Cerebral Blood Flow Autoregulation in the Rat

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SUMMARY Cerebral blood flow autoregulation (CBFA) to changes in perfusion pressure has not been previously reported in the rat. A modification of the Kety and Schmidt technique employing $^{133}$Xenon was used to measure cerebral blood flow (CBF) in paralyzed adult Sprague Dawley rats passively ventilated with 70% nitrous oxide and 30% oxygen. At a mean arterial blood pressure (MABP) of $121 \pm 19$ mm Hg, and a mean arterial PCO$_2$ of $36.2 \pm 2.9$ mm Hg, mean CBF was $103 \pm 22$ ml/min/100 gm of brain. CBF responses to hypercapnia were $4.9$ ml/min/100 gm per mm Hg change in arterial PCO$_2$. CBF was measured during steady state levels of hypo- and hypertension induced by phlebotomy, or by intravenous metaraminol, over the MABP range of 48-205 mm Hg. From a MABP of 80 to 160 mm Hg, CBF remained nearly constant, indicating the presence of CBFA. However, when MABP exceeded 160 mm Hg, CBF became pressure dependent, indicating a "breakthrough" of autoregulation in acute severe hypertension.

CEREBRAL BLOOD FLOW autoregulation (CBFA) denotes the adaptive reaction whereby blood flow to the brain is maintained nearly constant despite variations in perfusion pressure. In most mammalian species, including man, CBFA has been characterized within the mean arterial blood pressure (MABP) range of approximately 70 to 150 mm Hg. However, cerebral circulatory responses to blood pressures beyond this range in acute hypertension have not been extensively studied. Some investigators have recently reported that cerebral autoregulatory mechanisms fail at high perfusion pressures, with a so-called "breakthrough" of CBFA taking place.

Most studies of the cerebral circulation have been carried out in man or in large experimental animals. More recently, CBF has also been studied in the most widely used laboratory animal, the albino rat, by a variety of methods, and values for basal CBF under conditions of normotension have been reported. However, pressure-flow relationships for the cerebral circulation have not been defined in this species. In the present study we have measured CBF in rats during induced alterations in arterial blood pressure. We have examined a wide range of systemic pressures, paying particular attention to the state of cerebral circulation during acute hypertension.

Animal Preparation

Sprague-Dawley rats of both sexes, weighing 300-600 gm, were employed in this study. Each rat was rapidly tracheotomized under ether anesthesia, paralyzed with tubocurarine (0.5 mg/kg, administered subcutaneously), and passively ventilated with a mixture of 70% nitrous oxide and 30% oxygen by a small animal respirator. Atropine (0.05 mg/kg) was injected subcutaneously to minimize tracheobronchial secretion. Ventilatory rate and tidal volume were adjusted to ensure arterial blood normoxia and normocarbria. The caudal artery was exposed and catheterized with a 30 gauge Teflon catheter. This catheter served to monitor arterial blood pressure with a pressure transducer (Statham, Model P23Dc), and to allow anerobic withdrawal of arterial blood samples (200 µl) for determination of pH, PCO$_2$, and PO$_2$ by a blood gas apparatus (Radiometer, Model Mk 2). An external jugular vein was also catheterized for the administration of drugs.

Body temperature was monitored by means of a rectal thermocouple and maintained at 37° with a hot water blanket. Arterial blood pressure and MABP were continuously monitored on a recorder (Beckman, Model R411).

With the rat's skull firmly affixed in a stereotaxic frame, and after subcutaneous infiltration with procaine, a small longitudinal scalp incision was made over the sagittal suture. A burr hole 1 mm in diameter was then drilled directly into the posterior limb of the sagittal suture and continued until the roof of the underlying superior sagittal sinus was punctured. Immediately, a non-beveled, hubless 20 gauge stainless steel cannula was lowered into the sinus with an electrode carrier. The cannula was tightly sealed in place by sliding a silicone rubber sleeve firmly between the cannula and the skull. The cerebral venous cannula and the caudal artery catheter were less than 2 cm in length, so that dead space was minimal, and corrections for smearing of blood samples were not required. To prevent clotting before the CBF determinations, the arterial catheter and cerebral venous cannula were filled with heparin in saline solution.

Measurement of CBF

A modification of the Kety and Schmidt technique employing $^{133}$Xenon as indicator was utilized to determine CBF. This method has recently been described by Ekblf et al., and was implemented in this study with only minor modifications.

