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# Blood-Brain Glucose Transfer in Spreading Depression

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**Abstract:** Spreading depression in rat brain cortex is associated with a twofold increase of cerebral blood flow. It is not known whether this increase is coupled to increases of cerebral metabolic rate and glucose transport from blood to brain. During the passage of a single spreading depression, we measured blood-brain glucose transport and glucose metabolism in rat cerebral cortex by single intravenous injection of tracer glucose. Blood flow and tissue content of glucose were measured as well. Reduction of tissue glucose and the consequent increase of net transfer of glucose from blood to brain were consistent with a threefold increase of the consumption of glucose before the increase of blood flow. There was no increase of unidirectional blood-brain transfer. **Key Words:** Glucose transport—Spreading depression—cerebral blood flow. Gjedde A. et al. Blood-brain glucose transfer in spreading depression. *J. Neurochem.* 37, 807–812 (1981).

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Leão's spreading depression (SD) (1944) occurs in a variety of mammals, including man. Its elicitation requires some chemical, electrical, or mechanical stimulation (Bureš et al., 1974). The chief feature is reduction of electroencephalographic activity, spreading from the site of elicitation at a speed close to  $3 \text{ mm min}^{-1}$ , accompanied by marked changes of blood flow (Hansen et al., 1980), extracellular ion concentrations (Vyskočil et al., 1972; Nicholson and Kraig, 1975; Kraig and Nicholson, 1978; Hansen, 1979), extracellular space volume (Phillips and Nicholson, 1979; Hansen and Olsen, 1980), tissue oxygen tension (Tsacopoulos and Lehmenkühler, 1977), and tissue metabolites (Křivánek, 1958, 1961; Quistorff et al., 1979).

The relationship between blood flow, capillary glucose transport, and cerebral metabolic rate during SD is unknown. In order to determine this relationship we measured the three variables simultaneously by single intravenous injection of labeled tracers (Gjedde, 1980; Gjedde et al., 1980).

## MATERIALS AND METHODS

Attempts to measure the change of glucose consumption during an event that lasts only a few minutes must

take into account the turnover rate of unphosphorylated glucose in brain (Savaki et al., 1980). Changes of glucose metabolism do not reach steady state for less than 5–10 half-times, or 20–40 min. In SD, the rate of glucose consumption must continue to change, and the loss of glucose to the metabolic pool(s) may differ from the net rate of glucose translocation across the cerebral capillary endothelium. In short, methods which require steady state cannot be applied directly.

In the present study, we estimated the net rate of glucose translocation across the cerebral capillary endothelium from the unidirectional rates in both directions. The unidirectional rates were measured by intravenous injection of labeled glucose and determination of glucose uptake by brain after 10 s. The injection was performed during the spread of SD through the left parietal cortex of rat, as previously described (Hansen et al., 1980a). Ten seconds after the injection, the brain was frozen *in situ* by application of liquid nitrogen, sliced coronally in the frozen state (Quistorff and Chance, 1980), and the necessary variables (see below) determined in both hemisphere halves of the cortical tissue of each slice.

## Animal Preparations

Nine male Wistar rats were anesthetized with an initial dose of pentobarbital ( $60 \text{ mg kg}^{-1}$ ) and supplementary doses as required later. Catheters were placed in the fem-

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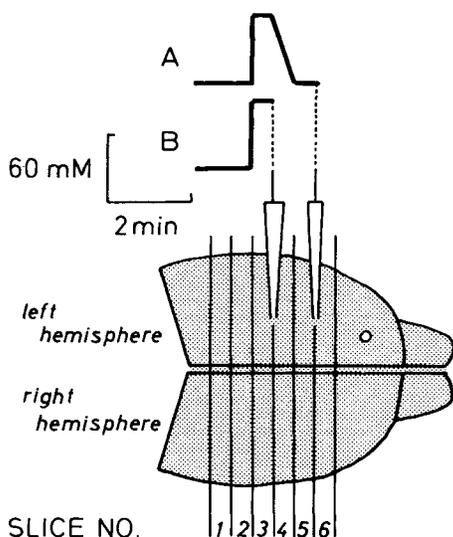
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Abbreviation used: SD, Spreading depression.

