Deleterious Effect of Glucose Pretreatment on Recovery from Diffuse Cerebral Ischemia in the Cat

I. Local Cerebral Blood Flow and Glucose Utilization

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SUMMARY Diffuse cerebral ischemia was created in pentobarbital-anesthetized cats by basilar and bilateral carotid artery occlusions and hypotension. Local cerebral blood flow (LCBF) was assessed autoradiographically with 14C-antipyrine, and local cerebral glucose utilization with 14C-2-deoxyglucose. In animals without glucose pretreatment, 15 min of ischemia led to a homogeneous reduction of post-ischemic cerebral perfusion to 31% of control; ischemia of 30 min produced post-ischemic perfusion heterogeneities in the cerebral cortex and deep gray structures. In animals pretreated with dextrose, 1.5 gm/kg intravenously, heterogeneous cerebral perfusion was observed following only 15 min of ischemia, and a severe global impairment of cerebral reperfusion occurred after the 30 min insult. Deoxyglucose autoradiograms in the latter animals were remarkable for a complete suppression of tracer uptake in the cerebral cortex and a paradoxically increased tracer concentration in the cerebral white matter. Mean plasma glucose in the treated animals exceeded 1000 mg/100 ml. Large glucose loads prior to ischemia dramatically impair post-ischemic cerebral perfusion.

Ours understanding of the factors which prevent the brain from recovering from episodes of ischemia remains incomplete. Myers and coworkers have provided evidence that the fed or fasted condition of an animal may dramatically affect its response to cerebral ischemia: Food-deprived juvenile rhesus monkeys subjected to 14-min periods of cardiac arrest characteristically showed good neurological recovery and preserved ability to perform visual discrimination tasks; at neuropathological examination, their brains were either intact or exhibited injury restricted to brain stem nuclei, Purkinje cells, and hippocampus. By contrast, 2 animals which had received glucose infusions immediately prior to cardiac arrest developed fasciculations, myoclonic activity, decerebrate rigidity and fixed, dilated pupils; their brains showed cerebellar tonsillar herniation and microscopic evidence of widespread necrosis involving cerebral cortex and basal ganglia. These findings were subsequently confirmed in a larger series. In a similar study, Siemkowicz and Hansen examined the effect of varying blood glucose levels on clinical survival in rats exposed to 10 min of complete brain ischemia by compression of neck vessels with a pneumatic cuff. Normoglycemic animals survived chronically with minor neurologic deficits, whereas hyperglycemic rats remained comatose and died within 24 h.

Because of the potentially important implications of these observations, we were prompted to investigate this phenomenon in a standardized experimental model of global cerebral ischemia developed in our laboratory, in which the hemodynamic, metabolic, and neuropathological consequences of graded cerebral ischemia have already been extensively documented. Preliminary findings have been reported in abstract form.
Methods

Thirty-three cats of either sex, weighing 3–4 kg, were anesthetized with sodium pentobarbital, 40 mg/kg intraperitoneally. Following tracheal cannulation, animals received gallamine triethiodide, 20 mg intravenously, and breathed mixtures of 30% oxygen and 70% nitrogen delivered by a Harvard ventilator. Additional doses of gallamine were given intravenously as needed to overcome resistance to mechanical ventilation; the total dose given was 16.9 ± 1.9 mg/kg per experiment (mean ± SEM). Catheters were placed in the femoral arteries and veins. Two pairs of brass screw electrodes were attached parasagittally over each hemisphere for bipolar EEG recording. Arterial blood pressure (measured with a Statham transducer), end-tidal CO₂ concentration (measured with a Beckman infrared analyzer), and EEG tracings were recorded on a Grass polygraph. Arterial blood gases were assayed at intervals (Radiometer). The rectal temperature was maintained at 37°C by a thermostatically controlled heating lamp.

The method of inducing cerebral ischemia consisted of occlusion of the basilar artery and both common carotid arteries, plus mild systemic hypotension (systolic blood pressure under 100 mm Hg), produced by arterial hemorrhage. The basilar artery was exposed in its mid-portion by a transoral approach through the clivus. Arteries were occluded with miniature Mayfield clips. (Details of this procedure were presented in a previous publication.) In some animals, studies were performed following 15 or 30 min of cerebral ischemia. In other animals, a 90-min period of normotensive recirculation was permitted by removing the vascular clips and reinflusing the shed blood. Sodium bicarbonate was administered during the late insult and early recovery periods (range 0–3 mEq) to correct the metabolic acidosis arising during the ischemic insult.

