Poly(ADP-ribose) polymerase is affected early by thyroid state during liver regeneration in rats

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Poly(ADP-ribose) polymerase (PARP), a nuclear enzyme involved in DNA synthesis, DNA repair, and cell replication and transformation, also plays a role in the early steps of liver regeneration induced by partial hepatectomy (PH). PARP and DNA topoisomerase I (Topo I) activities and de novo DNA synthesis were studied during liver regeneration in rats with altered thyroid state. Hepatic PARP activity, evaluated as [³²P]NAD incorporated into isolated liver nuclei, was inhibited in hyperthyroid rats and increased in hypothyroid animals. In both euthyroid and hyperthyroid rats PARP activity was rapidly stimulated, peaking 6 h after PH. In hypothyroid animals, an early decrease in activity was found, at a minimum of 6 h after PH, followed by an early onset of DNA synthesis. An inverse relationship between PARP and Topo I activities was a shared feature among euthyroid, hypothyroid, and hyperthyroid rats. Together these data show that, in replicating hepatocytes, thyroid hormones exert a regulatory role on PARP activity, which reflects the control of a number of nuclear proteins involved in DNA metabolism.

Poly(ADP-ribose) polymerase is affected early by thyroid state during liver regeneration in rats. Am J Physiol Gastrointest Liver Physiol 279: G1219–G1225, 2000.—Poly(ADP-ribose) polymerase (PARP), a nuclear enzyme involved in DNA synthesis, repair, and cell replication and transformation, also plays a role in the early steps of liver regeneration induced by partial hepatectomy (PH). PARP and DNA topoisomerase I (Topo I) activities and de novo DNA synthesis were studied during liver regeneration in rats with altered thyroid state. Hepatic PARP activity, evaluated as [³²P]NAD incorporated into isolated liver nuclei, was inhibited in hyperthyroid rats and increased in hypothyroid animals. In both euthyroid and hyperthyroid rats PARP activity was rapidly stimulated, peaking 6 h after PH. In hypothyroid animals, an early decrease in activity was found, at a minimum of 6 h after PH, followed by an early onset of DNA synthesis. An inverse relationship between PARP and Topo I activities was a shared feature among euthyroid, hypothyroid, and hyperthyroid rats. Together these data show that, in replicating hepatocytes, thyroid hormones exert a regulatory role on PARP activity, which reflects the control of a number of nuclear proteins involved in DNA metabolism.


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roid rats compared with euthyroid controls. In all cases, the changes in PARP activity were transient and preceded the beginning of de novo DNA synthesis.

Furthermore, in an attempt to find possible effects of poly(ADP-ribose)ylation, we evaluated the activity of DNA topoisomerase I (Topo I; E.C. 5.99.1.2) during liver regeneration in rats with altered thyroid state. DNA topoisomerase activity modulates DNA supercoiling by introducing transient strand breaks that reduce the superhelical tension that would accumulate during DNA replication (20) or transcription (2). Topo I can be inhibited or activated by poly(ADP-ribose)ylation (14, 18) or phosphorylation (13, 25), respectively. Our results show that during liver regeneration in rats with altered thyroid state there is a negative correlation between PARP and Topo I activities.

METHODS

**Chemicals and reagents.** Propylthiouracil (PTU), T3, T4, β-NAD, phenylmethylsulfonyl fluoride (PMSF), histone H1, agarose, calf thymus DNA, and supercoiled pBR322 DNA were all purchased from Sigma (St. Louis, MO). [Adenylate-32P]NAD (specific activity 800 Ci/mmol) and [methyl-3H]thymidine (3H[TdR; 60 Ci/mmol) were from NEN Life Science Products (Cinisello Balsamo, Italy). Hoechst 33258 dye (bisbenzimide) was purchased from Calbiochem (Inalco, Milan, Italy). Bicinchoninic acid-based reagent was from Pierce Chemical (Rockford, IL). Ketamine (Ketalar) was from Parke Davis. Serum levels of T3 and T4 were measured by the enhanced chemiluminescence (ECL) enzymatic immunoassay (DPC, Los Angeles, CA).

