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Local Variations in the Cerebral Microcirculatory Response to Hypercapnia and Haemorrhage

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Summary

This study evaluates local variations of the cerebral vasomotor responses to hypercapnia and haemorrhagic hypotension in a pig model. Four laser Doppler flow probes were used in each pig. There was considerable variation in laser Doppler signals between the four probes in baseline recordings. The increases in flow after CO_2 administration in 7 pigs had a mean coefficient of variation of 0.43 ± 0.31, and the flow changes after blood loss in another 7 pigs had a mean coefficient of variation of 0.45 ± 0.34. The range of flow changes within each animal was large; the probe with the highest CO_2 response showed on the average a $273\% \pm 157\%$ larger CO_2 response than the probe with the lowest CO_2 response. Correspondingly, the probe with the best preserved blood flow after blood loss had on the average a flow value of $93\% \pm 12\%$ of the baseline value, while the probe that changed most with haemorrhage had a flow value of $44\% \pm 24\%$ of the baseline value. Single laser Doppler recordings have been used for the monitoring of cerebral blood flow in neurosurgical critical care, but our results suggest that a single laser Doppler flow probe is not an adequate method to monitor vasoreactivity in neurosurgical patients because flow signals from one probe may be unrepresentative for other sites in the brain.

Keywords: Laser Doppler flowmetry; cerebral blood flow autoregulation; carbon dioxide reactivity.

Introduction

Cerebral vasomotor responses to changes in partial pressure of arterial carbon dioxide and to changes in arterial blood pressure serve protective functions. These reactions preserve blood flow to the brain during hypotension, respiratory failure, or tissue ischaemia by increasing the delivery of oxygen and the removal of acid metabolites. Loss of vasomotor reactivity leads to a poor outcome in neurosurgical patients under intensive care, especially in patients with reduced cerebral perfusion pressure [18]. The consequences of reduced perfusion pressure are greatly accentuated during failure of autoregulation, when cerebral blood flow is reduced in proportion to the fall in perfusion pressure instead of remaining within normal limits until a critical lower value is reached. Accordingly, the survival after severe head injuries correlates with the integrity of cerebral vasomotor reactivity [18, 29]. Laser Doppler flowmetry is currently being evaluated for the monitoring of cerebral blood flow in neurosurgical critical care [2, 14, 15, 24, 32]. An important aspect of this monitoring is the evaluation of the integrity of CO_2 reactivity and autoregulation [2, 5, 29, 31].

Laser Doppler flowmetry measures blood flow by recording the number and velocity of cells in the tissue [27]. This technique does not give values as volume per unit time, but it allows continuous recording of relative changes in microcirculatory flow. Laser Doppler flowmetry is therefore useful for the study of acute changes in perfusion caused by changes in arterial carbon dioxide pressure or arterial pressure variations [6, 11]. The laser Doppler flowmeter measures the microcirculation in a 1–3 mm^3 tissue volume, and the recordings are thus highly localized [8, 12, 13, 23]. The advantages of laser Doppler flowmetry are that changes in local circulation can be followed instantaneously, and changes in flow can be measured in any part of the brain [17]. The drawback of the method is that the measuring volume is so small, and the measurements are so localized that the signals may be unrepresentative for the total brain, or even unrepresentative within a region of the brain.

We have developed an experimental model for measuring local intracerebral microcirculatory

changes by microfibre laser Doppler flowmetry, and considerable local flow variations have been observed [17]. The aim of this study was to evaluate site to site variation in the cerebral vasomotor responses to hypercapnia and haemorrhagic hypotension, and to evaluate the usefulness of laser Doppler flowmetry as a tool for the monitoring of cerebral vasoreactivity.

