Role of Central Aminergic Fibers in Experimental Cerebral Ischemia in Stroke-Prone SHR

Relation to Anesthetic Effect

Ichiro Akiguchi, M.D., Ryoichi Horie, M.D., and Yukio Yamori, M.D.

SUMMARY Dopamine and norepinephrine fluorescence in the nucleus caudatus and putamen and cerebral cortex was markedly depleted along with rCBF reduction in symptomatic stroke-prone spontaneously hypertensive rats (SHRSP) with bilateral carotid artery ligation under light pentobarbital anesthesia. An accumulation of fluorescence at the intima of blood vessels, especially in the nucleus caudatus and putamen, was noted in some SHRSP under the same experimental conditions. These changes were hardly seen in deeply anesthetized SHRSP, as well as in normotensive Wistar-Kyoto (WK) rats. It may be possible, therefore, that released cerebral amines in acute brain ischemia accelerate the vasoconstriction and permeability of cerebral arteries, which further decreases the blood supply to these areas. Also, a barbiturate protective effect against the release of central dopamine and norepinephrine during acute brain ischemia was noted in deeply anesthetized SHRSP.

Several years ago the pathogenetic importance of brain monoamine in experimental cerebral ischemia was repeatedly proposed. Ligation of the unilateral common carotid artery in gerbils causes a remarkable and fairly selective decrease of brain DA and NE in the affected hemisphere. Furthermore, 24 hours after ligation brain H-catecholamines derived from intraventricularly administered H-dopamine are markedly decreased ipsilateral to the lesion in animals exhibiting clinical signs of stroke. Within brain regions known to receive dopaminergic projections, common carotid ligation is associated with a selective decrease in the concentration of H-deaminated metabolites. It is suggested that under severely ischemic conditions, central aminergic neurons release their neurotransmitters, and that these compounds, unable to escape directly into the systemic circulation, act locally to extend the pathological process. The present histochemical fluorescence study on central aminergic neurons in adult SHRSP with bilateral carotid artery ligation indicates the possible involvement of cerebral amines in the development of cerebrovascular lesions. Also, a barbiturate protective effect against the release of cerebral amines during acute brain ischemia was postulated.

Materials and Methods

Nineteen young SHRSP and 10 Wistar-Kyoto rats (WK) were lightly or deeply anesthetized with an intraperitoneal injection of pentobarbital (30 mg/kg or 75 mg/kg, respectively), and both common carotid arteries were doubly ligated after careful separation of the cervical-vago-sympathetic trunks by microsurgical technique. The resulting neurological manifestations and pathological alterations were examined 1 and 3 hours after ligation as previously described.

For histochemical observations, rats were sacrificed 1 to 3 hours after ligation, and central DA and NE
fibers in the nucleus caudatus and putamen, nucleus accumbens and frontal paramedian cortex were examined by the conventional Falk-Hillarp (FH) fluorescence method and the glyoxylic acid-formaldehyde (GA-FA) fluorescence method modified by Kimura et al. Animals under ether anesthesia were perfused through the heart at constant flow (35 ml/min) for 5 to 7 minutes with approximately 200 ml of ice-cold 2% glyoxylic acid in Krebs-Ringer solution for 5 minutes, frozen in liquid propane, freeze-dried for several days, and, finally, treated with 50% humid paraformaldehyde vapor for one hour at 80°C.

The basilar artery and mesenteric arteries were isolated and prepared for histochemical study of adrenergic innervation according to a modification of the glyoxylic acid fluorescence methods. Highly concentrated glyoxylic acid solution (14%) was dissolved in Krebs-Ringer bicarbonate buffer, adjusted to 7.4, and chilled to 0°C. Immediately after isolation, the arteries were immersed in the solution from 7 to 10 minutes, stretched flat on slide glass, quickly dried with hot air, and then mounted in liquid paraffin for fluorescence microscopy.

rCBF in the paramedian frontal cortex of young SHRSP and WK was measured with implanted electrodes before and 30 minutes and 2 hours after ligation by a modified hydrogen clearance method.

