Effect of Cyclosporine A on Hepatic Compensatory Growth: Role of Calcium Status

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ABSTRACT

Cyclosporine A (CsA) has been reported to positively influence hepatic compensatory growth (HCG) in normal animals. The role of calcium in the CsA-mediated influence on HCG was studied in normal and in chronically hypocalcemic rats, a model in which HCG is perturbed. CsA (3.33 mg/kg/day for 10 days) was administered before 2/3 partial hepatectomy (PHx). CsA did not influence serum Ca\(^{2+}\) but significantly increased concentrations of the vitamin D hormone calcitriol. After PHx in normal animals, CsA accelerated DNA synthesis without influencing liver weight restitution, suggesting that its main effect was to mediate an accelerated progression through the cell cycle G\(_0\) to G\(_1\)/S phase(s). In hypocalcemic rats, CsA did not influence DNA synthesis, but normalization of circulating calcium alone accelerated DNA synthesis but abrogated the stimulatory effect of CsA, indicating that CsA could not superimpose its stimulatory effect on the calcium effect. In vitro investigation on the CsA mechanisms of action revealed a dose-dependent increase in hepatocyte basal resting cytoplasmic Ca\(^{2+}\) and an increase in inositol-1,4,5-trisphosphate-sensitive Ca\(^{2+}\) pool, which was dependent on the presence of normal extracellular Ca\(^{2+}\) during CsA exposure. CsA also mediated a significant increase in cellular Ca\(^{2+}\) mobilization by phenylephrine, vasopressin, and epidermal growth factor (EGF) in the presence of extracellular Ca\(^{2+}\) concentration. Our data, therefore, demonstrate that CsA accelerates HCG after PHx by, in part, increasing the cellular Ca\(^{2+}\) pools and the response to EGF and Ca\(^{2+}\)-mobilizing hormones known to be comitogens for hepatocytes.

The structurally unrelated immunosuppressants cyclosporine A (CsA) and tacrolimus (FK506) have both been proposed as positive modulators of the compensatory growth process after partial hepatectomy (PHx) in the rat, mouse, and dog (García-Alonso et al., 1989; Francavilla et al., 1990; Kahn et al., 1990; Mazzaferrro et al., 1990; Tanaka et al., 1993). However, CsA has been shown to lead to either cell proliferation, cell cycle arrest, or cell death in different cell types or organs, but its mechanisms of action have not yet been fully elucidated and the dual potential of CsA on cell cycle progression remains a paradox.

Present literature suggests that the apparent stimulatory effect of CsA on normal compensatory growth seems to be largely confined to the liver. Indeed, Francavilla et al. (1990) in a study on hepatocytes, kidney, and intestine were unable to identify any influence of the drug on the hyperplastic response of either the contralateral kidney after unilateral nephrectomy, or on the small intestine after partial resection. However, despite numerous reports indicating an increase in the hepatic regeneration phenomenon induced by CsA, several studies have noted that the liver weight restitution was not influenced by the drug at 3 or 7 days after surgery (Makowka et al., 1986; Kahn et al., 1990). It has also been suggested that CsA might have a “priming”-like effect on the liver because in the unhepatectomized rat, parameters indicative of putative regeneration processes such as increases in the hepatic content of ornithine decarboxylase, thymidine kinase, and the estrogen receptor have been observed (Kahn et al., 1990). Moreover, a study carried out in our laboratory has indicated that at the critical time of greatest loss in liver mass, CsA has only a selective influence on the biotransformation of cytochrome P450-dependent activities (Provencher et al., 1999). This observation indicates that the effect of CsA on the regeneration process does not translate into an overall accelerated recovery of the hepatic drug-metabolizing function.

