Cerebral Blood Flow in Rats During Physiological and Humoral Stimuli

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SUMMARY  The technique for estimating cerebral blood flow (CBF) in anesthetized rats by injecting 133Xe into the internal carotid artery represents a potentially useful and inexpensive model for screening cerebral vascular responses to pathophysiological and pharmacological stimuli. We have examined associated neuropathology, the validity and the reproducibility of the method, and made comparisons of initial slope estimates of CBF with those obtained by stochastic analysis. Initial slope estimates (CBF = 1.62 ± 0.04 ml min⁻¹·g⁻¹, X ± SE, N = 38) were linearly related to stochastic measurements (CBF = 1.42 ± 0.09 ml min⁻¹·g⁻¹, N = 6), and overestimated mean CBF by about 15%. A reactivity to CO₂ of 0.05 ml min⁻¹·g⁻¹ per mm Hg, and an autoradiographic range of 70 to 180 mm Hg were found. CBF responses to the intra-arterial infusion of amine drugs were determined before and after opening of the blood-brain barrier with hypertonic urea. Serotonin reduced CBF after, but not before, the administration of urea. Acetylcholine increased CBF when the barrier was intact, the effect being augmented when the barrier was disrupted; these responses were reduced by atropine. Histamine increased CBF only after barrier opening, and this response was attenuated by the H₁-receptor antagonist, metiamide. These studies indicate that initial slope estimates of CBF derived in rats from intracarotid 133Xe injection, which represents an inexpensive and simplified approach for screening cerebral circulatory adjustments, may facilitate the characterization of stimuli affecting CBF.

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The determination of physiological and pharmacological mechanisms in the cerebral circulation represents an important experimental step in evaluating therapeutic regimes for treating cerebrovascular disorders. The screening of drugs that may affect cerebral blood flow (CBF) would presumably be facilitated in future studies by obtaining estimates of CBF in a rapid and inexpensive manner. Such methodology appears available with the technique of Hertz et al.1, 2 in which estimates of CBF are obtained in anesthetized rats from the initial slope of the clearance curve of 133Xe injected into the carotid artery.5 With certain precautions implemented, this method purportedly excludes extracranial structures6 and permits rapid assessment of CBF several times in the same rat.2, 3

Pharmacological studies have produced substantial evidence describing amine influences in the cerebral circulation.8 For many of the endogenous substances examined to date, it appears that blood-brain barrier (BBB) mechanisms limit access of neurohumoral substances such as norepinephrine, 5-hydroxytryptamine and prostaglandins from blood to cerebral vascular smooth muscle. Although potently vasoactive in peripheral vascular beds, these substances have little or no influence on CBF during carotid arterial infusion unless blood-brain barrier mechanisms are disrupted.7-10

The goal of the present study was to test and modify the method of Hertz et al.1 to: 1) examine the ability of the technique to detect responses of the cerebral circulation to changes in arterial pressure and PCO₂; and 2) determine changes in CBF during carotid arterial drug administration.

Methods

Animal Preparation

Experiments were performed with 53 male Sprague-Dawley rats weighing 250 to 400 g. Anesthesia was induced with 3.5% halothane and oxygen, and was maintained by artificial ventilation with a mixture of 0.4% halothane, 70% nitrous oxide, and 30% oxygen. Skeletal muscle paralysis was achieved with tubocurarine (0.75 mg kg⁻¹, i.p.) in the studies involving changes in arterial pressure and blood gases. Gallamine triethiodide (8 mg kg⁻¹, i.v.) was used for muscle relaxation in all drug experiments.

Polyethylene catheters were inserted into both femoral arteries to measure arterial blood pressure and to withdraw blood samples for analysis of hematocrit, hemoglobin concentration, blood gases and pH. For each experimental rat, another rat, anesthetized and ventilated in an identical manner, was used as a blood donor so that hematocrit and hemoglobin concentration could be maintained throughout the experiment. A femoral vein was cannulated for drug administration. Body temperature was monitored with a rectal probe and was maintained at 37 ± 0.5°C.

