A Venous Outflow Method for Measurement of Rapid Changes of the Cerebral Blood Flow and Oxygen Consumption in the Rat

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SUMMARY A technique for continuous measurement of cerebral venous outflow in the rat is described. The method involves cannulation of one retroglenoid vein close to its exit from the skull, and diversion of cerebral venous blood through a closed extracorporeal circuit with a drop recording device, the blood being returned to the central venous circulation via a catheter in the external jugular vein. Occlusion of the contralateral retroglenoid vein increases measured flow and minimizes extracerebral contamination of the diverted cerebral venous blood. The venous outflow system is not further isolated from cerebral or potential extracerebral collaterals. Thus, the mass of tissue drained cannot be exactly defined anatomically. However, the experiments involving changes of PP, arterial CO\textsubscript{2} tension, and induction of epileptic seizure activity, and simultaneous indirect measurements with radioactive tracer technique, indicate that significant extracerebral contamination does not occur and that in short term measurements the venous outflow represents cerebral blood flow (CBF) in a constant mass of (dorsal and central, mainly forebrain) cerebral tissue. Measurement of arterial blood pressure and pressure in the cisterna magna allows calculation of cerebral perfusion pressure (PP). By simultaneous measurement of arterial and cerebral venous oxygen content changes in cerebral oxygen consumption (CMR\textsubscript{O\textsubscript{2}}) can be calculated.

The method has been applied to document several situations of transient CBF and CMR\textsubscript{O\textsubscript{2}} changes.

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ALTHOUGH much information on cerebral blood flow (CBF) and oxygen consumption (CMR\textsubscript{O\textsubscript{2}}) can be obtained by indirect techniques mainly those using insert gases, there is a need for direct methods, especially for measurement of transients of blood flow and metabolic rate. The main difficulties in employing direct flow measurements arise from the fact that there are extensive communications between cerebral and extracerebral vessels, and between different cerebral vessels both at the arterial and venous side.

Most current techniques measure venous outflow. These preparations aiming at maximal isolation of the outflow venous system require rather extensive surgical procedures and have mainly been performed in dogs.

Methods for determining CBF and CMR\textsubscript{O\textsubscript{2}} in small laboratory animals like the rat have utilized the principles of indirect techniques. Thus, in our laboratory two modifications of the Kety and Schmidt technique, using \textsuperscript{133}Xenon, have been developed. When sampling of venous blood is performed by puncturing the superior sagittal sinus there seems to be insignificant extracerebral contamination, and the venous blood probably represents frontoparietal cortex. More recently, it was found that the main dorsal cerebral venous outflow vessel, the retroglenoid vein, lends itself to extracranial cannulation. Provided certain precautions are taken, particularly occlusion of the contralateral retroglenoid vein, the blood sampled via this cannula represents mixed dorsal cerebral tissue. The values for CBF and CMR\textsubscript{O\textsubscript{2}} with the retroglenoid vein as the source of cerebral venous blood are about 75% of those obtained when venous blood is sampled from the superior sagittal sinus, the latter representing mainly cortical tissue.

In the present study we describe a technique for continuous cerebral venous outflow recording for determinations of rapid changes of CBF (and CMR\textsubscript{O\textsubscript{2}}) in the rat brain. The method involves monitoring of the outflow volume from one retroglenoid vein with that on the opposite side being occluded. No craniotomy is required. In spite of the fact that no additional elimination of venous communicating channels is performed, the results show that admixture of extracerebral blood is insignificant, and that the venous outflow volume is fairly representative of a constant mass of brain tissue.

Methods

Preparation and Recording

Male Wistar rats (300–450 g) that had free access to pellet food and water until operation were anaesthetized with 2–3% halothane. When unresponsive to external stimuli the animals were tracheotomized and ventilated with a respirator of the Starling type (Braun, Melsungen). Immobilization was achieved with i.v. tubocurarine chloride (1 mg·kg\textsuperscript{-1}). Ventilation was continued with a gas mixture containing N\textsubscript{2}O:O\textsubscript{2} in the volume proportions 3:1. At this point halothane concentration was reduced to 0.6–0.8%. When the operative procedures were completed, halothane supply was discontinued. Body temperature was kept close to 37°C by means of intermittent external heating.

Catheters were placed in one femoral vein for donor blood infusion, and in both femoral arteries, one used for continuous blood pressure (BP) recording, the other for sampling of arterial blood.

