Effects of Intravenous Injection and Intraperitoneal Continual Administration of Sodium Propionate on Serum Cholesterol Levels in Rats

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Summary To examine the effects of sodium propionate on serum cholesterol levels, rats were given sodium propionate intravenously and intraperitoneally. Six-week-old male Sprague-Dawley rats were kept on a cholesterol-free semisynthetic diet for 2 weeks, fasted, and given 400 µl of saline solution intravenously supplemented with 0.01–10 mg sodium propionate. Three hours after injection of 1 mg of sodium propionate, the serum total-cholesterol level was significantly reduced (85.4 ± 4.0 mg/dl) compared with its starting level (102 ± 3.4 mg/dl), with the reducing effect lasting for 24 h. The intensity of the reduction increased proportionately with increased sodium propionate concentrations from 0.01 to 1 mg. Next, to evaluate the influence of continual sodium propionate administration on serum cholesterol levels, 6-week-old male rats were implanted with an osmotic pump intraperitoneally (ALZET® Model 2ML2, pumping rate: 5.0 µl/h; duration: 14 days; reservoir volume: 2,000 µl). At day 14, serum total-cholesterol levels were reduced by continual sodium propionate administration at both 0.12 and 1.2 mg/day. The maximum percentage change in the serum total-cholesterol level was 78.5 ± 6.7% of its starting level (111 ± 7.1 mg/dl), observed at 1.2 mg/day at day 7. These results indicate that sodium propionate can reduce serum total-cholesterol levels in vivo.

Key Words sodium propionate, short-chain fatty acid, dietary fiber, hypocholesterolemic effect, serum total-cholesterol

Undigested dietary constituents such as nonstarch polysaccharide (dietary fiber) and resistant starch escape digestion and absorption by the upper gastrointestinal tract and reach the colon. Since these resistant carbohydrates are well fermented by anaerobic bacteria in the colon, dietary fiber and resistant starch have been suggested to be main substrate for the production of short-chain fatty acids (SCFAs) such as acetate, propionate, butyrate, and other products in humans and animals (1–5). SCFAs are virtually totally absorbed in the intestine and have been
considered an energy source for the host (6).

In addition to providing energy, SCFAs may strongly influence normal gastrointestinal function affecting colonic blood flow (7), pancreatic secretion (8), sodium and water absorption in the colon (9), and intestinal mucosal growth (10). Moreover, Wright et al. reported that propionate can significantly inhibit cholesterol and fatty acid de novo synthesis in isolated rat hepatocytes (11). Nishimura et al. recently demonstrated that SCFAs cecal content correlated negatively with plasma total-cholesterol levels in rats (12).

In this study, to examine the effects of sodium propionate on serum cholesterol levels, both intravenous injection and intraperitoneal continual administration were performed in rats.

MATERIALS AND METHODS

1. Reagents and chemicals. All reagents and chemicals used were of analytical grade. Sodium pentobarbital was purchased from Dainabot Co., Osaka. Sodium propionate, DL-methionine, choline bitartrate, and other reagents and chemicals were purchased from Wako Pure Chem. Ind. Ltd., Osaka.

2. Rats. Six-week-old male Sprague-Dawley rats (Jcl: SD) were purchased from CLEA Japan Inc., Osaka. The animals were housed in a room with controlled lighting (20:00–8:00 h) and temperature (23±1°C), kept individually in stainless steel cages with wire mesh bottoms. Rats were fed ad libitum a purified diet contained (g/kg diet): corn starch, 418.4; sucrose, 209.3; casein, 209.3; corn oil, 93; AIN-76 mineral mix, 35; cellulose, 20; AIN-76 vitamin mix, 10; DL-methionine, 3; and choline bitartrate, 2 (13). After a 2-week acclimation period, three experiments (Experiments 1, 2, and 3) were performed using well grown rats (body weight: 300±3.3 g).

3. Experiment 1. To examine the time course changes in serum cholesterol levels after intravenous sodium propionate injection, ten rats were fasted for 16 h and given 400 μl saline supplemented with or without 1 mg sodium propionate in the right jugular vein. Blood samples were taken from right jugular vein at 0, 1, 3, 5, and 24 h after injection. Intravenous injection and blood sampling were performed under consciousness using a small animal retainer (Model MAC-1S, CFK Laboratory, Tokyo) (14). The concentrations of serum total-cholesterol and HDL-cholesterol were measured.