When a steady ventilatory state was achieved, a respirometer containing 15 mCi of $^{133}$Xe (New England Nuclear) in 70% N$_2$O and 30% O$_2$ was connected to the inlet of the respirator. A hematocrit was measured at the onset of the saturation period. The outlet of the respirator was connected to a large Douglas collecting bag, to permit safe disposal of expired gas. Inhalation of the gas mixture was continued for 20 minutes, and equilibration of indicator with brain established by obtaining serial paired arterial and cerebral venous samples during the latter part of the $^{133}$Xe inhalation period (at 15, 17, and 20 minutes). All arterial and venous samples were obtained anerobically, in 20 µl non-heparinized glass capillary tubes, after allowing at least 50-60 µl of blood to flush through them. The latter volume exceeded that contained by the arterial catheter or cerebral venous cannula, and ensured that there were no dead space
artifacts. The cerebral venous blood sample was obtained by gentle aspiration at the rate of approximately 3 μl/sec. Blood samples were placed immediately in tightly-stoppered glass containers. At the end of the 20 minute saturation period, ventilation with 70% N2O-30% O2 was resumed, and paired arterial and cerebral venous samples obtained at 0.25, 1, 2, 3, 4, 6, 8, 10, 15 and 20 minutes, following the onset of desaturation. An arterial blood sample was analyzed for pH, Pco2, and Po2 after the 4th minute of desaturation.

The sealed vials containing arterial and venous blood samples were counted in a deep-well scintillation counter (Packard Instrument Co., Model No. 5213) with the appropriate background subtractions based on arterial and venous blood blanks. The results were then tabulated, plotted, and desaturation curves drawn as best fits to the individual points. CBF was calculated by the trapezoid rule according to the formula

\[
\text{CBF} = \frac{100 \cdot C_i}{\int_0^{20} (C_v - C_a)dt}
\]

Where \( C_i \) equals tissue indicator concentration at the end of the saturation period, and

\[
\int_0^{20} (C_v - C_a)dt
\]

is the integrated arteriovenous difference over the 20 minute desaturation period. \( C_i \) was not measured directly but was derived from the indicator concentration in cerebral venous blood at the end of saturation, and from the partition coefficient (\( \lambda \)) expressing the ratio of indicator concentrations in brain tissue and in blood at equilibrium.

The values for \( \lambda \) were obtained in a separate group of rats by the method described by Nilsson and Siesjo. Each rat was prepared as described above, except for the placement of the superior sagittal sinus cannula. Instead, a 30 cc bottomless plastic cup was positioned directly onto the cranium and secured by suturing the resected scalp flaps closely to the cup. After 20 minutes of ventilation with a mixture of \(^{133}\text{Xe} \) in 70% N2O and 30% O2, liquid nitrogen was poured into the plastic cup for approximately 8 minutes. This time period was sufficient to freeze the underlying cerebral cortex. The rat was decapitated and the head immersed in liquid nitrogen for approximately 5 minutes. Chips of parasagittal cerebral cortex were carefully obtained under liquid nitrogen, placed in tared, tightly-sealed vials, weighed, and counted. Reference arterial blood samples were also obtained in 20 μl capillaries at the end of the saturation period with \(^{133}\text{Xe} \), and counted. The ratio of radioactivity between brain and blood (Ci/Ca) at saturation was calculated. This ratio, or \( \lambda \), was determined in 6 rats, and a mean value of 0.81 ± 0.09 (SD) obtained. This value was utilized to calculate CBF according to formula 1 of this section.

**Determination of Autoregulation**

CBF was measured during graded, stable hypotension, achieved by withdrawing small amounts of blood (2-6 ml). Steady states of arterial hypertension were induced by intravenous pump infusion of metaraminol (0.041-0.82 mg/min/kg) begun in the last 10 minutes of saturation with \(^{133}\text{Xe} \), and continued through at least the fifth minute of desaturation. Hematocrits during hypo- or hypertension were repeated at the sixth minute of desaturation in order to account for any hemococoncentration or dilution occurring during the induced alterations in MABP. Animals that developed cardiac arrhythmias during the administration of metaraminol, were excluded from the study.

All CBF values were corrected for changes in arterial Pco2. The correction factor was determined from the results of a linear regression analysis of CBF values obtained from rats under conditions of graded hypo- and hypercarbia induced by altering both tidal volume and respiratory frequency.

CBFA was characterized under conditions of hypo-, normo-, and hypertension, and CBF plotted as a continuous function of MABP, by a least squares curve derived by means of a trapezoidal equation.

