Effect of Pantethine on Fatty Acid Oxidation in Microvessels of Rat Brain

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Morisaki, N., Matsuoka, N., Shirai, K., Sasaki, N., Saito, Y. and Kumagai, A. Effect of Pantethine on Fatty Acid Oxidation in Microvessels of Rat Brain. Tohoku J. exp. Med., 1983, 141 (1), 41-45 — Fatty acid oxidation in brain microvessels decreased greatly when persistent hypertension developed in spontaneously hypertensive rats (SHR). Treatment of SHR with pantethine [D-bis-(N-pantothenyl-β-aminoethyl) disulfide] in vivo for 4 weeks restored their fatty acid oxidation activity to the control level. The mechanism of the activating effect of pantethine on fatty acid oxidation was investigated in brain microvessels. Pantethine and its metabolites (pantetheine and 4'-phosphopantetheine) activated three steps of fatty acid oxidation, i.e., acyl-CoA synthetase, carnitine acyltransferase and intramitochondrial oxidation. The relation between changes in fatty acid oxidation activities and injuries of brain microvessels and the protective effect of pantethine against such injuries is discussed. —— brain microvessels; fatty acid oxidation; angioneerosis; pantethine

Rat brain microvessels, which were prepared by a modification of the method of Brendel et al. as reported previously (Brendel et al. 1974; Morisaki et al. 1982; Sasaki et al. 1982) and which were not contaminated with brain parenchymal tissue, showed activities of various enzymes involved in lipid metabolism.

Pantethine, produced by formation of a disulfide bond between two pantetheine molecules, is thought to be a precursor of coenzyme A (Hoagland and Novelli 1954) and is known to increase fatty acid oxidation activity in rat liver (Kameda and Abiko 1980). So the in vivo effect of pantethine on fatty acid oxidation activity in brain microvessels and the mechanisms of its activating effect on fatty acid oxidation were examined in brain microvessels.

Materials and Methods

Chemicals

Adenosine 5'-triphosphate (ATP) bovine serum albumin (Fraction V, free from free fatty acid), CoASH and palmitoyl-coenzyme A were obtained from Sigma Chemical Co. (St. Louis, Mo.). Carnitine and octanoylcarnitine were gifts from Otsuka Pharmaceutical Co. (Tokushima). Pantethine, pantetheine and 4'-phosphopantetheine were gifts from Daiichi Pharmaceutical Co. (Tokyo). Potassium cyanide and reduced glutathione were received for publication, November 26, 1982.
obtained from Wako Pure Chemical Industries (Tokyo). NCS was obtained from Amersham Co. (Arlington Heights, Ill.). [1-14C]Palmitate and DL-[methy-14C]carnitine hydrochloride were purchased from the Japan Isotope Association.

**Animals and feeding conditions**

Spontaneously hypertensive rats (SHRs): SHRs (Aoki-Okamoto strain) (Okamoto and Aoki 1963; Yamori 1977) were obtained from the Japan Rat Association. Their blood pressure began to increase at 7 weeks of age, reached about 180 mmHg at 14 weeks of age and then remained at this high level, whereas that of controls was about 130 mmHg.

Diet containing 0.2% pantethine was administered per os for 4 weeks to 16-week-old SHR to determine the in vivo effect of pantethine on fatty acid oxidation in brain microvessels.

**Preparation of brain microvessels**

Rats were starved overnight and killed by decapitation. Then brain microvessels were obtained by a modification of the method of Brendel et al. (1974). Namely, the cerebrum was removed, separated from basilar vessels and gently compressed between two glass plates. Brain microvessels were picked out, freed from perivascular tissue under a microscope and sonicated mildly for 5 sec. Then the brain microvessels were washed with ice-cold saline and their purity was confirmed microscopically and immunologically (Sasaki et al. 1982). The tissues were used without homogenization for assay of fatty acid oxidation. For enzyme assay, the tissues were homogenized and centrifuged for 10 min at 600 × g and the supernatant was used, as previously reported for aorta (Shinomiya et al. 1979).

**Fatty acid oxidation activity**

The reaction mixture, consisting of 1 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 3% bovine serum albumin, 10 mM ATP, 0.75 mM [1-14C]palmitate and tissue was incubated for 2 hr at 37°C under 5% CO₂ in O₂. The reaction was stopped by adding 0.5 ml of 6 N H₃SO₄ and the mixture was incubated further for 30 min. The CO₂ evolved was trapped in NCS and its radioactivity was measured.

**Acyl-CoA synthetase activity**

Enzyme activity was measured as described previously (Morisaki et al. 1980).

**Carnitine acyltransferase activity**

Enzyme activity was determined by the method of McGarry et al. (1978), by measuring the rate of conversion of palmitoyl-CoA and carnitine to palmitoylcarnitine and CoASH. Reaction mixtures contained 12.5 mM Tris-HCl buffer (pH 7.4), 156 mM KCl, 3.1 mM KCN, 6.2 mM reduced glutathione, 50 µM palmitoyl-CoA, 200 µM L-carnitine, 0.625 µCi DL-[methyl-14C]carnitine and enzyme solution. Reactions were started by adding the enzyme solution. After incubation for 20 min at 37°C, the reaction was terminated by adding 1 ml of 1.2 N HCl and then 1 ml of butanol. Reaction mixtures were then homogenized in a vortex mixer and centrifuged, and 0.5 ml of the butanol layer was transferred to another tube containing 0.1 ml of water and 0.5 ml of water-saturated butanol. The phases were mixed and centrifuged, and the radioactivity of 0.2 ml of the butanol phase was counted.