Two animal conditions were employed. In one animal group, glucose, 1.5 gm/kg, was infused intravenously as a 50% dextrose solution over a one-hour period, commencing after completion of the basilar artery exposure; the ischemic insult was begun immediately upon terminating the infusion. In other animals, a comparable waiting period was carried out without dextrose infusion; in some of the latter animals, isotonic saline was infused during this period in a volume comparable to that of the dextrose infused in the first group (9 to 13 ml).

To assess local cerebral blood flow (ICBF), 250 μCi of 14C-antipyrine dissolved in 3.5 ml of saline was infused intravenously over a 45-second period while blood samples were taken from a freely flowing arterial catheter at 5-sec intervals.* Animals were sacrificed with intravenous potassium chloride, and their brains were rapidly removed and frozen. Autoradiograms of coronal brain sections were subsequently prepared and subjected to densitometry, from which ICBF values were obtained. This method has been described previously in detail.

In 2 animals of this series (with 15 min of ischemia and 90 min of normotensive recirculation), an antipyrine study was carried out while the brain was being frozen in situ for regional metabolite assay. In this procedure, described in detail in the accompanying report, liquid nitrogen was applied to the exposed calvarium for 10–15 min while animals were ventilated and maintained normal blood pressure. 14C-antipyrine (250 μCi) was injected intravenously over a 3-min period, and brain freezing commenced 2 min following the beginning of the antipyrine infusion. The frozen cerebral hemispheres were sectioned into 3 blocks. Autoradiographic sections were prepared from one face of a block, while the mirror-image face of the adjacent block was used for regional metabolite assay. Local blood flow could not be quantitated in these studies inasmuch as the freezing technique precluded abrupt animal sacrifice. Nonetheless, the autoradiograms provided a qualitative map of ICBF trends which could be compared with corresponding regional metabolite data.

In 9 animals of this series, local cerebral glucose utilization was assessed autoradiographically during the last 30 min of the post-ischemic recirculation period. Two hundred fifty μCi of 2-deoxy-D-(1-14C) glucose (specific activity approximately 50 mCi/mmole, New England Nuclear Corp., Boston) was injected intravenously as a bolus. Arterial blood samples were obtained over the following 30 min; plasma 2-deoxyglucose concentration was determined by liquid scintillation counting, and plasma glucose concentration was assayed by means of an automated glucose analyzer (Beckman). These data were acquired in the event that quantitation of glucose utilization in the post-ischemic brain was desired and considered feasible at a future time. The animals were sacrificed with intravenous potassium chloride 30 min later, and brain autoradiograms were prepared. The theory and use of this method have been described in a comprehensive publication.

Results

Physiological Data. Arterial blood pressure and blood gases are presented in table 1. No statistically significant differences in these data were demonstrable among animal groups (analysis of variance). The arterial hypotension induced during the ischemic insult period was successfully reversed in all animal groups. The EEG tracings of the present series were comparable to those previously reported at the commencement of the insult period, the EEG became isoelectric; during post-ischemic recirculation, no significant return of EEG activity occurred.

Cerebral Blood Flow Studies. Four control animals received a dextrose infusion, and 4 did not.
The brain was not frozen for simultaneous metabolite assay and, hence, quantitative autoradiography could be performed, many cerebral structures exhibited a great heterogeneity of ICBF values, ranging from zero to the mildly hyperemic; these ranges contrasted with the narrower ICBF ranges observed in the corresponding noninfused animals (table 2).

Extensive heterogeneities of local blood flow were also observed on autoradiograms of the 3 animals not receiving dextrose but subjected to 30 min of severe ischemia followed by 90 min of recirculation (figs. 1 G and H). The cortical mantle contained multiple irregular areas of decreased to absent blood flow alternating with irregular zones of normal or hyperemic flow. These heterogeneities were most marked in doral cortical regions but were also observed in the central thalamus. Quantitation of these autoradiograms confirmed wide ranges of ICBF values within individual cerebral structures, extending from the severely ischemic to the markedly hyperemic (table 2).

