Regional Cerebral Blood Flow, Glucose Metabolism, Protein Synthesis, Serum Protein Extravasation, and Content of Biochemical Substrates in Stroke-Prone Spontaneously Hypertensive Rats

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SUMMARY

Stroke-prone spontaneously hypertensive rats with arterial blood pressure above 210 mmHg were taken for the present study after appearance of neurological symptoms. Regional cerebral blood flow, glucose metabolism, and protein synthesis rate were evaluated on the same brain section by means of triple-labelled autoradiographic techniques. Consecutive sections were used in the pictorial presentation of glucose, ATP, and serum protein extravasation. In addition, NADH-fluorescence was recorded.

Two different patterns of hypertension-induced brain lesions could be distinguished: In two animals sharply demarcated cysts were visible in the cortical grey matter. In these animals no regional inhomogeneities of flow and metabolism were present remote from the infarct. In contrast, in three animals cysts were located in the white matter, leading to pronounced hemodynamic and metabolic disturbances throughout the brain. It is concluded that edema-induced brain swelling was the main cause for reduction in blood flow and metabolism.

One of the most important systemic risk factors for stroke is hypertension.1 In 1974 an animal model became available which allows the study of hypertension-induced ischemic brain lesions under controlled conditions: the stroke-prone spontaneously hypertensive rat.2,3 In this strain of animals stroke occurs spontaneously with high incidence, and the pathogenetic similarities of the animal model with humans have been well-established.4 In particular, the role of hypertension could be clearly defined: when blood pressure was lowered, the incidence of stroke also decreased.5

Stroke-prone spontaneously hypertensive rats have been extensively studied in respect to the pathology, morphology and prevention of brain lesions.2-4,6-10 However, little information is available about the metabolic changes involved during development of stroke. This is due to the fact that neither onset nor localisation of the ischemic lesion are foreseeable, and that for this reason it is difficult to decide when and from which region tissue samples should be taken for the biochemical study. Recently, several biochemical imaging techniques have been developed which lend themselves to overcoming the topographic problems involved. Using these methods, it is possible to evaluate the regional pattern of biochemical disturbances on intact brain sections, and to obtain an accurate picture of the interrelationship between biochemical and morphological alterations.

In the present communication, stroke-prone spontaneously hypertensive rats were investigated with a triple-labelled autoradiographic technique for simultaneous measurement of blood flow, glucose consumption and protein synthesis,11 combined with bioluminescent and fluoroscopic procedures for evaluation of regional glucose, ATP and NADH content12-14 and an immunoaautoradiographic approach for determination of serum protein extravasation.15 With this battery of methods it was possible to obtain, for the first time, precise information about the regional biochemical accompaniments of spontaneously occurring stroke.

Material and Methods

Animal Material

Experiments were carried out in 5 stroke-prone spontaneously hypertensive rats (SHR-SP). The rats originated from a strain of animals which was raised in the laboratories of the Japan Stroke Prevention Centre (Izumo, Japan) according to the procedure described by Yamori et al.2 Selected animals with tail arterial blood pressure above 210 mmHg had been kept on a Japanese stack laboratory diet (SP diet, Funahashi Farm, Japan) until, after 4 to 10 months of age, neurological symptoms of stroke appeared. They were immediately shipped by air freight to the laboratory in Cologne, FRG, and after two days of rest during which they had free access to tap water and standard rat diet (sniff R, Versuchstier-Diäten GmbH, Soest, FRG), examined for hemodynamic and biochemical alterations as described below.

For comparison, a group of 3 normotensive BD-IX rats was also investigated. These animals were raised in the Cologne laboratory and were fed with standard rat diet ad libitum. The hemodynamic and biochemical studies were carried out in the same way as in the experimental group.

Surgical Procedure

Animals were anesthetized with 0.8% halothane. Both femoral arteries and veins were catheterized for recording of systemic blood pressure, tracer applica-
tion, and sampling of arterial blood. Following tracheotomy, animals were immobilized and artificially ventilated. Arterial blood gases and arterial pH were controlled intermittently and, if necessary, adjusted to physiological levels by appropriate setting of the respiration pump and infusion of sodium bicarbonate. Body temperature was kept constant at 37°C.

