JOURNAL OF APPLIED PHYSIOLOGY
Vol. 33, No. 5, November 1972. *Printed in U.S.A.*

An indicator-dilution technique for study of blood-to-brain solute passage in the rat

JOEL ELISABETH MURRAY AND AUDRIUS PLIOPLYS
Section of Neurology, University of Chicago, Chicago, Illinois 60637

MURRAY, JOEL ELISABETH, AND AUDRIUS PLIOPLYS. *An indicator-dilution technique for study of blood-to-brain solute passage in the rat.* J. Appl. Physiol. 33(5): 681–683. 1972.—Details are given of a method adapting single-injection indicator-dilution techniques for use in the study of blood-to-brain solute transport in vivo in the rat. Glucose extraction by brain is shown to be saturable under conditions of high circulation plasma glucose concentrations. Investigation of functional vascular anatomical relationships was made with a view toward how effectively the brain of the rat may be isolated as an "organ" for indicator-dilution studies. Indicator-dilution curves obtained during simultaneous collection of venous effluent from the confluens of sinuses and the external jugular vein suggest that blood from the sinus does not contain significant admixture of extracerebral venous return and may therefore be relied on to provide information about solute extraction by brain in the rat.

capillary permeability; glucose transport; blood-brain barrier; cerebral vasculature; cerebral venous drainage; saturable transport

---

INDICATOR-DILUTION TECHNIQUES have been used in vivo in large mammals to study the movement of solutes into brain during a single passage through the cerebral circulation (1, 5, 6). The general method has proven a promising one for elucidation of mechanisms regulating the selective distribution of metabolites between the central nervous system (CNS) and the vascular space. An adaptation of such techniques for use in the rat, the details of which are presented here, offers both theoretical and practical advantages.

In previous studies of this type it has been assumed that isolation of the cerebral circulation is accomplished by cannulation of the internal carotid artery and the confluence of the sinuses. The possible existence of functional vascular connections between cerebral and extracerebral venous drainage, which would preclude meaningful estimation of brain solute extraction, has not been explored. Evidence is presented that, in the rat, venous blood sampled at the confluence of the sinuses does not contain significant admixture of extracerebral return and may therefore yield reliable information concerning solute extraction by brain.

Single injection indicator-dilution techniques have been used in mammals other than the rat to support the hypothesis that CNS capillaries may play more than a passive role in blood-to-brain glucose transport. Thus, in the dog (4), rabbit (1), and cat (6), high plasma glucose concentrations reduce the extraction of labeled glucose during a single cerebral circulatory passage. Similar findings, predictable in the case of the rat, are presented here as evidence for reliability of the techniques developed. Although some microsurgical skills are required, the method is a relatively simple one and has an additional advantage: that of economy in costs for animals and supplies over studies using large mammals.

METHODS

Fasting male Sprague-Dawley rats weighing 200–500 g were anesthetized by intraperitoneal injection of a fentanyl-droperidol solution (Innovar-Vet), 0.001 ml/kg. This agent produced rapid and smooth induction of analgesia, without depression of respiration or blood pressure, and the animal could be maintained in this state up to 3 hr with small supplementary doses. Additional local anesthesia at the site of skin incisions was required in some animals, and was administered routinely in all by injection of 1% Xylocaine solution subcutaneously. The animals breathed room air through a tracheostomy, a 0.5-cm piece of no. 90 polyethylene (PE) tubing. The animal was secured on an operating board warmed by an electric light bulb to ensure maintenance of body temperature during the procedure. The femoral artery was cannulated for monitoring of blood pressure or withdrawal of arterial blood samples.

Cannulation procedures. The carotid cannula consisted of 30 cm of PE-10 tubing filled with 0.15 M NaCl containing heparin 4 IU/ml. The proximal end of the cannula was fitted over a 27-gauge needle, the hub of which accepted a stopcock or Luer-Lok syringe. The carotid vessels on the right side were exposed by division of the omohyoid and stylohyoid muscles and removal ot a portion of the hyoid bone. Under operating microscope visualization the external carotid artery was freed of surrounding tissue by gentle blunt dissection and ligated at the level of the lingual artery (approximately 1 cm distal to the carotid bifurcation). The artery was cannulated in retrograde fashion through an incision just proximal to the ligature and the tip of the cannula was positioned opposite the mouth of the internal carotid. When possible, control of bleeding during cannulation was accomplished by occluding only the external carotid with a temporary proximal ligature; in other instances, the common carotid was compressed for a maximum of 30 sec to prevent blood flow. With the cannula tied loosely in place, several 0.1-ml bolus test injections were made of the heparin in saline solution. The arterial wall was thin enough that final adjustment of the catheter position could be made by observing the test bolus as it was swept into the internal carotid by the force of the common carotid pulse pressure. The cannula was secured with two tight ligatures and the neck wound closed with surgical clips. Each animal received 10 IU of heparin/kg during the arterial cannulation procedure.

