Inhibition of Induction of Rat Liver Tyrosine Aminotransferase by D-Galactosamine

ROLE OF URIDINE TRIPHOSPHATE*

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SUMMARY

1. An injection of D-galactosamine at the same time as an injection of dexamethasone inhibits the increase of rat liver tyrosine aminotransferase activity normally observed following injection of dexamethasone alone. The galactosamine-mediated inhibition is dose dependent: a 50% inhibition of the increase of enzyme activity is observed at 10 mg of galactosamine HCl per 100 g of body weight, and a total inhibition is observed at 15 mg per 100 g of body weight. Following a single injection of 37.5 mg of galactosamine-HCl per 100 g of body weight, the basal level of tyrosine aminotransferase activity does not change for at least 16 hours.

2. Induction of the enzyme by glucagon or dibutyryl cyclic AMP is only partially inhibited by a dose of 37.5 mg of galactosamine-HCl per 100 g of body weight.

3. The total inhibition of the dexamethasone-induced enzyme increase is specific for galactosamine. A high dose of D-glucose or D-galactose has no inhibitory effect, and a high dose of D-glucosamine or 2-deoxy-D-galactose has only a partially inhibitory effect.

4. The inhibition by galactosamine of the dexamethasone-induced enzyme increase can be prevented by simultaneous injection of uridine. Determination of the uridine phosphate levels following injection of different doses of galactosamine reveals a partial inhibition of the enzyme induction below 100 nmoles of UTP+UDP per g of liver and a total inhibition of enzyme induction below 40 to 50 nmoles of UTP+UDP per g of liver.

5. There is little or no relationship between levels of galactosamine metabolites in the liver and inhibition of enzyme induction.

6. Unlike actinomycin D, injection of galactosamine at various times after injection of dexamethasone does not result in a superinduction of tyrosine aminotransferase.

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The intraperitoneal injection of D-galactosamine into adult rats leads to biochemical and morphological liver damage similar to viral hepatitis (1, 2). Among the early biochemical events which occur following the injection of galactosamine are an accumulation of galactosamine-1-phosphate, UDP-hexosamines, and UDP-N-acetylhexosamines (see Reference 3), a marked decrease in the concentrations of UTP+UDP, UDP-glucose, and UDP-galactose (4, 5), and a decrease in the rate of incorporation of [U-14C]uridine into RNA (6, 7) and of [U-14C]leucine into protein (6-8). The reduction of the rate of incorporation of [U-14C]uridine into RNA is assumed to be due to a reduction of the UTP pool, caused by a trapping of uracil nucleotides by galactosamine (4, 5, 9). The cause for the reduced rate of [U-14C]leucine incorporation into protein is less clear. As several of the metabolites of galactosamine have been shown to inhibit UDP-glucose pyrophosphorylase (9) and UDP-glucose dehydrogenase (9a) in vitro, it is possible that these metabolites may inhibit an enzymatic step involved in protein synthesis. On the other hand, the reduced pool of UTP+UDP may, by inhibiting the synthesis of RNA, result in a deficit of rapidly turning over messenger RNA necessary for the synthesis of new proteins.

As a means of distinguishing between these two alternatives, we have chosen to study the effects of administration of galactosamine on the induction of rat liver tyrosine aminotransferase (EC 2.6.1.5). The use of this enzyme is suitable for these studies due to the rapid turnover of both the protein (10) and of its messenger RNA (11). Also, induction of synthesis of this enzyme can be effected by glucocorticoids (12), glucagon (13), and cyclic AMP (14), thus allowing the utilization of several compounds which have different mechanisms of action in the induction of this enzyme (15).

A preliminary report of the present data appears elsewhere (16).

MATERIALS AND METHODS

Animals—Male Wistar rats (Ivanovas, Kissingen, Germany) were obtained at least 5 days prior to each experiment and housed three per plastic cage with wood shavings for bedding. The animals were maintained in a room with natural lighting supplemented by overhead fluorescent lights during the daytime. The animals had free access to water and to a 20% protein diet (Altromin R, Altromin GmbH, Lage/Lippe, Germany). Food was removed at 6:00 p.m. on the day preceding the experiment. Body weights, determined at the time of removal of food, ranged from 250 to 350 g.