A longitudinal scalp incision was made to expose the skull and temporal muscles, and the right temporal muscle and overlying skin were excised. The importance of soft tissue removal for cerebral blood flow studies involving external monitoring of inert gas clearance has been emphasized previously,11 although not incorporated in previous applications of this

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method,1, 2 and will be discussed further in this paper. According to the surgical procedures of Hertz and Bolwig,3 a longitudinal ventral neck incision was made to expose the right common carotid artery and its branches. The digastric muscle, hyoid bone and hypoglossal nerve were divided and retracted to facilitate dissection near the carotid bifurcation. Ligatures were secured around the occipital, pterygopalatine, superior thyroid and external carotid arteries.

Carotid Catheterization and Determination of CBF

The method used for determination of CBF from intracarotid injection of inert gas requires placement of a catheter near the origin of an artery supplying the brain. In preliminary experiments in 7 rats, we used the following method for catheterization of the right external carotid artery. The common carotid and internal carotid arteries were occluded simultaneously for about one minute while a catheter was inserted retrogradely into the external carotid artery for placement near the origin of the internal carotid artery. To minimize blood loss from surgical wounds, we did not give heparin to these rats. Subsequently, we made up to 20 injections of 133Xe to determine serial measurements of CBF while varying the arterial blood pressure and PCO2 between 50-180 mm Hg and 25-70 mm Hg, respectively. Total duration of the surgery and blood flow studies was 4 to 6 hours. We found from neuropathological analysis that neuronal cell damage was present in the brains of some of these rats (see Results). Suspecting that temporary cessation of blood flow in the internal carotid artery may have been a precipitating factor, we adopted a modified procedure for carotid catheterization in subsequent animals. In these rats, heparin (750 U kg⁻¹, i.v.) was administered for anticoagulation. During occlusion of only the external carotid artery, a polyethylene catheter was inserted in a retrograde manner so that its tip lay within 1 mm of the origin of the internal carotid artery. For injection of 133Xe, a fine needle, with an outside diameter slightly smaller than the inside diameter of the catheter, was inserted through the external carotid catheter until its tip lay at the mouth of the catheter. The catheter and needle were secured in place with skin sutures. In these studies, we reduced the number of flow determinations in each rat, although arterial pressure and PCO2 were varied as above. Since brain lesions in a group of rats prepared in this manner, 1'3 and will be discussed further in this paper. Accord-
min after injection; 49.8 = correction factor for expression as ml min\(^{-1}\) g\(^{-1}\) including 0.83 as the partition coefficient for \(^{133}\)Xe in rat cerebral hemisphere; \(^{19}\) Area\(_t\) = total 5 min area of the clearance curve; and Background = 100 sec background counts determined immediately before \(^{133}\)Xe injection, converted to 5 min.

**Examination of Responses to Physiological and Humoral Stimuli**

**CO\(_2\) Reactivity**

Cerebral blood flow was measured a few minutes after production of arterial hypercapnia or hypocapnia. Arterial hypercapnia was produced by adding CO\(_2\) to the inspired gas mixture. Arterial hypocapnia was produced by increasing the tidal volume of the respiratory pump. Blood gases were determined before and after each series of blood flow measurements. For the group of rats, the range of arterial CO\(_2\) studied was 26 to 68 mm Hg; for each rat, however, the arterial CO\(_2\) was changed in one direction only.

**Autoregulation**

Cerebral blood flow was measured repeatedly during stable, graded alterations in arterial blood pressure in the range of 50 to 190 mm Hg. Arterial hypotension was produced by withdrawing small amounts of blood until a desired, steady level of pressure was obtained. Production of arterial hypertension was achieved by intravenous infusion of metaraminol (10 to 30 \(\mu\)g min\(^{-1}\)), angiotensin (5 to 20 \(\mu\)g min\(^{-1}\)) or a mixture of these agents. At least 2 determinations of blood flow were made at each level of arterial pressure.

**Carotid Arterial Infusion of Drugs**

A polyethylene catheter was inserted into the right axillary artery and advanced through the subclavian artery toward the bifurcation of the brachiocephalic and common carotid arteries. Injections of Evans blue dye (approximately 50 \(\mu\)l) were used to detect placement of the catheter in the common carotid artery.

Several determinations of CBF were made during control conditions and during infusion of drugs into the common carotid artery. Because all extracranial branches of the common carotid artery had been ligated, the drug was expected to course through the internal carotid artery to the brain. The rate of drug delivery was 45 \(\mu\)l min\(^{-1}\). Only one agonist was studied in each rat.