Results

In preparation for this study we performed preliminary examinations on about 20 SHRSP which showed various symptoms when recovered from light anesthesia (paw lifting, violent circling behavior, hopping fits, repetitive tonic convulsions, and severe cyanosis) and died of acute brain swelling or diffuse convulsions, repetitive tonic convulsions, and severe anesthesia (paw lifting, violent circling behavior, hopping fits, repetitive tonic convulsions, and severe anesthesia). On the other hand, 7 SHRSP showed various symptoms when recovered from light anesthesia, each of which always showed consciousness, or at most showed hypoactivity or hyporritability in response to various stimuli (table).

The results of fluorescence microscopic studies are shown in the table. Central DA and NE fluorescence in the nucleus caudatus and putamen and cerebral cortex and fluorescence in the sympathetic adrenergic fibers in the basilar artery and mesenteric artery were clearly depleted in all SHRSP with bilateral carotid ligation under light anesthesia. These changes were less detectable in WK, as well as in deeply anesthetized SHRSP under the same operative procedures.

In sections from the nucleus caudatus and putamen and the paramedian frontal cortex of sham-operated SHRSP and WK the number of distinguishable DA and NE fibers in that parenchyma treated by the GA-FA technique was greater than that seen by the FH technique. However, in the nucleus caudatus and putamen the whole system of DA fibers was more clearly visible as a fine meshwork-like background by the GA-FA technique. The system of perivascular sympathetic adrenergic fibers in the circle of Willis, the basal arteries and the mesenteric arteries, however, was equally well visible with both techniques.

In all 12 SHRSP with carotid ligation under light anesthesia, fluorescence of the whole system of the central DA fibers in the nucleus caudatus and putamen was mostly depleted, whereas depletion of fluorescence in the central NE and sympathetic adrenergic fibers was less prominent and remained visible even 3 hours after ligation (fig. 1). Moreover, an accumulation of fluorescence at the intima of small vessels in the nucleus caudatus and putamen was observed in some SHRSP ligated under light anesthesia, each of which always showed concomitantly a marked depletion in the parenchymal DA terminals (fig. 2).

In the whole mount preparations of the basilar and mesenteric arteries in control SHRSP, green fluorescence varicose fibers were fairly thick in diameter and had a high density compared to those in control WK. Findings essentially similar to those in control SHRSP and WK were obtained in both

<table>
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<th>Ratel</th>
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<th>Mode</th>
<th>Signs of Ischemia</th>
<th>Fluorescence depletion</th>
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<td>WK</td>
<td>(6)</td>
<td>Control L**</td>
<td>none</td>
<td>O***</td>
</tr>
<tr>
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<td>(4)</td>
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<td>WK</td>
<td>(6)</td>
<td>3 H* L</td>
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<td>O ~ +</td>
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<tr>
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<td>(4)</td>
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<td>mild ~ moderate</td>
<td>++</td>
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<tr>
<td>WK</td>
<td>(4)</td>
<td>3 H D**</td>
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<td>O ~ +</td>
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<tr>
<td>SHRSP</td>
<td>(7)</td>
<td>3 H D</td>
<td>none ~ mild</td>
<td>O ~ +</td>
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</table>

*Hours after bilateral carotid ligation.
**Light (L) or deep (D) anesthesia.
***O ~ none; + ~ mild; ++ ~ moderate; +++ ~ marked; ++++ ~ severe.
ROLE OF AMINERGIC FIBERS IN Cl/Akiguchi et al.

