Upsala J Med Sci 104: 145–162, 1999

Effects of Hyperglycemia on Small Cerebral Infarctions in Awake Rabbits

Magnus W. Roos

Department of Physiology, Uppsala University, Uppsala, Sweden

ABSTRACT

This study concerns the effects of hyperglycemia on small infarctions in the rabbit brain. Small ischemic foci were produced in both normoglycemic (6 mM) and hyperglycemic (25 mM) rabbits, by injecting small plastic beads into the left heart ventricle under short-acting anaesthesia. 2-deoxy-D-[^{14}C]glucose (2-DG) autoradiography was used to trace ischemic regions in which glucose uptake was increased, either shortly after, or six hours after, the embolization (in awake rabbits). A lesion was characterized by a 2-DG activity > 120%. The obtained freeze-dried sections were inspected for infarcts (with lost tissue structure and increased transparency to light).

In the short experiments (< 1 hour), lesions could be detected throughout the brains, indicating hypoxic regions with enhanced glycolysis. In some foci, mostly located in the basal ganglia (the region containing the largest lesions), a central dip could be seen in the 2-DG accumulation, suggesting a poor glucose supply to the ischemic core. The lesions in the basal ganglia of rabbits that were made hyperglycemic were smaller and did not show such dips. No infarcts could be found in the tissue sections.

In the long experiments (6 hours), both infarcts and lesions could be found. The impact of hyperglycemia on the infarction process in different brain regions was evaluated by measuring the infarct volumes, and by evaluating the fraction of infarcts – number of infarcts found in freeze-dried sections/number of foci (both lesions and infarcts) found in the 2-DG autoradiograms. Hyperglycemia reduced the fraction of infarcts in the cortex, and reduced the size of infarcted areas in the brain stem. In summary, this study shows that the impact of hyperglycemia on the ischemic outcome depends on where in the brain the ischemic focus is located. This adds interesting information as to what is known about the general effects of glucose on cerebral ischemia.

INTRODUCTION

Microemboli seem to account for a significant number of small infarctions in humans (19), which, in turn, are considered to contribute to dementia in the elderly (2, 29). The study of emboli-induced microinfarctions in rabbits could provide insights into the underlying pathophysiology and, hopefully, also suggest an appropriate treatment for this kind of cerebrovascular disorder.

Over decades, several studies on experimental cerebral microembolism have turned up in the literature (4, 27, 34, 47, 53, 54). In these studies, the description of the resulting ischemic regions have been poor. However, a minimally invasive model of multiple cerebral microischemia, employing plastic beads as emboli, has recently been introduced, and has been characterized with respect to several parameters in a number of papers (30, 32, 33).

Acute experiments on anesthetized rabbits, indicate that there is probably a lack of glucose in the very center of some ischemic foci in subcortical structures – mainly in the basal ganglia (30, 32). 24 h after embolization most of the infarcts can be found in these areas (30). From this, the question follows whether an insufficient supply of glucose to the ischemic core is a determinant of the outcome. In the setting of irreversible focal ischemia, caused through occlusion of the middle cerebral artery (relatively large ischemic regions), the effect of hyperglycemia seems equivocal (11, 16, 25, 28, 30, 51, 52). The influence of glucose on the outcome of small ischemic regions does not seem to have been studied at all. Thus, the aim of the present work was to evaluate the impact of hyperglycemia on the number and size of areas of damaged tissue (caused by plastic beads) in the brains of awake rabbits.

MATERIALS AND METHODS

Animals

New Zealand rabbits, 3-4 kg b.w., of either sex were used. Before the experiments, the animals were housed in individual cages in a specialized animal department where they were allowed free access to food and water. The experimental design has been approved by the local ethics committee.

Anesthesia and general preparations

The animals were awake throughout the experimental period, with the exception of the first few minutes to allow embolization (see below). Injections were made via the ear veins, and blood sampling and blood pressure measurements were made via an ear artery catheter (Xylocaine® was used as local anesthetic to allow insertion). Arterial blood gases and pH were mesured by an ABL300 acid-base laboratory, Radiometer.

One day prior to the experiments, the rabbits that would be made hyperglycemic were anesthetized with Hypnorm™ (approximately 0.25 ml/kg) and Dormicum® (approximately 1.5 mg/kg) and a central venous catheter (1.2 x 380 mm) was inserted to allow infusion of hypertonic glucose solution. The animals recovered within some hours, ready for the experiment the next day.

Embolization procedure

Black-dyed beads (Styrene/2%/Divinyl-benzene/170-200 mesh, from Bangs Laboratories, Inc.), size range 75-90 µm, were employed as emboli. About 120 mg of the delivered sphere suspension (10 mg/ml) was mixed with 12 g Macrodex® (60 mg/ml). To allow insertion of a needle into the heart, the rabbits were anaesthetized with Brietal® (methohexital, 10 mg/ml), 1 ml/kg, administered i.v. over about 20 s. Then, using a syringe and a needle (Ø=0.8 mm) connected to a length of tubing, about 0.67 ml/kg of the final suspension of beads was administered transthoracically into the left heart ventricle. Within five minutes of the Brietal® injection, the animals were awake, sitting upright and moving their heads and extremities.