When dextrose infusion was followed by 30 min of ischemia and 90 min of normotensive recirculation (2 animals), autoradiograms revealed a severe impairment of post-ischemic reperfusion (figs. 1 I and J). Minimal levels of cortical blood flow persisted only in isolated subpial sites (fig. 1 I). In the central thalamus, blood flow was markedly heterogeneous, with islands of normal flow.

In animals with 30 min of prior ischemia, we wished to assess whether the failure of cerebral reperfusion seen after 90 min of post-ischemic recirculation evolved during the recirculation period or, alternatively, was present during early recirculation. Thus,
Figure 1. $^{14}$C-antipyrine autoradiograms of cat brains. A: Control animal, not given dextrose. B: Control animal given dextrose, 1.5 gm/kg intravenously. C: During cerebral ischemia (basilar and bilateral carotid artery occlusions and hypotension). D: Animal with 15 min of ischemia and 90 min of recirculation, not given dextrose. E and F: Two animals with 15 min of ischemia and 90 min of recirculation, given dextrose prior to ischemia. G and H: Two animals with 30 min of ischemia and 90 min of recirculation, not given dextrose. I and J: Two animals with 30 min of ischemia and 90 min of recirculation, given dextrose prior to ischemia.
in 4 additional animals, ICBF studies were performed after 30 min of cerebral ischemia and only 10 min of normotensive recirculation. Two animals received a dextrose infusion prior to ischemia; 2 did not. In all 4 animals, brain autoradiograms showed extensive reperfusion defects. In both dextrose-infused animals, as well as one non-infused animal, there was almost complete failure of perfusion of the cortical mantle and patchy perfusion of the central thalamus. The remaining non-infused animal showed heterogeneities of reperfusion, with hypoperfusion of the dorsal fourfifths of the cerebral cortex.

Correlation of Local Blood Flow and Regional Energy Metabolites. In 2 dextrose-infused animals with 15 min of ischemia and 90 min of recirculation, regional metabolite levels and qualitative patterns of ICBF were simultaneously assessed. As figure 2 illustrates, a correspondence was observed between the extent of reperfusion and the degree of energy metabolite recovery. In zones of higher $^{14}$C-antipyrine concentration, ATP and phosphocreatine had recovered significantly from the ischemic insult, and lactate levels were only moderately elevated. In contrast, areas of lower $^{14}$C-antipyrine concentration corresponded to regions of significant persistent energy metabolite depletion and lactate elevation. Energy metabolite alterations resulting from cerebral ischemia with and without dextrose infusion are considered in the accompanying report.13

Deoxyglucose Studies. In 3 animals studied without prior ischemia, deoxyglucose autoradiograms revealed an even pattern of glucose utilization throughout the cerebral cortex and deep gray structures (fig. 3A). By contrast, in animals not receiving dextrose subjected to 30 min of ischemia and 90 min of recirculation, glucose metabolism appeared heterogeneous within cerebral gray structures. The cortical ribbon was mottled or striated, and mottling was also evident in the caudate nucleus and thalamus (fig. 3B). This appearance was striking in 2 brains and more subtle in the other 2. Finally, in the 2 animals receiving intravenous dextrose followed by 30 min of ischemia and 90 min of recirculation, there was a striking abnormality of glucose utilization involving the entire cerebral hemispheres. The cortical mantle of these animals was characterized by a virtually complete suppression of deoxyglucose uptake, and the underlying central white matter appeared, paradoxically,
FIGURE 2. 14C-antipyrine autoradiogram of cat receiving 1.5 mg/kg of dextrose intravenously prior to a 15 min period of cerebral ischemia followed by 90 min of normotensive recirculation. Regional metabolites were determined on the adjacent, mirror-image coronal block. In regions of reduced energy metabolites and persistent lactate elevations, reperfusion is impaired. (Metabolite levels for ATP, phosphocreatine (PCr) and lactate (Lac) are given in mmol/kg. Values for 14C-antipyrine uptake (14C) are expressed in cpm/mg.)

to have more isotopic uptake than is normally expected (fig. 3C). There was a paucity of isotopic uptake in the basal ganglia and in symmetrical portions of the thalamus (fig. 3C). We did not attempt toquantitate glucose utilization in the post-ischemic state inasmuch as data are still preliminary as to whether the lumped constant of the mathematical model describing the behavior of deoxyglucose is altered following ischemia.

