Mode of Action of Aspirin

EFFECT OF DIETARY ASPIRIN ON MITOCHONDRIAL PYRUVATE METABOLISM IN NORMAL AND THIAMINE-DEFICIENT RATS*

(Received for publication, August 13, 1970)

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SUMMARY

Diets containing 0.2% acetylsalicylic acid were fed to control and thiamine-deficient rats. Acetylsalicylic acid caused a significant decrease in body weight and adipose tissue weight in thiamine-deficient acetylsalicylic acid-treated but not in normal acetylsalicylic acid-treated rats. A large decrease in the total body ketone bodies in thiamine-deficient acetylsalicylic acid-treated rats was observed. A significant decrease in the blood $\beta$-hydroxybutyrate to acetoacetate ratio in both control and thiamine-deficient rats treated with aspirin was noted.

Rat liver mitochondria from both control, thiamine-deficient, and acetylsalicylic acid fed rats were incubated in the presence of ATP, Mg$^{2+}$, P$_i$, pyruvate, and radioactive bicarbonate, and synthesis of dicarboxylic acids was examined. It was found that in acetylsalicylic acid-treated rats, the pyruvate utilization and $^{14}$C$\text{CO}_2$ incorporation and organic acids formation were greatly decreased.

Addition of octanoylcarnitine greatly stimulated $^{14}$CO$_2$ incorporation into organic acids in thiamine-deficient and acetylsalicylic acid-treated rats. The percentage of pyruvate carboxylated in the presence of octanoylcarnitine was significantly increased in mitochondria from all animals.

Salicylates are known to inhibit glycogenesis (1) and amino-transferase enzymes, which may lead to decreased gluconeogenesis (cf. Reference 2). Administration of salicylates to diabetic, adrenalectomized, and hypophysectomized animals resulted in a decrease in blood glucose levels (3-5). A depletion of muscle and liver glycogen in normal animals given salicylates was also reported (6, 7). It has been suggested that these effects of salicylates on carbohydrate metabolism are associated with stimulation of oxygen consumption (8-10), presumably through the uncoupling of oxidative phosphorylation (11, 12).

This paper reports the investigation on the sites and possible mode of action of aspirin. Thiamine-deficient animals were used in these studies as a model system since thiamine deficiency is known to cause well defined biochemical lesions of carbohydrate metabolism (13-17).

EXPERIMENTAL PROCEDURES

Treatment of Animals—Male albino Sprague Dawley rats weighing 250 to 255 g were housed in individual stainless steel cages with wire screen bottoms and kept at 25 ± 2°C. All animals were fed ad libitum and had free access to tap water. A total of 24 animals were divided into four groups of six rats each, and fed two diets: (a) thiamine-deficient diet and (b) thiamine-deficient diet supplemented with 0.2% of aspirin.

Control animals, Group A without aspirin and Group B with 0.2% aspirin in the diet, were supplemented with 60 µg of thiamine hydrochloride administered orally three times a week. This dosage was found to be adequate for maintaining normal growth when rats were fed ad libitum. The deficient animals were fed the same diets unsupplemented with thiamine (Group C, without aspirin, Group D, with 0.2% aspirin in the thiamine-deficient diet). All experiments were performed on the 32nd day of thiamine deficiency after an overnight fast.

Procedures for Isolation of Mitochondria and Metabolite Analysis—The isolation of intact liver mitochondria, incubation, processing of samples, analysis for pyruvate, malate, citrate, $^{14}$CO$_2$ incorporation, acetoacetate, $\beta$-hydroxybutyrate, and mitochondrial nitrogen were performed according to the methods previously cited (18, 19). The blood glucose was analyzed by the glucose oxidase procedure (Calbiochem).

