Insulin Increases Glucose Transfer across the Blood-Brain Barrier in Man

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ABSTRACT The influence of insulin on unidirectional flux of glucose across the blood-brain barrier and on net uptake of glucose by the brain was investigated in seven fasting patients. The unidirectional extraction, E, of [^{14}C]D-glucose was determined using $^{36}Cl^-$ as an intravascular reference, by the indicator dilution method. 0.4 U insulin/kg body wt was infused intravenously over 30 min while blood glucose was maintained constant by glucose infusion. Six determinations were made in each patient, two before, two during insulin infusion, and two after. In connection with each blood-brain barrier study, arterial and cerebral venous samples were taken for measurement of glucose, oxygen, insulin, K^+, and phosphate. Cerebral blood flow (CBF) was measured in each patient.

The main finding was an increased extraction of glucose from 14 to 21% and a highly significant increase in unidirectional flux (CBF × unidirectional extraction × arterial glucose concentration) from 0.46 to 0.66 μmol/g × min during insulin infusion (plasma insulin ~ 1,500 μU/ml). The net brain uptake of glucose (CBF × arterio-venous difference for glucose) was unaltered during the investigation period of 45 min, which is too short a time for insulin to penetrate the barrier. It follows that the backflux of glucose from the brain was increased during insulin application. The effect of insulin might be a speeding up of the glucose carrier in analogy to heart muscle.

INTRODUCTION

The transport of glucose from blood to brain has been extensively reviewed (1) and it is a well established fact that glucose crosses the blood-brain barrier (BBB)[1] by facilitated diffusion (2–4), with characteristic saturability and stereo-specificity (5–9). As the movement of glucose across brain cell membranes is extremely rapid (1), it can be assumed that the supply of glucose to the brain is governed by transfer across the BBB. Thus, the BBB is of great importance as the brain depends almost entirely on glucose metabolism.

Insulin plays a central role in the regulation of glucose turnover, promoting synthesis of energy reserves such as glycogen and lipids, stimulating protein synthesis in general and increasing sugar transport into muscle and adipose tissue (10, 11). Whether insulin exerts any direct influence on the brain in vivo is a controversial matter. It penetrates the cerebrospinal fluid (and brain extracellular fluid) very slowly (12, 13) and any immediate regulation of transport and metabolism in the brain parenchyma is therefore excluded. However, insulin could have an effect once it has crossed the BBB as there are reports that insulin affects cerebral glucose metabolism (14, 15), glycogen synthesis (14, 16, 17) and amino acid synthesis (16). These changes in cerebral metabolism could exert a secondary effect on net uptake of glucose by the brain. Whether insulin has any effect on BBB transport mechanisms is an open question.

The aim of the present study in man was to determine both the unidirectional transport of glucose across the BBB, and the net uptake by the brain, before, during, and after a high dose of insulin, while keeping the blood sugar level constant.

METHODS

Seven patients hospitalized for cerebral disorders necessitating carotid angiography were studied in connection with the angiography, after informed consent had been obtained. Patients with cerebral lesions that might interfere with BBB function were not included. All patients were studied at 9 a.m. in the fasting state (no breakfast was given).

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[1] *Abbreviations used in this paper*: A-V, arterio-venous; BBB, blood-brain barrier; CBF, cerebral blood flow; CMR, cerebral metabolic rate; E, extraction; OGI, oxygen glucose index.

The BBB permeability for D-glucose was determined with the indicator dilution method (4, 18, 19). This technique involves intraarterial injection of a tracer together with an intravascular reference tracer, followed by rapid sampling of the venous effluent from the brain. In each patient the glucose transfer across the BBB was determined six times, twice before, twice during (at 20 and 30 min), and twice after (at 10 and 20 min) intravenous infusion of insulin.