Plasma Glucose Levels. Plasma glucose levels in control animals and in animals undergoing cerebral ischemia with and without prior dextrose infusion are summarized in figure 4. Of interest was the observation that, on the average, the plasma glucose values attained immediately following dextrose infusion, but before initiation of ischemia, were of the same magnitude as the stress-induced hyperglycemic levels observed during ischemia in animals not receiving prior dextrose (673 ± 25 vs. 687 ± 53 mg/100 ml, respectively). In animals receiving dextrose, plasma glucose rose to 1020 ± 51 mg/100 ml during ischemia. In all groups, plasma glucose fell during recirculation. It was not possible to correlate the severity of post-ischemic reperfusion deficits with plasma glucose levels in individual animals.

Discussion

The present investigation lends support to earlier observations that the administration of large amounts of glucose just prior to episodes of cerebral ischemia accentuates post-ischemic brain dysfunction. In addition, our studies have demonstrated, for the first time, the manner in which post-ischemic regional cerebral perfusion is affected by prior glucose loading. In the absence of exogenous glucose, shorter periods (15 min) of cerebral ischemia in this model lead to a uniform but subnormal restitution of local cerebral blood flow in the post-ischemic period, without focal perfusion impairments; by contrast, longer ischemic periods (30 min) induce striking post-ischemic heterogeneities of blood flow. In parallel metabolic studies, a corresponding regionally heterogeneous recovery of energy metabolites occurs following the 30-min, but not the 15-min insult. The present data

FIGURE 3. 14C-2-deoxyglucose autoradiograms of cat brains. A: Control animal. Brain shows uniform pattern of cerebral glucose utilization throughout the cortex and deep gray structures. B: Animal with 30 min of prior ischemia followed by 90 min of recirculation (no dextrose). A striated pattern of local glucose utilization is apparent in the cerebral cortex and a mottled heterogeneous pattern in basal ganglia and thalamus. C: Animal given dextrose, 1.5 gm/kg IV, followed by 30 min of ischemia and 90 min of recirculation. Isotopic uptake appears strikingly decreased throughout the cerebral gray matter but paradoxically increased in the central white matter.
demonstrate that when glucose is administered prior to ischemia its effects are 1) to cause the marked heterogeneities of cerebral perfusion normally seen only after 30 min of ischemia to appear after an ischemic insult as brief as 15 min; and 2) to produce a virtually complete impediment of cerebral reperfusion following 30 min of ischemia. Corresponding to the patchy restoration of cerebral perfusion in glucose-treated animals with 15 min of ischemia, there was a comparable heterogeneous derangement of energy metabolite recovery (fig. 2). The latter is considered in detail in the accompanying report. The 29% decrease in ICBF in the glucose-pretreated control animals remains unexplained but may be related to a slight increase in cerebrovascular resistance produced by the lower specific gravity and, hence, higher brain water content observed in this group.

We have reported previously that following 15 min of severe cerebral ischemia in this model, there is widespread ischemic cell change affecting neurons of the cerebral neocortex, hippocampus, basal ganglia and thalamus; after 30 min of ischemia, similar ischemic alterations are present, with somewhat more apparent regional accentuation in gyri near the longitudinal midline — the sites of greatest hemodynamic and metabolic impairment. We have not yet studied the histological sequelae of ischemia in animals with glucose pretreatment. However, since the hemodynamic pattern following 15 min of ischemia in the glucose-pretreated animals closely resembles that seen following 30 min of ischemia in the non-pretreated group, we would expect a comparably severe degree of ischemic cell change. Indeed, the profound interruption of post-ischemic cerebral circulation in the glucose-pretreated animals with 30 min of prior ischemia is almost certainly incompatible with neuronal survival.