FIGURE 1. Fluorescence of central dopaminergic fibers is mostly depleted (b), in contrast to that of central noradrenergic and sympathetic adrenergic fibers (c, d) in the same section from SHRSP 3 hours after bilateral carotid artery ligation under light (30 mg/kg) pentobarbital anesthesia.

a. nucleus caudatus and putamen, control WK under the same operative procedures, GAFA method, × 200.
b. nucleus caudatus and putamen, SHRSP, GAFA method, × 200.
c. nucleus accumbens, SHRSP, GAFA method, × 200.
d. the circle of Willis, SHRSP, GAFA method, × 200.

animals with bilateral carotid artery ligation when carried out under deep anesthesia (figs. 3 a, c, e), while under light anesthesia the same operative procedure resulted in a depletion of green fluorescence of the nerve fibers in both the basilar artery and the mesenteric artery (figs. 3 b, d, f). In the latter condition the fluorescence was more severely depleted in SHRSP than in WK (figs. 3 d, f).

The results of rCBF measurement in the paramedian frontal cortices of young WK and SHRSP whose carotid arteries were ligated under light anesthesia, were as follows: In WK, rCBF before ligation, 30 minutes, and 2 hours after ligation was 76.0, 30.6, 20.1 ml/100g/min., respectively. In SHRSP rCBF before ligation, 30 minutes, and 2 hours after ligation was 77.5, 4.9, and nearly 0 ml/100g/min., respectively. rCBF gradually diminished for 3 to 6 hours after ligation in WK, and then recovered slowly to the preoperative level within 12 or 24 hours. On the other hand, in SHRSP, within 2 hours after ligation rCBF was reduced to an unmeasurable level, and it did not recover.

Discussion

As one of the ideal models for cerebrovascular disease, SHR and SHRSP have been used for various experimental studies on acute brain ischemia. SHR had a marked increase in lactate and lactate-pyruvate ratio of the brain as well as a pronounced decrease in rCBF following bilateral common carotid artery ligation, and early mortality. In SHRSP rCBF was markedly decreased when blood pressure exceeded 200 mm Hg, and rCBF reduction preceded the development of cerebrovascular lesions. A decrease in ATP and phosphocreatinine levels and an increase in lactate were not significant even in asymptomatic SHRSP with reduced rCBF, but only signifi-
cant in symptomatic SHRSP with a marked reduction of rCBF. Norepinephrine in the brain was decreased in young SHRSP 3 hours after bilateral carotid artery ligation, as well as in SHRSP with spontaneous lesions.

We also studied the adrenergic innervation of the cerebral and peripheral blood vessels and central aminergic innervation in various ages of SHRSP using the GA-FA fluorescence method and confirmed ontogenical fluorescence augmentation in SHRSP, although not in WK. These findings suggest that a congenital alteration of central aminergic neuron systems may be present in SHRSP and these neuron systems may accelerate cerebrovascular lesions.

During the past several years, many studies have demonstrated the pathogenetic importance of brain monoamine in the progression of cerebral ischemia.

Although many chemical data have been reported, no histochemical evidence has been noted on the release of central aminergic neurons in experimental brain ischemia. In this study, we confirmed that central DA and NE as well as sympathetic NE fluorescence were markedly depleted in lightly anesthetized SHRSP with bilateral carotid artery ligation concomitant with extreme rCBF reduction. The accumulation of fluorescence at the intima of blood vessels in the nucleus caudatus and putamen was also noted in some of the SHRSP under the same experimental procedures, along with marked depression of the parenchymal DA fluorescence. These changes were hardly seen, though rCBF reduction occurred in lightly or deeply anesthetized WK and deeply anesthetized SHRSP. As Moskowitz et al. and Mrsuja et al. have already reported, our histochemical study suggests that severely ischemic neurons release their neurotransmitters, and that these compounds, unable to escape directly into the blood stream, accumulate mainly at the intima of the blood vessels and act locally to extend the pathological process.

