Present Paradoxes in the Environmental Control of Hepatic Proliferation

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Summary

Six "paradoxes" regarding hormonal control of normal hepatic proliferation in rats are defined; based partly upon preliminary studies described here, possible solutions to some of these problems are discussed. It is suggested that regulation of hepatic regeneration involves altered gene expression at both hepatic and extrahepatic sites.

Introduction

Hormones and low-molecular-weight compounds probably play major roles as environmental regulators of normal hepatic proliferation (7, 10). These "roles" are not, however, presently well defined, and paradoxical results have been reported (for discussion, see Ref. 13). Based partly upon a series of observations from in vitro and in vivo studies of hepatic proliferation control (see Ref. 10 for literature review; Refs. 12 to 14), we define here 6 "paradoxical" situations for which conceptual and some experimental solutions are offered.

Materials and Methods

All reagents, unless noted, were from Sigma Chemical Co., St. Louis, Mo. Insulin and glucagon were the gifts of W. Bromer (Lilly Research Laboratories, Indianapolis, Ind.). 125I-Monoiodinated insulin and glucagon (each about 100 mCi/μg) and carrier-free 32P, were from New England Nuclear, Boston, Mass. [G-3H]Hypoxanthine (2 Ci/m mole) was from Amersham/Searle, Arlington Heights, Ill. Prostaglandins of the A, B, E, and F series were gifts of The Upjohn Co., Kalamazoo, Mich.

Fischer 344 male rats (180 to 200 g) were used for in vivo studies. Blood hormone and VLDL3 (d ≤ 1.006) determinations, the stimulation and measurement of hepatic nuclear DNA synthesis in 70% hepatectomized and TAGH-infused rats, and the binding of 125I-labeled insulin and glucagon to hepatic membranes were performed as previously described (11, 12, 14). Except for chemical characterization, labeled hormone in vivo disappearance studies were performed using procedures described elsewhere (11).

In vitro DNA synthesis initiation assays were performed with 12- to 18-day-old differentiated monolayer fetal hepatocyte cultures (7, 10). Labeled or unlabeled compounds to be tested were added together with fresh medium, and assays [DNA synthesis (7, 10); formation of [3H]hypoxanthine, and/or 32P-derived acid-soluble metabolites (8, 9)] were performed 24 hr later.

Results and Discussion

Paradox 1. Stimulators are inhibitors. Insulin promotes hepatocyte DNA synthesis initiation in vitro under chemically defined conditions (9, 10, 13) and in totally pancreatectomized 70% hepatectomized rats (cited in Ref. 13). However, after partial hepatectomy (but not laparotomy), arterial insulin levels fall (12), as shown in Chart 1. Furthermore, if partially hepatectomized animals receive a peripheral venous infusion of insulin (and glucose), then hepatic DNA synthesis is suppressed (16).

Although inhibition of endogenous insulin secretion may account for declining arterial levels, increased hepatic insulin uptake also may occur soon after partial hepatectomy, as shown in Chart 2. This interpretation is preliminary because (a) it required the as yet unproved assumption that radioactivity is authentic hormone (or its degradation products), and (b) using the pulse-labeling method, significant differences between laparotomized and 70% hepatectomized rats were not observed until 40 min postoperatively. It also appears that, 80 min after the operations, "insulin" disappearance from venous blood is slower in 70% hepatectomized animals in comparison to laparotomized rats (Chart 3). Therefore, it would seem that within 60 min after partial hepatectomy, intrahepatic "insulin" availability increases, whereas extrahepatic availability decreases. [Other factors, such as purines, also may enhance insulin action (9); early hepatic changes, if any, in insulin binding to membranes have yet to be determined.] Because extrahepatic lipolysis may be required for promoting DNA synthesis initiation in the partially hepatectomized liver (13, 16), such hormonal changes could enhance lipolysis.

Iodothyronines also promote hepatocyte DNA synthesis initiation in vitro (9, 10, 13) and in the intact rat (15), and blood L-thyroxin levels also decline specifically after partial hepatectomy (11, 12) as shown in Chart 1. Additional evidence suggests that, in vivo, hepatic L-thyroxin utilization...
Hepatic Proliferation Control

Chart 1. Alterations of plasma insulin, glucagon, and L-thyroxin after 70% hepatectomy in adult rats. These experiments were performed exactly as described in Ref. 11, except that animals received food and water ad libitum. Each point (insulin, •; glucagon, □; and L-thyroxin, △) represents the mean of 3 to 4 animals with a range of values ± 10 to 15%.

increases within 6 hr after 70% hepatectomy (11). These changes may contribute to altered plasma L-thyroxin levels.