While deoxyglucose studies performed during ischemia and hyperglycemia must be interpreted with caution, we feel justified in concluding from the profoundly abnormal post-ischemic autoradiograms following 30 min of ischemia in the glucose-pretreated group (fig. 3C) that there was essentially no utilization of glucose by the cerebral gray matter. However, the surprisingly intense uptake of tracer by the cerebral white matter of these brains implies that there had been at least a transient antecedent period of minimal ICBF which sufficed to deliver the tracer to tissue. In support of this inference, we have demonstrated that it is only in white matter regions that substantial resynthesis of ATP and phosphocreatine occurred in the glucose-pretreated animals following 30 min of ischemia. It is possible that during the early phase of the deoxyglucose study (which was carried out between the sixtieth and ninetieth minute of the recirculation period), ICBF in the cerebral white matter may have been somewhat higher than that measured at 90 minutes (table 2). Proof that the intense deoxyglucose uptake in the white matter of the glucose-treated animals is related to an antecedent period of heightened glucose utilization (as opposed to mere trapping of deoxyglucose substrate) would require the direct demonstration of the metabolite — 2-deoxyglucose-6-phosphate — in white matter. Pulsinelli and Duffy carried out such an analysis in their study of the hypoxic-ischemic "Levine" preparation in the rat. They found that tracer concentration in the cerebral white matter exceeded that in adjacent gray matter regions and that approximately 90% of tissue tracer was in the form of the metabolite, 2-
deoxyglucose-6-phosphate — consistent with increased glucose utilization by hypoxic white matter. In a recent study, Miyaoaka et al. confirmed a 20-80% increase in local glucose utilization in the cerebral white matter of the conscious rat during hypoxemia.

The mechanism by which glucose loading adversely affects post-ischemic cerebral perfusion remains to be elucidated. As the accompanying study presents in detail, the status of energy metabolites during ischemia does not provide a strong clue as to mechanism: In animals both with and without glucose pretreatment, ATP and phosphocreatine levels are profoundly depressed during the insult. Regional brain lactate levels, while 30% higher in animals with glucose pretreatment, are extremely elevated even in those animals without glucose pretreatment (29-34 mmol/kg in animals without glucose; 38-45 mmol/kg in animals with glucose). Nonetheless, this difference may be a determinant of the additional injury resulting from glucose administration. Since the EEG became promptly isoelectric and the pupils maximally dilated during ischemia in both treated and non-treated groups, a severe degree of brain blood flow reduction was undoubtedly present in the glucose-treated animals during ischemia, comparable in magnitude to that documented autoradiographically in non-treated animals in our earlier study. Thus, while brains of glucose-treated and non-treated animals appear not to differ greatly during ischemia, glucose pretreatment in some manner sets the stage for striking failure of hemodynamic and metabolic recovery during subsequent recirculation.

Other than acting to increase brain lactate levels during ischemia, glucose may have exerted its deleterious effect by acting as an osmotic agent. The results of experiments designed to evaluate this possibility, however, do not clearly support this hypothesis. There have been few previous investigations of the metabolic effects of glucose administration prior to ischemia. In a study of brain ischemia in the rat reported in abstract form, glucose pretreatment led to a positive cerebral arteriovenous difference for ammonia in the post-ischemic period and to an apparent change in blood-brain glucose transfer from facilitated to simple diffusion. Hansen demonstrated that the duration of the initial 3-9 mM rise in brain extracellular potassium concentration following cardiac arrest is doubled by prior glucose administration and approximately halved by hypoglycemia. The significance of these observations is uncertain.

The impairment of brain recirculation promoted by glucose treatment is not due to a failure of systemic perfusion since post-ischemic blood pressure was comparable to preischemal values (table I). Rather, the non-recuperation appears to be due to factors primarily affecting cerebrovascular resistance. Our studies indicate that reperfusion failure may be well developed following as little as 10 min of recirculation. However, the post-ischemic decreases in NADH levels documented in the accompanying report suggest that there was probably a brief, initial return of circulation at the end of the ischemic period.

Without further studies, it would be hazardous to draw inferences from the present results as to the management of patients with ischemic stroke. The dose of dextrose administered in this study was exceptionally high. Whether smaller glucose loads administered during or following episodes of ischemia impair clinical outcome remains to be investigated.

References

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