Effect of Norethisterone Enanthate on Intestinal Digestive and Absorptive Functions in Protein Deficient Rat

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Abstract

The effect of norethisterone enanthate (NET; 2 mg/100 g body weight per week for four weeks) on the digestive and absorptive characteristics of the intestinal epithelium has been investigated in protein deficient female rats. The drug did not cause significant changes in the absorptive functions with respect to amino acids or calcium but significantly augmented the intestinal uptake of glucose. This steroidal contraceptive had no effect on the activities of the brush border membrane enzymes, alkaline phosphatase and leucine aminopeptidase, but significantly decreased the specific activities of sucrase and maltase in pair-fed animals. In contrast, NET significantly increased the activities of these enzymes in protein deficient animals. The changes in sucrase activity may be attributed to an alteration in Vmax by the drug.

Key words: Norethisterone enanthate, nutrient absorption, small intestinal functions, protein deficiency, brush border membrane.

Introduction

Medroxyprogesterone acetate (MPA) and norethisterone enanthate (NET) are long acting steroidal contraceptives. The former has been shown to exhibit glucocorticoid-like effects on carbohydrate metabolism and drug-metabolizing enzymes while the latter is devoid of these properties. Earlier, we have shown that MPA also has a glucocorticoid-like action on intestinal absorptive and digestive functions in female rats fed ad libitum normal diet4 and in protein deficient rats.5 The response of absorptive functions to MPA was similar in nature in pair-fed as well as in protein deficient rats. However, following MPA administration activities of intestinal brush border membrane digestive enzymes, sucrase and leucine aminopeptidase, were depressed in pair-fed animals and elevated in protein deficient rats.5 The present work evaluates the influence of NET on intestinal absorptive and digestive functions in protein deficient female rats.

Material and Methods

Two groups of 40 female albino rats each (150-175 g, Wistar derived) of the Institute Colony were used. Protein deficiency was produced in one group by feeding a synthetic diet containing 8% casein. The diets were made isocaloric by replacing the protein with carbohydrate. After keeping the animals for 13 days on their respective diets, each group was divided equally into control and experimental subgroups.

The experimental groups were injected intramuscularly NET (Noristerat, Schering AG, Berlin-Bergkamen) at a dose of 2 mg/100 g body weight in a vehicle (poly-L-ornithine 80—0.237 mg; propylparaben—0.1349 mg; propylparaben—0.0140 mg; propylene glycol 4000—2.852 mg; sodium chloride—0.8567 mg per 0.1 ml), and the controls received an equal volume (0.1 ml) of vehicle per week on days 0, 7, 14 and 21. The animals were killed on day 24 after overnight fast. The entire small intestine was removed, flushed with cold saline and everted. The uptake of D-glucose, L-leucine, L-alanine and calcium was determined by tissue accumulation method.6 Intestinal rings were incubated in 2 ml oxygenated Krebs-Ringer buffer, pH 7.4 containing 5 mM D-glucose or L-leucine or L-alanine with a trace of (U-14C) D-glucose or amino acid, for 5 min at 37°C in a shaking water bath. Calcium uptake in the duodenum was determined using 5 ml of Tris-HCl buffer, pH 6.8 (140 mM NaCl, 6 mM KCl, 4 mM Tris), containing 2 mM calcium along with trace amounts of 45Ca. The incubations were carried out at 37°C for 30 min. At the time of incubation, tissues were removed, blotted gently and weighed. The radioactivity taken up by the tissues was counted in an LKB 1215 Rackbeta Liquid Scintillation Counter. After correcting for extra-cellular space measured with 3H-inulin, the uptake rate was calculated.

Brush border membranes were prepared from the intestinal mucosal scrapings. The membranes were suspended in 50 mM sodium malate buffer, pH 6.8 and the purity was checked by marker enzyme sucrase. The preparation exhibited 18-20 fold purification. Brush border membrane disaccharidases, leucine aminopeptidase and alkaline phosphatase were assayed. Protein was estimated by the method of Lowry et al.12 Kinetic studies on sucrase were carried out and Km and Vmax values were calculated from the Lineweaver-Burk plot. Statistical analysis was done by Student's t test.

Results

Rats fed the protein deficient diet had significant reduction in body weight, serum total proteins and albumin as compared to pair-fed controls. However, NET treatment did not induce any significant change in these parameters (Table 1).
NET did not produce any significant change in the uptake of amino acids and calcium in pair-fed as well as in protein deficient rats. However, this drug significantly (p<0.01) elevated the uptake of glucose by intestinal segments (Table 2).