Tracing tissue hypoxia with 2-deoxy-D-[^{14}C]glucose

Unlike glucose, 2-deoxy-D-[^{14}C]glucose (2-DG) is not metabolized after being phosphorylated, and is therefore essentially trapped within the cells. Under certain conditions, this allows quantitative measurements to be made of the glucose consumption (42).

The idea behind using i.v. administered 2-DG to trace hypoxic regions is that, during hypoxia, glycolysis tends to be stimulated (Pasteur effect (15)). Accordingly, the cells increase their uptake of glucose, and also of 2-DG (32).

Short experiments

Normoglycemic animals:

Rabbits (n=5) were subjected to embolization and four minutes later, after awakening, an i.v. injection of about 125 μCi 2-DG (American Radiolabeled Chemicals, Inc.). After another 42 minutes, the animals were put to death by a massive i.v. dose of pentobarbital sodium. To ensure that the 2-DG reached the systemic circulation, an arterial blood sample was taken from an ear artery and analyzed for ^{14}C activity using a LKB scintillation counter. Four minutes later, the rabbit's head was submerged in hexane, chilled with dry ice. Then the head was severed from the body. The subsequent autoradiographic procedure is described below.

Hyperglycemic animals:

About an hour before embolization, infusion of 2 M solution of glucose through the central venous catheter was started, and was then continued throughout the experiment (n=5). By measuring the concentration of glucose in blood samples taken at regular intervals (using a glucose analyzer, YSI® Model 2700), the infusion rate was adjusted to keep the blood glucose concentration at about 25 mM. The mean rate of infusion was 8.1 ml/kg/h at the start of the glucose infusion, and 5.6 ml/kg/h at the end of the experiment. The rabbits were subjected to embolization and then given about 250 μCi of 2-DG four minutes after the bead injection (a higher dose was given than in the normoglycemic animals since glucose inhibits the tissue accumulation of 2-DG (42)). The subsequent procedure was the same as in the normoglycemic animals.

Long experiments

Normoglycemic animals:

Rabbits (n=4) were subjected to embolization as described above and, six hours later subjected to the same procedure as the untreated animalas in the short experiments.

Hyperglycemic animals:

About one hour before embolization, 2 M glucose solution was infused through the central venous catheter and maintained throughout the experiment (n=4). By

measuring the blood glucose concentration at regular intervals (using the glucose analyzer), the infusion rate was adjusted to keep blood glucose at about 25 mM. The mean rate of infusion was 10.0 ml/kg/h at the start of the glucose infusion, and 8.3 ml/kg/h at the end of the experiments. The rabbits were subjected to embolization and then given 250 μCi of 2-DG (American Radiolabeled Chemicals, Inc.) at six hours after the beads. Bicarbonate, 5%, was slowly administered i.v. (on average, about 35 ml/experiment) to keep blood pH as constant as possible (hyperglycemia causes lactacidosis – probably via the Crabtree effect (15). The rest of the procedure was the same as in normoglycemic animals.

Autoradiography and bead counting

The autoradiographic technique was according to Ullberg (49). Briefly, whole brains were sectioned *in situ* into 40 μm thick sections, very few sections being lost in the process. The consecutive series of sections was dehydrated through storage at -20°C and then, some days later, put on film at room temperature for exposure at -20°C for about six weeks. The exposed films were then developed, fixed and rinsed.

The beads were counted manually under a stereo microscope. The anatomical structures were identified with the aid of an atlas of anatomy (37).

Image analysis

The autoradiograms and tissue sections were analyzed using computer-assisted image analysis techniques. Autoradiographic [^{14}C] micro-scales (Amersham International plc) were used to define the relationship between optical density and radioactivity levels. No attempt was made to calculate the cellular glucose uptake.

A "lesion" was defined as a well-demarcated focus with a 2-DG activity > 1.2 times the activity of corresponding unaffected tissue. An infarct, seen in the tissue sections, was defined as an area in which optical density was < 0.8 the density of corresponding unaffected tissue. The selected lesion/infarct was encircled interactively in the computer image of the autoradiogram/tissue section. Then, the region of interest was computer-analyzed with respect to 2-DG activity/optical density. Finally, the lesion volumes were calculated as:

(section thickness) x (sum of areas with a relative 2-DG activity > 1.2),

and the infarct volumes calculated as:

(section thickness) x (sum of areas with a relative optical density <0.8).

Statistics

Student's *t*-test (two-tailed, unpaired data), ANOVA + Scheffe's test or the binomial distribution with significance at $p < 0.05$ were applied.