The cerebral venous outflow method involves diversion of the retroglenoid venous flow through an extracorporeal, low resistance closed circuit with a chamber for drop recording as schematically shown in figure 1, (DRC). This preparation was achieved by the following procedure: First a soft catheter (inner diameter 1.5
FIGURE 1. Schematic illustration of the cerebral and extracerebral venous collateral system obtained after preparation for retroglenoid venous outflow measurement. DRC represents the retroglenoid to superior caval vein (SCV) drop recording circuit. ECV is the extracerebral venous system separated from the intracranial venous system by compression of the retroglenoid vein at X. ICV represents the intracranial collateral venous pathways. Through the thin needle at S slow sampling from streaming cerebral venous blood can be performed.

mm) with tip and side holes was introduced into the right external jugular vein and advanced into the superior caval vein to the level of the right atrium. By blunt dissection followed by sharp removal of the temporal muscle from the parietal bone the vein was exposed at its exit through the retroglenoid foramen. The left retroglenoid vein was exposed in similar fashion. On this side a catheter (inner diameter 0.75 mm) was inserted through a superficial venous branch and advanced in retrograde direction to the retroglenoid foramen. The end of the catheter had been cut obliquely to fit and "seal" the opening of the retroglenoid foramen. A ligature was placed around the catheter close to the exit of the vein at the foramen, above the branch from the temporo-mandibular joint.

The head of the animal was then fixed with a clamp. In some experiments electrodes for EEG recording were inserted (bone screws or needle electrodes). For measurement of cisterna magna pressure the neck muscles were somewhat retracted from the occipital bone, and a needle, connected to a closed manometer system, was advanced through the muscles and the atlanto-occipital membrane to enter the cisterna. After this preparation the animal was heparinized (300 I.U.·kg⁻¹, i.v.).

The extracorporeal venous flow system was then connected. First, the jugular catheter was connected to the drop recording chamber via a catheter (inner diameter 3 mm), which together with the lower part of the chamber were filled in a retrograde direction with blood from the animal. An equal volume of donor blood (about 1.5 ml) was simultaneously infused in the femoral vein. The retroglenoid catheter was then connected to the upper end of the drop chamber (via a catheter with inner diameter 2 mm). When flow was established through the circuit the contralateral retroglenoid vein was compressed by a rubber pellet (fig. 1:X). A thin needle was inserted into the retroglenoid vein catheter to allow intermittent sampling of cerebral venous blood (fig. 1:S). After compression, a stabilizing period of 10 min was usually allowed.

The photoelectric drop signal, as well as EEG, BP and ICP were continuously recorded with a polygraph (Mingograph, Elema-Schönander, Solna).

Measurement of Blood Gases and pH

Blood was sampled in glass pipettes from the femoral artery and from the side branch of the venous outflow catheter (sampling thus temporarily interrupting quantitative drop recording). Arterial PₐO₂, PₐCO₂, and pH, as well as arterial and cerebral venous total oxygen content (CₐO₂) were measured immediately upon sampling. PₐO₂ and PₐCO₂ were measured by microelectrodes operated at 37°C (Eschweiler and Co., Kiel). The electrodes were calibrated with gases of known O₂ and CO₂ contents before each measurement. The pH was measured with a microelectrode (Radiometer, Copenhagen). All PₐO₂, PₐCO₂ and pH values were corrected for any difference in temperature between animal and electrode. CₐO₂ of arterial and cerebral venous blood was measured in 25 μl samples with the polarographic method of Fabel and Lübbers as described previously, allowing calculation of the arterio-venous difference for oxygen (AVDₐO₂).
**Quantification of Venous Outflow Volume**

The relationship between drop counts and flow rate was determined in model experiments: defined volumes of heparinized blood, taken from five animals at the end of the experiments, were perfused through the drop recorder using an infusion pump at speeds varying between 0.2 and 5 ml/min. On the basis of these results a diagram was constructed relating flow volume to number of drops per min.

**Calculations**

Cerebral blood flow was primarily expressed as outflow volume per min.

Cerebral perfusion pressure (CPP) was calculated as the difference between the mean arterial blood pressure and the pressure in the cisterna magna (ICP).