At 8 a.m. the patients were connected to an artificial beta cell; the Biostator (Miles Laboratories, Inc., Elkhart, Ind.). This consists of a glucose electrode that measures blood glucose level continually (giving a readout every 10 s, with an 80 s delay) and infusion pumps for insulin and glucose. At the time of the study insulin (insulin Leo neutral RI; Nordisk Insulin Laboratorium, Gentofte, Denmark) was infused intravenously in a dose of 0.40 U/kg body wt over a period of 30 min. Blood glucose was kept constant by means of variable infusion of 50% glucose (Fig. 1).

A polyethylene catheter was placed in the internal carotid artery on the side appropriate for the angiogram and another in the contralateral internal jugular vein. This was done under local anaesthesia with percutaneous punctures in the neck using the Seldinger technique. The tip of the venous catheter was placed in the superior bulb of the internal jugular vein and that of the arterial catheter in the internal carotid artery just below the syphon. Correct positioning of the arterial catheter was verified by noting absence of diffuse facial discoloration after a rapid injection of saline or Evans blue. At the end of the study the carotid catheter was used for the diagnostic angiogram.

For each determination of BBB permeability a 1- to 2-ml bolus containing the test compound, 20 μCi [^{14}C]D-glucose

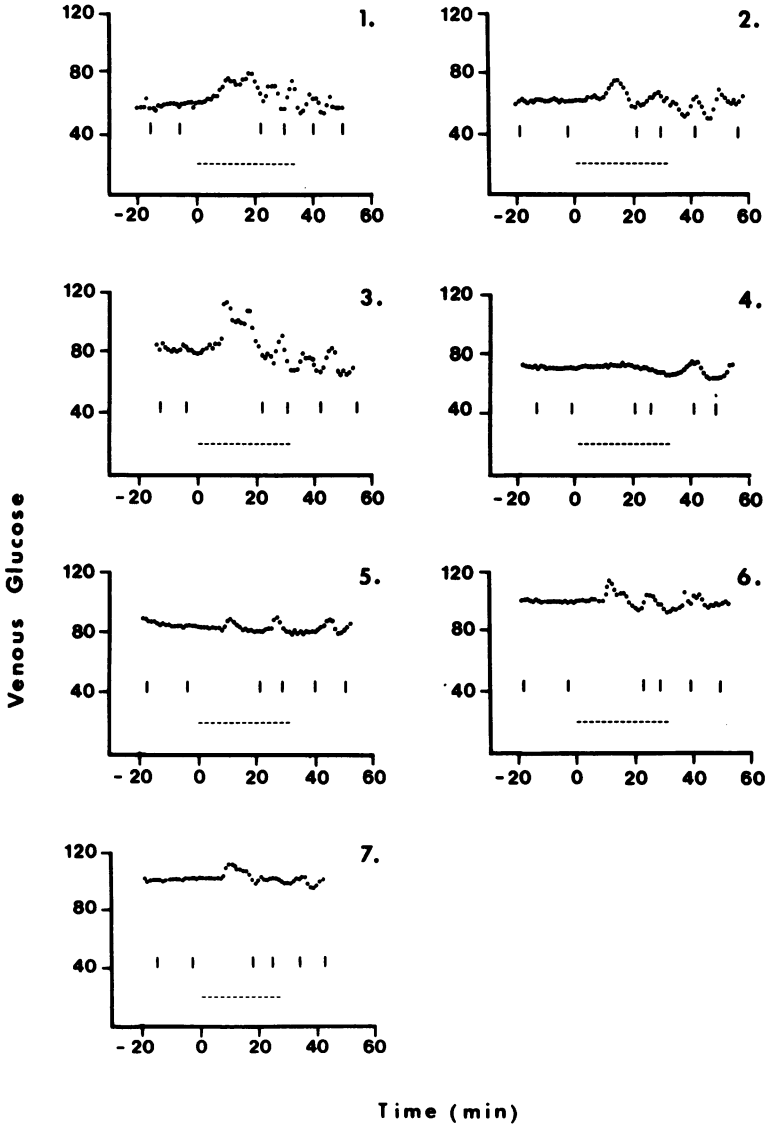

FIGURE 1 Venous glucose concentrations (mg/100 ml) in the seven patients at each minute during the investigations. Zero on the time scale indicates the start of insulin infusion. The vertical bars indicate each measurement of permeability.

