Effect of hypothyroidism and thyroid hormone replacement on the level of protein kinase C and protein kinase A in rat liver

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We investigated the influence of the thyroid hormone status on the levels of protein kinases C (PKC) and A (PKA) in the soluble fraction of rat liver. The immunodetectable PKC level in hypothyroid liver was elevated 7.5-fold, whereas the phorhol-ester binding capacity and the immunodetectable a-PKC level were increased 2.4- and 2.6-fold, respectively. Conversely, in hypothyroid livers the abundance of the regulatory type I and the catalytic subunits of PKA were lowered to 42% of the euthyroid level as determined by immunoblotting and by measuring the substrate specific phosphorylation rate of PKA. These changes in the PKC and PKA levels were reversible upon treatment with 0.5 μg T₄/100 g body weight for 2-21 days. The thyroid state dependent alterations in hepatic PKC and PKA levels may be responsible for the known changes in the response of hepatocytes to other hormonal stimuli in hypothyroidism.

Protein kinase C: Protein kinase A: Thyroid hormone: Rat liver: Hypothyroidism

1. INTRODUCTION

Thyroid hormones exert most, if not all of their actions by binding to nuclear receptors, thereby regulating the expression of specific genes [1]. It has been estimated that approximately 8% of the hepatic genes are regulated by thyroid hormones in vivo [2]. Recently, thyroid hormones have been shown to regulate the mRNA levels of specific G-protein subunits in adipose tissue and heart in vivo [3,4] providing evidence for a modulation of transmembrane signalling of other hormones by thyroid hormones [5]. The signal transduction regularly results in the activation of various protein kinases. The phosphorylation of serine, threonine and tyrosine residues by these enzymes has been demonstrated to be a common cellular mechanism for regulating gene transcription, cell division, membrane transport and metabolic pathways [6]. Thyroid hormones have been reported to stimulate the phosphorylation or dephosphorylation of multiple rat liver cytosolic proteins [7]. A 1.5-fold increase in type I cyclic AMP dependent protein kinase (PKA type I) activity in the liver of hyperthyroid compared to hypothyroid rats has been demonstrated by electrophoresis of cytosolic proteins followed by phosphorylation assays [8]. In contrast to the studies on PKA, no evidence has been presented on the influence of thyroid hormones on the PKC levels in any organ. Protein kinase C is a family of enzymes encompassing at least 7 subspecies, which has been shown to mediate and modulate transmembrane signalling and which is proposed to play a crucial role in governing cell metabolism and growth [9]. Choosing the liver as an important target organ of thyroid hormone action we investigated whether the levels of α-, β-, γ-PKC and PKA type I or II are altered in the liver cytosol of hypothyroid rats and whether these changes can be reversed by treatment with L-thyroxine (T₄).

2. MATERIALS AND METHODS

2.1. Animals

25 male Wistar rats (100-120 g) were randomized into 5 different treatment groups and were rendered hypothyroid by feeding an iodine depleted diet and by adding 1% KCIO₄ to the drinking water for 35 days. 0.1% 6a-propyl-2-thiouracil was added to the diet for the first 6 days (modified from [2]). Five control rats were maintained on the iodine depleted chow with 1 ppm potassium iodide added to the drinking water. The free T₄ and total L-triiodothyronine serum levels in the hypothyroid group were 1 ± 0.0% and 13 ± 4% of the euthyroid (iodide substituted) group, respectively. The hypothyroid animals of 4 of the 5 groups were daily substituted orally with 0.5 μg T₄/100 g body weight for 2, 5, 9 and 21 days, respectively. Vehicle was administered to one hypothyroid and the iodine-substituted euthyroid group for 11 and 22 days, respectively. While under a short ether anesthesia, the liver was excised and placed into ice-cold buffer. This protocol has been approved by the Committee on Animal Care and Use of the Canton Basel-Stadt.