The animal was then secured in a head holder and the posterior portion of the calvarium exposed and cleared of periosteum. A 1.2-mm burr hole was made in the center of the lambdoid process of the interparietal bone, which proved to be a reliable surface landmark overlying the region of the confluens sinuum. The outflow cannula was a heparin-filled 35-cm length of PE-90 tubing fitted over a 2.5-cm segment of a sawed-off 18-gauge needle. The blunt end of the needle fitted snugly into the burr hole and the

free end of the cannula was directed by hand for collection of 1-drop venous blood samples directly into scintillation counting vials each containing 1.5 ml H_2O. Prior to each arterial injection, the rate of flow from the venous catheter was adjusted to an average of 1 sample/sec in order to facilitate data comparison among experiments. This was done by raising or lowering the animal, with respect to the collection site, with a mechanical jack under the head holding board. In the hands of an experienced operator the time required for placement of both arterial and venous cannulas was 30–45 min. Blood loss prior to injection and sample collection was less than 5 ml.

Injection procedure. Injectate was prepared immediately prior to injection by mixing 0.5 ml of the animals' freshly drawn blood with tracer amounts of isotopically labeled indicator and test solute. In most experiments 15 μc of uniformly labeled inulin-^{3}H was used as the nondiffusable indicator, with 5 μc of a ^{14}C-labeled test solute. For each bolus injection, a 0.1-ml volume of the mixture was drawn into a 1-ml Luer-Lok syringe which was fitted into the hub of the needle at the proximal end of the arterial cannula, with precautions to avoid trapping of air bubbles. Injection was made by hand in less than 1 sec delivering a total volume of 0.08 ml of the isotope mixture into the arterial circulation, when corrected for that remaining in the carotid cannula. Venous outflow was collected for 30 sec following injection. Isotope first appeared in the distal end of the venous outflow cannula at about 6 sec following injection, and peak concentrations were reached 4–5 sec after initial appearance. When more than one injection was performed in a given preparation, 5 min were allowed to elapse between them, the carotid cannula was flushed, and the venous outflow cannula was replaced. The larger animals tolerated three injections without evidence of cardiorespiratory deterioration, and with a drop in Hct averaging only 10–20% maximum. The extraction values calculated from successive injections in a given preparation showed less than 10% variability, and thus the animal could be used as its own control. After the final collection period the animal was sacrificed with an overdose of pentobarbital.

Isotope assay. At the end of each experiment, triplicate 1-drop samples of injectate were added to water-containing scintillation vials and assayed in the same manner as the venous effluent samples. The problem of quench interference when counting samples containing hemoglobin was eliminated by the following method, derived empirically: Hyamine hydroxide, 0.05 ml, and 3% H_2O_2, 0.5 ml, were added to each vial for solubilization and decoloration of the blood samples. After mixing with a Triton/toluene liquid scintillant (9), the samples were allowed to stand

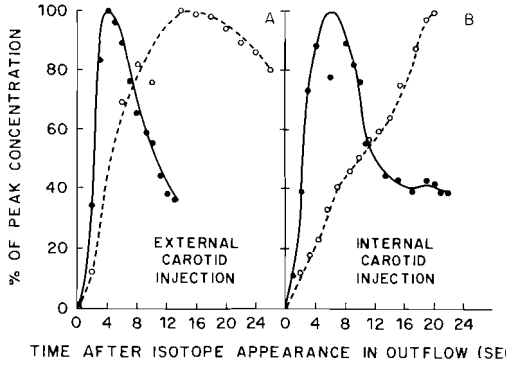

FIG. 1. Following carotid injection, emergence of inulin-^{3}H from confluence of the sinuses (●) and the external jugular vein (○) is plotted as a function of time. Appearance of isotope in outflow is taken as starting time in order to minimize possible transit time differences through collecting systems. In *A* and *B*, representative curves are shown for single injections in two animals.

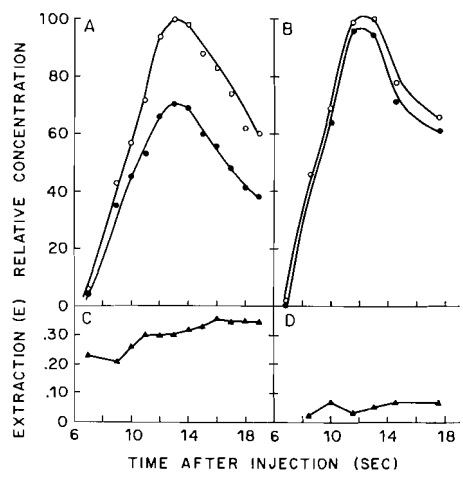

FIG. 2. *Upper panel:* emergence curves for two tracers (inulin-^{3}H: ——○, glucose-^{14}C: ——●) from confluence of the sinuses following simultaneous injection into the internal carotid. Percent of peak inulin-^{3}H concentration is plotted as a function of time. In *A*, a typical experiment in a fasting animal is shown together with a plot (*C*) of extraction (E) values for each sample (——▲). In *B*, elevation of blood glucose to 660 mg/100 ml prior to injection results in decreased E values (*D*). For each venous effluent sample, $E = (C_{ref} - C_{test})/C_{ref}$, where C_{ref} and C_{test} are concentrations of reference and test solute normalized with respect to their concentrations in the injectate (3).

in natural light for 6–12 hr for completion of decoloration. The samples were stored in the dark for one hour at −4 C prior to counting to allow disappearance of photoluminescence produced by the Hyamine-protein solution. Samples were counted in a Packard liquid-scintillation counter and corrections were applied for quench and background, as well as channel spillover, using the method of external standardization.