(uniform label; Amersham, Buckinghamshire, England) and the reference, 2 μCi $^{36}Cl^-$ (NEN, Dreieich, West Germany) was injected rapidly through the intracarotid catheter. The collection of samples from the venous catheter was started at the same time (1 sample/s). The glucose concentration of the injected solution was ~5 μmol/ml. The transit time is a few seconds and neglible amounts of glucose are metabolized in that period. A standard solution was prepared from each bolus by adding an aliquot of the injected solution to venous blood sampled before the injection. Background from previous runs was subtracted and never exceeded 1% of peak value. $^{36}Cl^-$ and ^{14}C were assayed in plasma using liquid scintillation counting (Instagel and Packard Tricarb 3375; Packard Instrument Co., Inc., Downers Grove, Ill.). Corrections for quenching and channel spillover were applied using the method of external standardization.

The unidirectional transcapillary fractional loss of glucose (the extraction, E) was determined relative to the intravascular reference substance Cl^- and calculated according to the equation:

$$E = \int_0^{40\% \text{ desaturation}} \frac{C_{ref} - C_{test}}{C_{ref}} dt,$$

where C_{ref} and C_{test} are the relative concentrations (count rate in blood sample divided by count rate in the standard solution) of the reference and of the test substance, respectively. The limits of integration were chosen so as to minimize the influence of intravascular separation of test and reference substance. The theoretical considerations concerning the choice of reference molecule and the calculation of E have been detailed earlier (21).

Additional measurements. Immediately after each run samples of arterial and cerebral venous blood were collected for determination of glucose concentration (glucose electrode; Radiometer, Copenhagen) oxygen content (microelectrode; Eschweiler, Kiel) (22), pH, P_{CO_2}, P_{O_2} (conventional microelectrodes). This sampling was performed over 15 s, the venous samples being started 10 s after the arterial in order to obtain matching samples and avoid the influence of spontaneous glucose fluctuations on arterio-venous (A-V) differences. Phosphate, K^+ and insulin (radio-immunoassay) were determined in venous blood after each run.

Cerebral blood flow (CBF) was measured in each patient using the ^{133}Xe intra-arterial injection method (23). The ^{133}Xe (3–5 mCi dissolved in 2–3 ml of isotonic saline) was injected rapidly into the internal carotid artery through the indwelling catheter. The wash out of the isotope was detected externally from the ipsilateral hemisphere. CBF was calculated from the initial slope of the semilogarithmically recorded clearance curves (24). It was assumed that the cerebral metabolic rate (CMR) for oxygen remained constant in each patient, and the CBF for each run was calculated from the A-V differences for oxygen.

RESULTS

During insulin infusion, unidirectional flux of glucose across the BBB increased significantly without any concomitant change in net uptake of glucose by the brain. The results are summarized in Table I, and the derived values for unidirectional flux, net uptake, and back flux

TABLE I

Insulin Concentrations, Glucose Concentrations, E of Glucose, CBF, and Cerebral A-V Oxygen Differences before, during, and after Insulin Administration