Materials—The NaH$^{14}$CO$_3$ was purchased from Tracerlab and was diluted with unlabeled KHCO$_3$ to give a final concentration of 0.22 µCi per µmol. The specific radioactivity of the solution used was determined as previously described (20). The enzymes

1 Diet composition: vitamin-free casein, 18%; sucrose, 67%; corn oil, 10%; salt mixture, 4%. Salt mixture (No. 2 USP XIII) contained: calcium phosphate, 19.35%; calcium lactate·2H$_2$O, 2.7%; potassium phosphate (dibasic), 23.38%; sodium diphosphate·2H$_2$O, 8.72%; sodium chloride, 4.33%. Vitamin mixture contained in grams per 100 pounds of diet: vitamin A (200,000 units per g), 4.5; vitamin D (400,000 units per g), 0.25; $\alpha$-tocopherol, 5.0; ascorbic acid, 45.0; inositol, 5.0; choline chloride, 75.0; menadione, 2.25; p-aminobenzoic acid, 5.0; niacin, 1.8; riboflavin, 1.0; pyridoxine HCl, 1.0; calcium pantothenate, 3.0; in milligrams per 100 pounds of diet: biotin, 20; folic acid, 90; vitamin B-12, 1.35. Diet was obtained from Nutritional Biochemicals.

* This research was supported by Grant AM-1357901 from the National Institutes of Health and by Army Research Grant DAHC 19-68 G0039.
RESULTS

Effect of Feeding Dietary Aspirin on Body Weight and Adipose Tissue Weight of Normal and Thiamine-deficient Rats—In normal animals, 0.2% aspirin in the diet had no effect on growth. On the other hand, Vaughan, Steele, and Korty (21) showed that normal animals fed 0.5% aspirin in the diet for 2 weeks significantly reduced weight gain. Animals fed deficient diet started to lose weight after the 18th day on the diet. The rate of weight loss was significantly larger (p < 0.01) in the deficient rats fed aspirin (Table I).

Adipose tissue is highly responsive to metabolic and hormonal (22) alterations of the animals. Results in Table I show that there was a significant decrease (p < 0.02) in the adipose tissue weight of thiamine-deficient rats. The decrease in adipose tissue weight in thiamine-deficient rats is consistent with the requirement for utilization of additional lipid for energy, because of impairment in carbohydrate metabolism. In deficient animals fed aspirin, the decrease in adipose tissue weight was more severe (p < 0.01). When adipose weights were expressed as grams per 100 g of body weight, a significant difference in weight among normal, deficient, and deficient fed aspirin was observed. Feeding of aspirin to normal rats had no effect on the adipose tissue weight.

Effect of Feeding Dietary Aspirin on Blood Glucose and Blood Ketone Bodies of Normal and Thiamine-deficient Rats—Results in Table II show the concentrations of blood glucose and ketone bodies of normal and deficient rats fed aspirin. A significant (p < 0.01) hypoglycemic effect of normal animals fed aspirin was observed. There were no differences in the blood glucose levels among normal, thiamine-deficient, and deficient aspirin-fed rats. Modulations in the blood levels of acetoacetate of β-hydroxybutyrate can reflect changes in the liver intramitochondrial oxidation-reduction state of pyridine nucleotides or the rate of metabolism of ketone bodies (23). A significant increase (p < 0.01) in the acetoacetate levels in normal aspirin-treated rats were observed (Table II).

The oxidation-reduction state of pyridine nucleotides in mitochondria, as represented by the β-hydroxybutyrate to acetoacetate ratios, is significantly decreased (p < 0.01) in both normal (from 3.64 ± 1.54 to 1.27 ± 0.30) and deficient (from 2.40 ± 0.27 to 1.33 ± 0.35) rats treated with aspirin. In the deficient rats, the ratio of β-hydroxybutyrate to acetoacetate was also significantly (p < 0.01) lower than that of normal rats (from 3.60 ± 0.54 to 2.40 ± 0.27). In deficient aspirin-treated but not in normal aspirin-treated rats, there was a highly significant decrease (from 1.430 ± 0.097 to 0.522 ± 0.009 μmoles per ml) in the total ketone bodies (p < 0.005).

Influence of Dietary Aspirin on Pyruvate Metabolism of Normal and Thiamine-deficient Rats—The incorporation of radioactive carbon dioxide into tricarboxylic acid cycle intermediates, mainly malate and citrate, in the presence of octanoylcarnitine from pyruvate, H14CO3, ATP, inorganic phosphate, and magnesium ions by intact liver mitochondria from normal, deficient, and aspirin-treated rats is shown in Table III.

