Participation of Type A Monoamine Oxidase in the Activated Deamination of Brain Monoamines Shortly after Reperfusion in Rats

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Abstract—Changes in monoamine levels during and after ischemia and effects of RS-8359, a type A monoamine oxidase (MAO-A) inhibitor, were studied in the cerebral cortex, hippocampus, and striatum of rats killed by microwave irradiation. The patterns of the changes in norepinephrine (NE), dopamine (DA), and serotonin (5HT) levels were similar during ischemia: All these monoamines decreased in all three regions. After reperfusion, however, the three monoamines showed different patterns of changes: NE, except in the striatum, decreased further; DA increased over the controls; 5HT remained suppressed in all three regions. With regard to the metabolites of the monoamines, the changes during and after reperfusion were almost similar in all regions: O-methylated metabolites, normetanephrine and 3-methoxytyramine, markedly increased during ischemia; After reperfusion, the elevated levels of normetanephrine and 3-methoxytyramine returned to normal, while deaminated metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid, homovanillic acid (HVA), and 3-methoxy-4-hydroxyphenylethylene glycol clearly increased. RS-8359 pretreatment (30 mg/kg, p.o.) at an hour prior to ischemia elevated the levels of NE in the cortex and hippocampus during ischemia and inhibited the increases in DOPAC and HVA levels and the decrease in 3MT levels at 30 min after reperfusion. These results suggest that deamination of NE, DA, and 5HT is activated by the increases in the substrates for MAO in all three regions, except the noradrenergic system in the striatum, and that MAO-A participates in the activated deamination after reperfusion.

Changes in monoamine metabolism in the brain are known to occur not only in patients with cerebral infarction (1–3) but also in experimental animals exposed to brain ischemia (4–9).

Excitatory amino acids, such as glutamate, are widely accepted to be toxic factors for ischemia-induced neuronal death (striatal cells, hippocampal CA1 cells, etc. (10)). Recently, ischemia-induced changes in monoamine metabolism have also been suggested to participate in the morphological and metabolic damage elicited by ischemia (7, 11–13).

In the previous studies, the changes in monoamine metabolism during and after reperfusion were not always consistent (4–6, 9). For instance, Globus et al. (7) reported an increase in 3,4-dihydroxyphenylacetic acid (DOPAC) levels after reperfusion which suggested that dopamine (DA) metabolism was involved in the damage induced by ischemia-reperfusion. Benfúé-Ferrer et al. (8), however, did not observe such an increase in DOPAC level after reperfusion.

When the metabolism of monoamines is accessed, the changes in tissue levels of the metabolites (e.g., 3-methoxytyramine (3MT) and DOPAC for DA metabolism) are more important than their parent monoamines (14, 15). In addition, rapid inactivation of enzymes involved in the metabolism by microwave irradiation is required for detection of the metabolite levels (16, 17). Nevertheless,
levels of monoamines and their metabolites have been studied in animals killed by decapitation (7, 8) or freezing topically (4–6) in most of the previous studies.

The aims of the present investigation were first to determine the levels of monoamines and their metabolites during and shortly after ischemia in the brain of rats sacrificed by microwave irradiation and secondly to examine the implication of type A monoamine oxidase (MAO-A) in the changes in the monoamine metabolism. For this purpose, we used RS-8359 ((±)-4-(4-cyanoanilino)-7-hydroxycyclopenta(3,2-e)pyrimidine), a new reversible and selective MAO-A inhibitor (18) that has some ameliorating effects on the morphological and metabolic damage caused by ischemia (19, 20).