		During insulin		After insulin	
	Rest	I	II	I	II
Plasma-insulin, *μU/ml*	13 (8–42) *n* = 7	1,400* (1,185–1,450) *n* = 7	1,650* (1,430–1,825) *n* = 7	540* (275–700) *n* = 7	220* (165–355) *n* = 7
Arterial glucose concentration, *μmol/ml*	5.13 (4.79–5.69) *n* = 7	5.15 (4.89–5.65) *n* = 7	4.97 (4.69–5.26) *n* = 7	4.95 (4.71–5.15) *n* = 7	5.51 (4.89–6.15) *n* = 7
A-V difference in glucose concentration, *μmol/ml*	0.50 (0.41–0.57) *n* = 7	0.51 (0.38–0.65) *n* = 6	0.60 (0.50–0.73) *n* = 6	0.51 (0.48–0.59) *n* = 6	0.65 (0.56–0.72) *n* = 6
E of glucose, %	14.1 (13.3–19.4) *n* = 7	21.3 (15.0–21.5) *n* = 7	20.9* (16.9–22.7) *n* = 6	17.9 (14.6–22.0) *n* = 7	15.9 (15.7–17.6) *n* = 6
CBF, *ml/g·min*	0.58 (52–62) *n* = 7	0.60 (56–67) *n* = 7	0.62 (51–65) *n* = 7	0.65 (54–69) *n* = 7	0.60 (54–62) *n* = 6
A-V difference in O_2 concentration, *μmol/ml*	3.21 (3.09–3.25) *n* = 6	3.18 (2.89–3.19) *n* = 6	3.09 (2.65–3.54) *n* = 6	3.09 (2.75–3.22) *n* = 6	3.22 (2.78–3.80) *n* = 6

The values given are the medians and quartiles, *n* the number of values.

* Significantly different from resting value, $P < 0.05$. Wilcoxon's test for pair differences was used.

are given in Table II. The resting values are average of two determinations, the other values single determinations.

In Fig. 1 the curves of the monitored venous glucose concentrations from the seven patients are shown. Except in patient No. 3 they are all fairly even. The monitoring of venous glucose made it possible to predict a time for BBB studies when glucose was as close as possible to its preinsulin level. Thus, the arterial glucose concentration at the time of the BBB studies was unchanged throughout the investigation and although the very last value showed some scatter, it was not statistically different from the resting state. Insulin concentration increased about 100-fold during the infusion and even 15 min afterwards, it was still 10–20 times the resting value.

The E of glucose increased with increasing plasma insulin concentration but only the value 30 min after infusion reached significance. Fig. 2 shows a representative venous outflow curve for glucose and the reference substance Cl^-, and the corresponding extraction curve.

The unidirectional flux (CBF × E × arterial glucose concentration) which is the amount of glucose that crosses the BBB from blood to brain (the expression is the same as the permeability surface area product multiplied by the average capillary glucose concentration) increased significantly during infusion of insulin, each patient showing an increase at both 20 and 30 min. After the infusion the median flux was still higher but was not statistically different from the resting value. This increased flux is not accompanied by an increase in the net uptake of glucose into the brain as calculated from the product of CBF and the A-V difference for glucose. Back flux (unidirectional flux-net uptake) was increased during insulin influence so that both in- and outflux were accelerated. These results are illustrated in Fig. 3. A-V differences for glucose and oxygen were remarkably constant (partly because precautions were taken to get corresponding arterial and venous samples), and only the last glucose difference was slightly higher, although not significantly so.

The oxygen-glucose index (OGI) (25) can be calculated from the A-V differences for glucose and oxygen OGI = (A-V)O_2/6 (A-V) glucose × 100%. When OGI = 100% all glucose is oxidized to CO_2 and H_2O, whereas when it is <100%, some of the glucose is converted to lactate or stored. The average OGI was 92% in the resting state, 90 and 85% during insulin infusion and 98% after insulin infusion.

Venous concentrations of K^+ and phosphate in the resting state were 3.8 and 1.04 mmol/liter, respectively. After insulin infusion had started K^+ decreased (as

TABLE II

Cerebral Fluxes and Net Uptake of Glucose before, during, and after Insulin Administration