**Intramitochondrial oxidation activity**

The reaction mixture for fatty acid oxidation assay described above was first preincubated for 15 min to allow fatty acid to be incorporated into mitochondria and then 10 mM octanoyl carnitine was added to stop the incorporation. Further incubation was carried out for 2 hr and the radioactivity of 14CO₂ formed from acyl-CoA, which had been incorporated into mitochondria during preincubation, was counted.

**Measurement of protein**

Protein concentration was measured by the method of Lowry et al. (1951).
RESULTS

Effect of pantethine on fatty acid oxidation activity of rat brain microvessels

Table 1 shows that oral administration of diets containing 0.2\% pantethine for 4 weeks increased fatty acid oxidation activity in brain microvessels of SHR of 16 weeks old to the control level.

<table>
<thead>
<tr>
<th></th>
<th>Wistar King (n=5)</th>
<th>SHR (n=5)</th>
<th>SHR+Pantethine+ (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity (× 10⁶ dpm/g tissue wet weight)</td>
<td>2.34±0.05</td>
<td>1.25±0.38*</td>
<td>2.11±0.54+ NS</td>
</tr>
</tbody>
</table>

Mean ± s.d.
* p<0.001 (compared with Wistar King rats), † p<0.05 (compared with SHR).
NS, Not significant (compared to Wistar King rats).
\+ Diet containing 0.2\% pantethine was given for 4 weeks.

Effects of pantethine, pantetheine and 4′-phosphopantetheine on fatty acid oxidation in rat brain microvessels

As shown in Table 2, pantethine and its metabolites increased acyl-CoA synthesis, the activity of carnitine acyltransferase, and also intramitochondrial oxidation.

<table>
<thead>
<tr>
<th>Addition</th>
<th>Acyl-CoA synth.</th>
<th>CAT</th>
<th>Intramitochondrial oxidation % activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>PaSS</td>
<td>165</td>
<td>123</td>
<td>118</td>
</tr>
<tr>
<td>PaSH</td>
<td>150</td>
<td>130</td>
<td>148</td>
</tr>
<tr>
<td>P-PaSH</td>
<td>340</td>
<td>212</td>
<td>178</td>
</tr>
</tbody>
</table>

PaSS, Pantetheine; PaSH, Pantetheine; P-PaSH, 4′-phosphopantetheine. Concentrations of PaSS, PaSH and P-PaSH are 50 μM.

DISCUSSION

It was recently reported from this laboratory that FFA oxidation is decreased in brain microvessels of SHR (Morisaki et al. 1982).

Table 1 shows that treatment of hypertensive rats with pantethine for 4 weeks restored their fatty acid oxidation activity to the control level.

Hypertension is the most frequent cause of cerebral bleeding (Kannel 1976) and cerebral bleeding may be related to fatty acid oxidation according to the following reasoning: One cause of cerebral bleeding is angionecrosis, which is
caused by cell necrosis (Ooneda et al. 1973). Cell necrosis in turn is partly caused by reduction of the energy supply necessary for transport of materials and membrane function (Saladino and Trump 1968; Trump and Arstila 1971; Trump et al. 1971; Hawkins et al. 1972). Therefore, reduction in fatty acid oxidation activity in brain microvessels in hypertension could well be a cause of cerebral bleeding.

If this is the case, pantethine might have a protective effect against cerebral bleeding. So, we next studied the mechanism of this effect of pantethine.

Pantethine is thought to be converted to coenzyme A. But, it is uncertain whether all the effects of pantethine are actually effects of coenzyme A. So, we examined the effects of pantethine and its metabolites, such as pantetheine and 4′-phosphopantetheine, on fatty acid oxidation.

The process of fatty acid oxidation can be divided into three steps, formation of acyl-CoA, catalyzed by acyl-CoA synthetase, incorporation of acyl-CoA into mitochondria, catalyzed by carnitine acyltransferase, and intramitochondrial oxidation. As shown in Table 2, pantethine and its metabolites increased all three steps of fatty acid oxidation in brain microvessels to various extents, as seen in liver mitochondria (unpublished results).

Our results suggest that not only coenzyme A derived from pantethine but also pantethine itself and its intermediate metabolites (pantetheine and 4′-phosphopantetheine) stimulate all three steps of fatty acid oxidation. Since these substances have been found in mitochondria (Tachizawa and Okazaki 1979), they may have direct effects on these steps of fatty acid oxidation, but their precise mechanisms of action remain to be examined. In addition, the contents and localizations of pantethine, pantetheine, 4′-phosphopantetheine and CoASH in the cells after oral administration of pantethine or its incubation with tissues must be determined to clarify the mechanisms of the effects of pantethine and its metabolites on free fatty acid oxidation.

References