**Results**

**Basal CBF During Normocarbia, Normoxia, and Normotension**

In 19 normotensive rats in which MABP averaged 121 ± 19 (SD) mm Hg, CBF was 103 ± 22 ml/min/100 gm of brain. Mean arterial blood pH in these rats was 7.388 ± 0.060, while mean Pco2 and Po2 values were 36.2 ± 2.9 and 99.8 ± 25.0 mm Hg, respectively. Figure 1 illustrates a typical cerebral desaturation curve of \(^{133}\text{Xe} \). As evidenced in this figure, cerebral desaturation of \(^{133}\text{Xe} \) is nearly complete within 5 minutes in the rat, reflecting the high rate of CBF normally encountered in this species, in this and other studies.

**CBF Responses During Hypo- and Hypercarbia**

CBF was measured in 33 rats during alterations in arterial CO2 tensions induced by hypo- and hyperventilation. Arterial Pco2 values obtained at the 4th minute of desaturation in this group ranged from 25 to 62 mm Hg, while arterial Po2 was always maintained above 70 mm Hg. MABP in these rats equaled 128 ± 15 mm Hg. Figure 2 illustrates the linear regression analysis of CBF values obtained at various levels of hypo-, normo-, and hypercarbia. CBF responded considerably to alterations in arterial CO2 tensions, increasing an average of 4.9 ml/min/100 gm of brain for every mm Hg change in arterial Pco2. Thereafter, all CBF measurements were normalized to an arterial Pco2 of 36.2 mm Hg, the mean value reported above under normocarbia, normoxic, and normotensive conditions.

**CBF Responses to Changes in MABP**

As shown in figure 3, CBF was measured in a total of 51 rats during various levels of hypo-, normo-, and hypertension. MABP ranged from 48 to 205 mm Hg. A curve for our data, derived by the least-squares method, is also shown in figure 3. This curve suggests that CBF remained rather constant in the MABP range from approximately 80 to 160 mm Hg. However, when MABP exceeded 160 mm Hg, a marked increase in the predicted CBF was observed, indicating an absence of autoregulation.

An index of cerebral vascular resistance was derived from the ratio MABP/CBF for all 51 rats. A plot of this ratio as a
function of MABP is shown in figure 4. A curve also obtained by the least-squares method, suggests that cerebral vascular resistance rose when MABP increased from approximately 60 to 160 mm Hg, but then fell considerably beyond this point, indicating the presence of autoregulation up to a resistance maximum at approximately 160 mm Hg.

During the arterial and venous sampling period in all normo-, hypo-, and hypertensive rats, MABP seldom dropped more than 3 to 5 mm Hg in the course of CBF measurement, indicating that the cumulative blood sample volume (0.75-1.00 ml, maximum) withdrawn was not sufficient to induce significant hypotension. Minimal hemodilution occurred during the induction of hypertension with metaraminol, and hematocrits taken during hypertension did not differ from those obtained during the control period by more than 5%. Thus, corrections for hematocrit in the individual blood brain partition coefficients used in calculating CBF were not necessary.14, 15

Discussion

The Kety and Schmidt15 method we have employed to measure CBF in this study is based on the indirect Fick principle and depends upon the rate of extraction of a diffusible
inert gas from the brain as a measure of blood flow per unit weight of cerebral tissue. This gives a valid measurement of CBF only if the following conditions are satisfied: 1) the cerebral circulation is the only route by which the indicator, in this case $^{133}$Xe, can enter or leave the brain; 2) the indicator is neither stored nor metabolized by the brain; 3) the venous blood samples are purely cerebral in origin and are not significantly contaminated with blood from extracerebral sources, and 4) CBF remains constant during the sampling period. In addition, several potential sources of experimental error related to length of saturation and desaturation times\cite{16, 20} and to blood hematocrit values\cite{16, 18} have been identified.

The modification of the Kety and Schmidt method by Eklof \textit{et al.}\cite{10, 11} has been shown to be valid over a wide range of experimental conditions, including hypercarbia.\cite{10} Other methods of measuring CBF in small laboratory animals, employing autoradiographic techniques,\cite{21} are known to underestimate CBF under these conditions, due to limited tracer diffusion.\cite{10} In contrast to intra-arterial indicator methods, this technique does not require assumptions regarding instantaneous tracer diffusion, or homogeneity of blood flow.\cite{22}

A major objection raised against the Kety and Schmidt technique relates to the possibility of incorporating significant contamination of cerebral venous blood samples with blood from extracerebral tissues. To avoid this potential source of error in the present study, cerebral venous blood was sampled from the superior sagittal sinus after resection of the scalp and periosteum. Blood from this site should reflect primarily cortical blood flow from parasagittal portions of the cerebral hemispheres. However, we cannot entirely discount the possibility of including a small amount of blood flow to subcortical areas in our CBF measurements.