Material and Methods

Animals and Operative Technique

The experiment conformed to the Norwegian Council of Animal Research Code for the Care and Use of Animals for Experimental Purposes. Fourteen juvenile Norwegian bred landrace pigs of either sex, weighing between 15 and 30 kg, were used. All animals were under veterinary supervision and fasted overnight, but were allowed free access to water. The animals were anaesthetized with pentobarbital intraperitoneally (25 mg/kg), and anaesthesia was maintained with pentobarbital in continuous infusion through an ear vein (15 mg/kg/h), supplemented with occasional intravenous injections of 1 mg/kg pentobarbital to keep pain reactions abolished. After tracheotomy, ventilation was established with 70% nitrous oxide and 30% oxygen by a servo-ventilator (Siemens 900B, Solna, Sweden) at 20 breaths/min and 5–7 l/min, adjusted to maintain normocapnia according to repeated blood gas measurements. Muscular paralysis was achieved with pancuronium (0.1 mg/kg intravenously in repeated doses as needed) after the surgical preparation had been completed. With the animal in the supine position, fluid filled catheters were inserted into the abdominal aorta through the right femoral and brachial arteries for arterial pressure recording and for blood sampling. All animals were catheterized for measurement of thermodilution cardiac output determination (Swan-Ganz 93A-131H-7F, American Edwards Laboratories, Santa Ana, CA, U.S.A.) through the right external jugular vein. Cardiac output was measured in triplicate by injection of 10 ml of glucose solution (50 mg/ml) at 0 °C and a cardiac output computer (model 9520A, Edwards Lab., Santa Ana, CA, U.S.A.). Blood temperature was measured through the thermistor probe of the Swan-Ganz catheter. Body temperature was maintained with the use of a heating pad. Indwelling urinary bladder catheter was inserted via cystostomy. Ringer-acetate solution was administered intravenously at a rate of 10 ml/kg/hour throughout the experiment.

These preparations having been made, the animal was turned to the prone position and a longitudinal midline incision extending from the glabella to the 7. cervical vertebra was made. Four holes, 5 mm in diameter, were made with a twist drill in the frontoparietal area of the skull, 10 mm from the midline on both sides, 10 mm in front of, and 10 mm behind the coronal suture. Four laser Doppler flow probes (PF319:0 L120, diameter 0.5 mm, Perimed AB, Stockholm, Sweden) were positioned into the cerebral tissue through small incisions in the dura, or on the dura mater without penetrating the dura. All probes measured from the cortical gray matter. Care was taken to assure mechanical stability of the probes, because the system is sensitive to movement and vibration. To achieve this, the probes were stabilised in relation to the skull by inserting them through a cork which was designed to fit the hole and glued to the bone with rapidly setting methyl-metacrylate. The probes were connected to a master probe (PF 318, Perimed AB, Stockholm, Sweden) with a screw coupling (Perimed AB, Stockholm, Sweden) which allowed the master probe to be changed from one probe to another during the experiment. Once a suitable placement was obtained with good signals, the probe was left in unchanged position during the experiment.

Catheters were connected to pressure transducers (model AE840, Sensonor, Horten, Norway) and the zero reference was levelled at mid-chest. Recordings were made on paper by a Gould ES2000 recorder (Gould, Cleveland, OH, U.S.A.).

Systemic arterial pressure and one laser Doppler signal were recorded continuously and signals from all other probes were measured intermittently. Mean arterial pressure was calculated as the diastolic pressure plus one-third of pulse pressure. Heart rate was obtained from the arterial pressure curve. Blood volume was calculated as 61 ml/kg body weight [1].

Laser Doppler Flowmetry

Measurement of cerebral microcirculation with laser Doppler flowmetry was performed with a Periflux PF 2B laser Doppler flowmeter (Perimed AB, Stockholm, Sweden). The flowmeter uses a 2 mW helium-neon laser light, led by an optical fibre to a single fibre probe with a diameter of 0.5 mm. The magnitude and frequency distribution of the Doppler shifted light reflect the number and the velocity of the blood cells moving through the illuminated tissue area. Movement direction is not detected. The flowmeter records signals from a tissue depth of 1–1.5 mm. It produces a relative and not an absolute value of the blood flow in a semispherical volume [26, 27]. The instrument was calibrated against a standarised latex solution, as recommended by the manufacturer (Perimed AB, Stockholm, Sweden). The analysing band width of the flowmeter was set at 12 kilohertz, the gain at 1, and the time constant of the output amplifier at 0.2. There is a measurable electrical signal also at cardiac arrest because of micromotion in dead tissue. The post-mortem signal was therefore considered as zero and substracted from the flow values. The Periflux PF 2B instrument is only capable of monitoring a single probe at a time. Measurement at different sites is possible by connecting the single fiber probes to the master probe one at a time. The flowmeter was connected to the Gould recorder. The Movement Artifact Filter of the flowmeter was not used.