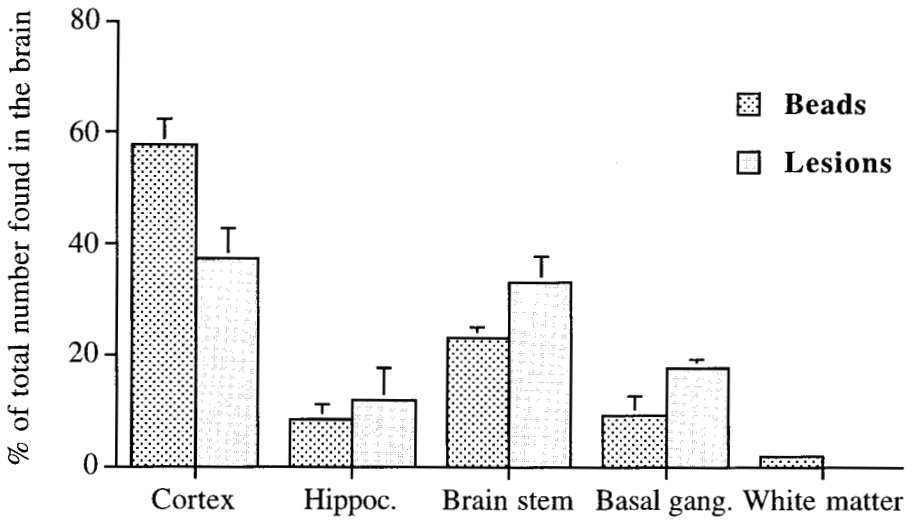

Fig. 1. Results from the short experiments. The distribution (means with S.E.) of beads and lesions (foci of increased 2-DG activity) in the brain regions studied (brain stem includes the diencephalon). The total number of beads and lesions were 177 and 135 respectively.

RESULTS

Short experiments

Untreated animals:

Fig. 1 shows the relative distribution of beads and lesions, and representative autoradiograms are shown in Fig. 2a-b.

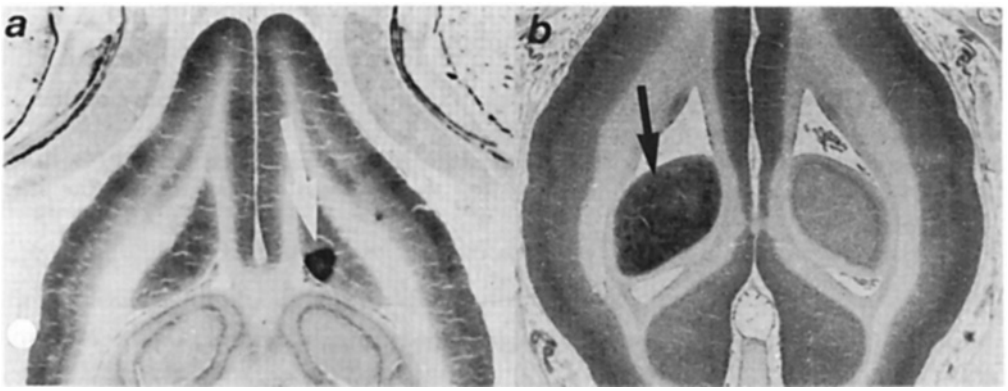

Fig. 2. Results from the short experiments. a) 2-DG autoradiogram, from a normoglycemic rabbit, showing one lesion (arrow) with decreased 2-DG accumulation in the very center. b) 2-DG autoradiogram, from the same group of animals as Fig. 2a, showing diffusely increased hippocampal 2-DG accumulation (arrow). This probably does not reflect ischemia, but rather increased cellular activity.

Fig. 3 shows that the mean ratio of lesions to beads for the cortex was significantly lower than $\mu_0 = 1$ (the null-hypothesis – what was expected), while the mean ratio for the other brain regions studied was not significantly different from 1.

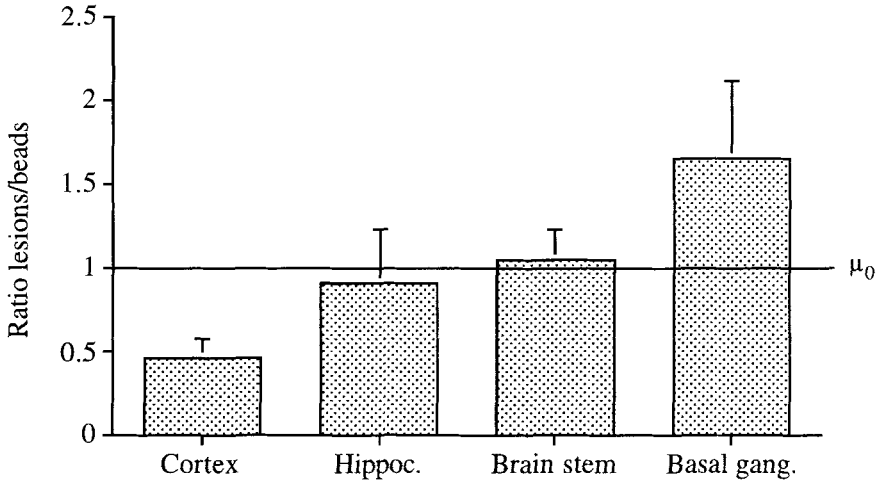

Fig. 3. Results from the short experiments. The ratio number of lesions/number of beads in the different structures studied (means with S.E.). For the cortex this ratio is significantly different from $\mu_0 = 1$(null-hypothesis). For the other structures in the figure this ratio is not significantly different from 1 (brain stem includes the diencephalon).