The major intermediates formed by liver mitochondria are malate and citrate, in the presence of octanoylcarnitine, β-hydroxybutyrate, and acetoacetate. In normal aspirin-treated rats there was a significant (p < 0.01) decrease in utilization of pyruvate. The mitochondria from deficient rats used less than half the amount of pyruvate of normal rats. In the deficient aspirin-treated rats the utilization of pyruvate was greatly impaired (p < 0.001). The incorporation of radioactive bicarbonate was significantly decreased (p < 0.05) in normal aspirin-treated rats. Pyruvate utilization was also decreased (p < 0.001). In the mitochondria from the deficient and deficient aspirin-treated rats, 14CO2 incorporation was significantly decreased (from 1705 ± 189 to 780 ± 48 μmoles per mg of phosphate per hour) (p < 0.005). The formation of products, citrate and malate, was decreased in normal and deficient mitochondria from aspirin-treated rats. The percentage of pyruvate carboxylated in the absence of fatty acid was the same in normal (65.7%) and deficient (65.3%) mitochondria and was significantly decreased in mitochondria from normal (45.5%) and deficient (41.3%) aspirin-treated rats. Fatty acids are known to inhibit oxidation of pyruvate in mitochondria from liver (18, 20, 24, 25). In the presence of 0.67 mM octanoylcarnitine, the utilization of pyruvate was significantly decreased in mitochondria from normal and normal aspirin-treated rats (Table III). In the mitochondria from deficient animals, especially in the deficient aspirin-treated group, pyruvate

Table I

<table>
<thead>
<tr>
<th>Parameters examined</th>
<th>No. of rats</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>Without aspirin, Group A</td>
<td>With aspirin, Group B</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>6</td>
<td>327 ± 16</td>
</tr>
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<td></td>
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<td>(p &lt; 0.02)</td>
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<tr>
<td>Adipose tissue wet weight (g)</td>
<td>6</td>
<td>4.16 ± 0.05</td>
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<td></td>
<td></td>
<td>(p &lt; 0.02)</td>
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<tr>
<td>Adipose tissue (g/100 g)</td>
<td>6</td>
<td>1.27 ± 0.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(p &lt; 0.01)</td>
</tr>
</tbody>
</table>

* Group A compared with Group C.  
* Group C compared with Group D.
Pyruvate Metabolism in B1-deficient Rats Fed Aspirin

Table II
Blood ketone bodies and glucose levels in normal and thiamine-deficient rats treated with aspirin

<table>
<thead>
<tr>
<th>Parameters examined</th>
<th>Normal</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Without</td>
<td>With aspirin,</td>
</tr>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>Blood glucose (mg per 100)</td>
<td>6</td>
<td>127 ± 10</td>
</tr>
<tr>
<td>Acetocetate (μmoles per ml)</td>
<td>6</td>
<td>0.236 ± 0.085</td>
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<tr>
<td>β-Hydroxybutyrate (μmoles per ml)</td>
<td>6</td>
<td>0.765 ± 0.185</td>
</tr>
<tr>
<td>Total ketones (μmoles per ml)</td>
<td>6</td>
<td>1.001 ± 0.091</td>
</tr>
<tr>
<td>Ratio β-hydroxybutyrate to acetocetate</td>
<td>6</td>
<td>3.64 ± 0.54</td>
</tr>
</tbody>
</table>

* Group A compared with Group B.
# Group A compared with Group C.
& Group C compared with Group D.
" NS, not significant.

Table III
Effect of dietary aspirin on pyruvate metabolism in rat liver mitochondria from normal and thiamine-deficient rats

The reaction mixture was made isotonic with 0.154 M KCl and contained, in 3.0 ml, 3.3 mM ATP, 10 mM MgSO4, 13.3 mM potassium phosphate, and 13.3 mM triethanolamine buffers, pH 7.3, 10 mM KH14CO3 (specific activity 185,000 dpm per μmole) and 9.7 mM pyruvate. Mitochondria from 0.5 g of liver contained 3.2 mg of nitrogen and was suspended in 0.25 M sucrose. Incubation time was 7 min at 37°C.