It has now been well documented that hypocalcemia and vitamin D deficiency are accompanied by modifications in calcium signaling in hepatocytes such as impairment in inositol-1,4,5-trisphosphate (IP\(_3\)) synthesis, IP\(_3\) receptor affin-
ity, and in the responsiveness of several Ca\(^{2+}\)-mobilizing hormones and growth factors, which seem related to low receptor affinity and/or to the size of the hormone/drug-sensitive intracellular Ca\(^{2+}\) pools (Gascon-Barré et al., 1997; Mailhot et al., 2000). Hypocalcemia has also been shown to retard the hepatic regenerative process (Éthier et al., 1990). These observations have raised the hypothesis that the regenerative process associated with hypocalcemia is due to defect(s) in the hepatocyte mitogenic signaling pathways (Sikorska et al., 1983; Rixon et al., 1989; Éthier et al., 1993; Goupil et al., 1997). Moreover, in several liver diseases where patients are candidates for liver transplantation, malabsorption of vitamin D and of calcium is suspected as being a major determinant of vitamin D depletion and of its accompanying poor systemic and cellular calcium metabolism. This contention is well illustrated by the increased incidence of secondary hyperparathyroidism and of bone diseases (osteo-malacia as well as osteoporosis) in these patients (Krawitt et al., 1977; Kato et al., 1982; Mawer et al., 1985).

The goals of the present studies were to investigate the 1) role of calcium status on the positive effect of CsA on liver regeneration in vivo by investigating whether CsA could reverse the delay in the regeneration process induced by calcium deficiency, and 2) effect of CsA on intracellular Ca\(^{2+}\) homeostasis in isolated rat hepatocytes in vitro to further understand its mechanism of action.

**Materials and Methods**

**In Vivo Studies**

**Animal Model and Drug Regimen.** Studies were conducted in normal rats (Charles River, St. Constant, QC, Canada) and in rats subjected to vitamin D depletion to induce a state of chronic hypocalcemia as described previously (Éthier et al., 1990). In a subset of hypocalcemic animals, oral calcium supplementation was achieved by providing a 3% calcium gluconate solution as drinking water to normalize circulating Ca\(^{2+}\) (Mailhot et al., 2000) and to investigate the interaction between CsA (Novartis, Montreal, QC, Canada) and the in vivo extracellular Ca\(^{2+}\) concentration. The rationale for the model used rests on previous reports showing that 1) hepatic regeneration is perturbed in vitamin D-depleted hypocalcemic animals but completely restored by either vitamin D\(_3\) or 1,25-dihydroxyvitamin D\(_3\) (calcitriol) administration (Éthier et al., 1990); and 2) CsA has been shown to decrease the IP\(_3\)-sensitive intracellular pools of Ca\(^{2+}\) (Mailhot et al., 2000) and to modulate the in vitro IP\(_3\)-sensitive cellular Ca\(^{2+}\) pools. Therefore, the effects of CsA were compared to those observed in normocalcemia in vivo (1.25 mM Ca\(^{2+}\)) or in Ca\(^{2+}\)-free medium.

**In Vitro Studies.** To investigate the influence of CsA on the hepatic compensatory growth process, hepatocytes were obtained from livers of nonfasting normal rats, as described previously (Gascon-Barré et al., 1997). The freshly isolated hepatocytes were equilibrated in William E medium (Invitrogen, Burlington, ON, Canada), and cell viability was assessed by trypan blue exclusion. The liver was then transfected with a calcium-sensitive dye. The cells were then transferred to a 10-μl plastic chamber on the stage of an inverted microscope (Nikon Diaphot; Nikon, Tokyo, Japan) and a refrigerated camera (C4880; Hamamatsu Photonics, Hamamatsu City, Japan), as described previously (Gascon-Barré et al., 1997; Mailhot et al., 2000).

**IP\(_3\)-Sensitive Ca\(^{2+}\) Pools.** IP\(_3\)-sensitive cellular pools were evaluated as described previously (Hofer et al., 1998). Briefly, CsA content was monitored using 5 μg of mag-Fura-2/AM (Molecular Probes, Eugene, OR) for 30 min at 20°C. Dye-loaded cells were then transferred to a 10-μl plastic chamber on the stage of an inverted microscope (Nikon Diaphot; Nikon, Tokyo, Japan) equipped for epifluorescence measurement. All test compounds were superfused at a rate of 3 ml/min in calcium-free Krebs-Henseleit solution, pH 7.4, equilibrated with O\(_2\)/CO\(_2\) (95:5, v/v). Intracellular calcium (Ca\(^{2+}\)) measurements were made at the single cell level using an MCID dual excitation spectrophotometer system (Imaging Research, St. Catharines, ON, Canada) and a refrigerated camera (C4880; Hamamatsu Photonics, Hamamatsu City, Japan).