Materials and Methods

Male Wistar rats (12–14 weeks) were used. Ischemia was introduced for 30 min by the method of Pulsinelli and Brierley (21), with some modifications (22). Vertebral arteries were electrocauterized bilaterally under pentobarbital anesthesia (40 mg/kg, i.p.). Three days later, bilateral carotid arteries were exposed under 1.5% halothane anesthesia and occluded with small clips. After the clips were removed, blood flow was verified visually. Rats were sacrificed by head-focused microwave irradiation (3.5 kW, 2.3 sec, Metabostat, New Japan Radio, Tokyo, Japan) at the end of the 30 min of ischemia or 30 min after reperfusion. After each brain was removed, the cerebral cortex, hippocampus, and striatum were dissected out and stored at -80°C. The frozen tissues were homogenized with 0.1 N perchloric acid (PCA) containing 0.1% ethylenediaminetetraacetic acid disodium salt (EDTA2Na) and 0.1% sodium bisulfite (Na2S2O5) and sonicated (30 sec, UR-20P, Tomy Seiko, Tokyo, Japan). The homogenates were centrifuged (10,000 rpm) for 20 min. The monoamine concentrations in the supernatant were determined by high performance liquid chromatography with electrochemical detection (HPLC-ECD). HPLC consisted of a pump (655 Liquid Chromatograph, Hitachi, Tokyo, Japan) and a column (EICOM PAK MA-ODS, 4.6×250 mm, EICOM, Kyoto, Japan). Citrate-sodium acetate buffer (0.1 M, pH 4.0) containing 3 mg/l EDTA2Na, 144 mg/l sodium octanesulfonate, and 17% methanol were used for the mobile phase, which was degassed (Model 545, Gasukuro Kogyo, Tokyo, Japan). The flow rate used was 0.8 ml/min. An electrochemical detector (EC-100, EICOM, Kyoto, Japan) with a graphite electrode (WE-3G, EICOM, Kyoto, Japan) was used, and 750 mV was applied vs. Ag/AgCl. Standard solutions of monoamines and their metabolites (noradrenaline (NE), dopamine (DA), serotonin (5HT), norepinephrine (NE), DA, serotonin (5HT), nor-metanephrine (NM), 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), 3MT, DOPAC, homovanillic acid (HVA), and 5-hydroxyindoléacetic acid (5HIAA), all purchased from Sigma) were prepared every day in 0.1 N PCA containing 0.1% Na2S2O5 and 0.1% EDTA2Na. The concentrations of monoamines in the supernatant were calculated by comparing the peak heights of the samples with those of standards. The standard peaks were separated from each other. However, sometimes it was difficult to determine the minor constituents (e.g., NM and MHPG) because a long running time on a column caused a slight loss of peak resolution. Because of this, NM and MHPG were determined when their separations were adequate for quantitative analysis.

The pellets were dissolved using 0.1 N NaOH, and the protein concentrations were determined by the method of Lowry et al. (23).

RS-8359 was suspended in 0.5% sodium carboxymethylcellulose solution, and was administered orally to rats one hour before carotid artery occlusion since the effect of RS-8359 attained the maximum at 1–2 hours after oral administration (18).

Data are expressed as mean±S.E.M. and were analyzed by the Mann-Whitney U-test.

Results

Changes in monoamine and metabolite levels during 30 min of ischemia and 30 min after ischemia and effects of RS-8359 on them are shown in Table 1.

During 30 min of ischemia

NE and the metabolites: During 30 min of ischemia, NE levels in the cerebral cortex, hippocampus, and striatum reduced signifi-
Means±S.E.M. (N=4–6). The data are expressed as pmol/mg protein. The ischemia groups were sacrificed at the end of the 30 min of ischemia, and the reperfusion groups were sacrificed at 30 min after reperfusion. RS-8359 (30 mg/kg) was administered orally at one hour before ischemia.

**NE**, norepinephrine; **NM**, normetanephrine; **MHPG**, 3-methoxy-4-hydroxyphenylethylenglycol; **DA**, dopamine; **3MT**, 3-methoxytryramine; **DOPAC**, 3,4-dihydroxyphenylacetic acid; **HVA**, homovanillic acid; **5HT**, serotonin and **5HIAA**, 5-hydroxyindoleacetic acid. *P<0.05, **P<0.01, as compared with the ischemia control group (Mann-Whitney U-test). #P<0.05, ##P<0.01, as compared with the corresponding reperfusion control group (Mann-Whitney U-test).