oral arteries and one femoral vein. The spontaneously breathing rats were placed in a head holder and the parietal bones removed by craniotomy. The rectal temperature was fixed at 37°C by a thermostat. Five of the 9 animals were allowed to remain normoglycemic, 2 were treated with glucose, and 2 with insulin to provide a full spectrum of plasma glucose concentrations for calculation of  $T_{\max}$  and  $K_m$ , as previously described (Gjedde, 1980).

### Experimental Procedures

The experimental design is shown in Fig. 1. When the animals were in respiratory steady state (see below), a single SD was elicited by a brief stab in the left frontal cortex, using a hypodermic needle. Preliminary experiments showed that only the left hemisphere was subject to SD by this procedure. The advance of the SD was monitored with two double-barrelled, potassium-sensitive microelectrodes with tip diameters of 1–2  $\mu\text{m}$ . They were passed through the intact dura and placed in the extracellular space of the left parietal cortex at a depth of approximately 0.5 mm (Hansen et al., 1980). As the anterior-posterior distance between the electrodes was approximately 3 mm (2 slice widths), the SD traveled between the electrodes in 1 min. Approximately 30 s after the appearance of the SD at the posterior electrode, we injected a 200- $\mu\text{l}$  intravenous bolus of a mixture of 10  $\mu\text{Ci kg}^{-1}$   $n$ -[1- $^{14}\text{C}$ ]butanol, 50  $\mu\text{Ci kg}^{-1}$  D- $^3\text{H}$ (N)glucose, and 50  $\mu\text{Ci kg}^{-1}$   $^{111}\text{InCl}$ , and arterial blood sampling was started simultaneously. The bolus of isotopes arrived to the brain approximately 5 s after the injection. Ten seconds after the injection, arterial sampling was discontinued, and the cortical circulation arrested by *in situ* application of liquid nitrogen. The parietal cortex was exposed to labeled butanol for about 5 s, estimating an arri-



**FIG. 1.** Experimental design. Diagrammatic representation of dorsal aspect of rat cerebral hemispheres. Two potassium-sensitive microelectrodes recorded traces A and B at time of freezing. Number and approximate position of coronal slices are shown in relation to potassium electrodes.

**TABLE 1.** Average physiological variables of rats used in the present study

Variable	Mean $\pm$ S.E.M. ( $n = 9$ )
Arterial plasma glucose ( $C_a$ , mM)	9.6 $\pm$ 1.4 <sup>a</sup>
Hematocrit	0.58 $\pm$ 0.01
Mean arterial blood pressure (MABP, mm Hg)	105 $\pm$ 22
Arterial oxygen tension ( $P_a\text{O}_2$ , mm Hg)	86 $\pm$ 9
Arterial carbon dioxide tension ( $P_a\text{CO}_2$ , mm Hg)	37 $\pm$ 2
Arterial pH	7.43 $\pm$ 0.01

<sup>a</sup>  $n = 5$  (normoglycemic animals).

val time of the bolus to brain of 4 s, and a freezing time of 1 s (Gjedde et al., 1980; Hansen et al., 1980).

### Sample Analyses

The frozen brain was divided into 1.2-mm-wide slices by an electric saw in a glove box at  $-25^\circ\text{C}$ . The cortical tissue was isolated, weighed, and extracted (Quistorff and Chance, 1980). Samples of the extracts were prepared for liquid scintillation counting, as was the arterial sample, including a separate sample of the last 5  $\mu\text{l}$  of arterial blood drawn into the syringe, representing the arterial concentration of tracers at the time of circulation arrest.