1 The abbreviations used are: cyclic AMP, cyclic adenosine 3',5'-monophosphoric acid; dibutyryl cyclic AMP, N4, O'-dibutyryl cyclic adenosine 3',5'-monophosphoric acid; dexamethasone, dexamethasone 21-monosodium phosphate.
between 140 and 200 g. All initial injections were given at 8:30 a.m. on the day of the experiment, and subsequent injec-
tions, if required, were given at the times indicated after 8:30 a.m. For determination of tyrosine aminotransferase activity, the rats were killed by decapitation 4 hours after the last injection, unless noted otherwise. The livers were quickly removed, chilled over ice, trimmed, weighed, and homogenized by an Ultra-Turrax homogenizer (Janke & Kunkel KG, Staufen im Breisgau, Germany) in 4 volumes (w/v) of 0.2 M sodium phosphate buffer (pH 7.3). The homogenates were centrifuged at 104,000 \( \times g \) for 1 hour, and the resulting supernatants were used for the tyrosine aminotransferase assay.

For determination of uridine phosphates and galactosamine metabolites, the livers were removed under pentothal anaesthesia (4 mg per 100 g) by tightly clamping in stainless steel tongs which had been cooled in liquid nitrogen.

**Chemicals**—The following chemicals, of highest purity available, were purchased from the following suppliers: \( \beta \)-galactosamine-HCl, 2-deoxy-\( \beta \)-galactose, and \( \alpha \)-ketoglutaric acid from C. Roth OHG (Karlsruhe, Germany); \( \beta \)-galactose, \( \beta \)-glucose, uridine and pyridoxal 5'-phosphate from E. Merck AG (Darmstadt, Germany); \( \beta \)-glucosamine-HCl and dibutyryl cyclic AMP from Sigma (St. Louis, Missouri); nucleotides and enzymes for the determination of uracil nucleotides from Boehringer Mannheim GmbH, (Mannheim, Germany); and crystalline glucagon from Eli Lilly GmbH (Giessen, Germany). Dexamethasone was a generous gift from Medice (Iserlohn, Germany) 12 hours in neonatal rat liver (21).

Determination of Galactosamine Metabolites and Uracil Nucleotides — The determination of galactosamine metabolites was performed according to Bauer et al. (3). The determination of uracil nucleotides was done according to Keppler et al. (19), except that the buffer solution used was pH 9.2 instead of pH 8.8. Using this method, UTP and UDP are determined together as the commercially available UDP-glucose dehydrogenase is contaminated with nucleoside diphosphate kinase.

**RESULTS**

**Inhibition of Enzyme Induction**—Preliminary experiments on the effects of galactosamine injection on the induction of tyrosine aminotransferase in rat liver indicated that a single injection of 37.5 mg of galactosamine-HCl per 100 g of body weight completely inhibited the normally observed induction of the enzyme 4 hours after an injection of 2 mg of dexamethasone per 100 g of body weight. The inhibition of induction was observed if the dexamethasone and galactosamine were given simultaneously or if the dexamethasone was given 3 hours after the galactosamine.

The data from a dose-response experiment (Fig. 1) indicate that a dose of 10 mg of galactosamine-HCl per 100 g causes approximately a 50% inhibition, and a dose of 15 mg per 100 g is sufficient to cause a total inhibition of the dexamethasone-mediated induction of the enzyme. This dose is below the minimum dose (20 mg per 100 g) to elicit galactosamine hepatitis (7). A dose of 37.5 mg per 100 g is far in excess of the dose required to inhibit the increase of tyrosine aminotransferase activity and is the dose which has been used to produce galactosamine hepatitis (1, 2). This high dose is used in all subsequent experiments in order to insure a definite inhibition of induction. If galactosamine alone is injected and the rats are killed at 2, 4, 8, 12, 16 and 24 hours later, there occurs neither an increase nor a decrease in the basal level of the tyrosine aminotransferase activity for at least 16 hours. However, at 24 hours after the injection of galactosamine, there occurs an approximate 2-fold increase in the level of tyrosine aminotransferase activity.

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This increase may be caused by a decrease in the level of galactosamine and its metabolites along with a hypoglycemia due to absence of food for 40 hours. It has been shown that fasting causes an increase in the level of tyrosine aminotransferase activity after 3 days in adult rat liver (20) and after as little as 12 hours in neonatal rat liver (21).