As shown in Fig. 4, the lesions were larger in the deeper structures. Of the 24 lesions found in the basal ganglia, 12 showed a centrally located "dip" in the 2-DG accumulation (see Fig. 2a). In the cortex, two out of 52 lesions, in the brain stem (diencephalon included) six out of 45 and in the hippocampi three out of 13 lesions showed such a dip. Three of the five rabbits showed diffusely increased hippocampal 2-DG activity (Fig. 2b). Diffusely increased 2-DG activity in the nuclei of the brain stem (diencephalon included) could often be seen. The blood glucose concentration was 6.15 ± 0.81 (S.E.) mM.

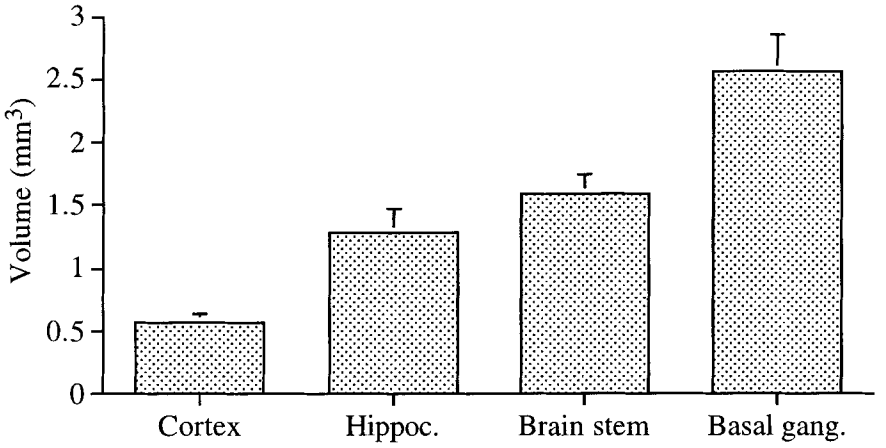

Fig. 4. Results from the short experiments. The lesion volumes (means with S.E.) found in different structures. The mean lesion volumes of the structures studied were significantly different from each other, except for the case of brain stem vs. hippocampus (brain stem includes the diencephalon).

Basal ganglia of the glucose-treated rabbits:

The purpose of these experiments was to determine whether the "dips" could be abolished by hyperglycemia. Because these dips appear mainly in the basal ganglia, attention was focused on this region. No centrally located dip was evident in any of the 15 lesions found, in comparison with the normoglycemic animals mentioned above, where 12 out of 24 lesions in the basal ganglia were found to have a dip in the 2-DG profile. Actually, no lesions with a centrally located dip could were found anywhere in the hyperglycemic brains. The mean lesion volume in the basal ganglia was 0.931 ± 0.138 (S.E.) mm^3, which is significantly lower than the value for the basal ganglia of the normoglycemic animals (2.568 ± 0.288 (S.E.) mm^3).

The blood glucose concentration was 7.3 ± 0.7 (S.E.) mM before the experiments, 26.5 ± 2.0 (S.E.) mM at the time when 2-DG was injected and 27.6 ± 1.8 (S.E.) mM at the end of the experiments. The glucose infusion appeared to make the rabbits sleepy.

Although the total number of lesions found in the brain, 105, was lower than in the normoglycemic group of animals, 135, the relative distributions of lesions between the brain regions studied were not significantly different (data not shown). Diffusely increased hippocampal 2-DG activity was seen in one of the animals.

Long experiments

In these experiments, the effects of hyperglycemia on infarct development were evaluated. Intravenous glucose treatment was associated with a significant reduction in the number of infarcts (detected in tissue sections (Fig. 5, right)) in the cortex (Fig. 6a). Moreover, the areas located in the brain stem, diencephalon included, were significantly reduced in size (Fig. 6b).

The blood glucose concentration in the normoglycemic animals was within the range 5.2-6.7 mM. In the hyperglycemic animals, blood glucose was kept within the range 19.0-28.0 mM, by adjusting the rate of infusion. The blood pressure and acid base parameters did not differ significantly between the two groups (data not shown).