4. Experiment 2. To estimate an effective dose of sodium propionate on the reduction in serum lipid levels, 38 rats were fasted for 16 h and given 400 μl saline alone or with 0.01, 0.1, 1, or 10 mg sodium propionate intravenously. Blood samples were taken at 0 and 3 h after injection. The concentrations of serum total-cholesterol, HDL-cholesterol, and triacylglycerol were measured. Likewise, another 31 unfasted rats were given 400 μl saline supplemented with or without 0.01, 0.1, and 1 mg sodium propionate intravenously, with changes in serum total-cholesterol and HDL-cholesterol levels examined.

5. **Experiment 3.** For the purpose of evaluating the efficacy of continual sodium propionate administration on serum lipid levels, an osmotic pump (ALZET® Model 2ML2, Alza Co., California, U.S.A.) was implanted intraperitoneally in rats. The reservoir volume of the pump is 2ml with solution delivered continuously (5µl/h) for 14 days (15). The pump was filled with 0, 1.9, 19.2, and 192mg sodium propionate 2ml saline solution (i.e. 0, 0.12, 1.2, and 11.5mg/day). At day 0, 20 rats were anesthetized with sodium pentobarbital (50mg/kg body weight). The pump was inserted into the peritoneal cavity. The muscle incision was closed with sutures (No. 5 suture, Natsume Seisakusyo, Tokyo), and the skin incision closed with wound clips (CHR. Diener oHG., Germany). The implantation-operated rats were kept for 21 days on a semisynthetic diet (see above). Blood samples were taken from the right jugular vein at days 0, 7, 14, and 21, with concentrations of serum total-cholesterol, HDL-cholesterol, and triacylglycerol measured.

6. **Measurement of serum lipid levels.** The concentrations of serum total-cholesterol, HDL-cholesterol, and triacylglycerol were measured by commercial diagnostic kits (enzymatic, colorimetric, endpoint method) purchased from Wako Pure Chem. Ind. Ltd., Osaka.

7. **Determination of statistical significance.** Differences between means were tested by Student's t-test after preliminary analysis of variance (ANOVA). The difference was considered significant when the probability was smaller than 0.05.

RESULTS

1. **Experiment 1**

Figure 1 shows the changes in serum cholesterol levels following intravenous saline injection supplemented with and without 1mg sodium propionate. In the sodium propionate-injected group, the concentrations of serum total-cholesterol from 3 to 24h were significantly lower compared with its starting level. At both 3 and 24h the percentage of changes in serum total-cholesterol levels in the sodium propionate group were significantly lower than in the saline group. The efficacy of sodium propionate on reducing serum total-cholesterol levels attained a maximum from 3h after injection (85.4±4.0mg/dl, 83% level of saline injection). On the other hand, the concentrations of serum HDL-cholesterol gradually reduced in both the groups, with no significant difference found between the two groups except for the percentage change at 1h.

2. **Experiment 2**

Table 1 shows the influence of intravenous sodium propionate injection on serum lipid levels in fasting rats. Sodium propionate produced a significant reduction of serum total-cholesterol levels at 1mg (90.3±4.7mg/dl) as compared with saline alone (107±4.6mg/dl). Although the intensity of the reduction increased with an increase in the propionate concentration of 0.01 (percentage of
change: $92.0\pm1.8\%$ to $1\text{mg}\ (82.9\pm1.5\%),$ $10\text{mg}$ sodium propionate no longer increased the intensity of reduction ($84.3\pm1.8\%)$ compared to $1\text{mg}$. Both the concentration and percentage of changes in HDL-cholesterol levels tended to decrease in all groups. The percentage of changes in serum triacylglycerol levels were significantly reduced by $0.01$ and $0.1\text{mg}$ sodium propionate compared with saline alone, but not by $1$ and $10\text{mg}$ sodium propionate.

Table 2 shows the effects of intravenous sodium propionate injection on serum cholesterol levels in unfasting rats. The concentration of serum total-cholesterol at the outset was $10$ to $25\%$ higher than in the fasting rats (Table 1). Though no statistical significance was found, the percentage of changes of total-cholesterol and HDL-cholesterol increased with increases in the sodium propionate concentrations of $0.01$ to $1\text{mg}$.

3. **Experiment 3**

Table 3 shows the concentration of serum lipid levels at day 0 (before osmotic pump implantation). No significant difference was found among the groups.

Figure 2 shows the changes in serum lipid levels for 21 days following implantation of an osmotic pump. Total-cholesterol levels reduced significantly following continual sodium propionate administration at $0.12$ to $1.2\text{mg/day}$, however, the intensity of the reduction at $11.5\text{mg/day}$ did not increase compared with $0.12$ to $1.2\text{mg/day}$. The maximum percentage of change in serum total-cholesterol by sodium propionate was $78.5\pm6.7\%$ observed at $1.2\text{mg/day}$ at day 7. The effect of

Table 1. Effects of intravenous injection of sodium propionate on serum lipid levels in fasting rats (Experiment 2).