Injection of dexamethasone, glucagon, or dibutyryl cyclic AMP brings about an increase in the activity of tyrosine aminotransferase, although the dibutyryl cyclic AMP is not as effective as dexamethasone or glucagon (Fig. 2). This may be due to the fact that only one time point after injection (4 hours) was
used, which may not have coincided with the peak of increase of enzyme activity. If galactosamine is injected alone or with dexamethasone, there is no increase in enzyme activity compared to the saline-injected control. However, if galactosamine is injected with either glucagon or with dibutyryl cyclic AMP, the activity level of tyrosine aminotransferase is increased about 2-fold over the control level. This indicates that galactosamine, at the high dose used, is unable to inhibit completely the increase in enzyme activity brought about by drugs which are reported to act at the translational level (15).

The specificity of the inhibition by galactosamine was determined by testing several other sugars for the inhibitory effect on induction of tyrosine aminotransferase by dexamethasone. Administration of glucose has been reported to result in a repression of tyrosine aminotransferase synthesis, although the glucose is usually given by intubation several times within a 24-hour period before the repressive effect is seen (22, 23). However, when the glucose is given in a single intraperitoneal injection along with dexamethasone and the activity level of tyrosine aminotransferase in the liver is determined 4 hours later, only a slight inhibition of induction of the enzyme is observed (Fig. 3), although the glucose is given in a 6.6-fold molar dose compared to the dose of galactosamine used. Glucose alone (200 mg per 100 g) has a slight stimulatory effect on the basal level of the enzyme. When glucosamine is injected with dexamethasone, there occurs a 48% inhibition of the increase of enzyme activity observed when dexamethasone is given alone. If a 10-fold molar dose (375 mg per 100 g) of glucosamine is given alone, there occurs a 55% inhibition of induction. Galactose has neither a stimulatory effect on the basal level nor an inhibitory effect on the induction of the enzyme. Injection of 2-deoxy-D-glucose alone increases the basal level 1.7-fold and, when injected with dexamethasone, inhibits the induction by 32%. Thus, among the sugars tested, only galactosamine does not affect the basal level of tyrosine aminotransferase when injected alone but still produces a total inhibition of the dexamethasone-mediated induction of the enzyme.

Role of Uracil Nucleotides—It has been reported that galactosamine acts as a uridine-trapping agent, resulting in a significant decrease in the hepatic level of UTP+UDP, UDP-glucose, and UDP-galactose (4, 5, 9). Therefore, the levels of these compounds in the liver were determined as a function of the dose of galactosamine given. As shown in Table I, the level of UTP+UDP is reduced to about 50% of the normal level 1 hour following an injection of 5 mg of galactosamine·HCl per 100 g of body weight and is further reduced to 34% of normal at 10 mg per 100 g. At these doses, the inhibitions of dexamethasone-mediated induction of tyrosine aminotransferase are 18 and 47%, respectively (Fig. 1). Increasing doses of galactosamine lower the level of UTP + UDP only slightly (Table I). UDP-glucose and UDP-galactose are reduced to about one-third and one-half, respectively, of normal values at a dose of 5 mg of galactosamine·HCl per 100 mg, with little additional decrease at doses above 10 mg per 100 g.

If the action of galactosamine in inhibiting the dexamethasone-mediated induction of tyrosine aminotransferase is due to the reduction of uridine phosphates or UDP-sugars, or both, then administration of uridine to the rats should at least partially relieve the galactosamine-mediated inhibition because the administration of uridine has been shown to restore temporarily the decreased pool of uridine phosphates (5). As shown in Fig. 4, a dose of uridine which is only one-half the molar dose of the galactosamine given does indeed produce a slight relief of the galactosamine inhibition of dexamethasone-mediated enzyme induction. At equimolar and 3-fold molar doses of uridine, the relieving of the galactosamine inhibition is even more apparent, with a level of 60% normal induction of the enzyme following injection of the 3-fold molar dose of uridine along with dexamethasone and galactosamine. It should be noted, however, that this high dose of uridine alone results in a substantial increase of the level of tyrosine aminotransferase. Thus, the increase of the enzyme level following injection of all three compounds may be partially due to the effect of uridine alone on increasing the enzyme level. If the uridine-mediated induction is taken into account, then the induction of the enzyme following injection of uridine, dexamethasone, and galactosamine is about 60% of normal instead of 90%.