Experimental Protocol

The following variables were recorded: Laser Doppler flow values from 4 probes, arterial pressure, heart rate (these continuously measured variables taken as the average of 1 min recording), cardiac output, blood gases, and blood temperature. Baseline recordings were taken after a stabilising period of 60 minutes. In seven pigs, recordings were taken after 10 minutes with 10% CO_2 added to the gas mixture in the ventilator. In another seven pigs recordings were taken 10 minutes after the withdrawal of approximately 25% of the blood volume.

Statistical analyses were performed with the paired Student's t-test and Pearson's correlation coefficient. Values are given as mean ± SD.

Results

The reason for using transdural and intracerebral measurements is that both methods have been used in

Table 1. *Physiological Variables During Hypercapnia or Haemorrhagic Hypotension in 14 Pigs*

	10% CO_2 in ventilator mixture (n=7)		Loss of 25% of blood volume (n=7)	
	Baseline	Hypercapnia	Baseline	Blood loss
MABP (mmHg)	97 ± 32	86 ± 25	95 ± 14	64 ± 21[b]
HR (beats/min)	123 ± 39	151 ± 40[a]	136 ± 27	162 ± 48
CO (liters/min)	2.5 ± 1.2	2.6 ± 0.8	2.7 ± 0.8	1.9 ± 0.4[a]
P_aCO_2 (kPa)	4.2 ± 0.9	11.8 ± 1.6[b]	4.9 ± 1.5	4.3 ± 1.5[a]
LDF (rel. to baseline)	1	1.72 ± 0.58[a]	1	0.69 ± 0.25[b]

Values given as means ± SD. MABP: mean arterial blood pressure, CO: cardiac output, HR: heart rate, LDF: laser Doppler flowmetry signal (mean of 4 probes), n: number of animals.
[a]Significantly different from baseline: $p<0.03$, [b]$p<0.001$.

Table 2. *Variation of the Cerebral CO_2 Reactivity Between Different Sites in the Brain in 7 Pigs*

Mean	Range	SD	Coefficient of variation
1.09	0.78–2.5	0.81	0.74
1.57	1.13–1.9	0.32	0.20
2.76	2.5–3	0.21	0.08
1.37	1.26–1.67	0.19	0.14
1.96	0.73–4.29	1.6	0.82
1.26	0.63–2.38	0.84	0.67
2.02	1.45–3	0.74	0.37

Cerebral CO_2 reactivity: Blood flow following 10% CO_2 in the inspiratory gas mixture divided by baseline blood flow.
Mean: mean of four probes in one animal.

clinical practice. We found no systematic differences between the signals from differently positioned probes, and the results were therefore pooled.

There were considerable interindividual differences in baseline blood pressure in the hypercapnia experiments, but all baseline blood pressures were within what would be expected to be the normal range in anaesthetized animals. Hypercapnia caused no change in mean arterial pressure or cardiac output but the heart rate increased (Table 1). The mean arterial carbon dioxide pressure (P_aCO_2) increase of 7.6 kPa caused a 1.72 relative increase of the laser Doppler signal. Haemorrhage caused a decrease in mean arterial pressure, cardiac output, P_aCO_2 and laser Doppler flowmetry signal, and tended to increase the heart rate (Table 1).

In baseline recordings there was a considerable variation in laser Doppler signals between the four probes within each animal with a mean coefficient of variation of 0.51 ± 0.21 in the CO_2 response group and 0.70 ± 0.32 in the blood loss group.

The variation of the response to CO_2 inhalation between the four probes in each animal was considerable (Table 2) with a mean variation coefficient of 0.43 ± 0.31, and in three animals one probe showed a paradoxical decrease in signal during increased P_aCO_2. The probe with the highest CO_2 response showed on the average a 273% ± 157% larger CO_2 response than the probe with the lowest CO_2 response.

