Increasing density of glucagon receptors in liver from endurance-trained rats

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Légaré, A., R. Drouin, M. Milot, D. Massicotte, F. Péronnet, G. Massicotte, and C. Lavoie. Increased density of glucagon receptors in liver from endurance-trained rats. Am J Physiol Endocrinol Metab 280: E193–E196, 2001.—The binding properties of glucagon receptors were determined in plasma membranes isolated from liver of untrained (n = 6) and swimming endurance-trained Sprague-Dawley male rats (n = 7; 3 h/day, 5 days/wk, for 8 wk). Plasma membranes were purified from liver by aqueous two-phase affinity partitioning, and saturation kinetics were obtained by incubation of plasma membranes (10 μg of proteins/150 μl) with 125I-labeled glucagon at concentrations ranging from 0.15 to 3.0 nM for 30 min at 30°C. Saturating curve analysis indicated no difference in the affinity of glucagon receptors (0.57 ± 0.06 and 0.77 ± 0.09 nM in untrained and trained groups, respectively) but a significant higher glucagon receptor density in liver from untrained vs. trained rats (3.09 ± 0.12 vs. 4.28 ± 0.19 pmol/mg proteins). These results suggest that the reported increase in liver glucagon sensitivity in endurance-trained subjects (Drouin R, Lavoie C, Bourque J, Ducros F, Poisson D, and Chiasson J-L. Am J Physiol Endocrinol Metab 274: E23–E28, 1998) could be partly due to an increased glucagon receptor density in response to training.

Methods

Animals and training. The experiment was conducted on 13 male Sprague-Dawley rats (Anilab, Ste-Foy, QC, Canada) randomly assigned to the untrained (n = 6) or trained group (n = 7). Body mass at the time of death was not significantly different in trained (418 ± 28 g) and untrained rats (433 ± 9 g). The animals were kept in individual cages at 20°C and 55% relative humidity, with a 12:12-h light-dark cycle, in a facility that met the Canadian Council of Animal Care guidelines. They had access to a standard rat chow and water ad libitum. The protocol was approved by the Animal Care Committee of the Université du Québec à Trois-Rivières.

The rats were trained 5 days/wk for 8 wk in a 60 × 90-cm tank filled with 50 cm of water at 37°C. The duration of each training session, which took place between 8:00 and 11:00 AM, was progressively increased to reach 3 h over the first 2

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Table 1. CS activity, plasma insulin and glucagon concentrations, and properties of liver glucagon receptors from untrained (n = 6) and trained (n = 7) animals

<table>
<thead>
<tr>
<th></th>
<th>Untrained</th>
<th>Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS activity in rectus femoris, μmol·min⁻¹·g⁻¹</td>
<td>20.7 ± 2.5</td>
<td>27.9 ± 1.6*</td>
</tr>
<tr>
<td>Plasma insulin, pmol/l</td>
<td>326.9 ± 13.0</td>
<td>271.4 ± 24.0*</td>
</tr>
<tr>
<td>Plasma glucagon, nmol/l</td>
<td>33.5 ± 2.7</td>
<td>30.6 ± 1.2</td>
</tr>
<tr>
<td>( B_{max}, ) pmol/mg proteins</td>
<td>3.09 ± 0.12</td>
<td>4.28 ± 0.19*</td>
</tr>
<tr>
<td>( K_d, ) nM</td>
<td>0.57 ± 0.06</td>
<td>0.77 ± 0.09</td>
</tr>
</tbody>
</table>

Values are means ± SE. CS, citrate synthase; \( B_{max}, \) maximal density, \( K_d, \) affinity, of glucagon receptors. *Significantly different from untrained rats (P < 0.05).

wk. The animals assigned to the untrained group were handled daily.

Tissue sampling and plasma membrane purification. The animals were anesthetized (pentobarbital sodium 50 mg/kg ip) at 8:00 AM, after an overnight with free access to food and water. After the last bout of exercise for the trained group, Blood (3 ml) was collected on EDTA via the abdominal aorta. After centrifugation at 3,000 g, the plasma was removed, and stored along with the rectus femoris at –80°C until analysis.

Liver plasma membranes were prepared according to the procedure developed by Persson and Jergil (19). The liver was immediately chilled in ice-cold 0.25 M sucrose in 5 mM Tris-HCl at pH 8.0, cut into small pieces, and transferred to a Dounce homogenizer containing an aqueous two-phase system. This system was prepared to contain a final concentration of 5.7% (w/v) Dextran T-500 (Pharmacia Biotech, Piscatway, NJ), 5.7% (w/v) polyethylene glycol 3350 (Sigma-Aldrich, Oakville, ON, Canada), 0.15 mM Tris-HCl at pH 7.8, and 2.5% HSA in HEPES buffer (50 mM, pH 7.6). The entire purification procedure was performed at 4°C. The recovery of plasma membrane, assessed by the membrane marker 5’-nucleotidase (Sigma-Aldrich), averaged 63.5 ± 7.0%. The protein content in pellets used for the binding assay was determined by the method of Bradford (Bio-Rad Protein Assay, Bio-Rad Laboratory, Mississauga, ON, Canada).