The following drugs were used in this series: 5-hydroxytryptamine, acetylcholine and the muscarinic antagonist, atropine, histamine dihydrochloride and the histamine H\(_2\)-receptor antagonist, metiamide.

Because blood amines have difficulty penetrating the blood-brain barrier, \(^{18}\) urea was used as a hypertonic agent (2 \(M\), 1 ml injected into carotid artery over 30 sec) in some studies in order to disrupt the blood-brain barrier, \(^{17}\) thereby allowing penetration of the drug to cerebrovascular smooth muscle and brain.

CBF measurements were obtained approximately 5 min after the start of drug infusion when arterial pressure was stable. When urea was used to disrupt the BBB, drugs were infused 15 to 20 min after urea injection. Antagonists were administered 10 min before the infusion of agonist.

**Statistical Analysis**

Changes in CBF were analyzed statistically using standard procedures. \(^{18}\) Linear regression analysis was applied to data for derivation of line equations, and the paired \(t\)-test was used for matched comparisons. Results were accepted as statistically significant at \(p < 0.05\).

**Neuropathology**

After completion of the blood flow studies in several rats, the animals were fixed by perfusion according to the method of Brierley et al. \(^{16}\) Briefly, the animals were placed in a supine position and the thorax was opened at the sternum. A cannula was introduced into the ascending aorta via the left ventricle, and 2 ml of heparin (2000 U) was injected. After incising the right atrium, about 100 ml of FAM fixative (40% formaldehyde: glacial acetic acid: absolute methanol = 1:1:8) was injected over 1 min at approximately the level of normal arterial pressure. The head of the animal was stored in FAM for a minimum of 24 hours and, after removal, the brain was immersed in FAM for an additional 24–48 hours. Coronal sections 3 mm thick were embedded in paraaffin wax and slices 7 \(\mu\)m thick were stained with hematoxylin and eosin, and by a method combining cresyl violet and Luxol fast blue. The sections were examined by conventional light microscopy and any abnormalities were recorded on a series of line diagrams.

**Results**

**Preliminary Experiments and Neuropathological Studies**

In our initial studies, we used a procedure for catheterization of the right external carotid artery that involved simultaneous clamping of the common carotid and internal carotid arteries during insertion of a catheter in the external carotid artery. Seven animals were perfusion-fixed for neuropathological examination. As judged by uniform blanching and hardness of the specimens, satisfactory fixation was achieved in all animals. Cytological artifacts such as "dark" and "hydropic" cells were not seen. In 5 out of 7 animals there was microscopic evidence of selective neuronal necrosis of the type described previously for FAM-fixed material. \(^{16},^{17}\) Focal lesions were widely distributed in the cortex, hippocampus and thalamus ipsilateral to carotid surgery and injection. The blood flow data collected from the 5 rats with brain damage were discarded because these animals had poor CBF responsiveness to physiological stimuli.

In subsequent studies, we avoided occlusion of the
common carotid and internal carotid arteries, administered heparin after all surgery was completed, reduced the number of flow determinations, and prepared several rats for neuropathological examination. Of the 12 brains examined using this modified preparation, there were 5 with lesions in the ipsilateral cortex or hippocampus, and 7 in which we saw no abnormality. In the 5 animals with identifiable brain damage, we detected in 3 brains one to 3 small lesions in hippocampus and/or parietal cortex. In the other 2 rats, we found larger lesions in the hippocampus of one animal, and in the parietal cortex of the other animal. Because the CBF responsiveness to physiological stimuli in these rats was not different from others, we included the data in our analyses.

Cerebral Blood Flow during Control Conditions

Repeated measurements of CBF were obtained in 38 rats during stable, control conditions. Physiological parameters for control CBF determinations were (means ± se): mean arterial pressure = 106 ± 4 mm Hg; arterial Po₂, pH and Pco₂ = 113 ± 3 mm Hg, 7.36 ± 0.01 units and 38 ± 0.4 mm Hg, respectively; hemoglobin concentration = 11.1 ± 0.4 mg %; and hematocrit = 41 ± 0.6%. Under these conditions, CBF determined from 169 estimates from initial slope analysis was 1.62 ± 0.04 ml min⁻¹ g⁻¹ (coefficient of variation = 10.8%). Mean half-time of the plotted clearance curves was 20.5 ± 1.3 sec.