NET treatment did not affect the brush border alkaline phosphatase and leucine aminopeptidase activities, but a significant decrease in the activities of sucrase and maltase in pair-fed rats and an increase in their activities in protein deficient rats was observed in response to NET treatment (Table 3). Lactase activity was augmented in NET treated rats fed protein deficient diet. The kinetic studies conducted with brush border sucrase showed change in Vmax without altering the affinity constant (Km=2mM). The Vmax

Table 1: Effect of NET on body weight and serum total protein and albumin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pair-fed control</th>
<th>Pair-fed treated</th>
<th>Protein deficient control</th>
<th>Protein deficient treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g) (n = 15)</td>
<td>163±6.7</td>
<td>168±5.8</td>
<td>162±6.8</td>
<td>167.5±7.1</td>
</tr>
<tr>
<td>Final body weight (g) (n = 15)</td>
<td>168±8.1</td>
<td>171±7.2</td>
<td>153±6.6</td>
<td>155.5±5.8*</td>
</tr>
<tr>
<td>Gain or loss (g) in body weight (g) (n = 15)</td>
<td>5.0±3.1</td>
<td>3.0±1.8</td>
<td>(-)9.4±4.8**</td>
<td>(-)12±5.7</td>
</tr>
<tr>
<td>Serum total protein (g/dl) (n = 6)</td>
<td>6.7±0.1</td>
<td>6.3±0.88</td>
<td>4.9±0.95*</td>
<td>5.3±0.77</td>
</tr>
<tr>
<td>Serum albumin (g/dl) (n = 6)</td>
<td>3.5±0.44</td>
<td>3.8±0.67</td>
<td>2.2±0.63</td>
<td>2.6±0.61</td>
</tr>
</tbody>
</table>

Values are mean±SD. p<0.05 **<0.001 as compared to pair-fed control p<0.01 as compared to initial body weight.

Table 2: Effect of NET treatment on intestinal uptake of nutrients

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Pair-fed control</th>
<th>Pair-fed treated</th>
<th>Protein deficient control</th>
<th>Protein deficient treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Glucose</td>
<td>5.4±0.83</td>
<td>4.9±0.64</td>
<td>5.12±0.68</td>
<td>6.3±0.88*</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>2.8±0.47</td>
<td>2.4±0.41</td>
<td>2.5±0.52</td>
<td>2.6±0.42</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>3.0±0.48</td>
<td>2.6±0.37</td>
<td>2.6±0.41*</td>
<td>2.4±0.46</td>
</tr>
<tr>
<td>Calcium</td>
<td>1.8±0.31</td>
<td>2.0±0.42</td>
<td>1.2±0.23**</td>
<td>1.38±0.29</td>
</tr>
</tbody>
</table>

Values are mean±SD of 8 observations. Uptake of glucose and amino acids = μ moles/5 min/g wet tissue. Uptake of calcium = μ moles/hr/g wet tissue.

p<0.05 **<0.01 as compared to pair-fed control p<0.01 as compared to protein-deficient control.

Table 3: Effect of NET treatment on brush border membrane enzyme

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Pair-fed control</th>
<th>Pair-fed treated</th>
<th>Protein deficient control</th>
<th>Protein deficient treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrase</td>
<td>1216.5±141.8</td>
<td>808±90.6*</td>
<td>1304±153.1</td>
<td>2100±192.82</td>
</tr>
<tr>
<td>Lactase</td>
<td>47.9±3.8</td>
<td>49.4±4.1</td>
<td>42.3±5.1</td>
<td>65.±10.4</td>
</tr>
<tr>
<td>Maltase</td>
<td>8216.6±700.1</td>
<td>6053±540.3**</td>
<td>7680±101.8</td>
<td>9870±70.11</td>
</tr>
<tr>
<td>Leucine aminopeptidase</td>
<td>1066.2±133.4</td>
<td>903±78.6</td>
<td>778.5±64.5*</td>
<td>644±83.70</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>6560±687.2</td>
<td>6382±722.5</td>
<td>6104±572.6</td>
<td>5909±742.4</td>
</tr>
</tbody>
</table>

One Unit is equal to μ mole of the substrate hydrolysed per minute under standard assay conditions. Values are mean±SD of 3 brush border membrane preparations.

p<0.05 **<0.01 as compared to pair-fed control p<0.01 as compared to protein deficient control

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values in pair-fed and protein deficient controls were found to be the same (3333 units). NET treatment reduced this value to 2000 units in the pair-fed group while it increased to 5000 units in the protein deficient treated group.

Discussion

Unlike MPA,6,8 NET did not produce significant changes in the intestinal absorptive functions. The elevated elevation in the uptake of glucose along in protein deficient animals may either be a consequence of the drug action or of increased levels of the glucocorticoids generally observed in protein deficient state.10 Thus NET per se may not influence appreciably the intestinal uptake of various nutrients.

Dietary factors (carbohydrate and protein) appear to be responsible for the opposite effects of the drug on sucrose and maltose in pair-fed and protein deficient rats. The relatively high levels of carbohydrate in the protein deficient diet, along with this drug, may stimulate the activities of these enzymes. It has been reported that the substrate sucrose along with glucocorticoids enhances the activity of sucrose.11 This differential effect may also be attributed to differences in pharmacokinetics of the drug arising out of differences in the levels of drug metabolizing enzymes in pair-fed and protein deficient rats.

Long term therapy with steroidal contraceptives is known to be responsible for a number of metabolic disturbances.13 One of the factors which can contribute to these disturbances may be the rate at which nutrients appear in circulation, which in turn is dependent upon the digestive and absorptive functions of the intestine. It has been reported that a high rate of carbohydrate and amino acid infusion results in disturbances of metabolic homeostasis.16,17

The present studies indicate that, in comparison with MPA,6,8 NET was less effective in influencing the intestinal digestive and absorptive functions in protein deficient rats. It may partly explain the greater potency of MPA in causing disturbances in metabolic homeostasis as compared to NET.18

References