The variation of the change in blood flow after haemorrhage between the four probes in each animal is shown in Table 3. The range of values was large and the mean coefficient of variation was 0.45 ± 0.34. The probe with the best preserved blood flow after blood loss had on the average a flow value of 93% ± 12% of the prehaemorrhage value, while the probe that changed most with blood loss had a flow

Table 3. *Variation of Autoregulatory Response of the Cerebral Circulation Between Different Sites in 7 Pigs*

Mean	Range	SD	Coefficient of variation
0.51	0.18–1	0.50	0.97
0.77	0.55–1	0.23	0.30
0.86	0.63–1	0.16	0.19
0.60	0.31–0.83	0.24	0.40
0.92	0.83–1	0.08	0.09
0.32	0.114–0.67	0.29	0.90
0.72	0.5–1	0.23	0.33

Autoregulatory response: Blood flow after loss of 25% of the total blood volume divided by baseline blood flow.
Mean: mean of four probes in one animal

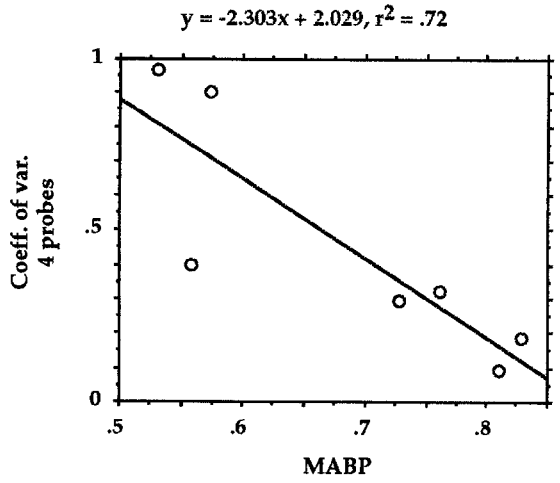

Fig. 1. Scatter plot of the variation in the autoregulatory response in the cerebral circulation after loss of 25% of the blood volume plotted against the change in mean arterial blood pressure (*MABP*). *Coeff. of var. 4 probes*: Coefficient of variation between 4 laser Doppler flowmetry signals

value of 44% ± 24% of the prebleeding value. The differences between laser Doppler signals from the four probes increased with decreasing mean arterial pressure, as the coefficient of variation was correlated with the change in mean arterial pressure ($r = 0.85$, $p < 0.02$) (Fig 1).

Discussion

This study demonstrates considerable spatial variation of the cerebral vasoreactivity. Consequently, a single laser Doppler flowmetry probe might be unreliable in assessing the integrity of CO_2 reactivity and autoregulation. The variation of the response to blood loss was greater with greater changes in mean arterial pressure.

Vasomotor responses to changes in arterial pressure and to changes in P_aCO_2 have been considered to be uniform reactions of the brain vasculature, although it is well recognised that local impairment of the vasomotor responses may occur in damaged or ischaemic tissue [6, 22, 25, 30]. Regional differences in cerebral blood flow have been extensively studied by a variety of methods [3], and highly localized spatial variation of the microcirculation in the cerebral cortex has also been found [4]. There are few studies of the regional or spatial variations of the important cerebrovascular responses to hypotension or hypercapnia, probably because adequate methods for continuous monitoring have been lacking until recently. The laser Doppler flowmetry technique allows continuous monitoring of cerebral blood flow, and should therefore be well suited for the study of changes in blood flow such as produced by P_aCO_2 variation or arterial pressure variation. The method has, however, some disadvantages. These include the lack of quantitation and the lack of multiple simultaneous recordings by most of the equipment currently in use. In addition, there are several sources of artifacts. The probes must be stable, because movement disturbs the signals. Vascular reactivity could be impaired by implanted probes or by tissue reactions in the vicinity of the probe tip. The intracerebral positioning of electrodes has, for several decades, been an established technique for estimating changes in cerebral blood flow. Few studies are available, however, concerning the influence of implantation of measuring probes or electrodes on flow estimates, and therefore we do not know if the probes produced any serious damage to the tissue studied, affecting the blood flow or local vasoreactivity. However, several reports have demonstrated preservation of vasoreactivity with similar invasive techniques [9, 10, 20, 28, 33], and clinical use of intracerebral laser Doppler probes have been readily accepted [2, 14, 24, 34].