Blood, tissue, and injectate sample activities of  $^3\text{H}$ ,  $^{14}\text{C}$ , and  $^{111}\text{In}$  were determined by  $\beta$ - and  $\gamma$ -scintillation spectrometry in appropriate counters, as previously described (Gjedde and Rasmussen, 1980a).

Arterial blood samples were assayed for pH,  $\text{PO}_2$  and  $\text{PCO}_2$  with Radiometer (Copenhagen, Denmark) electrodes. The animals were considered to be in respiratory steady state when the  $P_a\text{CO}_2$  of samples taken at least 10 min apart agreed within 10%. Arterial blood pressure was continuously recorded with a strain-gauge transducer. Hematocrit was determined by centrifugation.

Plasma concentrations of glucose were measured in freshly sampled arterial plasma by the glucose oxidase method (Christensen, 1967). The glucose content of the tissue extracts was determined as described by Lowry and Passonneau (1972).

Preparations of D- $^3\text{H}$ (N)glucose,  $n$ - $^{14}\text{C}$ ]butanol and  $^{111}\text{InCl}$  were obtained from the New England Nuclear Corporation (Germany) with specific activities of 18 Ci  $\text{mmol}^{-1}$ , 1.86 mCi  $\text{mmol}^{-1}$ , and 7 mCi  $\text{ml}^{-1}$ , respectively.

### Calculations

The calculation of some of the experimental variables has been described in detail previously (Gjedde and Rasmussen, 1980a). The calculation of the remaining variables has been set forth in an appendix. In brief, the cerebral plasma content was calculated as the ratio between brain and arterial plasma sample contents of  $^{111}\text{In}$ . The extravascular brain content of labeled glucose was calcu-

lated as the product of plasma content of brain and the labeled glucose concentration in arterial plasma at the time of circulation arrest. The clearance of labeled glucose by brain ( $K_{in}$ ) was calculated as the ratio between the extravascular brain content and the arterial time-concentration integral, estimated from the arterial plasma sample content of labeled glucose. The local cerebral plasma and blood flows were determined from the ratios between the brain and arterial plasma and blood sample contents of labeled butanol (Hansen et al., 1980). The apparent permeability  $P_1^1A$  was calculated from Eq. 2 (see Appendix),  $T_{max}$  and  $K_m$  from Eq. 8; the apparent permeability  $P_2^1A$  from Eq. 5; and  $J_{net}$  from Eq. 6. The cerebral glucose consumption was estimated from Eq. 9.

## RESULTS

### Right (Control) Hemisphere

The physiological variables of the 9 rats included in the present study are shown in Table 1.

Table 2 summarizes the results of the unaffected (control) right hemisphere. Note the marked reduction of cortical blood flow and net glucose transfer, due to pentobarbital anesthesia (Gjedde and Rasmussen, 1980b).

The kinetic analysis of unidirectional tracer glucose transfer from blood to the unaffected right hemisphere brain tissue is shown in the form of a flux ( $P_1^1AC_a$ ) versus apparent permeability ( $P_1^1A$ ) plot in Fig. 2. The result of the regression analysis is shown in Table 2. It yielded the average  $T_{max}$  and  $K_m$  in the range of concentrations studied. A concomitant reduction of blood flow and  $T_{max}$  is evident upon comparison with values reported previously for the awake rat (Gjedde and Rasmussen, 1980b).