		During insulin		After insulin	
	Rest	I	II	I	II
Unidirectional flux, *μmol/g·min*	0.458 (0.421–0.726) *n* = 7	0.679* (0.550–0.847) *n* = 7	0.657* (0.557–0.770) *n* = 6	0.569 (0.479–0.683) *n* = 7	0.639 (0.461–0.754) *n* = 6
Δ Unidirectional flux, *μmol/g·min*	—	0.040* (0.03–0.14) *n* = 7	0.117* (0.11–0.14) *n* = 6	0.084 (0.04–0.15) *n* = 7	0.093 (−0.13–1.07) *n* = 6
Brain net uptake, *μmol/g·min*	0.302 (0.26–0.36) *n* = 7	0.324 (0.26–0.34) *n* = 7	0.348 (0.32–0.38) *n* = 7	0.325 (0.27–0.35) *n* = 7	0.397 (0.30–0.43) *n* = 5
Δ Brain net uptake, *μmol/g·min*	—	−0.04 (−0.02–0.07) *n* = 7	0.02 (−0.02–0.13) *n* = 7	−0.01 (−0.06–0.03) *n* = 7	0.16 (−0.09–0.16) *n* = 5
Back flux, *μmol/g·min*	0.228 (0.10–0.40) *n* = 7	0.339 (0.27–0.52) *n* = 7	0.275 (0.17–0.38) *n* = 6	0.280 (0.23–0.31) *n* = 7	0.186 (0.18–0.30) *n* = 5
Δ Back flux, *μmol/g·min*	—	0.094 (0.04–0.22) *n* = 7	0.077 (−0.04–0.26) *n* = 6	0.113 (−0.01–0.12) *n* = 7	0.036 (0.04–0.07) *n* = 5

Medians and quartiles are given.

* Values significantly different from the resting value $P < 0.05$.

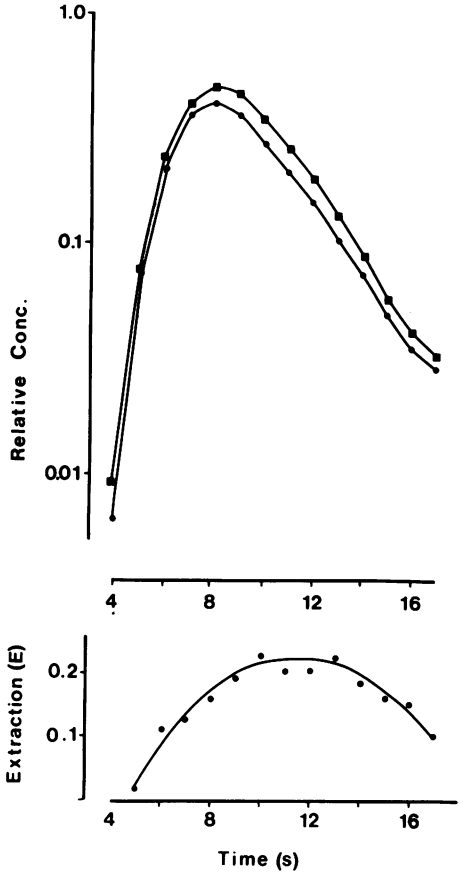

FIGURE 2 Cerebral venous outflow curves showing D-glucose (squares) and the reference substance Cl^- (circles). Below the corresponding glucose extraction. Note that back diffusion (evidenced by falling extraction) sets in rather late.

would be expected), during insulin infusion the values were 3.2 and 3.1, afterwards they were 3.0 and 3.1 mmol/liter. Phosphate behaved in a parallel manner falling to 0.83 and 0.75 during insulin infusion and 0.65 and 0.62 mmol/liter afterwards.

DISCUSSION

When discussing the effects of insulin on movement of glucose into the brain, a clear distinction must be made between unidirectional flux of glucose across the BBB and the net uptake of glucose by the brain. The unidirectional flux reflects BBB transport, whereas net uptake is more a function of brain metabolism and the maintenance of equilibrium between cerebral and plasma glucose concentrations. It is necessary to separate the action of insulin from the effects of the hypoglycemia that it induces. When plasma glucose is suddenly changed, the cerebral glucose concentration will eventually reach a new steady-state level, but only after a time-lag during which there is a change in net uptake of glucose by the brain. Thus, uptake is only equivalent to metabolism during steady state. During nonsteady state an increased uptake may occur without any change in cerebral metabolism, the glucose being used to increase the brain glucose concentration.