Reproducibility of Measurement

At least 2 measurements of CBF were obtained and compared from 20 rats during stable conditions over a wide range of arterial CO₂ (fig. 2). Flow measurements were approximately 4 min apart. The results indicate that the method was reproducible (r = 0.95, p < 0.05; paired t for comparison was not significant).

We also made repeated determinations of CBF from initial-slope analysis during control conditions over one hour. Values (in ml min⁻¹ g⁻¹) for every 15 min beginning at time 0 were: 1.68 ± 0.05, N = 20; 15 min: 1.69 ± 0.05, N = 20; 30 min: 1.66 ± 0.08, N = 11; 45 min: 1.69 ± 0.11, N = 7; 60 min: 1.65 ± 0.07, N = 14. These results indicate that the control measurement of CBF with this method is stable over time.

Comparison of Cerebral Blood Flow Estimates Derived from Initial Slope and Stochastic Methods

During control conditions in 6 rats, mean arterial pressure was 91 ± 3 mm Hg and arterial Pco₂ was 39 ± 0.5 mm Hg. Fourteen measurements of blood flow were made during these conditions. With the initial slope method, CBF was 1.66 ± 0.05 ml min⁻¹ g⁻¹, and the stochastic calculation was 1.42 ± 0.09 ml min⁻¹ g⁻¹. When the arterial Pco₂ was varied to alter CBF (10 measurements of CBF, Pco₂ range = 27 to 72 mm Hg), the comparison of CBF determined by the 2 methods gave a linear relationship with a line equation of y = 1.14X + 0.13, and a correlation coefficient of 0.90 (fig. 3).

Responses to Changes in Arterial CO₂

Measurements of CBF were made from initial slope analysis in 19 rats during arterial hypocapnia or hypercapnia. Arterial Po₂ was maintained above 90 mm Hg, and mean arterial pressure was between 90 and 135 mm Hg in all experiments. Figure 4 shows the CBF response to altering the Paco₂. Between a Paco₂ of 35 and 65 mm Hg, the values for CBF fit a linear regression. In this range, results indicate a CO₂ reactivity of 0.05 ml min⁻¹ g⁻¹ per mm Hg change in arterial Pco₂.

Responses to Changes in Arterial Pressure

CBF was determined from initial slope measurements in 12 rats during steady-state alterations in arterial pressure between 55 and 188 mm Hg. In the pressure range of approximately 70 to 180 mm Hg, CBF was constant (fig. 5). Below and above these limits, however, CBF varied directly with arterial pressure (fig. 5).
Responses to Carotid Arterial Infusion of Drugs

5-Hydroxytryptamine (5-HT). During conditions of normal arterial blood pressure and blood gases in 5 rats, infusion of 5-HT in the dose range of 0.02 to 0.1 μg min⁻¹kg⁻¹ had no effect on CBF. During control conditions, CBF (initial slope) was 1.65 ± 0.06 ml min⁻¹g⁻¹, and during 5-HT infusion CBF was 1.68 ± 0.09 ml min⁻¹g⁻¹ (p > 0.05). Mean arterial pressure (control = 119 ± 6 mm Hg) was not affected by the 5-HT infusion. One ml of 2 M urea was injected into the carotid artery to osmotically disrupt the blood-brain barrier to 5-HT. We have found in parallel experiments that the urea injection itself increases CBF transiently for approximately 15 min (unpublished observations). Therefore, 15 min following urea injection, 5-HT was infused again into the carotid artery (0.1 μg min⁻¹kg⁻¹). CBF decreased during 5-HT infusion by 29 ± 8% (fig. 6).

Acetylcholine (ACh). In four rats, carotid arterial infusion of ACh (3 μg min⁻¹kg⁻¹) increased CBF by 60 ± 6% (fig. 6). CBF (initial slope) increased from 1.77 ± 0.10 ml min⁻¹g⁻¹ during the control measurements to 2.84 ± 0.16 ml min⁻¹g⁻¹ during infusion of ACh. Mean arterial pressure decreased from 121 ± 8 mm Hg during control to 83 ± 6 mm Hg during ACh infusion. Fifteen minutes following injection of urea, CBF increased by 95 ± 12% with the same dose of ACh. The increase in blood flow induced by ACh was attenuated (+25 ± 3%, subsequent to urea injection) by atropine (500 μg kg⁻¹, i.v.) (fig. 6).