**Animal treatment.** Male Wistar rats weighing 120–140 g at the start of the experiment were used. The animals were housed at 27°C on a 12:12-h light-dark cycle and had free access to water and food. The animals’ maintenance and treatment were carried out according to national guidelines for animal care and use. Hypothyroidism was chemically induced by a 6-wk treatment with 0.05% PTU added to the drinking water as described elsewhere (11). Hyperthyroidism was induced by daily intraperitoneal injection of T3, given at the dose of 15 μg/100 g body weight, for 1 wk before PH. PH (68–70%; median and left lateral lobes excised) was carried out between 9:00 AM and 12:00 PM according to Higgins and Anderson (16) with minor modifications (7). Complete anesthesia was induced by ketamine given intraperitoneally at the dose of 15 mg/100 g body wt. Sham-operated animals were submitted to anesthesia, laparotomy, and liver manipulation without tissue resection. Postoperative care included a 24-h fasting period with free access to water. The time course of hepatic activity of PARP, Topo I, and DNA synthesis was studied in different groups of rats killed at 0, 1.5, 3, 6, 9, 12, 15, 18, 21, 24, 30, 36, 42, and 48 h after PH or after 3, 4, 6, and 8 days of liver regeneration. Serum T3 and T4 levels were measured in blood samples collected during death and allowed to clot. Detection limits for T3 and T4 were 0.35 and 4 ng/ml, respectively, as determined by the ECL enzymatic immunoassay. To compare liver weights during compensatory growth at 7, 14, and 21 days, groups of intact age-matched PH rats were killed. Livers were rapidly removed, rinsed in ice-cold physiological saline, blotted, and weighed. The average liver mass was calculated on the basis of liver weights of sham-operated rats and intact age-matched controls and weights of regenerating livers from hepatectomized rats killed at the scheduled times. The regeneration index (RI) was calculated by the formula: RI = [1 – (NL – RL)/NL], where NL is average liver weight of sham-operated controls and RL is average weight of regenerating livers at the scheduled times after PH.

**PARP activity.** The active fraction of PARP was determined by incubating aliquots of liver nucleus suspension with the reaction mixture containing the labeled substrate. Briefly, liver nuclei were prepared by pressing a weighed portion of tissue on a stainless steel net (400 mesh). This step allows very low contamination by nonparenchymal cells. Crude nuclei were then suspended at a final concentration of ~0.5–1 × 108 cells/ml in modified Merchant’s solution containing (in mM) 140 NaCl, 2.7 KCl, 1.47 KH2PO4, 0.5 EDTA, 8.1 Na2HPO4, 1 diethiotheritol (DTT), and 0.5 PMSF, pH 7.5. For [32P]NAD incorporation, 10 μl of nuclear suspension were added to 100 μl of reaction mixture containing 100 mM Tris-HCl, pH 8.0, 10 mM MgCl2, 10% glycerol, 1 mM DTT, and 100 μM [adenine-2,8-32P]NAD (specific activity 1,500 cpm/pmol). The incubation was carried out at 37°C, aliquots of 25 μl were withdrawn at 4 min, spotted on glass fiber disks, and processed as detailed elsewhere (7). The enzymatic activity (in pmol·min⁻¹·mg DNA⁻¹) defines the incorporation rate of labeled ADP-ribose into acid-insoluble product. The total level of the catalytic protein was determined in liver nuclear extracts by biochemical assay or by activity gel technique including PAGE separation, reaction with [32P]NAD, autoradiography, and densitometric analysis as described elsewhere (6).

**DNA Topo I activity.** DNA Topo I activity was assayed on liver nuclear extracts and normalized for protein content essentially according to Keller (19). The protein content was determined by the bicinchoninic acid method (26). DNA topoisomerases, obtained by reaction of nuclear extracts on pBR322 supercoiled DNA, were separated by electrophoresis on 0.8% agarose gel at 5 V/cm for 6 h in TPE buffer (in mM: 30 NaH2PO4, 1 EDTA, 36 Tris-HCl, pH 7.8). A photograph of the agarose gel separation was taken under ultraviolet exposure after staining with 2 μg/ml ethidium bromide. The film was then analyzed by PC-assisted densitometry, and Topo I activity was calculated on the basis of the amount of negatively supercoiled and relaxed plasmid DNA in 15 min at 37°C and expressed as an activity ratio against control.

**DNA synthesis and total DNA content.** The time-dependent pattern of DNA synthesis was evaluated, at the scheduled times of liver regeneration, by pulse incorporation of [3H]thymidine (2 μCi/g body wt) injected intraperitoneally 1 h before death. Crude liver DNA was isolated from nuclei, precipitated on GF/C filters, and acid washed, and [3H]TdR incorporation was measured by liquid scintillation counting (7). DNA content in nuclear preparations was determined by fluorimetry according to Cesareno et al. (5).

