We devised the present experiments to assess the effects of ischemia on the production of dopamine in the caudate nucleus of spontaneously hypertensive stroke-resistant rats. Ringer's solution was continuously perfused at a rate of 10 μl/min through 0.2-mm-diameter dialysis tubing implanted in the rat's caudate nucleus. After bilateral occlusion of the common carotid artery, perfusate was collected at 20-minute intervals for 120 minutes and was analyzed for monoamines and their metabolites using high-performance liquid chromatography and an electrochemical detection system. The extracellular concentration of dopamine increased abruptly approximately 3 minutes after the ischemic insult, reached a maximum at between 20 and 40 minutes after the insult, and subsequently decreased. During the 120 minutes, 3,4-dihydroxyphenylacetic acid and 5-hydroxyindole-3-acetic acid concentrations decreased significantly, whereas 5-hydroxytryptamine was not detected. Our results indicate that during cerebral ischemia a large increase in extracellular dopamine concentration in the caudate nucleus occurs, probably as a result of energy failure of the cell membranes. This leakage of dopamine may be a causal factor in the neuronal damage associated with cerebral ischemia.

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Several methods for detecting intracerebral monoamines associated with ischemic insult have been reported.1-13 One method, in vivo voltammetry, showed by direct evidence that dopamine was massively released into the extracellular space of the ischemic striatum of rats.14 The selectivity of in vivo voltammetry for detecting monoamines is limited due to the poor specificity of electrical signals from biologic material.15

Recently, measurement of extracellular monoamine concentrations using an in vivo dialysis method coupled with high-performance liquid chromatography and electrochemical detection (HPLC-ECD) has been developed.16,17 This method measures the concentrations of certain extracellular neurotransmitters or neuromodulators in various pathologic states such as ischemia and hypoxia.

We report sequential changes in the concentrations of dopamine (DA), 5-hydroxytryptamine (5-HT), and the amine metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindole-3-acetic acid (5-HIAA) in stroke-resistant spontaneously hypertensive rats (SHR-SR) in the caudate nucleus after severe ischemic insult.

Materials and Methods

We performed all experiments using SHR-SR aged 26-30 weeks. Cerebral ischemia was produced by bilateral occlusion of the common carotid artery under 100 mg/kg i.p. isomital anesthesia according to the method of Fujishima et al.18

Five millimeters of dialysis tubing (Asahi Medical Inc., Tokyo, Japan) with an outer diameter of 0.2 mm and molecular cut-off of 50,000 was implanted into the right caudate nucleus,19 and both ends were connected to PE-20 tubing for perfusion with Ringer's solution (147 mM Na+, 4.0 mM K+, 4.5 mM Ca2+, 155.5 mM Cl-, pH 6.5) pumped at a rate of 10 μl/min. For baseline values, five perfusate samples were collected before occlusion into plastic tubes containing 10 μl of 0.4N perchloric acid. During the first 20 minutes after occlusion (early period), samples were collected every minute, and thereafter samples were collected at 20-minute intervals over 120 minutes.

To assay monoamines and their metabolites by HPLC-ECD, we used a Waters Series 510 pump in conjunction with an LC-4B electrochemical detector (Bioanalytic Systems, West Lafayette, Indiana) and a 5 C18 reverse-phase column (150×4.0 mm) filled with Partisphere C18 (Whatman Inc., Clifton,
Changes in chromatographic pattern after implantation of dialysis tubing into caudate nucleus of stroke-resistant spontaneously hypertensive rats. A, B, and C, chromatograms obtained during first, second, and third 20 minutes, respectively. 1, 3,4-dihydroxyphenylacetic acid; 2, dopamine; 3, 3-methyl-4-hydroxyphenylethylenglycol; 4, 5-hydroxyindole-3-acetic acid; 5, homovanillic acid; 6, 5-hydroxytryptamine.

New Jersey). The mobile phase consisted of 0.05 M monochloroacetate sodium buffer (pH 3.5) with 2 mM ethylenediaminetetraacetic acid and 0.25 mM l-octanesulfonic acid. The detector potential was set at 680 mV against an Ag/AgCl reference electrode. The detection limit was 50 fmol/sample. The perfusate samples were analyzed immediately after collection by direct injection of a 20-μl aliquot onto the chromatography column without further purification.

We measured cerebral blood flow (CBF) using a laser Doppler flowmeter (Periflux, Canon, Tokyo, Japan) to indicate the degree of ischemia over the left parietal lobe of SHR-SR during in vivo dialysis. At the end of the experiment, SHR-SR were killed under deep anesthesia with 75 mg/kg i.p. nembutal. The brains were fixed in 10% buffered formalin and examined histologically to confirm implantation of the dialysis tubing in the caudate nucleus.

Results

Before in vivo dialysis, the dialysis tubing was bathed in a solution containing 1 ng/ml of each monoamine and metabolite, and dialysate was collected every 20 minutes. Absolute recovery rates calculated from the height of each peak were 10-15%. From these recovery rates, extracellular concentrations in the caudate nucleus were calculated to be 2.26×10^-8 M for DA, 2.26×10^-6 M for DOPAC, 3.60×10^-7 M for 5-HIAA, 2.20×10^-8 M for 3-methoxy-4-hydroxyphenylethylenglycol. These values match those of Zetterstrom et al.20

Figure 1 shows the chromatographic pattern during the first 60 minutes after implantation of the dialysis tubing. Because of minor tissue damage, DA and 5-HT were detected during the early period.