Determination of unidirectional fluxes may also be influenced if there is nonsteady state between blood and brain (1). The glucose concentration of an injected bolus may change considerably during the capillary transit. If the glucose concentration of a bolus is well below that in plasma, the direction of net transport is reversed and the bolus glucose concentration increases during passage through the cerebral vessels. Since average capillary glucose concentration enters into the calculation of flux, erroneous results will be obtained. These pitfalls have not been avoided in most early studies.

Net uptake. Butterfield et al. (26) measured net glucose uptake in human muscle and brain 10–20 min after intravenous insulin during nonsteady-state conditions (i.e., continually falling blood sugar) and found that cerebral glucose uptake was reduced. This can be attributed to hypoglycemia, which reduces glucose influx into the brain while the glucose efflux is still high due to the relatively high cerebral glucose concentration. Consequently no direct effect of insulin on the brain can be inferred from their data.

Gottstein et al. (27, 28) made extensive investigations of the effect of insulin on the glucose uptake of the human brain and tried to avoid hypoglycemia by glucose infusion, although it was not possible to keep the blood-sugar constant and it increased by about 200%. They found that when the blood-sugar level was raised by glucose infusion, brain glucose uptake increased, but the increase was much more pronounced when insulin was infused together with the glucose. The 50% increase in net uptake of glucose after an insulin dose and timing similar to ours contrasts with the unchanged net uptake of glucose in the present study. This discrepancy can probably be explained by the rising blood-sugar and consequent nonsteady-state conditions in their study. The difference in cerebral glucose uptake observed by Gottstein et al. when glucose was infused with or without insulin, however, suggest an influence of insulin on the BBB glucose transfer. Their resting CMR for glucose of 0.29 μmol/g $\times$ min, as well as their OGI of 91%, correspond well with our values of 0.30 and 92, respectively.

Unidirectional transfer. Few investigators have attempted to determine whether insulin influences unidirectional transfer of glucose into the brain. Crone (3) included a few experiments with insulin injections in his original study with the indicator dilution method, but uncontrolled blood glucose may account for the lack of effect on E of glucose.

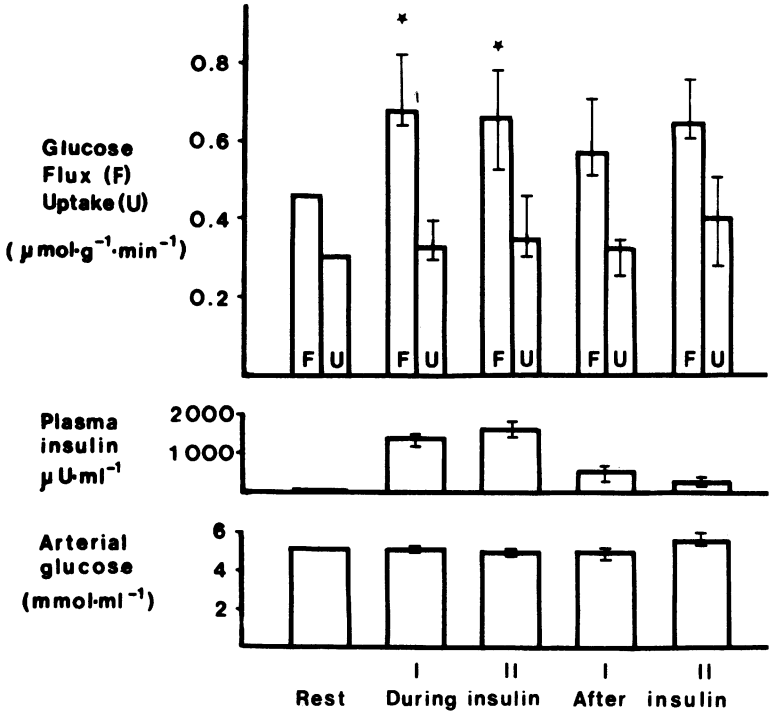

FIGURE 3 The unidirectional flux and the net uptake of glucose into brain are shown in relation to plasma insulin and arterial glucose concentration. The columns are the median values, the bars indicate the quartiles of the difference between the resting and experimental values.