Binding assay. The receptor binding assay used is a modification of the technique described by Frandsen et al. (12). Purified membranes (10 μg proteins/150 μl), in triplicate, were incubated with \(^{125}\)I-labeled glucagon (NEC Life Science Products, Boston, MA) at concentrations ranging from 0.15 to 3.00 nM in HEPES buffer (50 mM, pH 7.6) containing 1% human serum albumin (HSA; Sigma-Aldrich). Incubations were carried out at 30°C for 30 min in a total volume of 150 μl. Aliquots of 100 μl were added to microfuge tubes containing 200 μl of cold (4°C) 2.5% HSA in HEPES buffer (50 mM, pH 7.6). Free and membrane-bound \(^{125}\)I-glucagon were separated by centrifugation at 10,000 g for 5 min, and the supernatant was discarded. The membrane pellet was washed once with 200 μl of cold HEPES buffer containing 2.5% HSA, and the membrane-bound radioactivity was determined (gamma counter, Wallac 1470 Wizard, Wellesley, MA). The nonspecific binding was measured in presence of 3 × 10⁻⁸ M glucagon (Sigma-Aldrich). In the present study, based on preliminary experiments, an incubation time of 30 min with membrane protein concentration of 10 μg/150 μl was selected to ensure saturation kinetic sensitivity. The maximal density (\( B_{max}, \)) and the apparent affinity (\( K_d, \)) of glucagon receptors were obtained through rectangular hyperbolic regression of the specific binding curve (GraphPad Software, San Diego, CA).

Analysis. Plasma insulin (Medicorp, Montreal, QC, Canada) and glucagon (Diagnostic Products, Los Angeles, CA) concentrations were measured by radioimmunoassays with commercially available kits. The activity of citrate synthase...
was determined in the rectus femoris according to the method suggested by Srere (22).

Statistics. Data are presented as means ± SE. Comparisons were made using Student’s t-tests, and significance was set at the $P < 0.05$ level.

RESULTS

As shown in Table 1, citrate synthase activity in rectus femoris was significantly 27% higher in trained than in untrained rats, whereas plasma insulin concentration was significantly 17% lower. In contrast, no significant change was observed for plasma glucagon concentration.

The amount of $^{125}$I-glucagon bound to purified liver plasma membranes plateaued with glucagon concentrations ranging between 1.5 and 3.0 nM in both groups. A significant larger binding was observed in trained rats (0.21 ± 0.02 vs. 0.16 ± 0.02 nM in untrained rats; Fig. 1). A Scatchard plot of saturation curves for plasma membranes purified from liver of trained and untrained rats is shown in Fig. 2. The $K_d$ of glucagon receptors for the ligand was similar in liver plasma membranes for both groups, but $B_{max}$ was higher in liver plasma membranes from trained than untrained rats (Fig. 2 and Table 1).

DISCUSSION

Drouin et al. (10) showed that endurance training was associated with an increased liver glucose production in response to glucagon infusion in humans. In their study, sedentary and endurance-trained subjects were studied at rest. When endogenous insulin and glucagon secretions were suppressed by somatostatin and replaced to achieve similar physiological concentrations in both groups, liver glucose production was 53% higher in trained vs. untrained subjects (15.8 ± 2.8 vs. 7.4 ± 1.6 mol·kg$^{-1}$·min$^{-1}$, respectively). Preliminary data from our laboratory (11, 15) and from Cheeks et al. (5), with the use of in situ liver perfusion, indicate that, upon glucagon stimulation, glycogenolysis and gluconeogenesis are both significantly higher in liver from endurance-trained rats.

Results from the present experiment are in line with these observations in both animals and humans and suggest that the increased liver sensitivity to glucagon associated with training could be due, at least in part, to an increased glucagon receptor density in liver. Indeed, 8 wk of swimming endurance training in rats increased by ~28% the density of glucagon receptors measured by use of a radiolabeled binding assay in plasma membranes from the liver, with no change in the affinity of the receptors. The density and affinity of glucagon receptors measured in purified liver plasma membranes from untrained rats (3.09 ± 0.12 pmol/mg proteins and 0.57 ± 0.06 nM, respectively) were within the range of values previously reported: from 1.8 to 3 pmol/mg proteins and from 0.1 to 1.2 nM, respectively (8, 16, 21). In purified liver plasma membranes from trained rats, the affinity of glucagon receptors was in the same range (0.77 ± 0.09 nM), but their density was much higher (4.28 ± 0.19 pmol/mg proteins).

The only direct evidence of changes in properties of liver glucagon receptors is a reduction in density reported in streptozotocin-induced diabetes in rats (~40% over 5 days) (3, 9) and in healthy rats after acute (~20% within 5–60 min after a single bolus) (2) or chronic glucagon administration (~33% over 7 days) (3). The reduction in liver glucagon receptor density in diabetes could be responsible for the lower glucagon sensitivity shown by Ørskov et al. (18) in type 1 diabetic subjects and could explain, in part, the defective counterregulatory mechanisms to hypoglycemia observed in diabetic patients (7, 18, 24). As for the effect of exercise, the only indirect evidence suggesting that exercise training could increase glucagon receptor density and/or affinity in the liver is a study by Nieto et al. (17). In that experiment, the increase in adenylate cyclase activity over a wide range of glucagon concentrations was higher in purified liver plasma membranes from endurance-trained than from untrained rats.

Results from the present experiment extend the observations by Authier et al. (2), Bhathena et al. (3), and Dighe et al. (9) and indicate that, in addition to streptozotocin-induced diabetes and glucagon administration, training also modifies the binding characteristics of liver glucagon receptors. However, the mechanisms by which the density of glucagon receptors in the liver could be modified in various situations remain poorly understood. The expression of glucagon receptor mRNA in vitro has been shown to increase when oxygen (14) and glucose concentrations (1, 4, 20, 23) increase and to decrease when cAMP concentration increases (1). Data from Authier et al. and Bhathena et al. also indicate that glucagon can downregulate the density of its receptors both quickly (5–60 min after a single bolus) and chronically (24 h after 7 days of twice daily glucagon injection). These and other factors could be modified after chronic regular swimming exercise. It remains, however, difficult at the present time to speculate on the mechanisms by which 8 wk of swimming endurance training increased the density of liver glucagon receptors in rats.

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