Histamine. Infusion of histamine (20 μg min⁻¹kg⁻¹) had no significant effect on CBF before urea injection. During the control period, CBF (initial slope) was 1.64 ± 0.06 ml min⁻¹g⁻¹ and CBF was 1.55 ± 0.06 ml min⁻¹g⁻¹ during histamine infusion (p > 0.05). Histamine infusion decreased mean arterial pressure from 94 ± 7 to 75 ± 7 mm Hg. After urea, CBF increased during histamine infusion by 44 ± 6% in 6 rats (fig. 6). Attenuation of the increased flow to histamine infusion was achieved with metiamide (5 mg
The results from these experiments indicate that estimates of CBF in rats by the intracarotid 133Xe method are reproducible and sensitive for detection of cerebral vascular responses to physiological stimuli and to arterial infusions of drugs.

In the surgical preparation of animals in these studies, we modified the procedure of Hertz et al. to include removal of skin and muscle overlying the region of brain that would be viewed by the scintillation crystal. This modification represents a precaution for eliminating the possibility of 133Xe entering non-cerebral structures which may be partially supplied by anomalous anastomoses of the internal carotid artery. If extracranial vessels were involved in the CBF measurement, interpretation of CBF responses to physiological and pharmacological stimuli would be complicated. To test for this possibility, we determined CBF before and during continuous carotid arterial infusion of 5-hydroxytryptamine (5-HT) (at 0.1 µg min⁻¹kg⁻¹) via a catheter in the right subclavian artery, as described above. This dose of 5-HT is known to constrict extracranial arteries, including the extracranial portion of the internal carotid artery, but does not alter CBF. Infusion of 5-HT did not decrease CBF when the blood-brain barrier was intact (fig. 6). These results suggest that the CBF measurement was not affected when the extracranial vessels were probably constricted.

We also used a smaller volume for bolus injection of the 133Xe than the 50 µl injection volumes originally used by Hertz et al. Our use of 10 µl volumes, and the smaller volumes used more recently by Hertz and colleagues are less likely to cause artifactual changes in CBF. Recent studies in rats have demonstrated that intracarotid injection of volumes greater than 10 µl can transiently disturb cerebral hemodynamics.

It is important to discuss the limitations and advantages of the intracarotid 133Xe technique in comparison with other methods for measurement of CBF in rats. Several previous studies of rat CBF have involved use of a modified Kety-Schmidt technique. This method permits several measurements of CBF and brain oxygen metabolism in a given experiment. It does, however, require additional surgery to cannulate a representative cerebral vein, and involves a long, steady-state washout period for determination of arteriovenous tracer differences. Control CBF values with this technique during nitrous oxide anesthesia are about 1.0 ml min⁻¹g⁻¹. The use of labeled microspheres for CBF measurement in the rat affords the advantages of regionality and multiple determinations of flow, but is technically difficult in small animals. Blood flows for cortex with this method were 1.0 to 1.3 ml min⁻¹g⁻¹. Tissue sampling or autoradiography of brain after systemic administration of freely diffusible tracers provides for regional assessment of CBF in rats. The method has the limitation, however, of permitting only one determination of CBF in a given experiment. In rats anesthetized with nitrous oxide, values for cortical blood flow obtained with ethanol and iodoantipyrine were 1.2 and 1.0 ml min⁻¹g⁻¹, respectively. In conscious rats in which iodoantipyrine was used, blood flow in cortex was approximately 1.8 ml min⁻¹g⁻¹. The above values compare with the mean control CBF of 1.6 ml min⁻¹g⁻¹ obtained in the present experiments, from analysis of the initial slope of 133Xe clearance.

The intracarotid 133Xe method of Hertz et al. has several advantages for rat CBF studies. The method allows repeated determinations of CBF in one rat, the CBF estimate is yielded quickly, and cerebral oxygen consumption can be measured concurrently.