Table 1. Changes in monoamine levels during and after ischemia and the effects of RS-8359 pretreatment

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Ischemia</th>
<th>Reperfusion</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>RS-8359</td>
<td>Control</td>
</tr>
<tr>
<td><strong>Cortex</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>NE</td>
<td>27.3±1.2**</td>
<td>19.1±0.6</td>
<td>22.0±0.3**</td>
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<tr>
<td>NM</td>
<td>0.14±0.07**</td>
<td>0.45±0.03</td>
<td>0.44±0.11</td>
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<td>MHPG</td>
<td>0.18±0.06*</td>
<td>0.43±0.04</td>
<td>0.30±0.02</td>
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<tr>
<td>DA</td>
<td>4.47±0.97</td>
<td>3.51±0.24</td>
<td>5.85±0.48**</td>
</tr>
<tr>
<td>3MT</td>
<td>0.02±0.02*</td>
<td>0.24±0.02</td>
<td>0.24±0.07</td>
</tr>
<tr>
<td>DOPAC</td>
<td>0.51±0.11</td>
<td>0.64±0.06</td>
<td>0.40±0.08*</td>
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<tr>
<td>HVA</td>
<td>0.53±0.04</td>
<td>0.56±0.07</td>
<td>0.31±0.12</td>
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<tr>
<td>5HT</td>
<td>29.4±1.6**</td>
<td>16.9±1.0</td>
<td>16.7±0.7</td>
</tr>
<tr>
<td>5HIAA</td>
<td>12.4±0.8**</td>
<td>15.6±0.2</td>
<td>10.5±0.5**</td>
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<td><strong>Hippocampus</strong></td>
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<tr>
<td>NE</td>
<td>44.4±3.2**</td>
<td>23.7±1.3</td>
<td>29.3±2.6*</td>
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<tr>
<td>NM</td>
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<td>0.72±0.07</td>
<td>0.71±0.10</td>
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<td>MHPG</td>
<td>0.68±0.17</td>
<td>0.59±0.05</td>
<td>0.57±0.06</td>
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<tr>
<td>DA</td>
<td>3.54±0.36</td>
<td>3.48±0.25</td>
<td>5.46±0.38**</td>
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<tr>
<td>3MT</td>
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<td>0.27±0.04</td>
<td>0.14±0.05</td>
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<tr>
<td>DOPAC</td>
<td>0.44±0.07*</td>
<td>0.82±0.09</td>
<td>0.45±0.07*</td>
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<tr>
<td>HVA</td>
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<td>0.22±0.02</td>
<td>0.18±0.05</td>
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<td>5HT</td>
<td>50.9±2.4**</td>
<td>25.8±0.7</td>
<td>38.9±3.7**</td>
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<tr>
<td>5HIAA</td>
<td>32.3±3.7</td>
<td>36.2±0.8</td>
<td>29.5±2.3*</td>
</tr>
<tr>
<td><strong>Striatum</strong></td>
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<td></td>
<td></td>
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<tr>
<td>NE</td>
<td>13.1±0.9*</td>
<td>9.7±0.9</td>
<td>9.2±0.8</td>
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<tr>
<td>DA</td>
<td>873±45*</td>
<td>655±46</td>
<td>796±59</td>
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<tr>
<td>3MT</td>
<td>0.21±0.07**</td>
<td>13.70±0.70</td>
<td>12.30±1.90</td>
</tr>
<tr>
<td>DOPAC</td>
<td>65.4±3.9**</td>
<td>140.3±13.2</td>
<td>56.4±7.7**</td>
</tr>
<tr>
<td>HVA</td>
<td>25.5±2.7</td>
<td>26.0±1.9</td>
<td>15.8±2.1*</td>
</tr>
<tr>
<td>5HT</td>
<td>49.4±2.0*</td>
<td>35.3±2.2</td>
<td>41.1±3.6</td>
</tr>
<tr>
<td>5HIAA</td>
<td>32.8±1.6</td>
<td>38.1±1.8</td>
<td>30.0±2.2*</td>
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</tbody>
</table>

Means±S.E.M. (N=4–6). The data are expressed as pmol/mg protein. The ischemia groups were sacrificed at the end of the 30 min of ischemia, and the reperfusion groups were sacrificed at 30 min after reperfusion. RS-8359 (30 mg/kg) was administered orally at one hour before ischemia. NE, norepinephrine; NM, normetanephrine; MHPG, 3-methoxy-4-hydroxyphenylethylenglycol; DA, dopamine; 3MT, 3-methoxytryramine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 5HT, serotonin and 5HIAA, 5-hydroxyindoleacetic acid. *P<0.05, **P<0.01, as compared with the ischemia control group (Mann-Whitney U-test). #P<0.05, ##P<0.01, as compared with the corresponding reperfusion control group (Mann-Whitney U-test).