TABLE 2. Average circulatory and transport variables of right hemisphere parietal cortex in normoglycemia

Variable	Mean $\pm$ S.E.M. (n = 5)
Plasma volume ( $V_{pl}$ , ml 100 g <sup>-1</sup> )	1.11 $\pm$ 0.20 <sup>a</sup>
Blood flow (CBF, ml 100 g <sup>-1</sup> min <sup>-1</sup> )	48 $\pm$ 5
Glucose content ( $M_e$ , $\mu$ mol g <sup>-1</sup> )	3.4 $\pm$ 0.4
Tracer glucose clearance ( $K_{in}$ , ml 100 g <sup>-1</sup> min <sup>-1</sup> )	11.9 $\pm$ 1.5
Blood-brain barrier glucose permeability ( $P_1^1A$ , ml 100 g <sup>-1</sup> min <sup>-1</sup> )	16.5 $\pm$ 2.0
Glucose consumption ( $CMR_{glc}$ , $\mu$ mol 100 g <sup>-1</sup> min <sup>-1</sup> )	34 $\pm$ 5
Maximal transport capacity ( $T_{max}$ , $\mu$ mol 100 g <sup>-1</sup> min <sup>-1</sup> )	277 $\pm$ 15
Half-saturation constant ( $K_m$ , mM)	6.8 $\pm$ 0.7

<sup>a</sup> (n = 4)

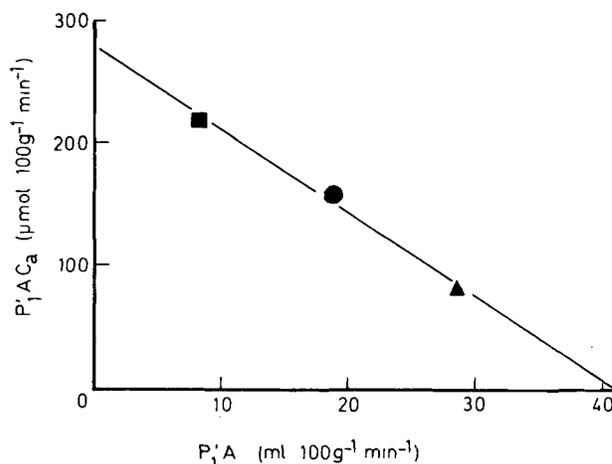


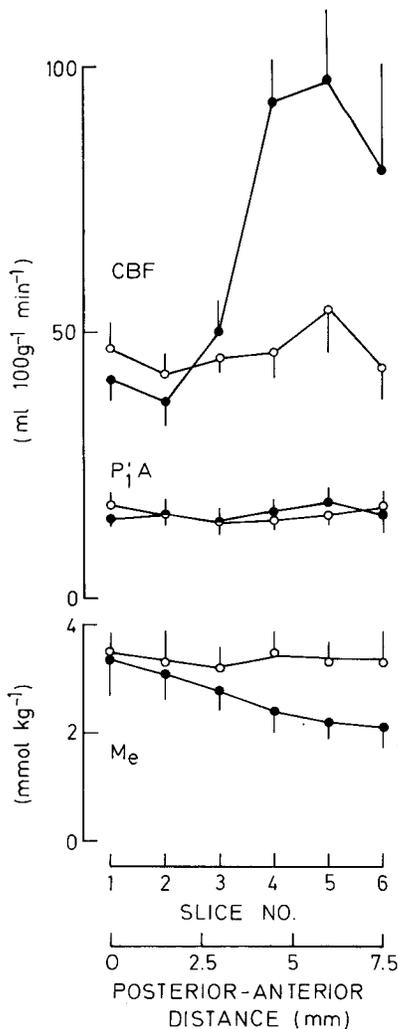
FIG. 2. Flux ( $P_1^1AC_a$ ) versus apparent permeability ( $P_1^1A$ ) plot of unidirectional tracer glucose transfer into right hemisphere parietal cortex in pentobarbital anesthesia. Points represent averages of 2–5 rats in hypoglycemia (▲), hyperglycemia (■), and normoglycemia (●).

### Left (SD) Hemisphere

The observed relationship between local cortical blood flow, apparent glucose permeability of the two hemispheres, and the tissue glucose contents are shown in Fig. 3. Each slice was approximately 1.2 mm wide and therefore represented half a minute at a speed of the potassium wave of 3 mm min<sup>-1</sup>. It is evident that no change of apparent glucose permeability occurred with the marked changes of blood flow and tissue glucose.

