Regional Cerebral Blood Flow in the Anesthetized Mouse as Measured by Local Hydrogen Clearance

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SUMMARY Platinum microelectrodes were used to measure H₂ clearance in mouse brain, and the clearance curves were used to calculate regional cerebral blood flow (rCBF). The curves were usually biexponential, whether or not electrode placement was confined to the cortex. When calculated by the height over area method, rCBF in anesthetized mice averaged 37 ± 14 and 49 ± 15 ml/100 gm per minute in two successive groups where cortical placement had been made. After CO₂ breathing, which raised Paco₂, to 77 ± 18 torr, the mean rCBF of the latter group was elevated to 70 ± 36 ml/100 gm per minute. Our basal rCBF values are lower than literature values for rats or mice, when compared with data obtained by other techniques. However, our data are comparable to rat rCBF data obtained by others using H₂ electrodes and are comparable also to data for whole brain CBF obtained by a variety of methods in larger anesthetized mammals. It is possible that H₂ electrodes provide low values for supposedly "cortical" rCBF in the very small mouse brain, because in such brains the electrode is usually close enough to a slow clearing compartment for the electrode reading to be influenced by that compartment. At the same time one cannot rule out the possibility that other techniques when applied to small rodents may, on occasion, produce spuriously high values for CBF. Indeed, while some studies using the latter techniques do show unusually high values for cortical flow in these animals, other studies using similar methods do not.

THE HYDROGEN CLEARANCE TECHNIQUE has been used to measure cerebral blood flow (CBF) in a variety of species including rats and monkeys. ¹ ² By utilizing small electrodes, it can measure regional cerebral blood flow (rCBF) in small volumes of brain tissue. Since the technique requires neither bulky isotope detectors nor puncture of a vessel to monitor the inert gas being cleared, it is ideally suited to measurements of rCBF in a small brain like that of the mouse. Such measurements do not appear to have been reported previously, except in one investigation which utilized an unconventional technique based upon the rate at which labeled water penetrated the mouse brain.³ As we have shown in a long series of studies, investigation of murine cerebral microcirculation can yield much useful data relevant to control of cerebral circulation in health and disease. In view of the paucity of flow data in the literature, it seemed valuable to add studies of murine CBF to our armamentarium, and we elected to use the hydrogen clearance technique because of its ready adaptability to the small mouse brain and its high degree of acceptance in the literature.

Methods

We used white male mice, IRC strain (Dublin Farms), weighing 18 to 40 gm. These were anesthetized with urethane (2 mg per gram intraperitoneally) or sodium pentobarbital (6 mg/100 gm intraperitoneally, with more if needed). Preliminary studies failed to show a difference between rCBF obtained with either anesthetic in small groups of mice. The final study was performed with urethane.

The mice had a tracheostomy. A small area of bone was removed from the calvarium and the dura stripped from the underlying brain. A platinum, glass-sheathed microelectrode with a bare tip about 3 μ in diameter (Transidyne Corporation, Ann Arbor, Michigan) was inserted into the brain. The reference electrode was an Ag-AgCl electrode inserted under the skin. The electrode was polarized at either 0.2 or 0.3 volt. Potentials generated by H₂ were recorded on a picometer in a circuit designed to maintain a constant polarization current at all times (Microsensor; Transidyne Corporation). The electrode's response to H₂ was linear in saline. Animals were given air or a mixture of air and oxygen to breathe. CO₂ could be varied in the inspired mixture. When H₂ was added to the mixture, the electrode recorded a rising H₂ current until equilibrium between blood and tissue was reached. Sometime after this point was reached, the H₂ was shut off. Flow was measured, using the height over area technique, after ten minutes of clearance following the cutoff of H₂. In addition, representative curves were analyzed using compartmental analysis.⁷

An initial series of studies was performed after placement of electrodes in widely varied locations within the cerebral hemisphere. Later studies were performed with the electrode depth controlled by a micromanipulator to a point 400 to 600 μ deep. Histological study verified that this placement kept the electrode within the parietal cortex.