Cerebrocortical NE, DA, and 3MT levels were lower, and those of NM and MHPG were higher, significantly to 70, 53, and 74%, respectively, of those in the sham-operated animals. On the contrary, NM, an O-methylated metabolite of NE, increased up to levels approximately 3 and 18 times of those in sham operated animals in the cerebral cortex and hippocampus, respectively. MHPG, a deaminated and O-methylated metabolite of NE, in the cerebral cortex also increased significantly (2.4 times of those in sham-operated animals) during ischemia, although the level in the hippocampus did not change.

DA and the metabolites: DA level reduced significantly in the striatum (75% of sham-operated animals), but not in the cerebral cortex or hippocampus. 3MT, an O-methylated metabolite of DA, however, increased significantly to 12 and 65 times of those in sham-operated animals in the cerebral cortex and striatum. In the hippocampus, the level of 3MT could not be detected in sham-operated animals, while it increased to...
a detectable level (0.27 pmol/mg protein) during ischemia. A deaminated metabolite of DA, DOPAC, also increased in the hippocampus and striatum significantly (approximately 2 times of those in sham-operated animals), but not in the cerebral cortex. HVA, an O-methylated and deaminated metabolite of DA, however, was little affected by ischemia in all three areas examined.

**5HT and the metabolite:** 5HT levels in the cerebral cortex, hippocampus, and striatum significantly decreased to about 60, 50, and 70%, respectively, of those in sham-operated animals. On the other hand, 5HIAA increased slightly (125% of sham-operated animals) but significantly in the cerebral cortex, while the changes in the other two regions were not statistically significant.

**Thirty minutes after reperfusion**

**NE and the metabolites:** Thirty minutes after reperfusion, NE levels in the cerebral cortex and hippocampus decreased further to about 70% of those in the ischemia control group, while the change in the striatum was not statistically significant. On the other hand, increased NM levels during ischemia tended to recover, while the levels of MHPG increased further in the cerebral cortex and hippocampus (approximately 2 times of that in the ischemia control group).

**DA and metabolites:** In contrast to NE, DA in the cerebral cortex, hippocampus, and striatum increased up to those of sham-operated animals after reperfusion (P<0.01). 3MT levels, which increased more than 10 times in all three regions examined during ischemia, recovered to the levels in sham-operated animals after reperfusion in all of these areas. On the contrary, DOPAC increased further up to 2–5 times of those during ischemia in all three regions after reperfusion. Similarly to DOPAC, HVA also increased up to 3.5–7.4 times of those during ischemia in all regions after reperfusion.

**5HT and the metabolite:** Although 5HT levels in these three regions changed little after reperfusion as compared with those during ischemia, the levels of its metabolite 5HIAA increased in all three regions (130–140% of those in sham-operated animals).

**Effects of RS-8359**

**During ischemia:** In animals pretreated with RS-8359 (30 mg/kg, p.o.) at an hour prior to ischemia, significant increases (20–55%) in NE, DA, and 5HT were observed in the cerebral cortex and hippocampus except for 5HT in the cerebral cortex during ischemia. In the striatum, however, the changes in NE, DA, and 5HT were not statistically significant. DOPAC (about 40–60%) and 5HIAA (about 70–80%) were significantly reduced by RS-8359 in all areas examined in comparison to those of the ischemia control. By pretreatment with RS-8359, HVA in the striatum reduced to 60% of that in the ischemic controls, although it was not affected significantly in the cerebral cortex and hippocampus.

**After reperfusion:** NE levels in the hippocampus of animals pretreated with RS-8359 increased significantly to 118% of that of the reperfusion control group, although the levels in the cerebral cortex and striatum were little affected.

In animals pretreated with RS-8359, NM levels in the cerebral cortex and hippocampus after reperfusion were significantly higher (6–7 times) than those in the corresponding control animals.

Although the elevated levels of DA in all regions after reperfusion were not significantly influenced by pretreatment with RS-8359, the DOPAC level in the striatum significantly reduced to less than 1/2 of that of the reperfusion control group, and HVA levels in the 3 regions tested were also decreased in animals pretreated with RS-8359. The elimination of 3MT levels was clearly inhibited by pretreatment with RS-8359 in the hippocampus and striatum.

Furthermore, the 5HT level in the hippocampus increased significantly in animals treated with RS-8359, while the changes in the cerebral cortex and striatum were not statistically significant. 5HIAA levels in the treated animals were significantly smaller (70–80%) than those in the reperfusion control group in all three regions.