To test the upper limits of autoregulation, we induced hypertension with metaraminol. Periods of steady state hypertension of at least 15 minutes, including the second 10 minutes of saturation and the initial 5 minutes of desaturation, were achieved by using escalating rates of metaraminol infusion. Steady states of hypertension generally prevailed 1–2 minutes after the administration of metaraminol started, and in all experiments MABP was stable before desaturation began. Since cerebral vascular adjustments to hypertension are known to be rapid,\cite{22} steady states of CBF at the outset of measurement were assured under the present hypertensive conditions. Ideally, hypertension would have been maintained stable throughout the periods of saturation and desaturation in all experiments. Unfortunately, long periods of hypertension induced with metaraminol, in the dose range we employed were not always attainable as tachyphylaxis sometimes occurred and MABP declined gradually by 10–20 mm Hg. However, cerebral desaturation in the rat has been shown to be nearly complete within 5 minutes, at normal or above-normal CBF rates.\cite{16} In all experiments reported here, steady state hypertension was achieved for a minimum of 5 minutes following the onset of desaturation. Therefore, the requirement that MABP must remain constant for valid measurement by the Kety and Schmidt method was met.

In the present study we have confirmed that blood brain partition coefficients for $^{133}$Xe in rats are similar to those obtained for human cortical tissue.\cite{16, 18} Nilsson and Siesjö\cite{22} have also recently reported that $\lambda$ for this indicator in rat cerebral cortex is comparable to that in humans.

The present CBF values in Sprague-Dawley rats, under normocarbic, normoxic, and normotensive conditions, are similar to those reported in rats of the Wistar strain.\cite{16} Likewise, the cerebral vascular sensitivity to changes in arterial CO$_2$ tension, appears to be in agreement with that noted by these authors. Gjedde \textit{et al.}\cite{16} have also measured CBF in Wistar rats. Although basal CBF was 98 ± 6.4 (SEM) ml/min/100 gm in their study, at an arterial Pco$_2$ of 40 ± 1.3, our values for CO$_2$ responsiveness are appreciably greater than theirs. They reported an alteration in CBF of 2.3 ml/min/100 gm per mm Hg change in arterial Pco$_2$, less than half the value of 4.9 we report here. The differences may be attributable to the method employed in sampling cerebral venous blood, since we obtained superior sagittal sinus blood, while they sampled from the transverse sinus.

Cerebral venous blood from the latter site includes a larger portion of the brain, incorporating venous drainage from both gray and white matter. It is also possible that their lower CO$_2$ response values reflect some degree of contamination from extracerebral sources since there appear to be anastomoses between the transverse sinus and the extra-cerebral vascular bed in the rat.\cite{22}

Matsumoto \textit{et al.}\cite{11} have also measured CBF in Wistar rats by a method employing intravenous infusion of $^{133}$Xe. However, these investigators measured whole hemisphere blood flow, incorporating both gray and white matter, rather than predominantly gray matter as we have in the present report. Perhaps for this reason, their values for CBF and CBF responses to hypercarbia are lower than ours, being 86 ± 15 (SD) and 3.46 ml/min/100 gm per mm Hg change in arterial Pco$_2$.\cite{22}
The results of the present study indicate that in the rat, CBF is maintained nearly constant despite wide variations in MABP (fig. 3), by adjustments in cerebral vascular resistance (fig. 4). The range of MABP over which autoregulation of CBF can be observed is similar to that of other species, including nonhuman primates.6,7 and man.2

Our results also suggest that when MABP exceeds approximately 160 mm Hg, the cerebral vascular bed is unable to compensate by further increases in resistance, and CBF rises passively with MABP. This phenomenon, recently named the "breakthrough" of autoregulation2 has already been reported in baboons6 and humans,8 when MABP is increased above 150 mm Hg. Some investigators have hypothesized that "breakthrough" of CBFMA may be the cause of hypertensive encephalopathy in humans. They have suggested that when the "breakthrough" point is exceeded, a mechanical failure in the segments of the cerebral vascular bed occurs, leading to injury of the blood brain barrier and extravasation of plasma fluids into the brain.5,6,8 This increase in brain extracellular fluid may ultimately result in compression of small blood vessels, with accompanying ischemia and subsequent neuronal lesions.