Recently, barbiturate attenuation of ischemic symptoms and pathologic lesions resulting in cerebral protection was reported. This protection has been demonstrated in a variety of animal models with regional and global ischemia or hypoxia when the drug is administered before, during and after these events. Doses of the barbiturates to provide protection were fairly large, and different in each study, i.e.,

Figure 2. Accumulation of fluorescence at the intima of small vessels in the nucleus caudatus and putamen, concomitant with marked depletion in the parenchymal DA terminals (a).

a. SHRSP 3 hours after bilateral carotid artery ligation under light anesthesia, FH method, \( \times 200 \).

b. SHRSP 3 hours after bilateral carotid artery ligation under deep anesthesia, FH method, \( \times 200 \).
FIGURE 3. Whole mount preparations of the basilar and mesenteric arteries of SHRSP and WK 3 hours after bilateral carotid artery ligation. In SHRSP ligated under deep anesthesia green fluorescence varicose fibers in each arterial specimen are fairly thick in diameter and have high density (c, e), as compared to those in WK ligated under deep anesthesia (a). Green fluorescence in the specimens from lightly anesthetized SHRSP and WK, though more severely depleted in SHRSP in both arterial specimens (d, f), is also slightly depleted in WK (b).

a. WK bilateral carotid artery ligated under deep anesthesia, basilar artery, \( \times 200 \).
b. WK bilateral carotid artery ligated under light anesthesia, basilar artery, \( \times 200 \).
c. SHRSP bilateral carotid artery ligated under deep anesthesia, basilar artery, \( \times 200 \).
d. SHRSP bilateral carotid artery ligated under light anesthesia, basilar artery, \( \times 200 \).
e. SHRSP bilateral carotid artery ligated under deep anesthesia, mesenteric artery, \( \times 200 \).
f. SHRSP bilateral carotid artery ligated under light anesthesia, mesenteric artery, \( \times 200 \).
methohexital 5 mg/kg, pentobarbital 25 mg/kg with thiopental 40 mg/kg, pentobarbital 90–120 mg/kg,
48 mg/kg, 350 mg/kg, mepobarbital 50–100 mg/kg. As mechanisms of protection all of the following have been suggested: 1) depression of the cerebral metabolic rate of oxygen (CMRO); 2) reduction of cerebral blood flow; 3) anticonvulsant effect; 4) anesthetic effect; and others. However, other anesthetics which also reduce CMRO do not provide any apparent protection. Some investigators suggested that the fall in CBF is the natural and apparent protection. In our fluorescence histochemical study in SHRSP, 75 mg/kg of pentobarbital administered intraperitoneally about 30 minutes before ligation showed a protective effect against central DA and NE depletion as well as development of ischemic symptoms. The anesthetic effect seemed to diminish about 1–2 hours after ligation under both light (30 mg/kg) and deep (75 mg/kg) anesthesia. Thus, our results strongly suggest that the barbiturate protective effect is distinct from the anticonvulsant effect and is bound to the stereospecific receptor for both protection and anesthesia.

In our fluorescence histochemical study in SHRSP, 75 mg/kg of pentobarbital administered intraperitoneally about 30 minutes before ligation showed a protective effect against central DA and NE depletion as well as development of ischemic symptoms. The anesthetic effect seemed to diminish about 1–2 hours after ligation under both light (30 mg/kg) and deep (75 mg/kg) anesthesia. Thus, our results strongly suggest that the barbiturate protective effect against central DA and NE depletion is not only a simple anesthetic effect or depression of CMRO, but a protective effect against the release of brain catecholamines.

Furthermore, fluorescence of the central DA fibers in the nucleus caudatus and putamen was mostly depleted, and much less than central NE in the same sections of rat brain 3 hours after carotid ligation. The quantitative difference in the susceptibility of DA and NE fibers to brain ischemia in gerbils with unilateral carotid ligature has been mentioned by Zervas et al. They considered that the major difference between DA and NE release in these experimental conditions resulted from the hemodynamic derangement of DA and NE cell bodies, or the difference in the susceptibility of DA and NE neurons to ischemia. It is suggested that DA is stored mainly outside the vesicles in synaptic terminals; in contrast, NE is stored mainly in the vesicles. Thus, the susceptibility of DA and NE release under various anesthetic and ischemic conditions may be different in each varicos terminal. This could result in severe DA depletion, concurrent with relative preservation of NE fluorescence in severe brain ischemia.