Thus, RS-8359 clearly prevented the changes in the levels of monoamines and their metabolites shortly after reperfusion.

**Discussion**

During ischemia, the following changes
were observed in the rat brain: (a) drastic increases in 3MT in all 3 brain regions examined, (b) remarkable increases in NM in the cerebral cortex and hippocampus, (c) increases in DOPAC in the cerebral cortex and striatum; increases in MHPG and 5HIAA in the cerebral cortex, (d) decrease in DA in the striatum. Thus the marked increases in the levels of metabolites by catechol O-methyltransferase (COMT), NM and 3MT, suggest that catecholamines are preferentially metabolized by COMT probably because of the retardation of MAO activity during ischemia. Increase in DOPAC and HVA implies that MAO is not completely inhibited during ischemia.

At 30 min after reperfusion, the following changes were observed: (a) MHPG, DOPAC, and HVA increased up to 2–7.5 times higher levels than those during ischemia; (b) 3MT levels recovered to the normal ones in all brain regions examined and NM levels also tended to recover; (c) DOPAC, HVA, MHPG, and 5HIAA levels increased in all brain areas examined; and (d) levels of NE, DA, and 5HT showed more complex patterns, because NE decreased further and DA increased, while 5HT was unchanged in all 3 brain regions examined. Some of the present results are in accordance with those of the previous studies: for instance, increases in 3MT during ischemia (9) and increases in DOPAC and HVA after reperfusion (7, 9) have already been reported. It should be noted that the remarkably increased levels of the substrates for MAO, 3MT and NM, during ischemia were rapidly eliminated; and the metabolites by MAO, MHPG, DOPAC, HVA, and 5HIAA, accumulated shortly after reperfusion. This indicates that the deamination of monoamines is activated shortly (within 30 min) after reperfusion due to the increases in the substrates for MAO during ischemia. Although the changes in 3MT and DOPAC can be explained by the reduction of COMT activity, the increases in HVA can not. Damsma et al. (24), Phebus and Clemens (25), and Globus et al. (26) suggested that DA metabolism was activated after reperfusion from their studies using microdialysis techniques. The present results also suggest that the activated deamination of NE and 5HT as well as DA occurs shortly after reperfusion in the cerebral cortex, hippocampus, and striatum. Since DOPAC is suggested to be a parameter of intracellular metabolism (27), the activated deamination of DA through MAO appears to occur in the cell.

RS-8359 inhibited the decrease in HVA in the three regions tested, the decrease in DOPAC in the striatum, and the elimination of 3MT in the hippocampus and striatum after reperfusion. Furthermore, RS-8359 inhibited the elimination of NM in the cerebral cortex and hippocampus and the increases in 5HIAA in the three regions tested. Since RS-8359 is a selective MAO-A inhibitor (2220 times more selective for MAO-A against MAO-B: Ki value for MAO-A is 2.9×10^{-7} M) (18, 19), MAO-A has been shown to participate in the activated deamination of monoamines in this phase. However, contribution of MAO-B to the activated deamination can not be excluded, because RS-8359 did not completely inhibit the activated deamination of monoamines.

The activated deamination of monoamines, especially striatal DA which is most rich among the three monoamines in the three regions tested, may participate in the ischemia-induced neuronal damage via an excessive production of hydrogen peroxide by MAO (28) in a short period after reperfusion. In fact, an increase in hydroxy free radical production shortly after reperfusion has been shown, although whether MAO is involved in the reperfusion-induced hydroxy free radical formation or not is not known (29). Such a hypothesis is tentative but has been also proposed by other investigators (24–26).

On the other hand, NE is reported to increase local cerebral blood flow, cerebral oxygen consumption, and cerebral glucose uptake (13), and to prevent cell death induced by ischemia (12). In the present study NE levels declined during ischemia and decreased further after reperfusion in the hippocampus and cortex. The reduction of NE levels suggests that the ameliorating effect of endogenous NE on metabolic deficits and on cell death may be retarded during and after ischemia.

From these results, it is concluded that an
activation of deamination of NE, DA, and 5HT occurs in all three regions, except for the noradrenergic system in the striatum, and that MAO-A participates in the activated deamination after reperfusion.

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References