As applied in ref. 11 (see also Results, 4) the technique allows calculation of CBF and CMR\textsubscript{O\textsubscript{2}} in conventional terms, i.e. in ml/g-min, using the following procedure: CMR\textsubscript{O\textsubscript{2}} in the control situation has previously been determined using the inert gas technique applied to the present preparation for sampling of cerebral venous blood. This factor (0.076 ml.g\textsuperscript{-1}.min\textsuperscript{-1}) can be used in a situation of normal CMR\textsubscript{O\textsubscript{2}} to calculate the actual flow value from the arteriovenous difference in oxygen content (AVD\textsubscript{O\textsubscript{2}}) according to the equation:

\[
\text{CBF (ml.g\textsuperscript{-1}.min\textsuperscript{-1}}) \times \text{AVD}_{O_2} (\text{ml.ml\textsuperscript{-1}}) = 0.076
\]

The CBF value thus derived can be related to the venous outflow volume at the time of AVD\textsubscript{O\textsubscript{2}} measurement, and a constant (K) can thus be determined:

\[
K \times \text{venous outflow (ml.min\textsuperscript{-1}}) = \text{CBF (ml.g\textsuperscript{-1}.min\textsuperscript{-1}})
\]

The K varies to some extent between different preparations indicating differences of brain mass draining through the recording systems. These differences are evidently due to differences in the relations between the flow resistance of the extracorporeal circuit and that of the collateral venous channels. However, as will be shown, the K remains essentially constant during short term measurements in the individual experiment.

**Experimental Design**

The following experiments were designed to characterize and validate the venous outflow method.

**I. The Retroglenoid Venous Outflow Preparation for Sampling of Cerebral Venous Blood**

In 4 animals the preparation for venous outflow measurement was used for determination of CBF and CMR\textsubscript{O\textsubscript{2}} according to the modified Kety and Schmidt technique, i.e. the cerebral venous blood sampling was performed via the thin needle (see fig. 1) inserted into, and deviating a portion of the blood from one retroglenoid vein catheter. The animals were kept normotensive, normoxic and normocapnic.

**II. Constancy of Venous Outflow**

Since the position of the retroglenoid and jugular vein catheters may affect the resistance in the recording circuit it was required that, after the establishment of flow in the circuit, a stable outflow was found during steady state, i.e. during a 5-10 min period of constant BP and blood gases, otherwise the preparation was discarded.

In 5 animals the venous outflow and AVD\textsubscript{O\textsubscript{2}} were determined in the control situation. Then during 30 sec 10% CO\textsubscript{2} was added to the respiratory gases, inducing a marked, short increase of venous outflow. Three min after this transient disturbance venous outflow and AVD\textsubscript{O\textsubscript{2}} were again determined for comparison with the results of the first determination.

**III. Autoregulation and CO\textsubscript{2} Response**

A preparation for measurement of CBF should expose physiological vasomotor reactions, such as autoregulation to changes in CPP, or response to changes in arterial CO\textsubscript{2} tension. In 11 animals autoregulation was tested during reduction of BP by bleeding. The venous outflow volume was determined before and following the change of BP, allowing about 3 min of stabilization after the BP change.

After reinfusion of blood and normalization of BP, CO\textsubscript{2} was added to the respiratory gases during 2-4 min. Venous outflow volume was calculated before, and at steady state 1-4 min after the start of CO\textsubscript{2} administration. In both sets of tests AVD\textsubscript{O\textsubscript{2}}, as well as arterial P\textsubscript{0\textsubscript{2}}, P\textsubscript{CO\textsubscript{2}} and pH were determined at each flow level.

**IV. Venous Outflow vs. Quantitative CBF During Seizure Activation**

These experiments, previously published\textsuperscript{11} will be briefly represented: In 4 animals the venous outflow was monitored and AVD\textsubscript{O\textsubscript{2}} determined in the control situation. Then status epilepticus was induced by bicuculline and after 30 min AVD\textsubscript{O\textsubscript{2}} was again determined. The change of venous outflow, and the AVD\textsubscript{O\textsubscript{2}} value at 20 min of seizure activity were used to calculate the CBF and CMR\textsubscript{O\textsubscript{2}} were determined in the same animals using the \textsuperscript{133}Xe technique described\textsuperscript{6} and the results obtained with the two methods compared.\textsuperscript{11}

**Results**

I. The CBF values obtained by the \textsuperscript{133}Xe technique using the venous outflow preparation for sampling of cerebral venous blood were 76, 72, 80 and 83 ml/100g-min and CMR\textsubscript{O\textsubscript{2}} 7.2, 8.0, 7.9 and 8.4 ml/100g-min, respectively. The clearance curves were similar to those previously described,\textsuperscript{6} thus with no signs of extracerebral contamination i.e. with no components indicating slowly perfused (= extracerebral) tissues.