**RESULTS**

**Serum level of thyroid hormones.** Daily T3 administration to euthyroid rats for 1 wk before PH produced hypertriiodothyroninemia with a low T4 serum concentration (T3 = 1.9 ± 0.28 ng/ml; T4 < 4 ng/ml). The administration of PTU to euthyroid rats for 6 wk induced hypothyroidism with a low serum level of thyroid hormones (T3 < 0.35 ng/ml; T4 < 4 ng/ml). In euthyroid control rats, serum thyroid hormone levels were T3 = 0.62 ± 0.02 ng/ml and T4 = 56.26 ± 2.87 ng/ml.

The decrease in thyroid hormone levels resulted in impaired growth. Hypothyroid rats weighing 120–140
g at the start of the experiment reached 157 ± 1.86 g after 6 wk of exposure to PTU compared withagematched euthyroid controls weighing 322 ± 5 g.

**PARP activity in liver of rats with altered thyroid state.** The basal level of PARP activity in the liver of intact euthyroid, hypothyroid, and hyperthyroid animals is illustrated in Fig. 1A. The values are means of ~50 euthyroid, 20 hypothyroid, and 10 hyperthyroid rats.

The basal level of PARP activity (pmol·min⁻¹·pg DNA⁻¹) was 0.8 ± 0.1, 1.5 ± 0.15, and 0.45 ± 0.06 for euthyroid, hypothyroid (PTU treated), and hyperthyroid (T₃ treated) animals, respectively. The increased activity in PTU-treated rats is in line with the level observed in surgically athyrotic rats (8). Likewise, the drop in basal PARP activity in long-term T₃-treated animals is consistent with the data obtained after treatment with a single dose of T₃ (8).

The total level of the catalytic protein in liver nuclei was evaluated by activity gel analysis (Fig. 1B). As can be seen in Fig. 1, in all experimental conditions the total level of PARP remained unaltered, indicating that the protein turnover was not affected by the thyroid state.

**PARP activity and DNA synthesis during liver regeneration in euthyroid and hypothyroid rats.** Figure 2 shows the time course of PARP activity and DNA synthesis during liver compensatory growth in hypothyroid rats and euthyroid controls. PARP activity is expressed as a ratio to the corresponding values in sham-operated rats. In euthyroid rats a transient increase in PARP activity was already detectable 1.5 h after PH, followed by a 2.4-fold peak 6 h later and a return to control levels by 18 h. A subsequent long-lasting rise started from 24–30 h after PH, returning to basal levels at 6–8 days.

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**Fig. 1.** Poly(ADP-ribose)polymerase (PARP) activity in liver of hypothyroid, euthyroid, and hyperthyroid rats. A: basal level of PARP activity in liver from sham-operated hypothyroid, euthyroid, and hyperthyroid animals. B: total level of PARP activity. Autoradiography of the “activity gel” of nuclei extracts from liver of sham-operated animals is shown. Lanes 1, 2, and 3 refer to hypothyroid, euthyroid, and hyperthyroid rats, respectively.

**Fig. 2.** Time course of PARP activity and DNA synthesis during liver regeneration in control (euthyroid) and propylthiouracil (PTU)-treated (hypothyroid) rats. The enzyme activity is expressed as a ratio vs. control values. The patterns of PARP activity in hypothyroid and euthyroid rats as well as the incorporation of labeled thymidine in hypothyroid and euthyroid animals are shown. Each point represents the mean ± SD of triplicate assays of at least 6 different experiments. Statistical significance: PARP activity at 6–9 h hypothyroid vs. sham-operated rats, \( P < 0.01 \); DNA synthesis at 15 h hypothyroid vs. control rats, \( P < 0.001 \).
The return of PARP activity to control levels at 18 h after PH corresponded to the beginning of DNA synthesis, which showed a maximum 24 h later and a minor peak at 42 h. In hypothyroid rats, a rapid inhibition of basal PARP activity was observed with a minimum at 6–9 h after PH. Subsequently, the activity slowly regained the hypothyroid basal values, which were completely restored after 96 h. DNA synthesis started at 12 h and showed a maximum 15–18 h after liver resection. It should be noted that at 12 h, when DNA synthesis started, PARP activity was low, i.e., within the range of basal euthyroid values.

