Continuous Quantitative Local Cerebral Blood Flow Measurement

Calibration of Thermal Conductivity Measurements by the Hydrogen Clearance Method

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SUMMARY The capability of a miniaturized probe to measure local cerebral blood flow in a continuous and quantitative manner is described. The incorporation of thermal conductivity measurements using the isothermal principle with the hydrogen clearance method allows calibration of the thermal conductivity component in absolute terms. Evaluation of this system in 14 cats showed a linear relationship between both measurement methods. The major limitation of this combination probe system is the need for routine intermittent recalibration in order that changes of tissue thermal conductivity induced by physiologic alterations during the experimental procedure may be recognized and resolved.

MOST METHODS used for measuring cerebral blood flow (CBF) in experimental animals are derived from Kety and Schmidt's development of the Fick principle. Although a variety of freely diffusible gaseous and non-gaseous tracers have been used, many investigators favor measuring the clearance of the inert gas. Hydrogen can measure CBF in discrete regions, is available, and does not require handling radioactive materials. Irrespective of the character of the tracer used in the clearance techniques, these tracers have been shown to provide repeatable and quantitative results. However, these studies give mean values measured over varying periods of time and, therefore, cannot follow rapid changes in local tissue flow.

To seek a dynamic continuous record of tissue perfusion, heat clearance techniques have been widely investigated. Grayson, in 1952, demonstrated a linear relationship between blood flow and the thermal conductivity of tissue. Betz and associates measured cortical blood flow using a probe consisting of 2 small gold plates, one heated, one neutral. In some of these studies, authors verified a correlation between local CBF measured by gaseous isotope clearance techniques and the continuous heat clearance method. Carter and Atkinson, in 1973, revised the cortical surface probe using the Peltier thermoelectric effect as previously described by Brawley. Calibration of this probe was carried out with Xenon clearance curves which demonstrated a linear relationship between the radioactive diffusion-washout rCBF and thermal diffusion local CBF measurements. These investigators believed that the Peltier flow probe was preferable to the heat clearance methods proposed by Betz and associates because it could quantitatively record continuous changes in CBF with reproducible results. Muller-Schauenburg et al. subsequently used a microthermistor system for quantitative flow measurements obtained by simultaneous measurements of Krypton clearance. In 1975, McCaffrey and McCook reviewed evidence for the isothermal principle and described their application of this method for measuring local tissue flow. These investigators demonstrated that such a system could measure the thermal conductivity of tissue in the vicinity of a small heated thermistor maintained at a fixed temperature difference above a reference thermistor. The power consumption of the heated thermistor is proportional to the thermal conductivity of the tissue which is directly related to local blood flow. In this study, calibration was not performed and the possible variations of local blood flow induced by a constant temperature thermistor were not acknowledged. This device, however, was relatively insensitive to alterations of ambient temperature and did demonstrate a continuous qualitative estimation of local tissue blood flow with a greater rapidity than has been shown by other techniques.

These considerations suggested that a flow probe incorporating the advantages offered by the isothermal and the hydrogen clearance methods would permit the continuous quantitative measurement of CBF in contiguous discrete regions of cerebral tissue. This report is concerned with the development and evaluation of such a device in which hydrogen clearance techniques are used to calibrate the isothermal conductivity element in absolute terms.

Materials and Methods

Fourteen cats weighing 2.2 to 3.8 kg were anesthetized with intravenous thiamylal sodium (10 mg/kg). Following intubation, catheters were placed in the femoral artery and vein for the continuous monitoring of mean arterial blood pressure (MABP), drug infusions and arterial blood sampling. Ventilation was maintained by a small-animal respirator with a rate of 38-44/min and tidal volume of 35 cc. Respiratory rate was adjusted to keep arterial carbon di-
oxide (Paco₂) levels as close as possible to 36 mm Hg. Ringers solution to which ketamine hydrochloride 8 mg/kg/h and pancuronium bromide 0.008 mg/ml were added for maintenance of anesthesia and muscle paralysis, was infused intravenously by pump at 16 ml/h. Heating pad and lamps kept body temperature between 37.5° and 39°C.