In vitro, 200 nM iodothyronine levels inhibit DNA synthesis initiation (13). Therefore, iodothyronines may show bell-shaped dose-response curves.

Paradox 2. Inhibitors are stimulators. Glucagon antagonizes in vitro DNA synthesis promoted by insulin under chemically defined conditions (10). This observation was subsequently confirmed (cited in Ref. 13). However, in partially hepatectomized rats, arterial glucagon levels rise (12), as shown in Chart 1. Iodinated "glucagon" also behaves like 125I-labeled insulin with respect to its slower disappearance from regenerating liver (Chart 2). However, blood disappearance of labeled glucagon showed similar kinetics in both animal groups receiving a single bolus (Chart 3, top) with an "instantaneous" rate (Chart 3, bottom) significantly higher (P < 0.05) in the 70% hepatectomized group. The above observations imply that glucagon secretion is elevated (and/or that peripheral losses are decreased) and that 70% hepatectomized liver accumulates and/or sequesters glucagon more efficiently. These observations suggest different explanations for the paradoxical antagonistic in vitro effects of glucagon as discussed in detail elsewhere (13).

Glucagon may promote in vivo DNA synthesis initiation in many ways; histone phosphorylation is 1 intriguing possibility (cited in Ref. 13).

Glucagon may also reduce hepatic VLDL production after partial hepatectomy (14). This lipoprotein has recently been shown to specifically block serum-promoted in vitro hepatocyte DNA synthesis initiation (14). VLDL [but not low-density (1.006 < d > 1.050) or high-density lipoprotein (1.050 < d > 1.21)] falls rapidly after partial hepatectomy (14) in a dose-dependent manner (Chart 4). Choline-deficiency states also promote hepatic DNA synthesis initiation concomitant to altered hormone and VLDL changes similar to those observed in 70% hepatectomized rats (8). The TAGH-stimulatory infusion mixture (15) also lowers blood VLDL levels dramatically and stimulates, in our hands, hepatic nuclear DNA synthesis initiation in intact rats about 5-
fold (Ref. 14; Chart 5). Table 1 further shows that declining blood VLDL levels are a necessary but insufficient condition for DNA synthesis initiation to begin; in addition, heparin alone accounts for nearly 85% of the VLDL-depressing effect. These observations suggest that normal clearing mechanisms after partial hepatectomy involve participation of glucagon- and heparin-mediated processes (8, 13, 14). It would be interesting to know, therefore, whether heparin production by hepatic mast cells is glucagon or lipid mediated.

With respect to this latter possibility, glucagon is a potent lipolytic hormone in the rat. Prostaglandin E1 may be among the classes of free fatty acids in circulating lipids that are elevated locally during hyperglucagonemic states. This fatty acid (but not others in the A, B, and F series) promoted

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**Table 1**

Effects of TAGH infusate components upon serum VLDL levels and hepatic DNA synthesis

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Infusate</th>
<th>No. of animals</th>
<th>µg protein/ml</th>
<th>% above control</th>
<th>%HThymidine uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.15 M NaCl solution</td>
<td>4</td>
<td>154 ± 18</td>
<td>&lt;0.01</td>
<td>434</td>
</tr>
<tr>
<td></td>
<td>TAGH</td>
<td>4</td>
<td>11 ± 4</td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>2</td>
<td>0.15 M NaCl solution</td>
<td>10</td>
<td>120 ± 9</td>
<td>NS</td>
<td>264</td>
</tr>
<tr>
<td></td>
<td>Iodothyronine</td>
<td>10</td>
<td>131 ± 9</td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>3</td>
<td>0.15 M NaCl solution</td>
<td>10</td>
<td>126 ± 6</td>
<td>NS</td>
<td>11 NS</td>
</tr>
<tr>
<td></td>
<td>A (mix)</td>
<td>10</td>
<td>117 ± 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.15 M NaCl solution</td>
<td>10</td>
<td>130 ± 12</td>
<td>&lt;0.05</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Glucagon</td>
<td>10</td>
<td>98 ± 11</td>
<td></td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>5</td>
<td>0.15 M NaCl solution</td>
<td>10</td>
<td>151 ± 7</td>
<td>&lt;0.01</td>
<td>29 NS</td>
</tr>
<tr>
<td></td>
<td>Heparin</td>
<td>10</td>
<td>19 ± 3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* VLDL (d ≤ 1.006 g/ml) was purified from sera as described (14).

* DNA synthesis was measured by [%HThymidine uptake into hepatic nuclei 22 to 24 hr postoperatively (14). Control values (0.15 M NaCl solution infusate) were about 400 ± 64 (mean ± S.E.) cpm/mg DNA/2-hr pulse.

* NS, not statistically significant (11).