The change of blood flow, total brain glucose content,  $T_{max}$ , and the calculated net glucose transfer from blood to brain were converted to functions of time, using the rate of progression of the SD. The first event in time was an increase of the net rate of glucose transfer into the tissue, coinciding with a slight decrease of blood flow and a reduction of tissue glucose. Since no change of the apparent glucose permeability occurred, the increase of glucose transfer was a consequence of the decreased tissue glucose and must have succeeded an increased rate of glycolysis. The tissue glucose fell in the left hemisphere at a rate of 0.7  $\mu$ mol g<sup>-1</sup> every minute for the first minute of the passage of SD, indicating that glycolysis in this hemisphere exceeded the net influx of glucose by about 70  $\mu$ mol 100 g<sup>-1</sup> min<sup>-1</sup>. Later, the rate of decrease of tissue glucose slowed to 20  $\mu$ mol 100 g<sup>-1</sup> min<sup>-1</sup>. The increased rate of phosphorylation of glucose was estimated as shown in Fig. 4. The glycolytic rate reached a maximum of 141  $\mu$ mol 100 g<sup>-1</sup> min<sup>-1</sup> at the onset of normalization of  $[K^+]_e$ .

Altogether, four phases in the development of spreading depression were noted: (1) increased glycolysis and decreased tissue glucose, (2) increased extracellular potassium, and (3) increased

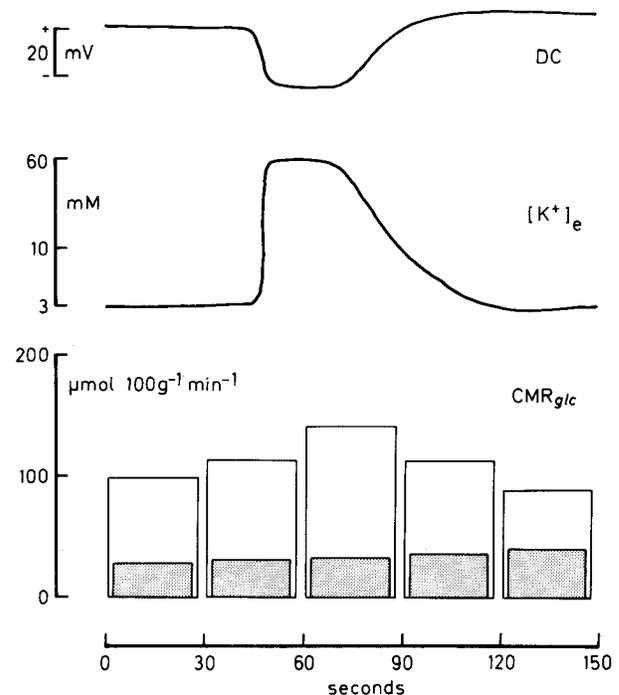


**FIG. 3.** Relationship between slice number and local blood flow (CBF), apparent permeability of glucose in blood-brain barrier ( $P_1A$ ) and tissue glucose ( $M_e$ ) during passage of SD. Open circles represent right hemisphere, closed circles left hemisphere. Bars indicate S.E.M. Where bars are not shown, they do not exceed limit of symbols.

blood flow. Last, a final increase of  $T_{max}$  occurred when the tissue glucose was maximally reduced.

### DISCUSSION

The present study had two important results: (1) The rapid changes of blood flow, glucose transport, and glucose consumption were not coincident but represented a sequence of events in time. (2) The first event observed during the passage of SD through a region of brain was increased glucose consumption by brain, and not the marked shifts of ions between the extra- and intracellular spaces. The change of glucose consumption could not be measured directly but was assessed from the net rate of glucose transfer into brain tissue and the glucose content of the tissue.



**FIG. 4.** Cortical glucose consumption during passage of SD. Changes are shown in relation to slow (DC) brain potential and  $[K^+]_e$ . Shaded histogram shows events in right hemisphere, open histogram events in affected left hemisphere.