It is shown in Table II that the injection of a 3-fold molar...
Effects of different doses of galactosamine on levels of uracil nucleotides

D-Galactosamine·HCl at a concentration of 100 mg per ml of saline was injected intraperitoneally in different volumes in order to result in the appropriate dose of galactosamine per 100 g of body weight. The injections were given at 8:30 a.m., and the rats were killed at 9:30 a.m. The values are the mean ± one standard deviation.

<table>
<thead>
<tr>
<th>Uracil nucleotide measured</th>
<th>Mg galactosamine·HCl per 100 g body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 g</td>
</tr>
<tr>
<td></td>
<td>n = 6</td>
</tr>
<tr>
<td>UTP+UDP</td>
<td>278 ± 14</td>
</tr>
<tr>
<td>UDP-glucose</td>
<td>355 ± 31</td>
</tr>
<tr>
<td>UDP-galactose</td>
<td>88 ± 12</td>
</tr>
</tbody>
</table>

Effects of different doses of uridine on levels of uracil nucleotides

p-Galactosamine·HCl at a concentration of 100 mg per ml of saline (0.46 molar) was injected intraperitoneally to give a dose of 37.5 mg per 100 g of body weight. The different doses of uridine used (1.4, 0.46, and 0.23 molar in saline) were injected intraperitoneally at the same volume as the galactosamine. The injections were given at 8:30 a.m., and the rats were killed at 9:30 a.m. The values are the mean ± one standard deviation.

<table>
<thead>
<tr>
<th>Uracil nucleotide measured</th>
<th>Molar dose of uridine injected compared to galactosamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3X</td>
</tr>
<tr>
<td></td>
<td>n = 6</td>
</tr>
<tr>
<td>UTP+UDP</td>
<td>168 ± 20</td>
</tr>
<tr>
<td>UDP-glucose</td>
<td>111 ± 22</td>
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<tr>
<td>UDP-galactose</td>
<td>77 ± 20</td>
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</tbody>
</table>

Effects of different doses of uridine on levels of galactosamine metabolites

The doses of galactosamine and uridine are given in Table II. The injections were given at 8:30 a.m., and the rats were killed at 9:30 a.m.

<table>
<thead>
<tr>
<th>Dose uridine injected compared to galactosamine</th>
<th>GaIN-1-P</th>
<th>UDP-HexN</th>
<th>UDP-HexNAc</th>
<th>HexNAc-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>3X Uridine</td>
<td>9.90</td>
<td>7.13</td>
<td>1.05</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>10.90</td>
<td>7.75</td>
<td>1.11</td>
<td>0.65</td>
</tr>
<tr>
<td>1X Uridine</td>
<td>11.00</td>
<td>8.30</td>
<td>1.15</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>7.29</td>
<td>5.76</td>
<td>0.68</td>
<td>0.51</td>
</tr>
<tr>
<td>0.5X Uridine</td>
<td>9.43</td>
<td>7.15</td>
<td>0.84</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>10.90</td>
<td>7.93</td>
<td>0.83</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>10.69</td>
<td>8.03</td>
<td>1.00</td>
<td>0.68</td>
</tr>
<tr>
<td>Control</td>
<td>11.85</td>
<td>8.80</td>
<td>0.84</td>
<td>1.14</td>
</tr>
<tr>
<td></td>
<td>11.10</td>
<td>8.20</td>
<td>0.80</td>
<td>1.02</td>
</tr>
</tbody>
</table>

* Abbreviations: GaIN, galactosamine; GaIN-1-P, galactosamine 1-phosphate; UDP-HexN, UDP-hexosamines; UDP-HexNAc, UDP-N-acetylhexosamines; HexNAc-P, N-acetylhexosamine phosphates.
of uridine partially relieves the inhibition of induction of tyrosine aminotransferase and partially prevents the depression of levels of uridine phosphates following injection of galactosamine, there is no effect on the level of total galactosamine metabolites. Similarly, there is no change in the level of galactosamine 1-phosphate with different doses of uridine. The levels of UDP-hexosamines are slightly increased and the levels of UDP-N-acetylhexosamines and N-acetylhexosamine phosphates are reduced to about one-half of the normal level following injection of the high dose of uridine. Thus, the data are not consistent with the possibility that the nonphysiological metabolites of galactosamine are solely responsible for the inhibition of the induction of tyrosine aminotransferase.