**Triple-labelled Autoradiography**

Regional blood flow, glucose consumption and protein synthesis were measured in the same animal with 131-I-iodo-antipyrine (specific activity 7.45 mCi/mg), 14C-2-deoxyglucose (specific activity 300–350 mCi/mm mol) and a mixture of 5 tritiated amino acids (total activity 0.5 mCi/100g), respectively. The isotopes were purchased from NEN Chemicals, Dreieichhain, FRG. First, a bolus of 14C-2-deoxyglucose (10 mCi/100g) was given intravenously for determination of glucose consumption according to Sokoloff et al. Twenty-five minutes later, 3H amino acids (0.5 mCi/100g) were injected intravenously for determination of protein synthesis according to Bodsch (in preparation) and 44 min after deoxyglucose application 131-I-iodo-antipyrine was infused intravenously at a constant speed for 1 min for assessment of regional cerebral blood flow, as described by Sakurada et al. After tracer injections arterial blood samples were withdrawn for measuring specific amino acid radioactivity and for determination of the concentration of 14C-2-deoxyglucose, glucose and 131I-iodo-antipyrine; the time intervals chosen were as described in the original procedures. At the end of the 131-I-iodo-antipyrine infusion the animals were decapitated into liquid nitrogen for rapid freezing of the brain.

Cryostat sections of 20 μm and 131iodine standards were immediately exposed for 24 hours on Kodak NMB film for recording of 131-I-iodo-antipyrine radioactivity. At the same time radioactivity of arterial blood samples was measured and blood flow was calculated according to the equation by Sakurada et al. After 131-I-iodo-antipyrine had decayed for 8 weeks, sections were again exposed with 14C-standards for 14 days on Kodak NMB film for recording of 14C-radioactivity. Plasma concentration of 14C-2-deoxyglucose and glucose was determined, and glucose consumption was calculated according to Sokoloff et al. Since brain hypoglycemia influencing the lumped constant was not detected in the brains examined (see below) glucose consumption was calculated using the lumped constant of control animals as described by Sokoloff et al. Finally, 14C-radioactivity and free 3H amino acids were removed from the section by washing it for 14 hours in a mixture of 10% trichloroacetic acid, 70% ethanol, 2% polyvinylpropilidone, 0.1% Nonidet-NP-40, and radioactivity of 3H amino acids incorporated into proteins was determined by exposure to tritium sensitive film for 3 weeks. Protein synthesis rate was quantified in small tissue samples (0.5 mg) taken from the block from which cryostat sections were cut. The procedure used is described elsewhere and includes the determination of specific radioactivity of free amino acids, of amino acids bound to tRNAs, and of protein-incorporated amino acids, using high performance liquid chromatography. The numbers obtained were used to calibrate the autoradiograms by plotting protein synthesis rate against optical density of the corresponding brain regions.

The degree of cross contamination of the three autoradiograms was tested by exposing sections with appropriate standards of all three isotopes, and was found to be neglectable.

**Serum Protein Extravasation**

Regional serum protein extravasation was evaluated by means of immunohistology as described elsewhere.

**Imaging of Biochemical Substrates**

The regional tissue content of ATP and glucose was estimated on freeze dried brain sections by means of substrate specific bioluminescence. 20 20 μm cryostat sections were freeze dried and covered by 60 μm thick sections of frozen solutions containing all necessary enzymes, coenzymes and cofactors involved in the bioluminescent reactions. Bioluminescence light emission was recorded on photographic film (Agfa Pan, 25 or 100 ASA). NADH concentration of the tissue sections was determined according to Welsh and Rieder by illuminating the tissue block with ultraviolet light and recording NADH fluorescence at 470 nm.

**Image Processing**

Autoradiograms and bioluminescent pictures were evaluated with an image processing system consisting of a rotating densitometer (Scandic 3, Joyce Loeb, Gateshead, United Kingdom), an image analyser (ID 22200 De Anza, Santa Clara, USA) and a laboratory computer (PDP 11/24, Digital Equipment, Maynard, USA). Areas of interest were identified, and quantitative data were calculated, using the appropriate operational equations (see above).

**Results**

Control measurements: regional cerebral blood flow, glucose consumption and protein biosynthesis were obtained in a group of 3 normotensive BD IX rats which were submitted to the same anesthesia and the same surgical procedure as the experimental group. Cortical blood flow amounted to 1.34 ± 0.13 ml/g/min, glucose consumption to 0.40 ± 0.02 μmol/g/min and protein synthesis rate (amino acid equivalents incorporated into proteins) to 85 ± 9 nmol/g/min. Systemic arterial blood pressure during tracer application was 114.7 ± 19.2 mm Hg.

