. Direct visual control suggests that lCBF values between 20 and 40 LD units are reflecting the microcirculation, whereas higher values are obtained from larger vessels. It turned out that ~50–55% of the data were found in flow classes between 20 and 40 LD units, corresponding to the microcirculation. It is worthwhile to note that low-flow areas, i.e., with readings below 20 LD units, were not found during the plateau phase of the pressure/flow relationship in the rat parietal cortex. The median lCBF (indicated by dotted vertical lines in Fig. 3) was similar (41–43 LD units) in the individual control histograms (Fig. 3, first four histograms;

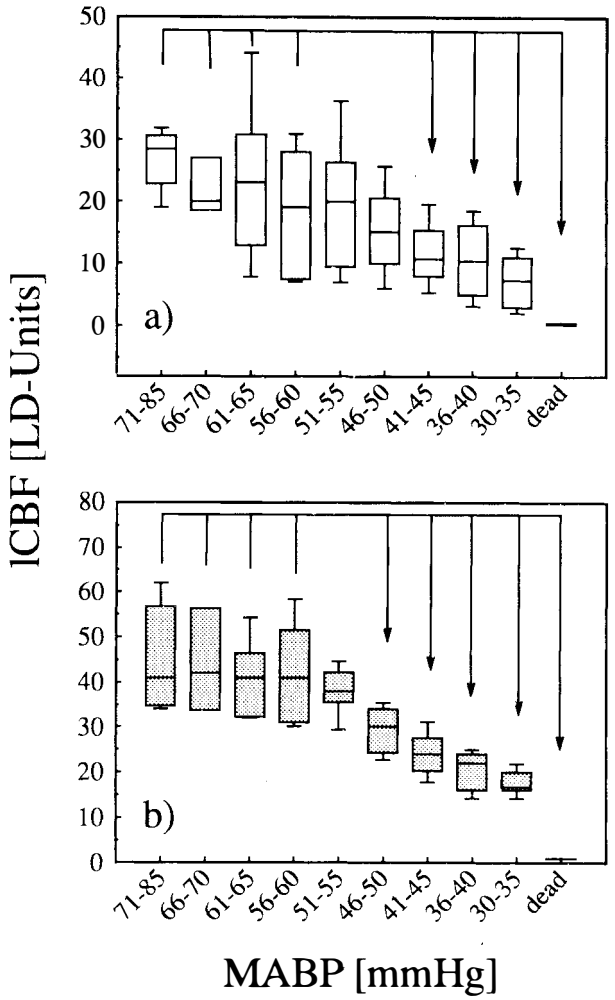

FIG. 2. Local CBF (lCBF) as assessed by stationary **(a)** and scanning **(b)** laser Doppler (LD). Data are shown as box plots (median with 5 and 95% percentiles) for all MABP classes, and biological zero as determined after death. Under conditions with MABP variations between 60 and 85 mm Hg, lCBF remained on a stable plateau. Below a threshold of <50 mm Hg, lCBF was reduced with decreasing blood pressure. Note the earlier statistical significance obtained with LD_{scan}. LD_{stat} values were lower than LD_{scan} since measuring sites free of visible vasculature had been chosen for the stationary probe, whereas LD_{scan} data were calculated from medians of scanned histograms. Arrows indicate statistically significant differences (analysis of variance on ranks for repeated measures, $p < 0.05$).

MABP 56–85 mm Hg). Hypobaric hypotension typically altered the shape of the histograms. The histogram at an MABP between 51 and 55 mm Hg indicates a transitional stage: The median lCBF was slightly reduced to 38 LD units, and lCBF readings occupying classes below 20 LD units—not observed during the plateau phase of the pressure/flow relationship—appeared (hatched bars). The further reduction of MABP below 50 mm Hg caused a more widespread decrease in lCBF, described by a significantly ($p < 0.001$) decreased median flow (Fig. 2b). In addition, hypoperfusion was evident as a shift of the frequency histogram to the left (Fig. 3). lCBF observations in low-flow classes <20 LD units (hatched bars) nearly tripled. At even lower MABP, the tendency of histograms to shift to the left continued with more frequently occurring low-flow areas. Below 40 mm Hg, 45% of the LD readings were <20 LD units (median 21 LD units). At MABP readings below 35 mm Hg, a median lCBF of 18 LD units was found, with 60% of the measurements below 20, and 4% below 10 LD units (Fig. 3). Biological zero (MABP = 0) was determined after death. One hundred percent of the flow values were found between 0 and 5 LD units (Fig. 2).

DISCUSSION

Hypobaric hypotension

The present results indicate that hypobaric hypotension is an excellent method with which to manipulate systemic arterial pressure. Any intended pressure level below the physiological MABP is adjustable. Possible side effects to blood cells or organs caused by the temporary venous blood pooling have not been described in the literature and were not found in the current study. Since the technique is efficient and not too complicated, it may be used for a variety of applications, particularly to induce global cerebral ischemia after occlusion of both carotid arteries as an alternative to hypotension induced by drugs or bleeding for the study of physiological or pathophysiological phenomena. Thereby, temporary global ischemia may be induced without heparinization, bleeding, or the additional occlusion of the vertebral arteries. Moreover, the degree of flow reduction can be regulated by the electronic control of the vacuum pump. MABP but also lCBF may be used as the independent variable.