These observations raise two questions: what factor(s) caused glucose consumption to rise before the change of extracellular ions, and why did the apparent glucose permeability of the blood-brain barrier not rise in proportion to blood flow?

Several previous studies have identified changes in SD that occur prior to the main changes of ion homeostasis. These studies include the works of Lukyanova and Bureš (1967) and Tsacopoulos and Lehmenkühler (1977), who noted an increase of oxygen tension 1/2 – 1 min before the rapid negative shift of the DC potential of brain and a marked increase of  $[K^+]_e$ , as well as our own observation of decreased ATP/ADP ratio and increased lactate/pyruvate ratio (Quistorff et al., 1979). These observations corroborated the finding of Lukyanova and Bureš (1967), who calculated decreased oxygen consumption prior to the main increase. The increased oxygen tension suggests that the increased rate of glycolysis is not due to tissue hypoxia. The increased oxygen tension may also explain the curious finding of the initial moderate decrease of cortical blood flow as a result of superoxygenation of the tissue. The mechanism of the early increase of glycolysis remains obscure. The late increase, however, is explained by the metabolic demand incurred by the return pumping of ions.

A net total of 600 μmol potassium and 2400 μmol sodium per 100 g brain were pumped in half a minute, as calculated from the potassium and sodium

contents and volume of brain cortex interstitial fluid under normal conditions and during SD. The  $[Na^+]_e$  fell from 150 to 60 mM, and the  $[K^+]_e$  increased from 3 to 60 mM, while the volume of the interstitial fluid shrank from 20 to 10 ml 100 g<sup>-1</sup> (Hansen and Olsen, 1980; Hansen and Zeuthen, 1981). Assuming a Na:ATP molar ratio of 3:1, the pumping of sodium required an equivalent of 1600 (800 × 2) μmol ATP 100 g<sup>-1</sup> min<sup>-1</sup>. Since the relative contributions of anerobic and aerobic glycolysis were unknown during this phase of the SD, it was possible to identify the glucose consumption as sufficient to cover this demand only if the anerobic glycolysis were assumed to make a minor contribution to the total glycolysis. Under this assumption, the excess glucose consumption of 100 μmol 100 g<sup>-1</sup> min<sup>-1</sup> in the left hemisphere regenerated about 3600 μmol ATP 100 g<sup>-1</sup> min<sup>-1</sup>, or more than sufficient for the requirement estimated above.

The second question concerns the absent proportionality between blood flow and the apparent permeability of glucose in the blood-brain barrier. Proportionality between the permeability of brain capillaries and cerebral blood flow was previously shown to exist both during reduction of blood flow by pentobarbital (Gjedde and Rasmussen, 1980b) or during postischemic hypoperfusion (Siemkiewicz and Gjedde, 1980), as well as during augmentation by seizures (Bolwig et al., 1977a,b). No such proportionality seems to exist in cases of "uncoupling" of the couple between flow and metabolism in brain, such as halothane anesthesia or hypercapnia (see Gjedde et al., 1980). The present study shows that similar uncoupling may take place in SD.

The association of uncoupling with nonparallel changes of cerebral blood flow and  $T_{max}$  points to two separate forms of regulation of the cerebral microcirculation. In one form of regulation, no change occurs of the cerebral capillary surface area available for diffusion of solutes. This form may be associated with gross regional control of arteriolar diameter rather than with capillary recruitment. In another form of regulation, blood flow and capillary surface area change in parallel. This form may be associated with intermittent capillary perfusion, controlled by microregional metabolic events. The former mechanism appears to be responsible for the flow increase in SD because the flow increase failed to result in a change of the apparent glucose permeability.