With the animal positioned Sphinx-like in a stereotaxic apparatus, bilateral one centimeter trephines were performed over the cerebral convexities. Following opening of the dura mater, a pair of the combination probes were inserted into the cerebral cortex to a 1.5 to 2.0 mm depth. The trephine openings were then sealed with gelatin solution. The probes were separated by 5 to 8 mm to allow one probe to serve as a heated measuring thermistor (Tm) and the other to function as the unheated reference thermistor (Tr). The thermal conductivity was then determined by measuring the power required to maintain a constant temperature difference (ΔT) between Tm and Tr. After the electrodes had stabilized for at least 2 hours, the thermal conductivity component was calibrated by correlation with hydrogen clearance measurements. The relationship between these 2 clearance methods was examined by evaluating their sensitivities to changes in Paco₂ induced by 5% CO₂ in air inhalation and hyperventilation. Following these evaluations, 3 to 4 CBF measurements by the hydrogen clearance method were obtained at normal MAP. Hypotension was then induced by an intravenous infusion of nitroprusside and elevation of the blood pressure was induced by infusion of metaraminol bitartrate. At the completion of these measurements the experiment was terminated by an overdose of barbiturates which permitted non-flow measurements of tissue thermal conductivity.

**Probe Design and Operation**

Figure 1 illustrates the combination isothermal/hydrogen clearance probe. The active portion of the hydrogen electrode is a platinum-iridium sphere measuring 0.25 mm in diameter. This platinum electrode is polarized with 300 mv against a Ag/Ag Cl reference electrode positioned in the paraspinal subcutaneous tissue. When tissue saturation is completed by the inhalation of hydrogen (6 to 7 vols %), the hydrogen is turned off and the decreasing current monitored on a strip chart recorder. The circuit of the instrumentation used for these hydrogen clearance determinations is essentially the same as described by Willis, Doyle and Ramirez, except that series resistance was increased to 1mΩ to accommodate the smaller platinum electrode. The exponential time constants of the hydrogen clearance curves were obtained by standard methods.

The thermistor portion of the probe consisted of a 0.13 mm diameter glass bead incorporated within the 0.356 diameter polyimide tube above the platinum sphere. The calibration of this thermal conductivity component was linear in the range of 0.6 to 2.0 cal/sec/cm/°C × 10⁻⁸ which is within the range of thermal conductivity for brain tissue. Although the instrumentation has a range of temperature variances available (0.25°C to 4°C), a temperature difference (ΔT) between Tm and Tr of 1°C was chosen to provide maximum sensitivity with minimum physiologic effects from heating on the surrounding tissue. The basic circuitry and calibration of the thermal component of the probe is based upon the methods and design described by McCaffery and McCook except that the resistors in the bridge circuit were adapted to the specific thermistors used in our probes.

**Results**

Control CBF measurements obtained with the hydrogen clearance method were a mean value of 58 ml/100 g/min with a standard error of ± 7 and consistently monoexponential. These findings indicate that the flows in the present experimental model were generally derived from the subcortical white matter. Spontaneous variations in the isothermal measurements were consistently less than 5%. Following these control recordings, the ability of the isothermal system to monitor changes in CBF induced by varying levels of Paco₂ is illustrated in figure 2. In this representative example CO₂ inhalation increased CBF as determined by the hydrogen clearance method from 45 to 55 ml/100 g/min (18%) while the isothermal measurement increased 24%. The CO₂ inhalation was stopped and flow allowed to return to baseline. Subsequent hypocapnia, induced by hyperventilation, caused the isothermal measurement to decrease 10% while hydrogen clearance-measured CBF decreased from 45 to 39 ml/100 g/min (14%). Generally, hypocapnia induced by CO₂ in air inhalation produced CBF increases, as measured by the isothermal component, in the range of 10% to 26% above control thermal conductivities. These values correlated closely with corresponding percentage changes determined by hydrogen clearance measurements. The changes in the isothermal measurement with hypocapnia were usually characterized by a gradual rise followed by a plateau. The return to baseline after cessation of CO₂ inhalation was usually a gradual event occurring over several minutes.
With controlled reduction of arterial pressure by nitroprusside infusion, the CBF remained unaltered until MABP was reduced below the 50-70 mm Hg range. At that point, CBF measured by the isothermal system began a rapid decline. With cessation of the nitroprusside infusion and infusion of metaraminol bitartrate there was concurrent recovery of MABP and CBF. Changes in the isothermal measurement were linear in correlation with changes in the hydrogen clearance measurements (Fig. 3). However, if the hypotensive episode was extensive or prolonged, subsequent hydrogen clearance measurements indicated that the isothermal calibration had changed suggesting a change in tissue thermal conductivity (Fig. 3). This group of animals all manifested loss of CBF autoregulation as shown by reactivity to mild hypertensive stimuli and loss of CO₂ responsiveness.