At the termination of experiments, blood from the carotid artery was sampled for analysis of CO₂ and O₂ in an IL ultramicro blood gas analyzer.

Results

Initial Studies

A single determination of flow (Fₐ) was made from the brain in each of 32 mice at arterial CO₂ tensions varying from 40 to 108 torr. The Fₐ was related to Paco₂ according to the equation y = -17 + 0.98x (r = 0.79; p < 0.01). We were surprised at the good fit of this straight line, since the sites being monitored differed from brain to brain, and the mice given CO₂ to breathe undoubtedly had widely varying rCBF's prior to their exposure to CO₂. Though a straight line relationship was obtained, the rCBF at a Paco₂ of 50 was surprisingly low (Fₐ = 33 cc/100 gm per minute). We assumed that volumes of white matter, known to have much lower flow than gray matter, may have been disproportionately represented in the volumes of tissue "seen" by

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the H₂ electrode. Such a hypothesis was compatible with the results of compartmental analysis of additional curves which showed at least two compartments in 18 of 19 curves analyzed by this technique. With pentobarbital, the slow flow (Fₛ) compartment showed a flow of 16 ± 3.8 ml/100 gm per minute and the fast flow (Fₚ) 49 ± 19 (N = 11); with urethane, Fₛ = 16 ± 6.0 and Fₚ = 43 ± 19 (N = 8). Consequently, we measured Fₛ₀ in a second series of mice in which the electrode placement was 400 to 600 μ beneath the surface of the brain, or essentially in the upper third of the cortex.

Studies of Flow From Cortical Placement of Electrode

Twenty-one mice were tested at a single site for each mouse. The Fₛ₀ was 49 ± 15 (mean ± SD). The mean Paco₂ was 51 ± 12 torr. The Fₛ₀ for 34 additional mice was measured before and after breathing a mixture enriched with varying amounts of CO₂. The mean Fₛ₀ before CO₂ breathing was 37 ± 14; the Paco₂ was not measured since only a single arterial sample could be obtained and this had to be taken at the end of the experiment when the animals were breathing higher levels of CO₂. After breathing CO₂ for at least five minutes prior to H₂ clearance, the animals had an Fₛ₀ of 70 ± 36, and a Paco₂ at the end of the H₂ clearance of 77 ± 18 torr. Thus, the increased arterial CO₂ was paralleled by an increased rCBF and, in fact, CO₂ breathing increased the Fₛ₀ in each of the 34 mice, the Fₛ₀ after CO₂ being 16% to 279% higher than the Fₛ₀ during the basal period (table 1) (only one mouse in the study failed to respond to CO₂, and data from this animal are not included with those from the other 34 animals).

Though CO₂ clearly raised Fₛ₀ in each individual mouse, we were not able in these studies of "cortical" flow to show the relationship between Fₛ₀ and Paco₂ for a whole group of mice simply by plotting one against the other as we did in the initial study employing random electrode placement. Presumably, our initial success was due to a fortuitous similarity of flows and CO₂ levels prior to CO₂ breathing, while in the later studies, basal flow and CO₂ level differed so from animal to animal that these differences obscured any within-group relationship between CO₂ levels and CBF after CO₂ breathing. The great variability in flow that can occur between mice also is reflected in the difference between mean flows of successively examined groups of mice. For example, in our initial group of animals with cortical electrode placement, the Fₛ₀ = 49 ± 15 while in the second group the Fₛ₀ = 37 ± 14.

Compartmental analysis of the clearance curves prior to CO₂ breathing revealed the curves to have two compartments in spite of the fact that the electrodes were placed only in the cortex.