**Results**

**Biochemical Analyses.** Serum 25-hydroxyvitamin D\(_3\) [25(OH)D\(_3\)] and calcitriol concentrations were measured using the 25(OH)D\(_3\) and 1,25-dihydroxyvitamin D\(_3\) assay kits (Incastar Corporation, Stillwater, MN) according to the manufacturer’s instructions. Serum Ca\(^{2+}\) concentrations were measured with an ICAZ ionized calcium analyzer (Radiometer, Copenhagen, Denmark).

**Parameters Indicative of Regeneration Process**

\[ {\text{IP}}_3 \text{-sensitive intracellular Ca}^{2+} \text{ pools} \]

**Cytosplasmic Ca\(^{2+}\) Measurements at Single Cell Level.** Hepatocytes were loaded with 2 μM Fura-2/AM (Molecular Probes, Eugene, OR) for 30 min at 20°C. Dye-loaded cells were then transferred to a 10-μl plastic chamber on the stage of an inverted microscope (Nikon Diaphot; Nikon, Tokyo, Japan) equipped for epifluorescence measurement. All test compounds were superfused at a rate of 3 ml/min in calcium-free Krebs-Henseleit solution, pH 7.4, equilibrated with O\(_2\)/CO\(_2\) (95:5, v/v). Intracellular calcium (Ca\(^{2+}\)) measurements were made at the single cell level using an MCID dual excitation spectrophotometer system (Imaging Research, St. Catharines, ON, Canada) and a refrigerated camera (C4880; Hamamatsu Photonics, Hamamatsu City, Japan), as described previously (Gascon-Barré et al., 1997; Mailhot et al., 2000).

**IP\(_3\)-Sensitive Ca\(^{2+}\) Pools.** IP\(_3\)-sensitive cellular pools were evaluated as described previously (Hofer et al., 1998). Briefly, Ca\(^{2+}\) content was monitored using 5 μg of mag-Fura-2/AM (Molecular Probes) as probe. After mag-Fura-2/AM loading (40 min at 20°C), hepatocytes were exposed to 100 μg of saponin/ml for a period of 1 min in a calcium-free buffer containing 125 mM KCl, 25 mM NaCl, 10 mM HEPES, 0.1 mM MgCl\(_2\), and 1 mM ATP, pH 7.3, at 37°C to achieve plasma membrane permeabilization. Ca\(^{2+}\) mobilization from internal pools was achieved by applying 10 μg of IP\(_3\) in the buffer described above but in the absence of saponin. Fluorescence signals were obtained at the single cell level using the imaging system described above to measure cytoplasmic Ca\(^{2+}\) concentrations. Data are presented as the signal ratios obtained at 340 and 380 nm.

**Statistical Analysis.** Results are expressed as means ± S.E.M. The qualitative evaluation of the time at which peak \[^{3}H\]thymidine incorporation into DNA occurred was done using a cubic spline equation. Statistically significant differences between group means
were evaluated by analysis of variance, or by the Student’s t test as indicated in the table and figure legends.

Results

Effect of CsA on Parameters of Vitamin D and Calcium Metabolism

As illustrated in Fig. 1, CsA significantly increased circulating calcitriol concentrations in normal rats at all time points during CsA exposure, whereas it significantly decreased that of its precursor 25(OH)D₃, particularly after 3 days of CsA exposure.