All 5 stroke-prone spontaneously hypertensive rats (SHR-SP) exhibited neurological symptoms before induction of anesthesia. The most prominent feature was a distinct reduction of spontaneous motor activity, combined with neglect of cleaning behaviour. In one animal spastic paresis of the left hindlimb and a marked weakness of the right hindlimb were present. Epileptic seizures were not observed. During 0.8% halothane anesthesia and after termination of the surgical procedure, arterial blood pressure was 126 ± 15.7
EDEMA-INDUCED BRAIN SWELLING IN SHR/Paschen et al

FIGURE 1. ATP-bioluminescence images of SHR-SP, representing regional cerebral ATP content in 5 individual animals. Note the high, homogenous bioluminescence in animals with cysts in the cerebral cortex (A, B) and the inhomogenously depressed bioluminescence in animals with edematous swollen white matter (C-E).

mm Hg. This value was not significantly higher than that of the normotensive control group, but lower than in the awake animals which in their home laboratory before shipment exhibited a blood pressure of more than 200 mm Hg.

Histological examination of the cryostat sections revealed structural alterations in all 5 animals, but the size and localization of these lesions varied considerably. Two different patterns could be distinguished (fig. 1): in two animals huge, sharply demarcated cysts were visible in the cortical grey matter, located near the borderzone between the anterior and middle cerebral arteries (fig. 1A, B). In the other 3 animals cysts were present in the deep cerebral white matter (fig. 1C-E). These animals exhibited severe cerebral edema as evidenced by massive swelling of perifocal white matter. In the cysts blood flow, glucose metabolism and protein synthesis had ceased, and ATP and NADH-fluorescence were absent (fig. 2, 3). Glucose-induced bioluminescence, however, persisted indicating high glucose content of the cystic fluid.

In the two animals exhibiting cortical lesions no regional inhomogeneities of flow, metabolism and biochemical substrates were present remote from the lesion (fig. 2). The content of glucose and ATP was high, glucose consumption and protein synthesis were similar to that found in normotensive control animals (table 1). There was, however, a slight decrease of cortical blood flow. The demarcation of the lesions was sharp, and there was no rim of hyperemia or hypermetabolism, as previously observed in acute infarcts. 20, 21

In the animals with white matter lesions, in contrast, pronounced disturbances were present throughout the brain (fig. 3). Blood flow, glucose metabolism, and the content of ATP were markedly and inhomogeneously depressed, particularly in the edematous white matter. In contrast, protein synthesis within the cerebral cortex was similar to that found in normotensive control animals (table 1). Glucose content was only slightly decreased (fig. 3E), indicating that depression of metabolism was not caused by insufficient glucose supply to the edematous tissue.

Inhibition of glucose metabolism correlated with the severity of brain swelling: in the brain of the animal exhibiting the most severe degree of brain swelling (fig. 1E) glucose metabolism was also most markedly reduced and amounted only to 30% of that of the normotensive control group.

Discussion

The strain of stroke-prone spontaneously hypertensive rats (SHR-SP) is a generally accepted model of cerebral infarction induced by chronic hypertension. In these animals hypertension leads to atherosclerotic alterations of the cerebrovascular system with ring-
like fat deposits in the circle of Willis and fibrinoid necrosis of the wall of intracerebral arterioles. The lesions are located mainly in the borderzones of the supplying arteries, indicating the importance of hemodynamic factors for the pathological process. This is also reflected by markedly reduced cerebral blood flow which can be improved by antihypertensive treatment.

In SHR-SP, mean arterial blood pressure rises above 200 mm Hg as a consequence of genetic predisposition in combination with a special salt-rich diet. In the rats used for the present investigation, average blood pressure amounted to only 126 mm Hg. This reduction was caused either by the transport or, more likely, by halothane anesthesia used during the surgical preparation. Although blood pressure was still within the autoregulatory range of SHR-SP, a reduction of cerebral blood flow cannot be excluded. The flow measurements obtained, however, did not differ from previously reported values, and we therefore consider the present observations as representative for the normal hemodynamic state of these animals.