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Fig. 1. Time course of the PKC level in the rat liver cytosol as detected by a polyclonal antiserum against PKC upon replacement of hypothyroid rats with 0.5 μg T4/100 g body weight/d over 2, 5, 9 and 21 days. (A) Autoradiograph of the immunoblot of 3 random samples from each treatment group. (B) Densitometric quantitation of all samples (n = 5) from each treatment group. The results were normalized to the hypothyroid group.

2.2. Preparation of cytosol and immunoblotting

Two grams of liver were homogenized in a buffer containing 0.3 M sucrose, 10 mM EGTA, 2 mM EDTA, 10% glycerol, 20 mM HEPES, 13 mM 2-mercaptoethanol, 2 μg/ml aprotinin, 50 μg/ml leupeptin, pH 7.5. Cytosol was prepared by centrifugation of the homogenate for 15 min at 1000 × g; the supernatant was centrifuged for 60 min at 100,000 × g. The cytosols were diluted to the same protein concentration. For SDS polyacrylamide gel electrophoresis (SDS-PAGE) of cytosolic proteins the samples were supplemented with 3% SDS and boiled.

SDS-PAGE (60 μg protein/lane) and immunoblotting were performed as described [10]. The characterization and immunopurification of the polyclonal antiserum against pig brain PKC have been described previously [10]. The monoclonal anti-PKC antibodies against the α, β and γ subspecies were purchased from Seikagaku Kogyo Co., Ltd (Tokyo, Japan) and used in 1:200 dilutions. Pure PKA regulatory type I, II and catalytic subunits (R₁, R₂, C) and the corresponding antisera have been characterized earlier [11]. The membranes were developed with the appropriate 125I-labelled second antibody. The autoradiographs were quantitated by scanning densitometry; if possible the optical density of the specific band was normalized to that of an unspecific band.

3. RESULTS

3.1. Analysis of PKC levels

In euthyroid rats α-PKC was the primary form of this enzyme detected by the isotype-specific monoclonal antibodies. Using a polyclonal antiserum the level of cytosolic PKC in the livers of hypothyroid rats was found to be increased 7.7-fold compared to the euthyroid group. The PKC level declined by 46% after administering T₄ to hypothyroid rats for 2 days.

Table I

<table>
<thead>
<tr>
<th>Time course of the PKC and PKA levels in the liver cytosol of T4-treated hypothyroid rats.</th>
<th>Hypothyroid</th>
<th>+2d T₄</th>
<th>+5d T₄</th>
<th>+9d T₄</th>
<th>+21d T₄</th>
<th>Euthyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-PKC (IA)</td>
<td>100 ± 8</td>
<td>71 ± 10*</td>
<td>72 ± 15*</td>
<td>70 ± 8*</td>
<td>66 ± 8*</td>
<td>39 ± 7*</td>
</tr>
<tr>
<td>PDBu binding</td>
<td>100 ± 10</td>
<td>81 ± 12</td>
<td>65 ± 9*</td>
<td>N.D.</td>
<td>58 ± 3*</td>
<td>42 ± 6*</td>
</tr>
<tr>
<td>PKA-C (IA)</td>
<td>100 ± 5</td>
<td>118 ± 15</td>
<td>131 ± 19</td>
<td>147 ± 17*</td>
<td>165 ± 32*</td>
<td>242 ± 24*</td>
</tr>
<tr>
<td>PKA-R₁ (IA)</td>
<td>100 ± 11</td>
<td>89 ± 5</td>
<td>125 ± 18*</td>
<td>145 ± 31</td>
<td>188 ± 17*</td>
<td>238 ± 29*</td>
</tr>
<tr>
<td>Kemptide assay</td>
<td>100 ± 7</td>
<td>140 ± 5*</td>
<td>104 ± 2</td>
<td>N.D.</td>
<td>137 ± 7*</td>
<td>155 ± 12*</td>
</tr>
</tbody>
</table>