Vitamin D depletion significantly decreased both 25(OH)D₃ and calcitriol concentrations. CsA administration, however, did not significantly influence the circulating concentrations of 25(OH)D₃ or calcitriol in vitamin D-depleted rats with serum calcitriol concentrations of 119 ± 2 pmol/l before CsA administration and 134 ± 28 and 165 ± 35 pmol/l after 9 and 12 days of CsA exposure. Serum 25(OH)D₃ concentrations were similarly unaffected by CsA with circulating concentrations of 5.3 ± 0.4 and 4.6 ± 0.3 µM before and after 12 days of CsA administration. Vitamin D depletion, however, led to frank hypocalcemia (0.86 ± 0.08 mM), which was fully normalized by oral calcium feeding (1.27 ± 0.03 compared with 1.3 ± 0.03 mM in normal rats, N.S.).

At the time of euthanasia, no signs of toxicity or intolerance (such as peritonitis) were observed in the CsA-treated animals. CsA administration did not significantly affect the circulating concentrations of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and ionized calcium. Body weight and liver weight were also unaffected by CsA pretreatment.

Effect of CsA on Regeneration Process

Figure 2A presents the effect of CsA on [³H]thymidine incorporation into hepatic DNA in normal rats. As illustrated, CsA administration did not affect [³H]thymidine incorporation into DNA as illustrated in sham-operated animals. After 2/3 partial hepatectomy, CsA administration accelerated [³H]thymidine incorporation into DNA. This apparent acceleration in DNA synthesis was entirely due to the magnitude of the response at the 20-h time point as indicated by the highly significant difference between the CsA- and placebo-treated groups (p < 0.003) (Fig. 2A). The acceleration effect of CsA on the regeneration process was also qualitatively evaluated by cubic spline analysis. It was estimated that peak DNA synthesis occurred 20.3 h after liver resection in CsA-treated compared with 22.9 in control rats. Liver weight restitution was found to be similar in both groups during the time frame studied (Fig. 2B).

As expected, hypocalcemia (Fig. 3A) significantly impaired the regeneration process, an impairment not restored to the level of normal placebo-controls by CsA pretreatment. Moreover, CsA administration did not accelerate [³H]thymidine incorporation into DNA nor did it lead to a significant or persistent effect on the magnitude of the response as judged by analysis of variance (Fig. 3A) as well as by the evaluation of the cumulative [³H]thymidine incorporation into DNA over the entire 48 h studied.

Normalization of the circulating calcium concentration alone in vitamin D-depleted rats accelerated DNA synthesis
compared with their hypocalcemic counterparts. Calcium supplementation, however, completely abrogated the stimulatory effect of CsA treatment on the pattern and magnitude of the DNA synthesis response (Fig. 3C). CsA had no significant effect on liver weight restitution compared with placebo-treated rats in either hypo- or normocalcemic vitamin D-depleted rats (Fig. 3, B and D, respectively).

Effect of CsA Exposure on Intracellular Ca\(^{2+}\)

**Cytoplasmic Ca\(^{2+}\) Concentrations.** Figure 4 presents the resting cytoplasmic calcium concentrations ([Ca\(^{2+}\)]\(_i\)) observed in normal rat hepatocytes exposed to CsA in normal extracellular Ca\(^{2+}\) concentrations ([Ca\(^{2+}\)]\(_o\)) (1.25 mM) (Fig. 4A) as well as in Ca\(^{2+}\)-free medium (Fig. 4B). As illustrated, in both cases, CsA led to a dose-dependent increase in basal resting cytoplasmic Ca\(^{2+}\) concentrations, which reached statistical significance at the 1- and 10-\(\mu\)g/ml doses of CsA. However, at the 10-\(\mu\)g/ml dose of CsA, the mean cytoplasmic Ca\(^{2+}\) concentration was found to be higher in calcium-free medium than in normal extracellular Ca\(^{2+}\) concentration, suggesting a translocation of Ca\(^{2+}\) from intracellular pools to the cytoplasmic compartment (p < 0.0001).