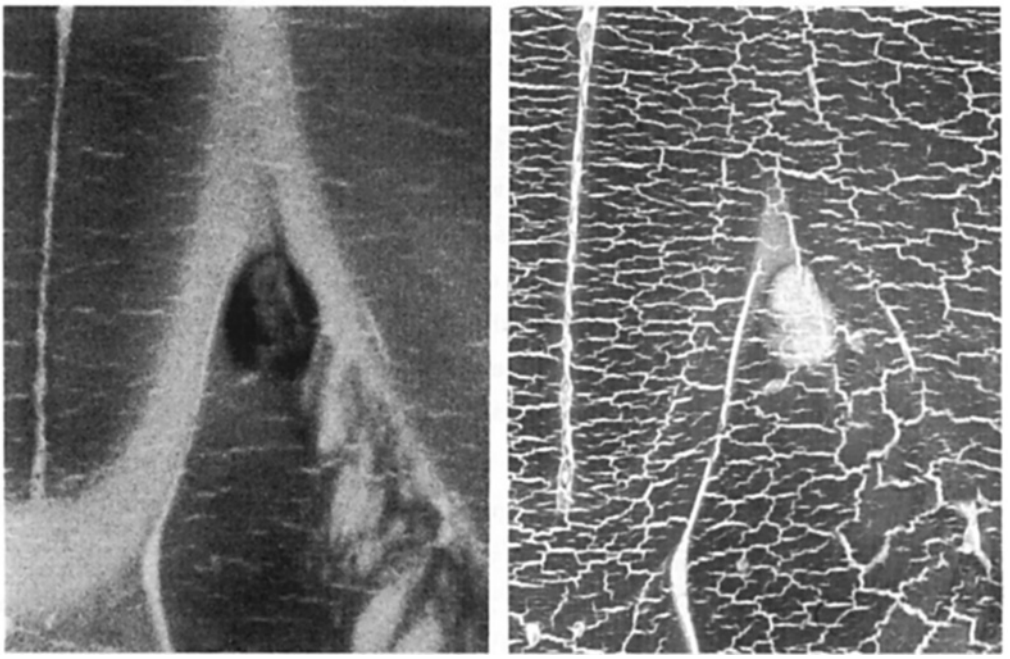

Fig. 5. A 2-DG autoradiogram, left, and the corresponding tissue section, right, from a normoglycemic animal of the long experiments.

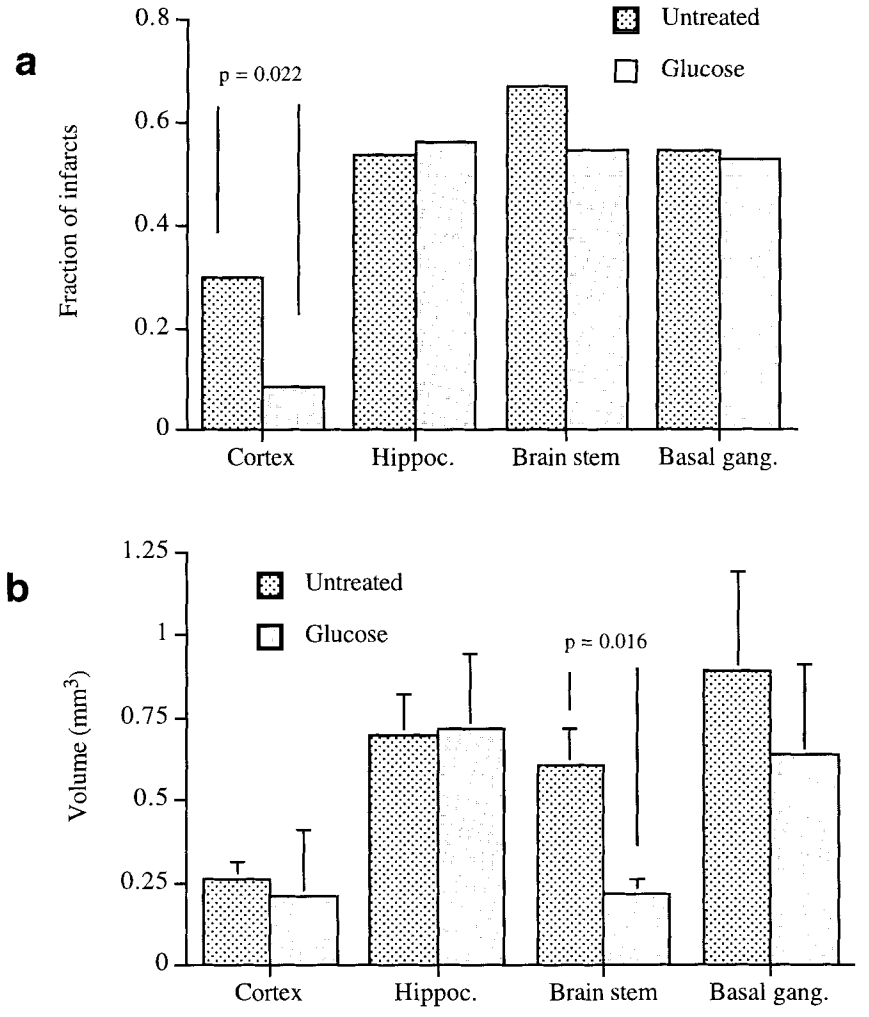

Fig. 6. Results from the long experiments. a) Fraction of infarcts (number of infarcts/ number of lesions and infarcts). In the cortex the fraction is significantly smaller for the glucose-treated group. White matter not shown because too few ischemic foci were found for statistical analysis. b) Infarct volumes. The infarcts located in the brain stem (diencephalon included) are significantly smaller for the glucose-treated group.

DISCUSSION

Small cerebral infarctions are often asymtomatic or cause symptoms that are not taken seriously – minor limb numbness, clumsiness etc. However, several such infarctions may together result in dementia (29). Unraveling the pathophysiology of this kind of infarction therefore has high priority.