<table>
<thead>
<tr>
<th>Sodium propionate injection</th>
<th>N</th>
<th>Total-cholesterol (mg/dl)</th>
<th>HDL-cholesterol (mg/dl)</th>
<th>Triacylglycerol (mg/dl)</th>
<th>Start</th>
<th>At 3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mg (saline)</td>
<td>10</td>
<td>109±4.6</td>
<td>96.8±5.4</td>
<td>83.5±5.9</td>
<td>84.3±5.7</td>
<td>72.5±3.7</td>
</tr>
<tr>
<td>0.01 mg</td>
<td>6</td>
<td>106±7.1</td>
<td>92.0±1.8*</td>
<td>81.8±4.7*</td>
<td>90.2±2.4</td>
<td>78.8±1.7</td>
</tr>
<tr>
<td>0.1 mg</td>
<td>6</td>
<td>109±7.7</td>
<td>90.3±4.7*</td>
<td>84.3±4.7*</td>
<td>90.3±4.7*</td>
<td>78.2±3.9</td>
</tr>
<tr>
<td>1 mg</td>
<td>10</td>
<td>109±7.7</td>
<td>90.3±4.7*</td>
<td>84.3±4.7*</td>
<td>96.5±5.0</td>
<td>73.0±7.5</td>
</tr>
<tr>
<td>10 mg</td>
<td>6</td>
<td>115±7.7</td>
<td>96.5±5.0</td>
<td>84.3±4.7*</td>
<td>88.5±3.1</td>
<td>77.0±7.0</td>
</tr>
</tbody>
</table>

Rats were fasted for 16h and given saline supplemented with or without sodium propionate intravenously. Mean±SE statistical comparisons were made against saline injection (*) and its starting level (**) (p<0.05).
Table 2. Effects of intravenous injection of sodium propionate on serum cholesterol levels in unfasting rats (Experiment 2).

<table>
<thead>
<tr>
<th>Sodium propionate injection</th>
<th>N</th>
<th>Total-cholesterol (mg/dl) Start</th>
<th>At 3 h</th>
<th>HDL-cholesterol (mg/dl) Start</th>
<th>At 3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 mg (saline)</td>
<td>8</td>
<td>119±2.4</td>
<td>117±2.9</td>
<td>59.8±3.1</td>
<td>62.1±2.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(100%)</td>
<td>(98.4±1.3%)</td>
<td>(100%)</td>
<td>(105±4.8%)</td>
</tr>
<tr>
<td>0.01 mg</td>
<td>8</td>
<td>126±6.7</td>
<td>124±6.6</td>
<td>65.6±2.2</td>
<td>65.6±2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(100%)</td>
<td>(98.4±2.1%)</td>
<td>(100%)</td>
<td>(101±4.4%)</td>
</tr>
<tr>
<td>0.1 mg</td>
<td>6</td>
<td>125±5.8</td>
<td>113±6.1</td>
<td>64.8±3.3</td>
<td>59.6±4.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(100%)</td>
<td>(91.0±3.1%)</td>
<td>(100%)</td>
<td>(92.3±5.8%)</td>
</tr>
<tr>
<td>1 mg</td>
<td>9</td>
<td>136±7.5</td>
<td>122±4.6</td>
<td>70.3±3.0†</td>
<td>63.3±3.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(100%)</td>
<td>(91.0±3.1%)</td>
<td>(100%)</td>
<td>(90.0±3.5%)</td>
</tr>
</tbody>
</table>

Unfasted rats were given saline supplemented with or without sodium propionate intravenously. Mean±SE: statistical comparisons were made against saline injection (†) (p<0.05).

Table 3. The concentrations of serum lipid at day 0 (Experiment 3).

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Total-cholesterol (mg/dl)</th>
<th>HDL-cholesterol (mg/dl)</th>
<th>Triacylglycerol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline alone</td>
<td>5</td>
<td>100±3.9</td>
<td>74.3±2.7</td>
<td>65.3±11.0</td>
</tr>
<tr>
<td>Sodium propionate (0.12 mg/day)</td>
<td>5</td>
<td>114±6.9</td>
<td>89.5±6.9</td>
<td>64.8±17.2</td>
</tr>
<tr>
<td>Sodium propionate (1.2 mg/day)</td>
<td>5</td>
<td>111±7.1</td>
<td>89.5±4.1</td>
<td>62.5±16.2</td>
</tr>
<tr>
<td>Sodium propionate (11.5 mg/day)</td>
<td>5</td>
<td>100±7.7</td>
<td>79.6±8.6</td>
<td>74.8±14.3</td>
</tr>
</tbody>
</table>

Blood samples were taken before operation. Mean±SE.