Previous morphological studies have revealed that infarcts in SHR-SP are mainly located in the cerebral cortex, whereas basal ganglia and thalamus are less frequently involved. Infarcts may lead to secondary lesions such as widespread rarefaction and cyst formation. In the present series of 5 experiments, only 2 animals exhibited this type of lesion. In the other 3 animals infarcts were located in the deep cerebral white matter. This difference of localization was associated with a different metabolic pattern. In animals with cortical defects glucose utilization and cerebral blood flow were normal or only slightly reduced and there were no inhomogeneities of either blood flow or metabolism. When infarcts were located in the white matter, on the other hand, considerable brain swelling occurred with gross reductions of blood flow, glucose utilization, and ATP content throughout the whole brain. Only glucose content remained normal, indicating that the metabolic disturbances were not due to a reduction of glucose availability.

The different metabolic pattern correlated clearly with the degree of edema development. Cerebral infarcts are associated with permeability disturbances of the blood-brain barrier causing extravasation of serum proteins. The break-down of the barrier does not depend on the localization of the lesion, but the spread of edema is much faster and more extensive in the white matter than in the cortex. The reason is the geometrical configuration of the extracellular channels which in the white matter are arranged in parallel, whereas in the cortex they form a complex tortuous network. It is, therefore, not surprising that in the present investigation white matter lesions caused more pronounced edema and were associated with more severe metabolic disturbances than lesions of the same size located in the cerebral cortex. This observation is in line with numerous earlier reports which also stress the preferential formation of vasogenic edema in the white matter (for review see Baethmann).

An alternative explanation of the two different pathobiocchemical patterns would be that these changes represent different time points of the same pathophysiological process. However, this explanation is unlikely because it is not conceivable that either the grey matter or the white matter lesions are reversible at a later stage.

The combination of reduced blood flow, glucose utilization and ATP in the presence of normal glucose

| TABLE 1 | Mean Arterial Blood Pressure (MABP), Cerebral Blood Flow (CBF), Glucose Consumption (CMRG), and Protein Synthesis Rate (PSR) in Halothane-Anesthetized, Normotensive BD-IX Rats (n = 3), and in Stroke-prone Spontaneously Hypertensive Rats with Cortical (n = 2) and White Matter (n = 3) Lesions |
|-----------|------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| MABP (mm Hg) | CMRG (µmol/g/min) | CBF (ml/g/min) | PSR (nmol/g/min) |
| BD-IX rats | 114.7 ± 19.2 | 0.40 ± 0.02 | 1.34 ± 0.13 | 85 ± 9 |
| SP-SHR with cortical lesion | 156.5 ± 9.2 | 0.39 ± 0.04 | 1.02 ± 0.02 | 89 ± 2 |
| SP-SHR with white matter lesion | 105.0 ± 9.8 | 0.23 ± 0.09 | 0.70 ± 0.05 | 87 ± 8 |

Protein synthesis rate is expressed as amino acid equivalents incorporated into proteins. Values are means ± SEM. Measurements were carried out in cortical grey matter.
content, as observed in the edematous brain tissue of animals with white matter lesions, is on the first sight difficult to interpret. Reduced blood flow in the presence of normal tissue glucose content should lead to activation of anaerobic glycolysis, as has been previously observed in other conditions of mild ischemia. 27, 28 The low glucose utilization in the present experiments, therefore, must have another reason. One explanation could be that prolonged state of brain edema causes inhibition of glycolytic activity despite continuing glucose supply. Such disturbances have, in fact, been previously observed in brain regions reperfused after prolonged state of cerebral ischemia. 29 Another explanation is the partial volume effect of edematous tissue. White matter edema around infarcts is of the vasogenic type. 23 It therefore can be expected to fuse after prolonged state of cerebral ischemia. 29 An- other explanation is that destroyed microvessels trapped blood and retained water, which may have contributed to the edema. This mechanism appears on the here presented images. However, even after exclusion of the vasogenic type, the edema remained dense enough to cause a decrease of the volume of the intracellular compartment. Since glucose utilization and ATP production are intracellular events, total tissue content of these substrates may decrease without any change of intracellular concentration. Such an effect does not apply to glucose content because glucose is distributed both extra- and intracellularly. The actual decrease of metabolic activity, therefore, is less severe than it appears on the here presented images. However, even with appropriate corrections made, perifocal edema remains a major complicating factor for metabolic disturbances following stroke, and therefore should be a main target for therapeutic interference.

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