There is a common notion that in man, spontaneously occurring subcortical infarctions are caused by "small vessel disease", which is closely coupled to high arterial blood pressure (5). However, cerebral microemboli have been suggested to be the cause of a significant fraction of these infarctions (6, 19).

Recently, a minimally invasive rabbit model of multiple cerebral micro-ischemia (most of the foci subcortically located), employing plastic beads as emboli, has been developed (30). The major aim of the present study was to utilize this model to evaluate the influence of hyperglycemia on the number and volume of the infarcts.

Short experiments

The idea of using 2-DG for detecting ischemic regions is that hypoxia gives rise to a deficiency of ATP, the result being an activation of glycolysis which results in increased 2-DG uptake (16, 32).

As previously reported (30), emboli reaching deeper brain structures seem to perturb the glucose metabolism more often than emboli reaching the cortex, probably because of a more efficient collateral vascular network in the cortex (28).

The lesions in the cortex were found to be smaller than those in other structures studied. The largest lesions could be found in the basal ganglia, where most of the lesions with a central dip in the 2-DG accumulation could also be observed.

When hyperglycemia was present, no lesions with a dip could be observed, suggesting that the dip is related to central lack of glucose. Further, the sizes of the lesions (as defined in the method section) in the glucose-treated animals were smaller. However this does not necessarily mean that the ischemic regions were actually smaller, since, for several reasons, increased tissue glucose will tend to flatten the profile of total 2-DG concentration. One reason is that increased glucose concentration will competitively inhibit 2-DG phosphorylation and thereby decrease the fraction of total 2-DG that is phosphorylated (i.e., the fraction that reflects glucose

uptake). Another reason might be that hyperglycemia inhibits ischemia-induced disturbances in ion homeostasis (*spreading depression*), which enhance cellular glucose utilization (23).

In view of their great size, the regions of increased 2-DG accumulation in the hippocampi (Fig. 2b) are probably not ischemic. Rather, they reflect increased activity, e.g., impaired inhibitory input or increased excitatory input, because of lesions elsewhere in the brain. Whether these observations reflect processes affecting important general brain functions remains to be studied. Such "activations" may well reflect processes of importance for the development of brain dysfunction, e.g., dementia. It is well known, for instance, that the hippocampus is of importance for memory.

Long experiments

With the above discussion in mind, as well as results reported by others (11, 25), I proceeded with longer experiments to examine whether an increased glucose supply impedes the development of irreversible tissue damage.

This work did not indicate that lack of glucose in the ischemic core is always of importance in the process of microinfarction (Figs. 6a-b). It is likely that improving the glucose supply stimulates glycolysis, which should reduce tissue pH. Thus, although the energy state might be improved by administering glucose i.v., the reduction in pH may well aggravate the condition. However, in the cortex and in the brain stem (diencephalon included), hyperglycemia reduced the damage significantly. One explanation might be that glucose inhibits the occurrence and propagation of ischemia-induced *spreading depression*, which has been suggested to be an important mechanism of recruiting the penumbra into the infarction process (12, 26). Thus, spreading depression might be of importance when the ischemia is located in the cortex and brain stem (diencephalon included), but not as important if the ischemia is located in the other structures studied. Unlike others (24, 51), we found no evidence that glucose is detrimental. However, in these previous studies hyperglycemia did not seem to affect blood pH. Accordingly no base infusion was needed.

In summary, it seems that hyperglycemia may inhibit the infarction process in many microischemic regions, at least in the rabbit cortex (provided blood pH is controlled). This finding may be of clinical relevance, and it should therefore be determined whether the results of this study hold for other species.