N.D. = not determined, IA = immunoreactivity. *P ≤ 0.05 vs hypothyroid (two-sided Mann-Whitney U-test). β-PKC levels were too low for reliable quantitation. γ-PKC and R₁ subunits could not be detected.
4, DISCUSSION

The presence of type I and II isoenzyme in rat liver has been demonstrated previously [13] and in the present study the R1, RII and C subunits of PKA were identified on immunoblots on the basis of their apparent molecular weight and the comigration with the pure subunits. In hypothyroid rats the content of R1 and C in the liver was reduced to 42% of the euthyroid level. Following T4 treatment the immunologically detected levels of regulatory type I and catalytic subunits as well as the kemptide phosphorylation rate increased in parallel (Table 1).

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4. DISCUSSION

Although the presence of PKC in the rat liver is well established [14] the increase in the PKC level in the liver cytosol of hypothyroid rats is a novel finding. The elevated PKC level was significantly reduced after 2 days of T4 treatment. The parallel changes in immunoactive α-PKC and PDBu binding as well as the lack of any detectable changes in β-PKC and the absence of γ-PKC suggest that mainly the α subseries is increased in hypothyroidism. While the α-PKC levels were increased 2.4-fold in hypothyroidism, the total PKC content as quantitated with a polyclonal antiserum directed against denatured PKC resulted in a 7.7-fold increase. This discrepancy may be due to the presence of additional forms of PKC (δ, γ-PKC) which has been demonstrated previously [13] and in the present study the R1, RII and C subunits of PKA were identified on immunoblots on the basis of their apparent molecular weight and the comigration with the pure subunits. In hypothyroid rats the content of R1 and C in the liver was reduced to 42% of the euthyroid level. Following T4 treatment the immunologically detected levels of regulatory type I and catalytic subunits as well as the kemptide phosphorylation rate increased in parallel (Table 1).

Preliminary evidence suggests, that changes in the PKC levels occur also in the brain, heart and kidney of hypothyroid rats as compared to euthyroid animals (data not shown). The functional consequences of an increased PKC content in hypothyroidism can only be speculated upon. Phorbol-myristate-acetate (PMA), an activator of PKC, is known to stimulate the expression of the proto-oncogenes c-myc and c-fos [15]. Increased expression of c-myc in the myocardiurn of hypothyroid rats has been described [16]; this change would be in accordance with increased PKC activity in this organ. An increased tissue content of PKC could inhibit transmembrane signalling, e.g. of α1 agonists, vasopressin, angiotensin II by desensitization of the phosphatidylinositol pathway, since PKC is known to down-regulate the signals which lead to its activation [15,17]. These considerations are supported by evidence demonstrating an inhibition of the effect of these hormones on rat hepatocytes after pretreatment with PMA [17-20]. The blunted glycogenolysis response to α1 agonists of hepatocytes from hypothyroid rats might be due in part to the homologous desensitization of the diacylglycerol/Ca2+ pathway by increased PKC levels [21].

Earlier studies have demonstrated the presence of the R1 and RII isotypes of PKA in the cytosol of rat liver [13]. In the present study, the R1 and C subunits of PKA and the kemptide phosphorylation rate increased with the duration of T4 replacement of hypothyroid rats. These thyroid state dependent alterations in PKA levels confirm earlier studies using different methods [8]. With the polyclonal antiserum used, the RII subunit could not be reliably detected in the liver cytosol; however, the close quantitative correlation between changes in the R1 and C subunits renders major alterations in the level of RII unlikely.

In conclusion, these results indicate that the hepatic levels of 2 protein kinases are dependent on the thyroid state of the animal; in hypothyroidism PKC levels are increased whereas PKA levels are decreased. These alterations may account for modified cellular responses to other hormonal stimuli during thyroid hormone deficiency. The mechanism by which thyroid hormones regulate the hepatic PKC and PKA content in vivo remains to be elucidated.

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REFERENCES