**PARP activity and DNA synthesis during liver regeneration in hyperthyroid rats.** The time course of PARP activity and DNA synthesis in the regenerating liver of hyperthyroid rats is shown in Fig. 3. PARP activity exhibited a sharp, transient increase peaking at 6–9 h after PH. The subsequent return to control values at 18 h preceded the onset of DNA synthesis, which revealed a broad peak 18–24 h later. When DNA synthesis started, PARP activity was about twofold higher with respect to that in the intact liver of hyperthyroid rats. This was still in line with the aforementioned data for euthyroid and hypothyroid rats; in fact, the basal PARP activity in the intact liver of control hyperthyroid rats is reduced to one-half that in euthyroid rats. Therefore, DNA synthesis started when PARP activity approached euthyroid control levels in this case also.

**PARP and DNA Topo I activity during liver regeneration in hypothyroid and euthyroid rats.** DNA Topo I is among the main targets of poly(ADP-ribosyl)ation. The patterns of PARP and Topo I activities during liver regeneration in hypothyroid rats are shown in Fig. 4. PARP and Topo I activities are expressed as a ratio with the respective control values. It can be seen that the activity of Topo I started to increase 3 h after PH, i.e., when PARP activity tended to fall. Furthermore, Topo I showed a maximum concomitant with a minimum in PARP activity 6–9 h after PH. The subsequent return of Topo I activity to the control range was associated with the partial recovery of PARP activity.

In euthyroid rats the time course of Topo I activity after PH was the mirror image of that of PARP activity (Fig. 5). Topo I activity started to decrease 3 h after PH, showed a minimum at 6 h, and returned to within the control range at 12–18 h. Subsequently, Topo I activity tended to increase concomitantly with the peaking of DNA synthesis (24 h).

**Effects of hypothyroidism on the regeneration of rat liver mass.** To check whether the changes in enzyme activity observed in the regenerating livers of hypothyroid rats were associated with an impaired recovery of liver mass, we evaluated the amount of regenerated tissue. The results, reported in Table 1, show that the regeneration process was delayed in hypothyroid rats compared with controls. In fact, 1 wk after PH, PTU-treated animals recovered only 46% of liver mass, whereas control animals regained 78%. Nevertheless,
3 wk after PH the liver mass was completely recovered in both euthyroid and hypothyroid rats. The DNA-to-protein ratio was in the range of 0.17 ± 0.03 in all conditions tested.

**DISCUSSION**

PARP activity is responsible for posttranscriptional changes of several nuclear catalytic and structural proteins. These modifications, in turn, can modulate primary cellular processes such as DNA replication and repair, gene expression, cell differentiation, and transformation (1, 27). Liver regeneration, induced by 70% PH in the rat, provides a useful model to study the factors involved in the regulation of cell proliferation. This experimental system was used previously to demonstrate that a rapid and transient increase in PARP activity precedes the onset of DNA synthesis during the early steps of liver regeneration (7). Moreover, it was reported that thyroid hormones can control the basal activity of PARP in rat liver. In fact, after a single injection of T3 in euthyroid animals a rapid and transient decrease in hepatic PARP activity was observed, whereas thyroidectomy induced an increase in enzyme activity in athyrotic rats (8).

In the present study we investigated whether changes in serum thyroid hormone levels may affect the pattern of PARP activity during rat liver regeneration. Hyperthyroidism was achieved by treatment of the animals with T3 for 1 wk before PH or sham operation. Liver nuclei from hyperthyroid rats displayed low levels of PARP activity compared with euthyroid controls, as previously observed after a single T3 injection (8). PTU-induced hypothyroidism was characterized by a net increase in hepatic PARP activity, confirming previous data in surgically thyroidectomized hypothyroid rats (8).

When hyperthyroid rats underwent PH, the pattern of hepatic PARP activity was essentially similar to that observed in regenerating liver of euthyroid animals. A maximum in the enzyme activity was observed 6–9 h after liver resection followed by a return to sham-operated hyperthyroid values within 20–24 h. DNA synthesis, evaluated as [3H]TdR incorporation, started when the level of PARP activity approximated the range of euthyroid controls, i.e., at 15–16 h, and reached a broad peak 18–24 h after surgery. These results seem to indicate that in the regenerating liver of both euthyroid and hyperthyroid rats DNA synthesis is hampered when PARP activity is high.

A thoroughly different pattern was observed during liver regeneration in hypothyroid rats when a rapid, marked decrease in PARP activity was observed. This drop was transient, showing a minimum 6–9 h after surgery and returning within 48 h to the range of sham-operated hypothyroid rats. It should be noted that the lowest PARP activity in hypothyroid rats and the highest activity in euthyroid and hyperthyroid rats occurred at the same time after PH. In any case, the regulation of PARP was rapid and was followed by a return to the level of sham-operated animals. Moreover, in partially hepatectomized hypothyroid rats the changes in PARP activity also preceded the onset of DNA synthesis, the latter exhibiting a peak 15–18 h after PH, i.e., earlier compared with the regenerating liver of euthyroid rats.