As illustrated in Figs. 5 and 6, in the presence of normal extracellular calcium, CsA exposure led to a significant increase in Ca\(^{2+}\) mobilization by phenylephrine (Figs. 5A and 6A), vasopressin (Figs. 5B and 6B), as well as EGF (Figs. 5C and 6C) at the 10-\(\mu\)g/ml dose. In the absence of extracellular calcium, however, Ca\(^{2+}\) mobilization was only increased by phenylephrine (Figs. 5D and 6D) to a level, however, 54% lower than that observed in the presence of normal Ca\(^{2+}\) in the extracellular milieu (p < 0.0001). CsA exposure had no effect on intracellular Ca\(^{2+}\) mobilization by vasopressin (Figs. 5E and 6E) and EGF (Figs. 5F and 6F) in the absence of extracellular Ca\(^{2+}\).

**IP\(_3\)-Sensitive Ca\(^{2+}\) Pools.** Evaluation of the IP\(_3\)-sensitive Ca\(^{2+}\) pools revealed that extracellular calcium did not influence the basal Ca\(^{2+}\) content of the IP\(_3\)-sensitive pools. CsA, however, increased the basal Ca\(^{2+}\) content of the IP\(_3\)-sensitive pools in the presence of extracellular calcium (Figs. 7A and 8A, a) but not in the absence of extracellular calcium (Figs. 7B and 8A, b). This observation is consistent with the observed lower agonist-stimulated Ca\(^{2+}\) mobilization presented in Figs. 5 and 6. The IP\(_3\)-mobilizable Ca\(^{2+}\) was shown to be increased at the 0.1-, 1.0-, and 10-\(\mu\)g/ml doses of CsA (Figs. 7A and 8B, a) in the presence of extracellular Ca\(^{2+}\), and at the 1.0- and 10-\(\mu\)g/ml doses of CsA (Figs. 7B and 8B, b) in the absence of extracellular Ca\(^{2+}\). Upon withdrawal of IP\(_3\) and application of 1 mM Ca\(^{2+}\), an expected increase in the cellular Ca\(^{2+}\) pools was observed in both groups (Fig. 7, A and B), illustrating the adequacy of the experimental preparations.
Discussion

Our data indicate that, in normal rats, CsA increased hepatic DNA synthesis after 2/3 partial hepatectomy. The most striking difference between CsA- and placebo-treated animals was, not only, the intensity of the response but also an acceleration and a sharpening in peak DNA synthesis. This observation clearly suggests that the drug mediated an increase in the progression through the G1/S phase(s) of the cell cycle and reinforce an earlier observation where CsA was shown to intensify the regeneration gradient (Garcia-Alonso et al., 1990). This phenomenon was, however, not observed in hypocalcemic animals in which a defective regeneration process and hyporesponsiveness to EGF have been shown to occur. Surprisingly, vitamin D-depleted rats fed a high-calcium diet were not responsive to CsA, despite a complete reversal of the EGF hyporesponsiveness by calcium feeding (Éthier et al., 1990; Bilodeau et al., 1995), and the reestablishment of normal DNA synthesis as shown in the present studies. These observations illustrate not only that calcium is an important mediator of DNA synthesis in the regeneration model associated with partial liver resection but also that CsA cannot superimpose its stimulatory effect on the calcium effect.

Although CsA has been shown to influence calcium metabolism in several ways, the mechanisms by which the ion mediates the action of CsA on compensatory hepatic hyperplasia is not known. In the present studies, CsA has been shown to influence calcium homeostasis in vivo by mediating an early and sustained increase in the concentrations of the calcium-regulating hormone calcitriol as well as by influencing the hepatocyte resting and stimulated cytoplasmic Ca2+ responses and the size of the mobilizable IP3-sensitive Ca2+ pools in vitro. Other investigators have also reported that CsA plays a role in calcium signaling as illustrated by an increase in the overall Ca2+ content in hepatocytes (Nicchitta et al., 1985), a reduction in the level of the calcitriol-depen-
Phenylephrine and vasopressin, at doses of 1.25 mM and 10 nM, respectively, were applied to hepatocytes isolated from normal rat livers and exposed in vitro to CsA in the presence of either 1.25 mM A–C or in the absence of D–F. Extracellular Ca\(^{2+}\) for a period of 30 min before cellular 

dent 28-kDa calcium binding protein despite increases in 
calcitriol synthesis (Stein et al., 1991; Steiner et al., 1996), a 
potentiation effect of known Ca\(^{2+}\) agonists such as vasopres-
sin and phenylephrine on the cellular Ca\(^{2+}\) response in kid-
ney mesangial cells (Skorecki et al., 1992) and C6 glioma cells 
(Ikesue et al., 2000), an enhancement at the IP3 receptor 