#### APPENDIX

The net rate of blood-brain glucose transfer equalled the difference between the unidirectional fluxes in the two directions:

$$J_{net} = P_1' A \bar{C}_c - P_2' A C_e, \quad (1)$$

in which  $J_{net}$  is the net rate of glucose transfer from blood to brain;  $P_1'$  the apparent blood-brain barrier permeability of glucose when crossing the cerebral capillary endothelium in the blood-to-brain direction;  $A$  the capillary surface area;  $\bar{C}_c$  the arithmetic mean of the arterial and venous concentrations ( $C_a$  and  $C_v$ );  $P_2'$  the apparent permeability of glucose when passing from brain to blood; and  $C_e$  the glucose concentration in the interstitial fluid of brain. Equation 1 is an expression of symmetrical carrier transport. The  $P_1' A$  product was determined from the transfer constant (clearance) of labeled glucose transfer from blood to brain in 10 s (Crone, 1963).

$$P_1' A = -F \ln [1 - (K_{in}/F)] \quad (2)$$

in which  $F$  is the plasma flow of the samples of brain, and  $K_{in}$  the transfer constant (clearance) of labeled glucose. The plasma flow was determined by means of labeled butanol, as described previously (Gjedde et al., 1980). The  $K_{in}$  was determined from the extravascular brain content of labeled glucose, as described in the same paper.

In order to calculate the mean capillary glucose concentration ( $\bar{C}_c$ ), it was necessary to know both  $C_a$  and  $C_v$ . Since  $C_v$  could not be determined directly, it was estimated from the relationship  $J_{net} = FC_a - FC_v$ . Although the relationship is only valid in the steady state, the time resolution of the present slice method (one slice represents a period of 30 s) is so low that differences between  $C_a$  and  $C_v$  caused by the mean plasma transit time of 1 s become of no consequence. Thus,

$$\bar{C}_c = C_a - (J_{net}/2F), \quad (3)$$

which upon insertion into Eq. 1 yields

$$J_{net} = \frac{P_1' A C_a - P_2' A C_e}{1 + (P_1' A/2F)}. \quad (4)$$

The  $P_2' A$  product was determined from the kinetic constants  $T_{max}$  and  $K_m$  (Lund-Andersen, 1979),

$$P_2' A = T_{max}/(K_m + C_e). \quad (5)$$

in which  $T_{max}$  is the maximal transport capacity for glucose, and  $K_m$  the half-saturation constant.

The interstitial fluid glucose concentration was estimated from the glucose content of brain cortex ( $M_e$ ), assuming glucose to be distributed evenly in a cortical water content of 79%. Equation 4 was therefore modified to read

$$J_{net} \sim \frac{P_1' A C_a - \left( \frac{T_{max}}{(K_m 0.79/M_e) + 1} \right)}{\left( 1 + \frac{P_1' A}{2F} \right)}, \quad (6)$$

from which equation  $J_{net}$  was calculated after sepa-

rate determination of all variables in each sample (slice) of brain.

In a non-steady-state period, the average glucose metabolism ( $CMR_{glc}$ ) in the period  $T$  (the time required for the SD wave to transverse one slice width, see below) can be inferred from the equation:

$$CMR_{glc} = \frac{J_{net}(n) + J_{net}(n+1)}{2} + \frac{M_e(n) - M_e(n+1)}{T}, \quad (7)$$

in which  $[J_{net}(n) + J_{net}(n+1)]/2$  is the mean rate of net influx in the period  $T$ , and  $M_e(n) - M_e(n+1)$  the change of glucose content from slice number  $n$  to slice number  $n+1$ .

The constants  $T_{max}$  and  $K_m$  were estimated from the relationship (Gjedde, 1980)

$$P_1'A \sim T_{max}/(K_m + C_a), \quad (8)$$

$$P_1'AC_a = -K_m P_1'A + T_{max}, \quad (9)$$

which represents a plot of flux versus apparent permeability, akin to the Eadie-Hofstee plot, for which the slope equals  $-K_m$ . The regression line intersects the ordinate at  $T_{max}$ .

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