II. The recording shown in figure 2 illustrates the dynamics of the CO\textsubscript{2} response induced by 30 sec of CO\textsubscript{2} inhalation. The absolute outflow volume in 5 animals before vs. 3 min after a transient CO\textsubscript{2} induced vasodilatation were: 1.1-0.8, 1.2-1.2, 1.2-1.3, 1.2-1.1 and 1.0-0.9 ml/min, respectively. The corresponding "CMR\textsubscript{O\textsubscript{2}}" values, i.e. the products of outflow volume and AVD\textsubscript{O\textsubscript{2}} were 0.100-0.088, 0.112-
FIGURE 2. Response to BP, ICP and venous outflow to a 30 sec period of CO2 addition to respiratory gases.

0.122, 0.117–0.112, 0.107–0.117 and 0.078–0.079 ml O2/min. The values of the repeated CMR02 determination, performed after a transient major CO2 induced change of CBF, deviated from that of the first within a range of -12 to +10%.

III. A. Autoregulation

The venous outflow volumes (“CBF”) obtained during reduction of BP in the range 150–50 mm Hg in 11 animals are shown in figure 3 (upper part). The calculated CMR02, expressed as the product of CBF and AVD02, is shown below the CBF results. Autoregulation was present, and the CMR02 was fairly constant within this range of BP changes. Arterial P CO2 in the whole group was 31—44 mm Hg, but in each experiment it remained within a range of 4 mm Hg.

B. CO2 Response

Figure 4 summarizes the response to hypercapnia induced during 2–4 min in 11 animals. A constancy of CMR02, similar to that found in II and III A was found. The BP was 100–130 mm Hg during normocapnia and 100–145 during hypercapnia. ICP increased from about 10 to 20–25 mm Hg during the hypercapnic vasodilatation. As a net result the CPP was essentially constant during the manoeuvre.

IV. Seizure Activation

As previously described11 the CBF and CMR02 determinations during seizures using the venous outflow technique (CBFvo) gave results in good agreement with those of the modified Ketty and Schmidt technique (CBFKS).9 Thus, during seizures the calculated CBFvo values increased to 317, 403, 242 and 293 ml/100 g·min and the corresponding CMR02 values were 19.5, 22.2, 30.4 and 20.0, respectively. The concomitantly measured CBFKS were 298, 423, 244 and 325 ml/100 g·min and CMR02 18.7, 23.5, 31.2 and 22.5 ml/100 g·min, respectively.

Discussion

As remarked in the introduction most techniques of direct CBF measurement have involved venous outflow recording. The main problems arise from the fact that there are extensive communications between the veins from different parts of the brain, and between cerebral and extracerebral veins1,12-15 Current tech-
niques used in larger animals, mainly dogs, have employed rather extensive surgery in order to isolate the venous outflow system. For example, the method of Rapela and Green is based on isolation of the outflow from the confluens sinuum in dogs by occlusion of the lateral sinus and cerebellar veins. A similar system developed by Michenfelder et al. involves cannulation of the superior sagittal sinus in anterior direction with obliteration of frontal and diploic extracerebral communications and occlusion of the sinus behind the point of cannulation. The present rat preparation is similar to that in dogs described by D’Alecy and Feigl. However, in the present technique no attempts were made to occlude the sigmoid sinus.

Figure 1 schematically illustrates the venous systems obtained in the present preparation. The dorsal brain drains mainly via the retroglenoid veins, i.e. through the drop recording circuit (DRC), since the contralateral vein is occluded. This occlusion also interrupts the main communication between the outflow system and extracerebral veins. Mainly ventral and vertebral veins are simply shown as ICV. For anatomical reasons certain regions preferentially drain via these systems, which, however, communicate with the veins draining via the artificial circuit. Instead of making further attempts to eliminate these venous communications, involving surgical trauma, we chose to investigate the validity of the method with these collaterals present.

Oclusion of flow through one retroglenoid vein increases flow through the other as regularly found in the present preparation. Previous tests as well as present findings (Results, I) demonstrate that, unless an unphysiologically high pressure gradient in the outflow system is created (as occurs when a wide catheter is open to air) significant extracerebral contamination does not seem to occur.

Our next concern was therefore the relationship between DRC and ICV i.e. the problem of the possible variations in the mass of cerebral tissue draining through these two systems. The experimental design was based on the concepts of figure 1. Using the symbols of the figure we can write the equation for total CBF as being composed of two fractions:

\[ CBF_{\text{tot}} = CBF_{\text{DRC}} + CBF_{\text{ICV}} \]

or

\[ CBF_{\text{tot}} = \frac{P_1 - P_2}{R_{\text{DRC}}} + \frac{P_1 - P_2}{R_{\text{ICV}}} \]

in which \( P_1 \) represents the cerebral venous pressure and \( P_2 \) the central venous pressure. Since the venous outflow system is closed and allows blood to return to the central venous circulation \( P_1 - P_2 \) gives the pressure head of both fractions. Thus, the masses of tissue delivering blood to the two parallel venous systems should be mainly determined by the relative resistances \( R_{\text{DRC}} \) and \( R_{\text{ICV}} \). Experimentally, the \( CBF_{\text{DRC}} \) was found to be constant in steady state and following a transient \( CO_2 \) induced increase of CBF (Results, II).