Considerations for using the intracarotid 133Xe method are: 1) it is important to exclude potential sources of extracerebral contamination of the 133Xe clearance curves. Several precautions in using the
method are necessary. These measures include ligation of extracerebral branches of the carotid artery, removal of soft tissues from the crystal viewing area, and accurate positioning of the crystal and collimator to minimize the viewing of non-cerebral sources of $^{133}$Xe, such as the trachea. Furthermore, in preliminary studies which included microscopic examination of brains for neuropathology, we found the importance of avoiding occlusion of the common and internal carotid arteries, using heparin for anticoagulation, and minimizing the number of $^{133}$Xe injections in a given animal. We emphasize that ischemic damage ipsilateral to bolus injections can occur despite precautions. Under these conditions, emboli by platelets or air are possible secondary factors producing lesions. Therefore, concurrent assessment for structural brain damage should be incorporated when using this technique. These potential associated problems, and neuropathological examination for brain damage in rats receiving carotid arterial injections for measurement of CBF, have not been reported in previous studies.1-2, 2) derivation of CBF from the initial slope of the clearance curve primarily reflects cortical flow,3, 4 and does not permit regional assessment of blood flow. In the present studies, our initial slope estimates were higher than values derived from stochastic analysis, because the height/area derivation represents mean blood flow through regions of the brain viewed by the crystal,5,6 and the initial slope value is heavily weighted toward blood flow in cortex;5, 6 and 3) the method cannot be applied in conscious animals.

Physiological Stimuli

Values for CO$_2$ reactivity in the rat cerebral circulation vary in the literature between 0.02$^{29}$ and 0.09$^{29}$ ml min$^{-1}$g$^{-1}$ per mm Hg. Our results demonstrate a CO$_2$ reactivity of 0.05 ml min$^{-1}$g$^{-1}$ per mm Hg which agrees with previously reported values obtained by this technique and the Kety-Schmidt method.5,25 Furthermore, we demonstrated that estimates of CBF with the intra-arterial $^{133}$Xe method were reproducible over a broad range of blood flows (fig. 2).

In our studies, the range of cerebral vascular autoregulation was approximately 70 to 180 mm Hg. These values correspond to previously reported values for cerebral autoregulation in rats.23 Furthermore, the shape of the autoregulation curve (fig. 5) is similar to previous results obtained in dogs and baboons at this Institute.20-22

Humoral Stimuli

Several recent studies in baboons have demonstrated the importance of blood-brain barrier mechanisms for amines in the cerebral circulation. Norepinephrine, prostaglandins and 5-hydroxytryptamine are without significant effect on CBF unless the blood-brain barrier is disrupted by hypertonic solutions or enzymatic inhibitors.7-15 In the present studies, similar results have been obtained in rats, viz.: for some agents, CBF is not changed significantly unless barrier disruption is first produced. After intracarotid administration of urea, 5-hydroxytryptamine and histamine produced considerable vasoconstriction and vasodilatation, respectively, while being without significant effect before urea injection. The reduction in blood flow with 5-hydroxytryptamine is consistent with previous results in the baboon.8 Descriptions in the literature of histamine's mechanism of action on cerebral vessels are equivocal.8 From our results, however, it appears that histamine had no effect during normal conditions when the blood-brain barrier was intact but when it had access to cerebrovascular smooth muscle following urea injection, it acted on histamine H$_2$-receptors mediating dilatation. These results are compatible with recent pial arteriolar studies demonstrating antagonism of histamine-induced vasodilatation by the H$_2$-receptor blocking agent, cimetidine.39

Cerebral vascular responses to intravascular acetylcholine have not been clearly defined. Despite an abundance of cholinergic nerves in cerebral vessels,44 most studies to date have reported little effect of acetylcholine when administered intravascularly.19 However, recent microsphere studies in rabbits receiving carotid arterial infusions of acetylcholine have shown that substantial regional increases in CBF result when cerebral oxygen consumption is unchanged, and the increase in CBF is blocked by atropine.46 The effect of acetylcholine administered intravascularly appears to be mediated by muscarinic receptors in vascular smooth muscle and is not associated with detectable changes in brain metabolism. Our studies in rats confirm the involvement of muscarinic cholinergic receptors in cortical vessels mediating vasodilator responses to intracarotid acetylcholine.

These experiments in rats support the concept of the blood-brain barrier having an important role in studies examining the pharmacology of the cerebral circulation. As demonstrated in this report, the intracarotid $^{133}$Xe injection method in rats is a reproducible and versatile technique that may constitute a useful and inexpensive approach for screening therapeutic programs for cerebrovascular disorders.

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