DOPAC concentration increased gradually throughout the 60 minutes.

Figure 2 shows the time course of the concentrations of DA, DOPAC, and 5-HIAA. After bilateral occlusion of the common carotid artery, DA concentration increased abruptly to 15,000% of baseline values, reached a maximum at 20-40 minutes, and then gradually decreased but remained high at 120 minutes after the ischemic insult. DOPAC and 5-HIAA concentrations decreased to approximately 50% of baseline values and reached minimums at approximately 60 minutes after the ischemic insult.

Figure 3 shows the sequential changes in DA, DOPAC, and 5-HIAA concentrations in the extracellular space during the early period after occlusion. DA concentration started to increase 3 minutes after the ischemic insult. Concentrations of both DOPAC and 5-HIAA decreased concomitantly over the early period.

Discussion

Our in vivo dialysis method coupled with HPLC-ECD for the measurement of extracellular monoamines and their metabolites is more sensitive and more selective than previously reported methods such as in vivo voltammetry, which was developed to measure ascorbic acid, catechols, and indoles by...
their electrical signals. A highly selective method for the determination of each monoamine and metabolite separately by specially coating the electrical probe in in vivo voltammetry has been developed, but even with this highly selective method it is not possible to simultaneously measure each monoamine and metabolite separately.

We have applied the in vivo dialysis method together with HPLC-ECD for the detection of monoamines and their metabolites in the caudate nucleus of SHR-SR after an ischemic insult produced by bilateral occlusion of the common carotid artery. DA was detected under deep anesthesia when CBF was very low. We could not detect any released DA in the extracellular space of the caudate nucleus during a reduction of CBF in the range 15-100%.

During the early period after the ischemic insult, extracellular DA concentrations started to increase approximately 3 minutes after the occlusion, when energy failure of the cell membranes occurred due to very low CBF. Extracellular DA concentration reached its maximum 20-40 minutes after the ischemic insult and decreased gradually over 120 minutes. At the end of our experiment, however, DA concentration remained high, approximately 5,000% of baseline values. Concentrations of the monoamine metabolites DOPAC and 5-HIAA decreased concomitantly after the ischemic insult. Our results differ from those of previous reports of DOPAC and 5-HIAA concentrations in the brain increasing after ischemia and suggest that DA accumulated in the extracellular space after the ischemic insult and was not metabolized. Ishikawa et al showed that DA accumulated near the ischemic area but disappeared after 2 hours. Our observations agree with their findings except that even after 120 minutes extracellular DA concentration remained high.

The role of DA accumulation in the extracellular space is still unclear. However, previous depletion of catecholamine concentration and inhibition of catecholamine resynthesis in the ischemic hemisphere might lessen or prevent cerebral damage. DA that accumulated in the extracellular space may penetrate into the cytoplasm of neurons and glia, where it would be exposed to monoamine oxidase and could produce H$_2$O$_2$ as a product of oxidative deamination. The surrounding brain area, in which there is incomplete cerebral ischemia, is especially likely to show this effect. Since H$_2$O$_2$ is neurotoxic, ischemic damage is probably enhanced by this mechanism.

References
damage in cerebral ischemia: Greater susceptibility of cate-
cholamine nerve terminals relative to serotonin nerve termi-

12. Welch KMA, Chabi E, Buckingham J, Bergin B, Achar VS,
Meyer JS: Catecholamine and 5-hydroxytryptamine levels in
ischemic brain. Influence of p-chlorophenylalanine. *Stroke*
1977;8:341–346

13. Zivin JA, Venditto JA: Experimental CNS ischemia: Sero-
tonin antagonists reduce or prevent damage. *Neurology*
1984;34:469–474

Togasaki D, Nieves-Rosa J, Maker H: Direct evidence of
acute, massive striatal dopamine release in gerbils with

15. Stamford JA: In vivo voltammetry: Promise and perspec-

16. Imperato A, Chatqa GD: Trans-striatal dialysis coupled to
reverse phase high performance liquid chromatography with
electrochemical detection: A new method for the study of
the in vivo release of endogenous dopamine and metabolites.
*J Neurosci* 1984;4:966–977

17. L’Heureux R, Dennis T, Curet O, Scatton B: Measurement
of endogenous noradrenaline release in the rat cerebral
cortex in vivo by transcortical dialysis: Effects of drugs
affecting noreadnergic transmission. *J Neurochem* 1986;
46:1794–1801

18. Fujishima M, Ishituka T, Nakatomi Y, Tamaki K, Omae T:
Changes in local cerebral blood flow following bilateral
carotid occlusion in spontaneously hypertensive and normo-

19. Paxinos G, Watson C: *The Rat Brain in Stereotaxic Coor-

20. Zetterstrom T, Ungerstedt U: Effects of apomorphine on the
in vivo release of dopamine and its metabolites, studied by

21. Ewing AG, Wightman RM: Monitoring the stimulated release
of dopamine with in vivo voltammetry. II: Clearance of
related dopamine from extracellular fluid. *J Neurochem*
1984;43:570–577

22. Astrup J: Energy-requiring cell functions in the ischemic

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