The constancy of the DRC fraction was further demonstrated in the autoregulation and \( CO_2 \) experiments (Results, III, A,B). Finally the validation was obtained by calculating changes of CBF and \( CMR_{O_2} \) during sustained epileptic seizures, and comparing the values with those obtained with the inert gas technique (Results, IV).

We conclude that despite remaining collaterals in the preparation, the changes in venous outflow recorded truly reflect alterations in CBF, at least during a time period of 20–30 min, i.e. the interval studied in the seizure series. However, it should be pointed out that over time there may occur shifts of the relative resistances that may change these conditions. Thus, a reliably optimal position of the catheters is required. Furthermore, during experiments over hours, we have found a tendency to a decrease of the portion of blood passing the drop recording circuit. We have also observed that elevation of the cisterna magna pressure by infusion may give rise to a slight increase in drop flow, probably due to compression of intrathecal, mainly vertebral veins. Thus, the technique is mainly suited for measurement of rapid changes of CBF and \( CMR_{O_2} \) in the same animal.

In conclusion, the technique described permits continuous measurement of cerebral venous outflow in the rat. No significant extracerebral contamination occurs. During rapid changes of CBF and \( CMR_{O_2} \), the venous outflow volume remains representative of the CBF within a fairly constant mass of brain tissue allowing quantitative measurements of short term changes.

The application of a venous outflow technique to a small laboratory animal has allowed studies of transient changes of CBF and \( CMR_{O_2} \), correlated to metabolic events and other regulatory mechanisms in several situations such as hypercapnia, hypoxia, ischemia, and seizures. The immediate effects of intracarotid drug administration, as well as the rapid effects of cyclo-oxygenase inhibition by i.v. indomethacin have been studied. The method has also been applied to studies of vasomotor reactions in spontaneously hypertensive rats.

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Regional Cerebral Blood Flow During Hypercapnia in the Anesthetized Rabbit

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SUMMARY These experiments were designed to test the hypothesis that increases in blood flow to the lower brainstem would be greater than forebrain regions during arterial hypercapnia. Total and regional cerebral blood flow (CBF) was measured via the tracer microsphere technique in seven anesthetized New Zealand white rabbits during normocapnia (arterial PCO₂ = 40 torr) and hypercapnia (arterial PCO₂ = 80 torr). During normocapnia average CBF was 0.77 ml/min/g, and regional measurements of blood flow indicated significantly greater flow to the cerebellum (0.86 ml/min/g) than either the medulla (0.52 ml/min/g) or the pons (0.49 ml/min/g). When arterial PCO₂ was increased average CBF increased 113%, and a significant linear regression was calculated for arterial PCO₂ vs CBF [CBF (ml/min/g) = 0.028 PCO₂ (torr) – 0.502]. The distribution of blood flow within the brain was similar to normocapnia except that blood flow to the cerebellum was now greater than any other brain region (1.97 ml/min/g for the cerebellum compared to 1.66 ml/min/g for the cerebrum). Absolute increases in blood flow to the lower brainstem were equal to or less than other areas of the brain. We conclude that ponto-medullary blood flow does not increase disproportionately to other areas of the brain during hypercapnia, but some redistribution of CBF does occur in that cerebellar blood flow increased significantly more than the cerebrum, medulla, or pons.

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ALTHOUGH INCREASES IN ARTERIAL CO₂ are known to increase both cerebral blood flow (CBF) and respiration, the interaction between these two phenomena has not been adequately studied. For example, stimulation of the medullary respiratory center occurs during hypercapnia resulting in a marked increase in breathing and may increase metabolism in this region of the brain. Since regional CBF is tightly coupled to increases in metabolism, one might expect that the local increase in medullary activity may lead to increases in flow above the level due to the CO₂ stimulus alone. Such a response could lead to a greater increase in blood flow to hindbrain regions than the forebrain during hypercapnia.

Previous studies from other laboratories have only provided indirect support for this hypothesis. Malik et al. stated that the percentage increase in infratentorial blood flow (cerebellum, pons, medulla, and cervical spinal cord) was greater than supratentorial flow in anesthetized dogs mechanically ventilated with 5% CO₂. The findings are somewhat difficult to interpret...
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