* A (mix), 300 mg complete amino acid mixture (14).
DNA synthesis initiation in vitro (Chart 6) and, of further interest, was elevated 2- to 3-fold (as measured by immuno-assay) in portal blood 12 hr post-70% hepatectomy (not shown).

Glucocorticoids also are usually thought to suppress in vivo hepatic G1-S transitions (cited in Ref. 10). This too may result from the fact that high levels (200 nM) inhibit in vitro DNA synthesis initiation, whereas low levels (20 nM) markedly potentiate stimulation by insulin (9, 10, 13). Thus, like the iodothyronines, glucocorticoids also may be hepatoproliferative regulators with bell-shaped dose-response curves.

Paradox 3. "Inactive" factors are active. The concept that cell populations, during development, "equilibrate" with a given environment which then defines the set of signals that "acquires" DNA synthesis initiation-promoting activity (i.e., signal specificity is relative) has been discussed (13). Simply stated, different factors can be made to limit DNA initiation in a single-cell population. Recent in vitro experimental evidence which supports this concept has been reported (6).

Receptor display also may influence signal requirements of proliferation. These changes can occur (12) with regard to what appears to be a loss of hepatic glucagon receptors during regeneration (Chart 7) and during fetal and neonatal development (2). Glucagon most probably affects early liver functions after partial hepatectomy, as discussed above; therefore, the significance of this putative, "fetal-like" change in a hormone receptor during regeneration is as yet unclear.

Paradox 4. Limiting factors are not limiting. It now appears that both insulin and glucagon limit the promotion of hepatic DNA synthesis initiation during normal regeneration (4). This limitation is difficult to "reveal," however, if partial hepatectomy is performed prior to pancreatectomy (3) or if maintenance infusions contained insulin (cited in Ref. 10).

Similarly, as stated elsewhere (10), surgical or chemical endocrine ablations do not appear to abolish entirely the DNA synthesis response during regeneration (cited in Ref. 10). These observations are consistent with the trivial explanation that residual factors remained in these animals, as well as with a multiple-factor hypothesis (10). It is now clear, however, that hormones implicated in hepatic growth control also may arise from ectopic sites throughout the body (1).

Paradox 5. Cyclic nucleotides regulate hepatic DNA synthesis initiation, but in vivo levels do not change during the early prereplicative period. Cyclic adenosine monophosphate and cyclic guanosine monophosphate have been implicated in hepatic growth control and in growth control of animal cells in general (discussed in Ref. 13). Their role is presently controversial. After 70% hepatectomy, neither increased nor decreased levels of either cyclic nucleotide were detectable in hepatic extracts during the 1st 90 min postoperatively (13).

Recently, Koch et al. (8, 9) and others (5) have shown that hepatocytes produce from [3H]hypoxanthine what appear to be a family of highly phosphorylated adenine-derived nucleotides. In vitro hepatocyte formation of these compounds is shown in Chart 8. Chart 8, A and B (radioautograms of phosphoethylenimine-cellulose plates) show 32P-labeled and [3H]hypoxanthine-labeled extracts from cultures before and after medium change (conditions which promote DNA synthesis initiation), whereas Chart 8C (DEAE Sephadex in 7 M urea) shows similar culture extracts from...
cells labeled only with \(^3\text{H}\)hypoxanthine (8, 9). Additional in vitro studies using DNA but not RNA synthesis inhibitors suggest that these novel nucleotides may play growth-regulatory roles. A working hypothesis has been presented (8). Cyclic nucleotides may not be alone with regard to nucleotide regulation of proliferation-related processes.

**Paradox 6.** Specificity is nonspecific. Many hormones that promote hepatic DNA synthesis initiation, including insulin, hydrocortisone, iodothyronine, glucagon, and parathyroid hormone, most likely affect growth of many cell types (cited in Ref. 13). In support of this statement, TAGH infusions have been reported to stimulate DNA synthesis in the exocrine pancreas, and a variety of fibroblast-like cell culture systems respond to insulin and steroid in the pres-
ence of additional polypeptide factors (cited in Ref. 13). We too found a partial hepatocyte-stimulatory effect by the cartilage-stimulatory factor somatomedin-C (10). No consistent evidence has yet been obtained, to our knowledge, for the existence of a single "hepatospecific" DNA synthesis initiation-promoting factor.

Specificity, therefore, may be accomplished by the removal of the putative inhibitory influence of VLDL, or it may be at the level of proliferation signal-receptor affinities and/or permutation of these "displays." In addition, the capacity of hepatocytes to synthesize a broad spectrum of functional plasma proteins may provide an endogenous source of "growth factors;" therefore, molecules like somatomedin-C may be a reasonable candidate.

Acknowledgments

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References

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