Venous drainage—evidence for regional separation. Ability to isolate the arterial supply and venous drainage of an organ is a fundamental requirement if solute extraction is to be studied using single injection indicator-dilution methods. In the rat, the internal carotid has an extensive extracerebral distribution, in addition to giving rise to the major cerebral vessels (7). If CNS solute extraction is to be studied following internal carotid injection, one must exclude the possibility that venous anastomoses "upstream" to the sampling point do not contaminate blood returning from the CNS with that from extracerebral sources, despite the inferences from anatomical evidence (7) to the contrary.

In an attempt to approach this problem, we collected venous blood from the confluence of the sinuses and the external jugular vein simultaneously, following bolus injection of inulin-^{3}H into either the internal or external carotid artery. Corrections were made for catheter delay. In both cases the emergence curves for the confluence of the sinuses sampling site were the same (Fig. 1). Comparison of the jugular vein emergence curves with those from the sinus shows that the latter appear earlier, are of shorter duration and differ in shape (i.e., steeper upslope and downslope prior to recirculation). The presence of a separable outflow curve, the slope of which is characteristic of an organ having high blood flow and low permeability to inulin such as brain and lung (3), is interpreted as evidence that blood collected from the confluence of the sinuses in the rat represents cerebral venous drainage. Thus, extraction values calculated from blood sampled at this site may be assumed with some confidence to exclude extraction by extracranial tissues.

Evidence for saturable glucose transport. The reliability of the method is supported by a series of experiments demonstrating saturability

of glucose extraction, predictable in the light of previous studies of a similar type in other mammals (1, 4, 6) and confirming the results of Bidder (2) whose experiments suggest that blood to brain glucose transport may be a carrier-mediated process in the rat. The extraction of a test solute during cerebral passage is its fractional loss relative to inulin, a dilutional indicator which does not leave the cerebral vascular space during a single passage. Figure 2 shows emergence curves for typical experiments using glucose-^{14}C as the test solute under conditions of high and low circulating plasma glucose. To minimize artifacts due to the Taylor effect, or laminar diffusion (8), an average was taken of the extraction values for samples lying within the top 40% of the reference curve, and this value, $\bar{E}$, used as the result for that experiment. In the presence of normal plasma glucose the mean $\bar{E}$ for 6 experiments was 0.21 ± 0.03. In experiments in which plasma glucose was elevated to approximately five times normal, by giving 100% glucose in saline, 0.1 ml/kg intraperitoneally, 10 min prior to arterial isotope injection, mean $\bar{E}$ was 0.09 ± 0.03 ($N = 5$).

This work was supported by Grant 1-R01-NB-09728 from the National Institutes of Health.

J. E. Murray was supported by Fellowship 1-F10-NS-02327 from the National Institutes of Health.

Received for publication 15 November 1971.

REFERENCES

1. Agnew, W. F., and C. Crone. Permeability of brain capillaries to hexoses and pentoses in the rabbit. *Acta Physiol. Scand.* 70: 168–176, 1967.
2. Bidder, T. G. Hemiinfusion of the brain in rats: blood-to-brain penetration. *J. Appl. Physiol.* 21: 1073–1077, 1966.
3. Crone, C. The permeability of capillaries in various organs as determined by use of the "indicator diffusion" method. *Acta Physiol. Scand.* 58: 292–305, 1963.
4. Crone, C. Facilitated transfer of glucose from blood into brain tissue. *J. Physiol., London* 181: 103–113, 1965.
5. Crone, C. The permeability of brain capillaries to non-electrolytes. *Acta Physiol. Scand.* 64: 407–417, 1965.
6. Cutler, R. W. P., and J. C. Sipe. Mediated transport of glucose between blood and brain in the cat. *Am. J. Physiol.* 220: 112–11868, 1971.
7. Greene, E. C. Anatomy of the rat. *Trans. Am. Phil. Soc.* 27: 183, 1935.
8. Lassen, N. A., and C. Crone. The extraction fraction of a capillary bed to hydrophilic molecules: theoretical considerations regarding the single injection technique with a discussion of the role of diffusion between laminar streams (Taylor's effect). In: *Capillary Permeability, Benzon Symposium II, Munksgaard, Copenhagen.* New York: Academic, 1970, p. 48.
9. Patterson, M. S., and R. C. Greene. Measurement of low energy beta-emitters in aqueous solution by liquid scintillation counting of emulsions. *Anal. Chem.* 37: 854–857, 1965.