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 CO₂ 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 (CMR₀₂) can be calculated.

The method has been applied to document several situations of transient CBF and CMR₀₂ changes. Stroke Vol 14, No 5, 1983

ALTHOUGH much information on cerebral blood flow (CBF) and oxygen consumption (CMR₀₂) 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.¹ 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.²⁻⁵

Methods for determining CBF and CMR₀₂ in small laboratory animals like the rat have utilized the principles of indirect techniques. Thus, in our laboratory two modifications of the Key and Schmidt technique, using ¹³³Xenon, 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.⁷ 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 CMR₀₂ 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,⁸ 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 CMR₀₂) 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 anesthetized 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⁻¹). Ventilation was continued with a gas mixture containing N₂O:O₂ 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

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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.6

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 \( P_{O_2} \), \( P_{CO_2} \), and pH, as well as arterial and cerebral venous total oxygen content (\( C_{O_2} \)) were measured immediately upon sampling. \( P_{O_2} \) and \( P_{CO_2} \) were measured by microelectrodes operated at 37°C (Eschweiler and Co., Kiel). The electrodes were calibrated with gases of known \( O_2 \) and \( CO_2 \) contents before each measurement. The pH was measured with a microelectrode (Radiometer, Copenhagen). All \( P_{O_2} \), \( P_{CO_2} \) and pH values were corrected for any difference in temperature between animal and electrode. \( C_{O_2} \) of arterial and cerebral venous blood was measured in 25 µl samples with the polarographic method of Fabel and Lübbers6 as described previously, allowing calculation of the arterio-venous difference for oxygen (AVD\( O_2 \)).
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⁻¹. 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 CMRO₂ in conventional terms, i.e. in ml/g·min, using the following procedure: CMRO₂ 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⁻¹·min⁻¹) can be used in a situation of normal CMRO₂ to calculate the actual flow value from the arteriovenous difference in oxygen content (AVD₂) according to the equation:

\[
\text{CBF (ml·g⁻¹·min⁻¹)} \times \text{AVD₂ (ml·ml⁻¹)} = 0.076
\]

The CBF value thus derived can be related to the venous outflow volume at the time of AVD₂ measurement, and a constant (K) can thus be determined:

\[
\text{K} \times \text{venous outflow (ml·min⁻¹)} = \text{CBF (ml·g⁻¹·min⁻¹)}
\]

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 extracorporal 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 Retroglonoid 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 CMRO₂ 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 retroglonoid vein catheter. The animals were kept normotensive, normoxic and normocapnic.

II. Constancy of Venous Outflow

Since the position of the retroglonoid 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 AVD₂ were determined in the control situation. Then during 30 sec 10% CO₂ was added to the respiratory gases, inducing a marked, short increase of venous outflow. Three min after this transient disturbance venous outflow and AVD₂ were again determined for comparison with the results of the first determination.

III. Autoregulation and CO₂ 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 CO₂ 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 5 min of stabilization after the BP change.

After reinfusion of blood and normalization of BP, CO₂ 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 CO₂ administration. In both sets of tests AVD₂, as well as arterial Pₐ, P CO₂ and pH were determined at each flow level.

IV. Venous Outflow vs. Quantitative CBF During Seizure Activation

These experiments, previously published, will be briefly represented: In 4 animals the venous outflow was monitored and AVD₂ determined in the control situation. Then status epilepticus was induced by bicuculline and after 30 min AVD₂ was again determined. The change of venous outflow, and the AVD₂ value at 20 min of seizure activity were used to calculate the CBF and CMRO₂ were determined in the same animals using the ¹³³Xe technique described and the results obtained with the two methods compared.

Results

I. The CBF values obtained by the ¹³³Xe technique using the venous outflow preparation for sampling of cerebral venous blood were 76, 72, 80 and 83 ml/100g·min and CMRO₂ 7.2, 8.0, 7.9 and 8.4 ml/100g·min, respectively. The clearance curves were similar to those previously described, 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 CO₂ response induced by 30 sec of CO₂ inhalation. The absolute outflow volume in 5 animals before vs. 3 min after a transient CO₂ 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 “CMRO₂” values, i.e. the products of outflow volume and AVD₂ 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 P₀₂ 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 described the CBF and CMR₀₂ determinations during seizures using the venous outflow technique (CBFᵥ) gave results in good agreement with those of the modified Kety and Schmidt technique (CBFₖ). Thus, during seizures the calculated CBFᵥ 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 CBFₖ 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 veins. Current tech-
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The constancy of the DRC fraction was further demonstrated in the autoregulation and CO₂ experiments (Results, III, A,B). Finally the validation was obtained by calculating changes of CBF and CMRO₂ 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 CMRO₂ 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 CMRO₂, 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 CMRO₂ 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.

Acknowledgments

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References

6. Kety SS, Schmidt CF: The nitrous oxide method for the quantita-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.

Occlusion 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:

\[
\text{CBF}_\text{tot} = \text{CBF}_{\text{DRC}} + \text{CBF}_{\text{ICV}}
\]

or

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

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 \((R_{\text{DRC}} \text{ and } R_{\text{ICV}})\). Experimentally, the \(\text{CBF}_{\text{DRC}}\) was found to be constant in steady state and following a transient CO₂ induced increase of CBF (Results, II).
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 PCO$_2$ = 40 torr) and hypercapnia (arterial PCO$_2$ = 80 torr). During normocapnia average CBF was 0.77 ml/min/g, and regional measurements of blood flow indicated significantly greater flow to the cerebelum (0.86 ml/min/g) than either the medulla (0.52 ml/min/g) or the pons (0.49 ml/min/g). When arterial PCO$_2$ was increased average CBF increased 113%, and a significant linear regression was calculated for arterial PCO$_2$ vs CBF [CBF (ml/min/g) = 0.028 PCO$_2$ (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.

ALTHOUGH INCREASES IN ARTERIAL CO$_2$ 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 CO$_2$ 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.$^2$ 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% CO$_2$. The findings are somewhat difficult to interpret.
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