**Table 1. Liver regeneration index in euthyroid and hypothyroid rats**

<table>
<thead>
<tr>
<th>Days after PH</th>
<th>Euthyroid</th>
<th>Hypothyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.77 ± 0.07</td>
<td>0.46 ± 0.05*</td>
</tr>
<tr>
<td>14</td>
<td>0.87 ± 0.04</td>
<td>0.73 ± 0.08</td>
</tr>
<tr>
<td>21</td>
<td>1.00 ± 0.03</td>
<td>1.00 ± 0.05</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 4 rats. PH, partial hepatectomy. *P < 0.01.
liver of euthyroid and hyperthyroid animals. Therefore, it is suggested that both thyroid hormone level and PH regulate PARP activity, which in turn triggers DNA synthesis.

It is worth noting that the initial decrease in PARP activity induced in hypothyroid rats by PH restored the enzyme level close to the range of euthyroid sham-operated controls and that DNA synthesis started when PARP activity was in this range. The latter observation was true also for partially hepatectomized euthyroid and hyperthyroid rats. On the basis of these data it can be argued that the rapid regulation of PARP activity after PH is essential to exert a stringent control over nuclear proteins before DNA replication. Therefore, a low level of PARP activity should be restored to set up DNA synthesis.

On a speculative basis, the increase in PARP activity observed in euthyroid and hyperthyroid animals after PH could be a signal for the presence of free ends on DNA to recruit the enzymes involved in DNA synthesis. Moreover, an increased poly(ADP-ribosyl)ation can inhibit the activity of DNA repair enzymes to stimulate replicative DNA synthesis.

Among the enzymes inhibited by poly(ADP-ribosyl)ation is Topo I (14, 18), which regulates DNA supercoiling during DNA replication (20) or transcription. Therefore, it can be suggested that a minimal rate of PARP should correspond to a maximum in Topo I activation. The increase in Topo I activity observed here at 6–9 h and the concomitant decrease in PARP activity appear to be in line with this hypothesis. A further confirmation of this view was obtained in the regenerating liver of euthyroid rats, in which the maximal inhibition of Topo I activity at 6–9 h corresponded to the highest PARP stimulation. In conclusion, on the basis of these data, an inverse correlation between PARP and Topo I activity can easily be drawn, suggesting a potential functional link between these nuclear activities.

Our data on the amount of the recovered liver and hepatic DNA and protein contents indicate that hypothyroid rats are able to regenerate the liver completely despite the decrease in overall body growth, the peculiar pattern of PARP activity, and the time shift in DNA synthesis. However, the process is significantly delayed in such animals, which recovered only about one-half of the total liver mass 1 wk after PH, when in normal rats regeneration was almost complete. These observations are in line with those reported by Canzanelli et al. (3) describing a lower regeneration rate in thyroidectomized rats and with the more recent paper by Maliekel et al. (21) reporting a reduced DNA synthesis in hypothyroid animals 22 h after hepatectomy. In conclusion, our results confirm that the alterations of the thyroid state do not jeopardize the completion of the liver regeneration process.

De novo DNA synthesis represents a basic event in the process of liver compensatory growth stimulated by PH, and the potential interfering factors that can affect this basic step can also influence the overall process. The early and transient activation of PARP observed in euthyroid and hyperthyroid rats, during liver regeneration and before the onset of DNA synthesis, is probably required to modulate and/or hinder the binding of nuclear structural and catalytic proteins to DNA, thus favoring the beginning of the replicative process, which, in turn, is triggered by a return of PARP activity within the basal range. This sudden increase is caused by an activation of silent PARP molecules and not by an increased amount of the catalytic protein as confirmed also by the PARP mRNA level previously reported (7). However, the mechanism of such an activation is at present unknown.

The thyroid state controls the basal level of liver PARP activity, which in turn has a regulatory effect on the early events of liver compensatory growth. It is known, in fact, that T₃ exerts a negative control on liver PARP activity (8), and changes in thyroid hormone level may result in an altered time course of compensatory growth.

In conclusion, the data reported here indicate a significant regulatory role of PARP activity in liver DNA synthesis and compensatory growth. However, we are still far from a reliable comprehension of the mechanism(s) by which PARP activity is controlled; paraphrasing an ancient Latin motto, the question posed is still, “Quis custodiet custodem?” (who will control the controller?).

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