REFERENCES

1. Alvarez, W. C.: Cerebral arteriosclerosis with small commonly unrecognized apoplexies. Geriatrics 1: 189–216, 1946.
2. Cummings, J. L.: Vascular subcortical dementias: Clinical aspects. Dementia 5: 177–180, 1994.
3. Erkinjuntti, T. & Hachinski, V. C.: Rethinking vascular dementia. Cerebrovasc Dis 3: 3–23, 1993.
4. Fieschi, C., Battistini, N., Volante, F., Zanette, E., Weber, G. & Passero, S.: Animal model of TIA: An experimental study with intracarotid ADP infusion in rabbits. Stroke 6: 617–621, 1975.
5. Fisher, C. M.: Lacunar strokes and infarcts: A review. Neurology 32: 871–876, 1982.
6. Fisher, C. M.: Lacunar infarction – a personal view. In: Lacunar and other subcortical infarctions (ed. G. A. Donnan, B. Norrving, J. M. Bamford & L. Bogousslavsky), pp. 16–20. Oxford university press, Oxford, 1995.
7. Futrell, N., Watson, B. D., Dietrich, W. D., Prado, R., Millikan, C. & Ginsberg, M. D.: A new model of embolic stroke produced by photochemical injury to the carotid artery in the rat. Ann Neurol 23: 251–257, 1988.
8. Futrell, N., Millikan, C., Watson, B. D., Dietrich, W. D. & Ginsberg, M. D.: Embolic stroke from carotid arterial source in the rat: Pathology and clinical implications. Neurology 39: 1050-1056, 1989.
9. Futrell, N.: An improved photochemical model of embolic cerebral infarction in rats. Stroke 22: 225–232, 1991.
10. Gautier, J. C.: Cerebral ischaemia in hypertension In: Cerebral arterial disease (R. W. Russel, ed.), Churchill Livngstone, Edinburgh. Pages 181– 209, 1976.
11. Ginsberg, M. D., Prado, R., Dietrich, W. D., Busto, R. & Watson, B. D.: Hyperglycemia reduces the extent of cerebral infarction in rats. Stroke 18: 570–574, 1987.
12. Hossman, K.-A.: Glutamate-mediated injury in focal cerebral ischemia: The excitotoxin hypothesis revised. Brain Pathol 4: 23–36, 1994.
13. Kobayashi, S., Okada, K. & Yamashita, K.: Incidence of silent lacunar lesion in normal adults and its relation to cerebral blood flow and risk factors. Stroke 22: 1379–1383, 1991.
14. Koennecke, H.-C., Mast, H., Trocio, S. S., Sacco, R. L., Thompson, J. L. P. & Mohr, J. P.: Microemboli in patients with vertebrobasilar ischemia. Association with vertebrobasilar and cardiac lesions. Stroke 28: 593–596, 1997.
15. Koobs, D. H.: Phosphate mediation of the Crabtree and Pasteur effects; Does a change in energy metabolism enhance the potential for malignancy? Science 178: 127-133, 1972.

16. Kraft, S. A., Larson Jr, C. P., Shuer, L. M., Steinberg, G. K., Benson, G. V. & Pearl, R. G.: Effect of hyperglycemia on neuronal changes in a rabbit model of focal cerebral ischemia. Stroke 21: 447–450, 1990.
17. Lowry, O. H., Passonneau, J. V., Hasselberger, F. X., & Schulz, D. W.: Effect of ischemia on known substrates and cofactors of the glycolytic pathway in brain. J Biol Chem 239: 18-30, 1964.
18. Matsuzawa, T., Hishinuma, T., Matsui, H., Meguro, K., Ueda, M., Kinomura, S. & Yamada, K.: Severe atrophy of amygdala and hippocampus in both Alzheimer's disease and multi-infarct dementia. Sci Rep Inst Tohoku Univ 37: 23–25, 1990.
19. Millikan, C. & Futrell, N.: The fallacy of the lacune hypothesis. Stroke 21: 1251-1257, 1990.
20. Miyake, K., Takeo, S. & Kaijihara, H.: Sustained decrease in brain regional blood flow after microsphere embolism rats. Stroke 24: 415–420, 1993.
21. Mohr, J. P.: Lacunes. Stroke 13: 3–11, 1982.
22. Narumi, S., Kiyota, Y. & Nagaoka, A.: Cerebral embolization impairs memory function and reduces cholinergic marker enzyme activities in various brain regions in rats. Pharmacol Biochem Behav 24: 1729–1731, 1986.
23. Nedergaard, M., & Astrup, J.: Infarct rim: Effect of hyperglycemia on direct current potential and [^{14}C]2-deoxyglucose phosphorylation. J Cereb Blood Flow Metab 6: 607–615, 1986.
24. Nedergaard, M.: Transient focal ischemia in hyperglycemic rats is associated with increased cerebral infarction. Brain Res 408: 79–85, 1987.
25. Nedergaard, M. & Diemer, N. H.: Focal ischemia of rat brain, with special reference to the influence of plasma glucose concentration. Acta Neuropathol 73: 131–137, 1987.
26. Nedergaard, M.: Spreading depression as a contributor to ischemic brain damage. In: Advances in Neurology, Vol. 71; Cellular and Molecular Mechanisms of Ischemic Brain Damage (ed. B. K. Siesjö, & T. Wieloch), pp. 75–84. Lipincott-Raven Publishers, Philadelphia, 1996.
27. Overgaard, K., Sereghy, T., Boysen, G., Pedersen, H., Høyer, S. & Diemer, N. H.: A rat model of reproducible cerebral infarction using thrombotic blood clot emboli. J Cereb Blood Flow Metab 12: 484–490, 1992.
28. Prado, R., Ginsberg, M. D., Dietrich, W. D., Watson, B. D. & Busto, R.: Hyperglycemia increases infarct size in collaterally perfused but not end-arterial vascular territories. J Cereb Blood Flow Metab 8: 186-192, 1988.
29. Román G. C.: The identity of lacunar dementia and Binswangers disease. Med Hypotheses 16: 389–391, 1985.
30. Roos, M. W., Sperber, G. O., Johansson, A. & Bill, A.: An experimental model of cerebral microischemia in rabbits. Exp Neurol 137: 73-80, 1996.
31. Roos M. W. & Ericsson, A. Magnetic resonance imaging of experimental microinfarctions in the rabbit brain. Exp Neurol 146: 295-297, 1997.
32. Roos, M. W. & Sperber, G. O.: A diffusion model of cerebral microischemia. Exp Neurol 147: 142-150, 1997.
33. Roos M.W. & Sperber G. O.: Effects of microemboli on local blood flow in the rabbit brain. Exp Neurol 149: 384-389, 1998.
34. Russell, R. W. R.: A study of the microcirculation in experimental cerebral embolism. Angiologica 3: 240-258, 1966.