Previous theories on the pathogenesis of hypertensive encephalopathy were largely based on Byrom's reports that segments of pial vessels in hypertensive rats sometimes exhibited localized spasm, taking on a "sausage-like" appearance.24 Although Byrom did not monitor CBF directly in these experiments, pial blood flow was measured by Meyer et al.26 under similar conditions. These authors reported that increases in systemic pressure of 75 to 100% in cats, resulted in considerable decreases both in pial arteriolar diameter and pial blood flow. Thus, the experiments of Meyer et al.26 corroborated Byrom's hypothesis that vasospasm was responsible for the ischemia underlying the neurological consequences of severe hypertension. Dinsdale et al.6 also measured regional changes in CBF in rabbits during acute angiotensin-induced hypertension. These authors reported mean increases in CBF of 34% above control levels during periods of maximum hypertension. However, when MABP returned to normal, they noted focal cortical areas of decreased CBF that persisted up to 60 minutes after hypertension. In the present study, direct measurement of CBF during acute hypertension failed to confirm such decreases in cerebral circulation. Methodological differences exist between our present report and the studies by Byrom24 and by Meyer et al.26 in that these authors employed chronically hypertensive animals, while we conducted our studies during acute pharmacogenic hypertension. The study by Dinsdale et al.6,28 measured regional changes in CBF, and only noted focal vasocostriction after normotension was restored. In the present study we measured blood flow derived from a larger, parasagittal region of cerebral cortex during, but not after, acute hypertension. Therefore, the experimental conditions of our study and that of Dinsdale et al. are not strictly comparable. We suggest that these differences do not fully account for the basic discrepancy in findings, since other investigators have recently reported evidence of autoregulation failure in both acutely and chronically hypertensive baboons.6,8 These authors also found no evidence of a primary reduction of CBF during severe hypertension. It would thus appear that large increases in systemic arterial pressure, whether originating from conditions of normotension, as in the present study, or from hypertensive levels, as in the study of Strandgaard et al.9 would elicit the "breakthrough" of CBF once the upper limits of autoregulation were exceeded.

The marked increase in CBF encountered in this study, of up to 300% of normal during severe hypertension, represents a degree of cerebral hyperemia encountered in only a few other abnormal conditions, e.g. epileptic seizures.27 However, the significance of this finding, and its potential role as an intermediary mechanism in hypertensive cerebral injury, awaits further study.

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References

Experimental Stroke in Gerbils: Correlation of Clinical, Pathological and Electroencephalographic Findings and Protein Synthesis

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SUMMARY Cerebral ischemia was produced in gerbils by ligation of the right common carotid artery and the resulting clinical manifestations and pathological alterations, along with electroencephalographic findings, were followed from 30 minutes to 24 hours. Protein synthesis was evaluated with brain slices in vitro and subsequent cellular and subcellular fractions. One group of animals developed clinical signs of cerebral ischemia and stroke very rapidly and often died within 12 hours. In these animals cerebral infarction was diffuse in the right side of brain within a few hours postoperatively and there was persistent suppression in the electroencephalographic recordings. Amino acid incorporation into proteins of subcellular fractions was decreased to 50% of the opposite side at 30 minutes and further declined to less than 10% in 8 to 10 hours. Another group of animals survived to 24 hours in spite of severe neurological manifestations, and protein synthesis was about 15% of the control side at 24 hours. The suppression of protein synthesis was observed both in the neuronal and neuroglial fractions indicating similar vulnerability of these cellular elements toward cerebral ischemia as shown with cerebral anoxia in the past. It was emphasized that the correlation of clinical manifestations and biochemical data is very important to extract meaningful information from biochemical investigations in this model.

Materials and Methods

During the early period of the present investigation mongolian gerbils (60 to 75 grams) were anesthetized with intraperitoneal injection of chloral hydrate (0.25 mg/kg) and supplementary inhalation of ether. Later, ether inhalation was the only anesthesia used, particularly when observation periods were shorter than 2 hours. The right common carotid artery was exposed through a midline skin incision in the neck. The artery was isolated from the vagus nerve, double-ligated with a 4-0 silk suture and cut between ligatures. A platinum needle electrode was inserted in the parietal area and a reference electrode in the retroauricular area. A platinum needle electrode was inserted in the parietal area and a reference electrode in the retroauricular area subcutaneously on each side. For histopathological examination with hematoxylin-eosin staining.

For biochemical investigation the brain was promptly cooled in crushed ice. Tissue slices, 0.3 mm thick, were prepared from each cerebral hemisphere and the thalamus with a McIlwain tissue chopper and incubated in buffered-electrolyte medium with 10 mM glucose and 10 μCi of L-leucine-4,5-[3H] (58 Ci/mmol from Amersham adjusted to 0.5 mCi/mmole) at 37° C for 30 minutes under oxygen at-
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