Discussion

So far as we can determine, the H₂ clearance technique has not been used previously to measure CBF in the mouse. In the rat, Haining et al. found cortical flow values like our own in pentobarbital-anesthetized animals. Scremin et al. and Rovere et al. used rats anesthetized with urethane and found that H₂ clearance gave mean cortical flows of 35 to 75 ml/100 gm per minute depending upon the group of rats used and/or the depth of anesthesia. The values found in our murine investigation fell within the range found by the latter authors.

However, while the values we have found are in agreement with those from H₂ clearance studies utilizing rats, these values are lower than those found for cortical flow in both small and large mammals when other techniques of measurement are used and are less than cortical flows recorded with the H₂ electrode in larger mammals.

There are several possible reasons for the discrepancy between cortical flow values obtained with H₂ clearance in small rodents and cortical values obtained by other methods. The cortical values provided by H₂ clearance resemble those found for whole brain in large mammals when other techniques are used. This suggests that even when a cortical placement of the electrode was made in mice or rats, a slow clearing compartment like white matter still provided a portion of the clearance value, and reduced the rCBF to a level resembling an overall CBF from whole brain. In a very small brain, the electrode is always close to white matter and/or close to the subarachnoid space, both of which are slowly clearing compartments. For example, in a mouse cortex 2-mm thick, a placement 1-mm deep would be within 1 mm of both the subarachnoid space and white matter, while a deeper or shallower placement brings the electrode

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<th>Table 1 Blood Flow (Urethane)</th>
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<td><strong>Fₛ₀ before CO₂</strong></td>
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The table shows values for blood flow (ml/100 gm per minute) obtained from microelectrodes placed in the cortex of 34 mice. A previous series of 21 animals similarly treated gave a basal flow of 49 ± 15 ml/100 gm per minute or about 50% higher than the group shown here (see text).
even closer to one of the slow clearing compartments. Thus, "cortical" rCBF may still be a mixed rCBF representing white and gray matter or subarachnoid space and gray matter. Deeper placements, in turn, may be near gray masses like diencephalon and neighboring white matter like corona radiata, internal capsule, or cerebral peduncle. It is of interest that compartmental analysis of our clearance curves revealed biexponential curves even from the "cortex," suggesting that our "cortical" rCBF was instead a mixture of rCBF from fast and slow clearing areas. Of course, biexponential curves are not absolute proof of two definable anatomical compartments. Such curves might reflect inhomogeneous flow (i.e., multiple compartments) within the gray matter alone. Nevertheless, it is often impossible to demonstrate inhomogeneous flow in cortex (see Discussion19). Certainly, in larger animals like primates or cats, the electrodes can be placed far enough from white matter to produce "pure" cortical flows that are often monoeXponential2 and do not differ in magnitude from those obtained with other techniques. Unfortunately, the H2 clearance curves from rat cortex have, for the most part, a monoeXponential form1-3. So that support is lacking for the suggestion that the relatively low flow values in the rat reflect a measurement from both a high and a low flow compartment.

While the H2 clearance technique gives comparable "cortical" flow values in rats and mice and while these values are also comparable to whole brain CBF in larger mammals, the values are considerably smaller than either cortical or whole brain blood flow obtained with other techniques in both rats and mice. Stated another way, the H2 technique fails to demonstrate the peculiarly high values recently reported for small mammals when isotope clearance or fractionation techniques have been used. One should consider whether the latter techniques when applied to small rodents give spuriously high values.

The literature contains too few data from mice to resolve this question. In the one murine study we have located, labeled water was used as the indicator for CBF measurement.8 The cerebrum showed a flow of 220 ml/100 gm per minute, more than twice that of rat cortex as determined by the same technique.10 This one murine value is more remarkable since it includes flow from the lower corona radiata, internal capsule, or cerebral peduncle. It is of interest that in their xenon clearance study Gjedde et al. pointed out that the hematocrit of their rats was reduced 12% because of blood sampling during the period of observation. It is possible that at least a portion of the high flow values they observed might be due to reductions in the red cell concentration.14 Other workers15,16 have noted that withdrawal of blood samples, required by isotope clearance studies of rat CBF, may have deleterious effects on the preparation, and have utilized transfusions to counteract these effects. One such study17 stated that transfusions were used to prevent flow from falling. One wonders if, in fact, the transfusions in some way supported a supranormal flow.