When hypertension was induced by metaraminol bitartrate without previous nitroprusside infusion, CBF was stable until MABP exceeded 200-225 mm Hg. Above this level CBF increased. Again the correlation between the isothermal and hydrogen clearance measurements was linear without indications of changes in isothermal calibration unless an excessive (> 250 mm Hg) and prolonged (> 20 minutes) hypertensive period had occurred.

By extrapolation from flow data and measurements made following sacrifice of the animals, the normal range of thermal conductivity of brain tissue was found to be $1.8 \times 10^{-8}$ to $2.0 \times 10^{-8}$ cal/cm/sec °C.

**Discussion**

The linear correlation found with thermal conductivity and hydrogen clearance-measured CBF in the present study agrees with prior studies which demonstrated a similar relationship between thermal diffusion and radioactive-diffusion washout CBF. In comparison to these other studies, the combination probe system has certain advantages. The size of the present probe (0.36 mm) limits flow measurements to relatively discrete regions as demonstrated by the consistently monoexponential hydrogen clearance curves noted in this study. Because the heat transfer coefficient of metal is significantly greater than the polyimide covering of the probe, the thermistor is most sensitive to the tissue volume surrounding the platinum-iridium component. These thermal conductivity properties permit flow measurements by both thermal and hydrogen methods from relatively con-
continuous measurement of local cerebral blood flow in laboratory animals. Additionally, the use of the isothermal principle in performing the thermal conductivity studies alleviates the sensitivity to changes in ambient temperature, potential sources of error with many other thermal techniques.

To avoid certain errors incurred by devices of this type, cautious data interpretation is necessary. Use of implanted electrodes results in varying zones of tissue damage surrounding the electrodes. The consequences of such tissue damage on electrode function, however, is usually minimal when the electrode does not exceed 0.5 mm in diameter. Although the combination probe fulfills this size restriction, flow measurements are sensitive to small variations in local vascular geometry. Investigators using similar implanted electrodes have resolved this difficulty by averaging the flow value obtained from varying montages of multiple electrodes.

In the present study, the major difficulty in achieving reproducible flow determinations resulted from local alterations in tissue thermal conductivity. Figure 3 shows this type of baseline shift occurring after prolonged cerebral ischemia. Previous calibration of the heat probe is, therefore, no longer adequate and repeat hydrogen clearance determinations are required. These considerations do not apply to those measurements obtained in minimally traumatized and non-ischemic brain tissue which shows a consistent response in local CBF to stimuli such as CO₂. During such periods, the thermal diffusion component of the measurement system maintains a constant responsivity which requires no recalibration. However, because changes of tissue thermal conductivity may be difficult to recognize, intermittent calibration should be used with this measurement system.

This study shows that incorporation of the thermal conductivity measurement method with the commonly used hydrogen clearance method offers a technique for accomplishing continuous measurement of local cerebral blood flow in laboratory animals.

The combination electrode described in this report is available from Robert A. Macur Inc. Route 2, Box 126-S, Mukwonago, WI 53149. Additionally, the authors can supply further technical information upon request.

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