LD scanning

LDF allows reliable, noninvasive, and continuous recordings of lCBF with a high temporal resolution (Skarphedinsson et al., 1988; Dirnagl et al., 1989). This is the first report to study the lower threshold of blood flow autoregulation by two different LD techniques. The combination of a conventional (stationary) LD with a high temporal resolution and the scanning LD characterized by a high spatial but lower temporal resolution helps to overcome the drawbacks of the LD technique, the small sampling volume and the absence of calibrated units. LD scanning represents an excellent tool with which to compare LD data from individual animals or different physiologic conditions. With a stable

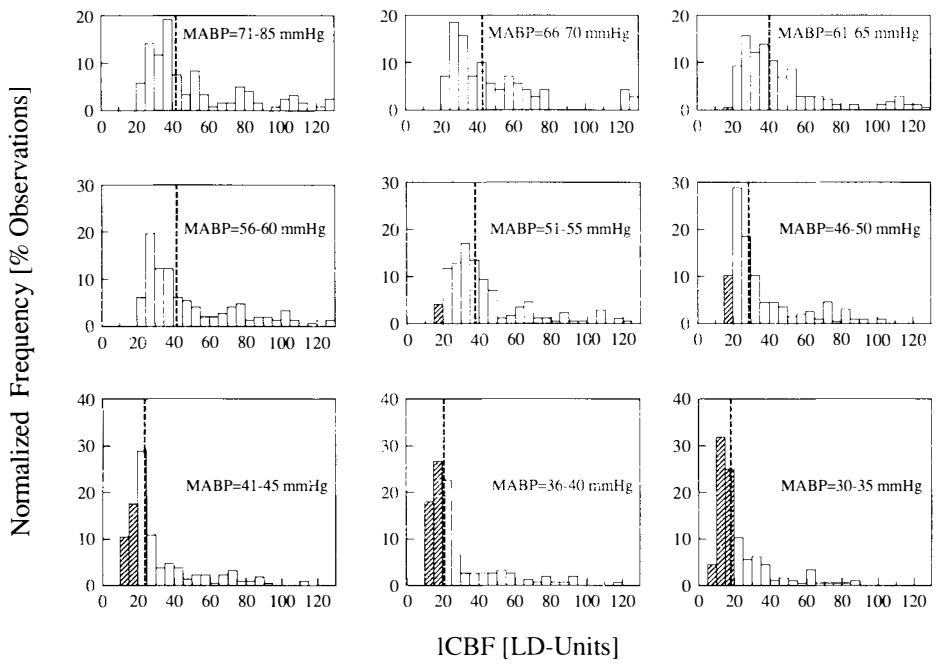

FIG. 3. Local CBF (lCBF) frequency histograms calculated from scanning laser Doppler (LD_{scan}) for all MABP classes. Histograms are obtained by assigning measurements to flow classes and counting observation frequencies for each class. Frequencies were then normalized to 100% for better comparison. The lCBF median for each histogram is indicated by a dotted line. The typical lCBF pattern without applying hypobaric hypotension (upper left histogram) is characterized by a left-shifted nonnormal distribution with a frequency maximum between 20 and 40 LD units. Note that there were no observations in 0–20 LD unit classes during the plateau phase of the pressure/flow relationship. Hypobaric hypotension induced changes of this pattern: At an MABP between 51 and 55 mm Hg, flow values below 20 LD units occurred, illustrated by hatched bars, and the median flow decreased. Further decreases of MABP induced shifts of the histograms to the left. Simultaneously, the frequency of low-flow areas (<20 LD units) increased.

and low biological zero (as shown here for the TSI LDF), histograms from repeated measurements in individual animals are well comparable under control conditions (Ulrich et al., 1983; Ungersböck et al., 1993*a,b*). The technique detects flow changes as alterations of the frequency distribution pattern and as shifts of the median flow. Scanning LDF allows for the detection of abnormal lCBF changes even in cases where control measurements are not possible. As a prerequisite, the normal lCBF pattern of a given anatomical region in the species of interest has to be known beforehand. With enough sampling points, statistically significant shifts of the frequency distribution may thus be obtained. The detailed analysis of the frequency histograms in the current study reveals a sudden occurrence of flow values below 20 LD units at the autoregulation threshold (51–55 mm Hg; Fig. 3). As soon as critical thresholds have been established using the scanning technique, even single scans may allow for the detection of critically low perfused areas. It should be stressed that to permit the recognition of an altered flow pattern, the scanning technique does not require a strictly linear relationship of LDF data with regional CBF data obtained by conventional techniques.