We found considerable variation in laser Doppler signal between different probes. The fact that the microcirculation of various organs and tissues show considerable site to site variation, has been repeatedly shown, and this variation has been considered to be caused by differences in the density of microvessels, or localized physiological variations of flow [16, 17, 19, 21, 26]. We have recently reported spatial variation of the cerebral microcirculation of a similar magnitude as the variation reported from skin, muscle, and bone [17]. The results from the present study confirm this observation, and in addition show that the variation of the response of the to hypercapnia and hypotension is substantial, and that the heterogeneity of flow increases as the arterial pressure decreases. There are several possible mechanisms for the spatial differences in cerebral vasoreactivity. There may be local variations in perfusion pressure and local variations in CO_2 concentration or hydrogen ion concentration. Alternatively, vascular smooth muscle reactivity may be unequal from site to site. The present study is the first report to analyse difference between several probes in the brain, and the data indicates the precence of local heterogeneity of functional responses of the microvascular bed. A micro-

vascular module (i.e., a local functional unit of the microvasculature) has been postulated [35], and we suggest that such units may react differently from one another to various stimuli. The flow measurements might also be influenced by volume changes in the brain. When the cerebral perfusion, and presumably the cerebral blood volume, changes, it is likely that some movement in the brain tissue occurs. The probe might therefore measure from slightly different volumes of tissue as the blood flow varies, and this might in part explain the blood flow variation. Most animals commonly used for experimental purposes, including pigs, differ from humans in the arterial inflow pattern to the brain because they have a carotid rete mirabilis, which supplies arterial blood from both external and internal carotid arteries. The large arteries at the base of the brain are similar in humans and pigs. More distal vessels, and the cerebral microvasculature, have similar architecture in all mammals [7]. The laser Doppler flowmetry technique measures flow in microvessels. It is not very likely that species differences in the arterial inflow pattern to the brain affects the laser Doppler measurements, or variation in laser Doppler signal between different probes.

The observation that flow changes are not uniform, suggests that a single laser Doppler recording may well be unrepresentative for the vasoreactivity of the brain. Our results further suggest that in patients with low cerebral perfusion pressure caused by arterial hypotension or intracranial hypertension, the likelihood of an unrepresentative recording is even greater. Careful monitoring of cerebrovascular reactivity is especially important in this patient group, because decisions concerning therapeutic regimes like hyperventilation or sedation with barbiturates may rely on studies of cerebrovascular reactivity. Laser Doppler flowmetry techniques have been evaluated for monitoring of cerebral blood flow in neurosurgical critical care and the attitude towards the new method from most groups have been enthusiastic [2, 14, 15, 24, 34]. A less favourable report stated, however, that the signals may be unpredictable in the clinical situation, and that "well behaved" signal patterns can be isolated by appropriate data selection [32]. The present report supports this view. In conclusion, our results suggest that a single laser Doppler flow probe is not an adequate method for monitoring vasoreactivity in neurosurgical patients. If laser Doppler flowmetry is to be used for this purpose, one must use several probes or use probes with larger measuring area than the ones currently in use.

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Comments

The authors performed an experimental study in pigs on cerebral vasoreactivity to hypercapnia and haemorrhagic hypotension. Changes in microcirculatory blood flow were recorded at four different sites with a laser Doppler blood flow monitor.

The results show a considerable intra-individual difference, and the authors conclude that single probe laser Doppler monitoring for changes in cerebral blood flow in neurosurgical patients is an inadequate method.

A heterogeneous change in cerebral blood flow (CBF) in this order of magnitude, 273% to hypercapnia and 49% with haemorrhagic hypotension is a previously unreported phenomenon.

The controversial results of this study are interesting.

P. Schmiedek

The authors evaluate in a pig model the cerebral vasomotor responses to hypercapnia and haemorrhagic hypotension using four laser Doppler flow probes in each animal. They demonstrated a considerable variation in laser Doppler signals between the four probes in the same animal. They conclude that a single laser Doppler flow probe as used in neurosurgical critical care may not be an adequate method for monitoring vasoreactivity of the brain in neurosurgical patients because of the small volume measured by the probe and the local heterogeneity of functional responses of the microvascular bed. If cerebral vasoreactivity is evaluated by Doppler flowmetry, probes with larger measuring era or several probes should be used. I think this is a very well done and critical paper and underlines the importance of investigating new methods critically in experimental situations before introducing them in clinical practice.

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