35. Sako, K., Kobatake, K., Yamamoto, Y. L. & Diksic, M.: Correlation of local cerebral blood flow, glucose utilization, and tissue pH following a middle cerebral artery occlusion. Stroke 5: 828-834, 1985.
36. Schuier, F. J., Vise, W. M., Hossman, K.-A. & Zülch, K. J.: Cerebral microembolization. II. Morphological studies. Arch. Neurol. 35: 264-270, 1978.
37. Shek, J. W., Wen, G. Y. & Wisniewski, H. M.: Atlas of the rabbit brain and spinal cord . Karger, Basel/ New York, 1986.
38. Siegel, B. A., Meidinger, R., Elliott, A. J., Studer, R., Curtis, C., Morgan, J. & Potchen, E. J.: Experimental cerebral microembolism. Multiple tracer assessment of brain edema. Arch Neurol 26: 73–77, 1972.
39. Siesjö, B. K.: Brain Energy Metabolism. John Wiley & sons, Chichester, 1978.
40. Siesjö, B. K.: Pathophysiology and treatment of focal cerebral ischemia, Part I: Pathophysiology. J Neurosurg 77: 169–184, 1992.
41. Siesjö, B. K.: Pathophysiology and treatment of focal cerebral ischemia, Part II: Mechanism of damage and treatment. J Neurosurg 77: 337–354, 1992.
42. Sokoloff L., Reivich, M., Kennedy, C., Des Rosiers, M. H., Patlak, C. S., Pettigrew, K. D., Sakurada, O. & Shinohara, M.: The [^{14}C]deoxyglucose method for measurement of local cerebral glucose utilization: Theory, procedure, and normal values in the conscious and anesthetized albino rat. J Neurochem 28: 897-916. 1977.
43. Steegmann, A. T., & de la Fuente, J.: Experimental cerebral embolism. J Neuropath Exp Neurol 18: 537–558, 1959.
44. Sugimori, H., Ibayashi, S., Fuji, K., Nagao, T., Sadoshima, S., & Fujishima, M.: Mild hyperglycemia and insulin treatment in experimental cerebral ischemia in rats. Brain Res Bull 40: 263–268, 1996.
45. Svensson, L. G., Robinsson, M. F., Esser, J., Fritz, V. U. & Levien, L. J.: Influence of anatomic origin on intracranial distribution of microemboli in the baboon. Stroke 17: 1198–1202, 1986.
46. Swank, R. L., & Hain, R. F.: The effect of different sized emboli on the vascular system and parenchyma of the brain. J Neuropathol Exp Neurol 11: 280–299, 1952.
47. Takeo, S., Taguchi, T., Tanonaka, K., Miyake, K., Horiguchi, T., Takagi, N. & Fujimori, K.: Sustained damage to energy metabolism of brain regions after microsphere embolism. Stroke 23: 62–68, 1992.
48. Tuszynski, M. H., Petito, C. K. & Levy, D. E.: Risk factors and clinical manifestations of pathologically verified lacunar infactions. Stroke 20: 990–999, 1989.
49. Ullberg, S.: The technique of whole body autoradiography. Cryosectioning of large specimens. In: Special Issue on Whole-Body Autoradiography (ed. O. Alvenfeldt) pp. 2-29. LKB Instrument Journal. Science Tools, Bromma, Sweden, 1977.
50. Vise, W. M., Schuier, F., Hossman, K.-A., Takagi, S. & Zülch, K. J.: Cerebral microembolization. I. Pathophysiological studies. Arch Neurol 34: 660–665, 1977.
51. Yip, P. K, He, Y. Y., Hsu, C. Y., Garg, N., Marangos, P. & Hogan, E. L.: Effect of plasma glucose on infarct size in focal cerebral ischemia-reperfusion. Neurology 41: 899–905, 1991.
52. Zasslow, M. A., Pearl, R. G., Shuer, L. M., Steinberg, G. K., Lieberson, R. E. & Larson, C. P.: Hyperglycemia decreases acute neuronal ischemic changes after middle cerebral artery occlusion. Stroke 20: 519–523, 1989.

53. Zivin, J. A., DeGirolami, U., Kochhar, A., Lyden, P. D., Mazzarella, V., Hemenway, C. C. & Henry, M. E.: A model for quantitative evaluation of embolic stroke therapy. Brain Res 435: 305–309, 1987.
54. Zivin, J. A., Fisher, M., DeGirolami, U., Hemenway, C. C. & Stashak, J. A.: Tissue plasminogen activator reduces neurological damage after cerebral embolism. Science 230: 1289–1292, 1985.

Correspondence to:
Magnus W. Roos
Department of Physiology, BMC, Uppsala University,
Box 572, 751 23 Uppsala