The stationary as well as the scanning LD detect the plateau lCBF within the classic limits of CBF autoregulation (Gross et al., 1981; Dirnagl and Pulsinelli, 1990). Figure 2 demonstrates, however, that LD_{scan} recognizes the breakdown of blood flow autoregulation with a sharp edge in the trend of median lCBF and also with better statistical accuracy than LD_{stat}: With the scanning technique, the end of the plateau phase of blood flow was determined at an MABP of 46–50 mm Hg, where median flow was significantly reduced as compared with lCBF between 56 and 81 mm Hg. With LD_{stat} statistically significant lCBF reductions were found only at an MABP of 41–45 mm Hg.

Taken together, the analysis of LD data by frequency histograms represents a useful tool, providing information on the regional CBF variability not available from a single stationary probe. Conventional, stationary LD, on the other hand, has a better temporal resolution that allows for the detection of rapid changes in lCBF. The spatial resolution, however, is limited to a volume of 1–2 mm^2. In consequence, CBF assessment by stationary LDF is highly dependent on the localization of the LD probe and the underlying anatomical substrate. Even minor unintentional displacements of the LD_{stat} probe (or the underlying tissue) preclude the comparison of data with preceding values. Problems related to probe/tissue displacement are, in fact, responsible for the variability of LD_{stat} data in Fig. 2a, causing the larger variability of lCBF as compared with LD_{scan}.

The scanning technique has meanwhile been successfully used in a variety of other pathophysiologic conditions such as carotid occlusion and flow stimulation by acetazolamide, yielding reproducible histogram shifts to the left and to the right, respectively (Ulrich et al., 1993). The significance of a more regional assessment of LD flow has also been recognized by Wårdell et al. (1993), who have introduced a dynamic light-scattering technique to create a perfusion image. Such an image is built up within 4 min with a resolution of 1,024 * 1,024 points. Therefore, the technique does not average flow values for each pixel nor allows one to observe vasomotion or slow respiration-dependent flow changes. Perfusion images may also be obtained by the scanning technique (Ungersböck et al., 1993*a*). Here, an averaging is possible. However, fewer locations are scanned per time unit than with the dynamic light-scattering technique.

For future studies, LD scanning as well as hypobaric hypotension are suggested as suitable methods, simplifying the investigation not only of the lower threshold of blood flow autoregulation but especially of global and focal cerebral ischemia.

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REFERENCES

DiResta GR, Arbit E, Lau N, Galicich JH (1987) Measurement of regional cerebral blood flow using a combination laser Doppler-hydrogen clearance probe. *J Cereb Blood Flow Metab* 7(suppl 1):S570

Dirnagl U, Pulsinelli W (1990) Autoregulation of cerebral blood flow in experimental focal brain ischemia. *J Cereb Blood Flow Metab* 10:327–336

Dirnagl U, Kaplan B, Jacewicz M, Pulsinelli W (1989) Continuous measurement of cerebral blood flow by laser-Doppler flowmetry in a rat stroke model. *J Cereb Blood Flow Metab* 9:589–596

Dirnagl U, Thorén P, Villringer A, Sixt G, Them A, Einhäupl KM (1993) Global forebrain ischemia in the rat: controlled reduction of cerebral blood flow by hypobaric hypotension and 2-vessel occlusion. *Neurol Res* 15:128–131

Gross PM, Harper AM, Graham DI (1981) Cerebral blood flow in rats during physiological and humoral stimuli. *Stroke* 12: 345–352

Haberl RL, Heizer ML, Marmarou A, Ellis EF (1989) Laser-Doppler assessment of brain microcirculation: effect of systemic alterations. *Am J Physiol* 256:H1247–H1254

Lindsberg PJ, O'Neill JT, Paakkari IA, Hallenbeck JM, Feuerstein G (1989) Validation of laser Doppler flowmetry in measurement of spinal cord blood flow. *Am J Physiol* 257: H674–H680

Skarphedinsson JO, Harding H, Thorén P (1988) Repeated measurements of cerebral blood flow in rats. Comparison between the hydrogen clearance method and laser-Doppler flowmetry. *Acta Physiol Scand* 134:133–142

Thorén P, Westling H, Skarphedinsson JO (1989) Effect of the calcium antagonists felodipine and nimodipine on cortical blood flow in the spontaneously hypertensive rat. *J Hypertens* 7(suppl 4):S153–S158

Ulrich P, Kroppenstedt S, Marchand C, Heimann A, Kempski O (1993) Reserve capacity tested by acetazolamide response of cortical microflow up to six weeks after permanent bilateral occlusion of the common carotid artery of the rat. *J Cereb Blood Flow Metab* 13(suppl 1):S210

Ungersböck K, Heimann A, Strecker U, Kempski O (1993*a*) Mapping of cerebral blood flow by laser-Doppler flowmetry. In: *Microcirculatory Stasis in the Brain* (Tomita M, Mchedlishvili G, Rosenblum WI, Heiss W-O, Fukuuchi Y, eds), Amsterdam, Elsevier Science, pp 405–413

Ungersböck K, Heimann A, Kempski O (1993*b*) Cerebral blood flow alterations in a rat model of cerebral sinus thrombosis. *Stroke* 24:563–570

Wårdell K, Jakobsson A, Nilsson GE (1993) Laser Doppler perfusion imaging by dynamic light scattering. *IEEE Trans Biomed Eng* 40:309–316