<table>
<thead>
<tr>
<th>Addition to system</th>
<th>No. of rats</th>
<th>Pyruvate used</th>
<th>14CO2 incorporated</th>
<th>Malate found</th>
<th>Citrate found</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Without octanoyl-carnitine</td>
<td>With octanoyl-carnitine (0.67 mM)</td>
<td>Without octanoyl-carnitine</td>
<td>With octanoyl-carnitine (0.67 mM)</td>
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<tr>
<td>Control</td>
<td>4</td>
<td>7.60 ± 0.43</td>
<td>4.40 ± 0.46</td>
<td>3.12 ± 0.23</td>
<td>3.12 ± 0.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(p &lt; 0.01)*</td>
<td>(NS)*</td>
<td>(p &lt; 0.05)</td>
<td>(NS)*</td>
</tr>
<tr>
<td>Deficient</td>
<td>4</td>
<td>3.26 ± 0.27</td>
<td>4.13 ± 0.38</td>
<td>1.71 ± 0.19</td>
<td>3.12 ± 0.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(p &lt; 0.001)*</td>
<td>(NS)*</td>
<td>(p &lt; 0.05)*</td>
<td>(NS)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.43 ± 0.09</td>
<td>3.13 ± 0.11</td>
<td>0.78 ± 0.03</td>
<td>3.15 ± 0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(p &lt; 0.001)*</td>
<td>(NS)*</td>
<td>(p &lt; 0.05)</td>
<td>(NS)*</td>
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</tbody>
</table>

* Group A compared with Group B.
# NS, not significant.
& Group A compared with Group C.
" Group C compared with Group D.

utilization and carboxylation were greatly increased. There was no difference in pyruvate utilization in the presence of octanoyl-carnitine between normal and deficient rats. The incorporation of 14CO2 into organic acids and formation of malate was the same for all groups in the presence of octanoyl-carnitine. There were large significant decreases in the amounts of citrate formed in normal and deficient aspirin-treated rats.

Results in Table IV show that when pyruvate carboxylation was estimated by summing up all products by the method previously described (26) there was a 50% decrease in normal aspirin-treated rats (from 4930 ± 191 to 2436 ± 129 mpmoles per mg of protein per hour). In the deficient aspirin-treated rats the pyruvate carboxylation was decreased by 63% when compared to deficient rats (from 2110 ± 154 to 573 ± 69 mpmoles per mg of protein per hour).
The pyruvate carboxylation in the presence of octanoylcarnitine in control aspirin-treated rats was decreased by 30% (from 4253 ± 94 to 3000 ± 176 mmoles per mg of protein per hour). There was no difference between the activities of normal and deficient rats. In the deficient aspirin-treated rats there was a 40% decrease in pyruvate carboxylation (from 3997 ± 402 to 2375 ± 94 mmoles per mg of protein per hour). The percentage of pyruvate carboxylated was greatly increased in the presence of octanoylcarnitine in all groups. In the aspirin-treated groups the increase in the percentage of pyruvate carboxylated was smaller than that of untreated groups. In the presence of fatty acid in both deficient (from 65.3 ± 1.3 to 92 ± 2.8%) and normal rats (from 4930 ± 191 to 2110 ± 154 mpmoles per mg of protein per hour), the pyruvate carboxylation was decreased by approximately 57976 from normal and thiamine-deficient rats. In the deficient aspirin-treated rats there was a 40% decrease in pyruvate carboxylation (from 3997 ± 402 to 2375 ± 94 mmoles per mg of protein per hour). The percent-}

**DISCUSSION**

The results presented in this study show that aspirin affects pyruvate metabolism. The importance of pyruvate metabolism in rat liver, kidney, and adipose tissue mitochondria for providing precursors for gluconeogenesis has been studied in great detail (18–20, 25–33). These studies in rats demonstrate that malate and aspartate formed in mitochondria from pyruvate carboxylation are transported to cytosol where they are used for gluconeogenesis.

Thiamine is a known important cofactor for metabolism of pyruvate and other α-keto acids (12–17). In addition fatty acids are known to exert "thiamine sparing effect" (34–37); but the exact mechanism by which fatty acids spare thiamine requirement still remains obscure. However, it is known that thiamine as thiamine pyrophosphate is involved in the decarboxylation of pyruvate to acetyl-CoA. Acetyl-CoA is a required allosteric activator of pyruvate carboxylase (EC 6.4.1.1, pyruvate:carboxyl-
action of aspirin on carbohydrate metabolism are in the mitochondria at the pyruvate carboxylation step, due to alteration of mitochondrial oxidation-reduction potential of pyridine nucleotides that can be controlled by fatty acids.

REFERENCES
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