Sodium propionate on reducing total-cholesterol levels decreased at day 21 (ALZET® Model 2ML2 pump delivers the solution for 14 days). Serum HDL-cholesterol levels decreased in all the groups. The percentage of changes at day 7 were 55.3±1.9% (saline) and 50.0±2.4% (1.2 mg/day sodium propionate). At day 14, HDL-cholesterol levels had recovered to 70.0±2.1% (saline) and 63.0±2.7% (1.2 mg/day sodium propionate). No similar tendency was observed in triacylglycerol levels. No significant difference was found among the groups in changes in body weight gain (66.8±3.9–86.8±3.7 g) during the test period.

**DISCUSSION**

Wright et al. reported that sodium propionate significantly inhibited cholesterol and fatty acid de novo synthesis in isolated rat hepatocytes (11). Since the cecal content of propionate can be altered by the content of dietary fiber, the effectiveness
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Fig. 2. Percentage changes in serum lipid levels for 21 days following an osmotic pump implantation (Experiment 3). Mean±SE (five rats/group): statistical comparisons were made against saline injection (') (p<0.05). ●, saline; ○, 0.12 mg/day sodium propionate; △, 1.2 mg/day sodium propionate; □, 11.5 mg/day sodium propionate.

of lowering serum lipid levels by dietary fiber is thought to be mediated by the action of propionate on the lipid metabolism in the liver. Recently, Nishimura et al. demonstrated that SCFA in the cecum correlated negatively with plasma total-cholesterol levels in rats (12). Furthermore, Chen et al. (16) and Illman et al. (17) demonstrated that the propionate-supplemented diet significantly reduced both serum and liver cholesterol levels in rats. In the present study, to obtain further evidence for the reducing effects of sodium propionate on serum lipid levels, both intravenous injection and intraperitoneal continual administration were conducted in rats. Rats were kept on a cholesterol-free semisynthetic diet (13) in order to exclude the effects of dietary cholesterol on serum cholesterol levels, and intravenous injection and intraperitoneal continual administration were performed.

Three hours following intravenous injection of 1 mg sodium propionate, the serum total-cholesterol level was significantly reduced compared with its starting level, with the reducing effect lasting for 24 h. The intensity of the reduction increased proportionately with increased sodium propionate concentrations of 0.01 to 1 mg. Still, the intensity of the reducing effect of sodium propionate on serum cholesterol no longer increased at 10 mg as compared to 1 mg. Illman et al. reported that portal vein concentrations of propionate (not sodium propionate) increase to 0.8 mM (0.06 mg/ml) in rats fed a variety of diets high in soluble fiber (17). Furthermore, as mentioned above, Wright et al. reported that the intensity of the inhibitory effect of sodium propionate on lipid metabolism no longer increased sodium propionate concentration more than 1 mM (0.10 mg/ml). The plasma volume of rats weighing 300 g is estimated to be 10 ml (3.26±0.09 ml/100 g) body weight reported by Yale et al. (18). Following intravenous injection of 1 mg
sodium propionate, the serum level increased 0.1 mg/ml. Thus, the incremental increase of serum sodium propionate levels following injection is higher than that of the above mentioned effective dose \((11, 17)\). Therefore, it is considered that the intensity of the reducing effect of sodium propionate on serum cholesterol reached a limit at 1 mg. Likewise, the percentage of changes in serum triacylglycerol levels were significantly reduced by 0.1 mg sodium propionate, with the reducing effect no longer enhanced at 1 or 10 mg.

Next, to elucidate the concern between propionate-mediated reduction of serum cholesterol and triacylglycerol levels and the hypocholesterolemic effects of certain soluble dietary fibers, intraperitoneal continual administration was examined. An osmotic pump was filled with a wide range of propionate concentrations \((0–11.5 \text{ mg/day})\) and was implanted in the rats as an artificial cecum. At day 14, total-cholesterol levels were significantly reduced by both 0.12 and 1.2 mg/day sodium propionate, as compared to saline alone. It is curious that HDL-cholesterol levels were reduced by saline similarly to that of sodium propionate. At day 21, 7 days after the osmotic pump was inactivated, the reducing effect of sodium propionate on serum cholesterol levels diminished. These observations suggest that intraperitoneal continual administration of sodium propionate can produce a significant reduction of serum total-cholesterol levels. Although serum triacylglycerol levels were significantly reduced by intravenous sodium propionate injection, a similar tendency was not observed following intraperitoneal continual administration. The difference between the two types of administration is obscure.

In conclusion, the present data indicate that sodium propionate can reduce serum total-cholesterol levels in vivo.

REFERENCES


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