Daniel et al. (29) reported that insulin increased net uptake of glucose by rat brain, without changing glucose flux. However, the technique used does not measure true unidirectional flux, rather it measures something between flux and uptake because the measurement is made over 1 min and ignores back flux. Furthermore, nonsteady-state conditions prevailed at the time of flux and uptake measurements. They tried to avoid the hypoglycemic effects by giving glucose injections 14 min after insulin and 2 min before uptake measurement, thus performing their uptake measurement at a time when brain glucose concentration is still subnormal because of the preceding 14 min of hypoglycemia, but blood glucose was high. The nonsteady-state conditions account for the increase in net uptake and probably disguise any effect on the influx values, as these include subnormal amounts of back flux.

The only comprehensive study of effect of insulin on unidirectional glucose transport across the BBB is that of Betz et al. (30) on isolated dogs' brain. With the indicator dilution method, which is quick enough to measure true unidirectional transfer and during steady-state conditions, they were unable to show any effect of insulin (800 μU/ml). In spite of the elaborate technique, a thorough review of their studies reveals several inconsistences: (*a*) The BBB must be abnormally leaky. Even with the small molecule $^{22}Na^+$ as a reference, the E for Cl^- was 21% (31), whereas, in the intact dog the BBB extraction of chloride is almost zero (4, 32), as is the case in humans (33). (*b*) CMR of O_2 is relatively low (1.8 [34] and 1.3 μmol/g·min [35] compared to other values in the dog, 2.5 [36] and 2.6 μmol/g·min [37]). Surprisingly, however, CMR of O_2 doubled when the perfusion rate was raised (in a random manner) from 40 to 100 ml/100 g·min (35). The only physiological explanation seems to be that there are large unperfused areas of brain in their normal experimental setup. (*c*) CMR of glucose is often (1970, 1977) very low 0.12 (34) and ~0.15 μmol/g·min (38) yielding OGI values of about 250%. However, in another study (1973) more normal values were found: CMR of glucose, 0.3 μmol/g·min yielding OGI of 72% (39). In conclusion the isolated dog brain preparation is physiologically unsound, the metabolism seems disturbed and the capillaries are probably leaky resulting in nonspecific glucose uptake large enough to mask any effect of insulin.

Isolated capillaries. Studies on isolated capillaries have failed to show an effect of insulin on glucose uptake, which otherwise seems to have the characteristics of BBB glucose transport (stereo-specificity and saturability) (40). Our data could be interpreted to mean that insulin speeds up the glucose carrier mechanism so that it works with increased velocity in both directions (in analogy with heart muscle [41]).

In that case, an increased glucose content in the endothelial cells would not be expected and so the lack of an insulin effect on isolated capillaries does not contradict our finding in vivo.

Concluding remarks. The physiological importance of the effect of insulin on BBB transfer of glucose is difficult to evaluate. It should be stressed that a very high insulin dose was used in the present study to disclose any effect that it might have. Under normal conditions the BBB is not rate-limiting for brain glucose metabolism (the rate of BBB-transport and the rate of phosphorylation is about 3:1) (1), and enhanced requirements for glucose can be met by increased CBF or by capillary recruitment (32). However, in extreme situations (prolonged seizures [42], recovery after hypoglycemia [43]) the vasomotor regulatory mechanisms are inadequate to meet metabolic demands and the BBB transport may then be decisive.

An abundance of insulin receptors have been demonstrated on cerebral microvessels (44), the endothelium of which forms the BBB. Binding to the vessels may account for the very high insulin concentration in brain homogenates (25 times higher than plasma insulin levels [45]).

Thus, it is possible that insulin could influence the BBB even under resting conditions, and perhaps insulin has a role in long-term regulation of transport, for example by influencing the number of carrier sites, as has been shown for adipocytes (46).

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