It is of interest that in their xenon clearance study Gjedde et al. did not relate high values for rat CBF to the small size of the animal and to an inverse relation of CMRO2 to body size with coupling of CBF to CMRO2. This hypothesis has been suggested by others.11 Rather, Gjedde et al.12 stated that rat brain has a greater proportion of gray to white matter, and they accounted for both the high CMRO2 and high flows on that basis. However, proof of a disproportionate amount of gray matter in the rat brain was not presented.

In view of the preceding comments and because the cortical flows observed in rats by Goldman and Sapirstein8 and by Norberg and Siesjö12 were similar to cortical flows observed with similar techniques in larger species, it appears to us that the assertion of an unusually high CBF in small rodents remains unproved. Meanwhile, H2 clearance, measured with electrodes implanted in brain, would appear to provide a convenient method of comparing groups of mice, or of assessing the effects of experimental manipulation on flow in a single mouse or in a group of mice with initially comparable flows.

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References

Relative Role of Factors Associated With Cerebral Infarction and Cerebral Hemorrhage
A Matched Pair Case-Control Study


SUMMARY
Comprehensive ascertainment of all possible new cases of stroke appearing between January 1, 1970 and June 30, 1971, and admitted to three major hospitals in Winnipeg, Manitoba, has been achieved by reviewing the Manitoba Health Services Commission claim reports. The medical records of these cases were reviewed, pertinent data were abstracted, and rigid criteria for diagnosis were followed. Also, data were obtained from death certificates, autopsy reports and long-term hospital records. A total of 606 ascertained cases (410 infarction, 137 hemorrhage, and 59 undetermined type) were matched for age, sex, residence and year of admission with 606 controls from admissions for other than cardio-vascular and cerebrovascular disorders. The data were analyzed for elucidating the possible risk factors for infarction (INF) and hemorrhage (HGE).

For Chronic Incurable Diseases
Successful control may be achieved only through primary prevention, and toward this end elucidation of the risk or causative factors is necessary. Unfortunately, the elucidation of such factors in the case of cerebrovascular disease (stroke) is far from complete, and repeated descriptive and analytic studies are required to uncover those factors and to establish the basis for preventive measures.

In previous communications the incidence of cerebrovascular disease by clinical type and by certain variables such as age, sex, residence, birthplace, occupation and religion was described in a total community including 700,000 residents in Manitoba, Canada. Certain etiological hypotheses for the two major types of stroke, infarction (INF) and hemorrhage (HGE), were generated. This article presents the results of an analytical case-control study in an attempt to elucidate further risk factors in stroke and to test the hypotheses generated from the previous studies. An outstanding feature of this design is that it deals only with new cases of stroke and it considers each of the two major entities of stroke (INF and HGE) separately, so that the natural history of each entity might be understood and the relative role of the factors associated with one entity versus the other might be clarified.

Methods
Virtually all residents in the province of Manitoba are registered with the Manitoba Health Services Commission (MHSC) — a governmental health care agency which organizes medical and hospital care at no cost for the residents. The patient has the freedom of selecting his own physician.

Since stroke is an acute condition of serious manifestations, and medical care is of no cost to the patient, almost every patient is expected to be brought to the attention of the physician and subsequently will be hospitalized, at least for establishing the diagnosis and for initial care. Thus the MHSC hospital claim reports provide a reasonable source for comprehensive ascertainment of all possible new cases of stroke appearing in the province during a specified period. From these reports, all cases diagnosed as cerebrovascular disease (ISCD rubrics 430-438 Eighth Revision) who were

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