Superinduction—If actinomycin D is injected at a time when the level of tyrosine aminotransferase is elevated through injection of a glucocorticoid, the level of the enzyme is further increased. This phenomenon, referred to as superinduction, has been observed in vivo (24) and in hepatoma cell culture (25). Since the present data indicate that galactosamine acts at the transcriptional level in inhibiting the induction of tyrosine aminotransferase, it was of interest to determine whether galactosamine could also produce superinduction of the enzyme. As shown in Fig. 5, no superinduction of the enzyme occurs at any of the time points tested. In fact, if galactosamine is injected at the peak of the enzyme activity (10 hours after injection of dexamethasone), there is a decrease in the activity of the enzyme instead of an increase. However, at 18 hours after injection of dexamethasone there occurs a slight reduction of the rate of loss of enzyme activity.

**Discussion**

Dexamethasone and similar glucocorticoids are thought to act on the induction of tyrosine aminotransferase at the transcriptional (26) or immediate post-transcriptional level (27). Glucagon, by activating adenyl cyclase (28), brings about an elevation in the concentration of cyclic AMP in liver cells (29). Cyclic AMP has been suggested to act at the translational level in inhibiting the induction of tyrosine aminotransferase activity (15), perhaps by aiding in the release of completed nascent protein from polysomes (30, 31). As shown in the present paper, the administration of galactosamine completely inhibits the induction of tyrosine aminotransferase by dexamethasone but only partially inhibits the induction by glucagon and dibutyryl cyclic AMP. The moderate increase in enzyme activity observed following injection of galactosamine plus glucagon or dibutyryl cyclic AMP (Fig. 2) may be the result of an increased rate of translation or release of messenger RNA for tyrosine aminotransferase which was formed prior to administration of the galactosamine. Agents which inhibit at the transcriptional level would prevent synthesis of additional messenger RNA for the enzyme and thus prevent a large increase in enzyme activity, as is seen if glucagon alone is injected. Thus, the data in Fig. 2 indicate that galactosamine exerts its action primarily at the transcriptional level of induction of tyrosine aminotransferase. However, the data presented do not exclude the possibility of an additional, although minor effect of galactosamine at the translational level.

**Determination of the concentration of the uridine phosphates and galactosamine metabolites following injection of galactosamine and uridine** (Table III) indicates that the galactosamine metabolites could be responsible for, at most, only a small inhibition of induction of tyrosine aminotransferase by dexamethasone. From Fig. 1 and Tables I and II, it can be concluded that a UTP+UDP concentration of about 60% of normal is necessary for the induction of the enzyme by dexamethasone. Below this concentration, an increasing inhibition of induction of tyrosine aminotransferase is observed. Total inhibition is observed at a concentration of 40 μmoles of UTP+UDP per g of liver. This corresponds to about 30 μmoles of UTP per g of liver, since about 70% of UTP+UDP is UTP (4). Therefore, we propose that the administration of galactosamine inhibits the induction of rat liver tyrosine aminotransferase primarily at the transcriptional level by decreasing the pool of UTP, thereby preventing the synthesis of new messenger RNA for the enzyme. Inhibition by galactosamine of the translation of preformed messenger RNA occurs only slightly, if at all. The observation that glucosamine and 2-deoxylgactose slightly inhibit the induction of synthesis of the enzyme (Fig. 3) is consistent with this proposal as these sugars also slightly lower the pool of UTP (4).

The fact that galactosamine did not result in a superinduction of tyrosine aminotransferase (Fig. 5) in a time period sufficient for actinomycin D to elicit superinduction (24) is inconsistent with the model of regulation of induction of tyrosine aminotransferase by glucocorticoids, as proposed by Tomkins et al. (27). Although actinomycin D and galactosamine inhibit the synthesis of RNA by different mechanisms, this difference by itself does not seem able to account for the lack of superinduction of the enzyme by galactosamine injection. Further experiments are necessary in order to resolve this discrepancy.

**References**


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1 From the observations of Kenney and co-workers (32-34), it is assumed that an increase in enzyme activity is equivalent to an increase in the concentration of the enzyme molecules.
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