Fluorescence depletion in sympathetic adrenergic fibers in the basilar and mesenteric arteries, as well as in central aminergic fibers, was also found in SHRSP with acute brain ischemia. However, the role of this depletion must be studied further.

In conclusion, we have found histochemical evidence on the depletion of central DA and NE fluorescence in experimental brain ischemia in symptomatic SHRSP under light pentobarbital anesthesia. Under deep anesthesia, however, this depletion is not noted in the brains of asymptomatic SHRSP despite use of the same operative procedures. It is suggested that the barbiturate protective effect against brain ischemia is bound not only to a simple anesthetic effect but also to a protective effect against release of central DA and NE.

Acknowledgment

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References

Hemodynamic Response to Profound Hypoxia in Intact Rhesus Monkeys

RONALD E. MYERS, M.D., PH.D., GARY S. KOPF, M.D., AND DAVID M. MIRVIS, M.D.

SUMMARY  Respiring rhesus monkeys with 2.5 or 4.5% oxygen greatly decreased their cardiac contractility, stroke volume and blood pressure but altered their total peripheral vascular resistance only slightly and inconsistently. All monkeys exposed to 15 minutes and 2 of 4 exposed to 30 minutes of hypoxia recovered and survived without brain injury. Though all animals recovered full cardiovascular function immediately after they were reoxygenated, 2 respired with 4.5% oxygen for 30 minutes began showing declines in blood pressure after a delay of 1 to 2 hours and both subsequently died in shock. Their reductions in blood pressure were associated with reductions in cardiac contractility and stroke volume. The hypotension the animals exhibited both during hypoxia and during development of shock afterwards resulted from pump failure rather than a reduced vascular resistance or an inadequate venous return.

RHECUS MONKEYS need to be exposed to magnitudes of hypoxia where the arterial blood oxygen pressures are reduced below 25 mm Hg for 20 or more minutes in order to develop brain injury or to die. Such animals often die from an apparent cardiac arrest before they are reoxygenated and during the recovery period. The present study investigates the hemodynamic changes produced by exposing monkeys to marked hypoxia both during the actual exposure and during the delayed development of shock.

Methods

Ten fasted 4.5 to 6.5 kg monkeys (M. mulatta) anesthetized with i.v. pentobarbital, 35 mg/kg, were intubated and ventilated with a Harvard respirator. Catheter-tip micromanometers (Millar Instruments, Houston, TX), positioned so their tips lay in the left ventricle and thoracic aorta, recorded the left ventricular and systemic blood pressures. Lead II of the ECG was also recorded while samples of arterial blood were analysed for Po2, PCO2, and pH. The respiratory gas values of arterial blood were brought within normal limits by regulating the respirator settings. Body temperature was maintained at 38°C.

Three animals were ventilated with 4.5 and 2.5% oxygen for 15 minutes and 4 animals with 4.5% oxygen for 30 minutes. Left ventricular pressure, central aortic pressure, ECG, and heart rate were recorded. Cardiac output, cardiac contractility as reflected in $\dot{V}_{\text{max}}$ values, and total peripheral vascular resistance were calculated at 3-minute intervals throughout the control, the hypoxic, and the recovery periods. Hypoxic exposure was terminated by substituting 100% oxygen and, later, room air for the hypoxic gas mixture in the respirator stream. The animals also were injected with 10 µg epinephrine and 10 mEq sodium bicarbonate. Additional sodium bicarbonate was administered to treat persisting acidosis.

The physiologic data were recorded on FM magnetic tape. Analogs were converted to digital data...
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