Fig. 6. Influence of CsA exposure on the response to calcium-mobilizing 
agonists in single rat hepatocytes isolated from normal rat livers and 
exposed in vitro to CsA in the presence of either 1.25 mM (A–C) or in the 
absence (D–F) extracellular Ca\(^{2+}\) for a period of 30 min before cellular 
Ca\(^{2+}\) measurements were made using the Fura-2/AM fluoroprobe. Cyto-
plasmic Ca\(^{2+}\) determinations were made in two to six rats per group with 
an average of 64 hepatocytes evaluated per group. Data represent the 
mean ± S.E.M. Significant differences between group means were eval-
uated by analysis of variance using the Bonferroni/Dunn test for all post 
hoc evaluations in relation to the 0 CsA dose. Phenylephrine was applied 
at a dose of 2.5 mM, vasopressin at a dose of 10 nM, and EGF at a dose of 
25 ng/mL. *, *p < 0.05; ** *p < 0.005; *** *p < 0.0001.

Whether CsA acts as a hepatomodulator of compensatory 
growth via a calcium-mediated mechanism is still uncertain 
but Ca\(^{2+}\) has been demonstrated to be essential for the 
normal cell cycle progression (Lu and Means, 1993), whereas 
several Ca\(^{2+}\)-mobilizing agents have been reported to posi-
tively influence hepatic compensatory growth. For example, 
norepinephrine has not only been shown to increase [Ca\(^{2+}\)]\(_i\) 
mobilization but also to stimulate the hepatic regeneration 
process via an \(\alpha_1\)-adrenergic receptor-mediated signaling 
pathway and as such to act as a comitogen in hepatocytes 
(Cruise et al., 1985; Michalopoulos and DeFrances, 1997). In 
addition, several growth factors (EGF/transforming growth 
factor \(\alpha\), hepatocyte growth factor, acidic fibroblast growth 
factor, which are considered complete liver mitogens and 
known cellular Ca\(^{2+}\) mobilizers) are essential for the hepatic 
compensatory growth process, but their role in the CsA-
mediated effect on DNA synthesis in the regenerating liver 
has not been investigated (Michalopoulos and DeFrances, 
1997). Mazzaferro et al. (1990) have suggested that CsA 
seems to have an effect similar to that of insulin, a permis-
sive but incomplete hepatic mitogen, an observation analogo-
gous to that proposed for \(\alpha_1\)-adrenergic agents. The data 
obtained during the present studies are entirely in agree-
ment with the latter suggestion because they unequivocally 
show that CsA does not have any effect on hepatic DNA 
synthesis in animals not subjected to partial hepatectomy, 

To investigate the potential role of changes in cellular Ca\(^{2+}\) 
homeostasis in the CsA-induced effect on the early hepatic 
regeneration process, an investigation of the IP\(_3\)-sensitive 
Ca\(^{2+}\) pools and of the response to Ca\(^{2+}\)-mobilizing agonists 
known to be either hepatocytic mitogen (EGF) or comitogens 
(vasopressin and phenylephrine) during the compensatory 
growth process was undertaken. Data show that CsA induced 
an extracellular Ca\(^{2+}\)-dependent increase in the IP\(_3\)-sensi-
tive Ca\(^{2+}\) pools. In addition, IP\(_3\)-mediated Ca\(^{2+}\) mobilization 
was significantly increased at all CsA doses in the presence of 
extracellular Ca\(^{2+}\), whereas IP\(_3\) induced significant Ca\(^{2+}\) 
responses at the 1- and 10- \(\mu\)g/ml doses of CsA in the absence 
of extracellular Ca\(^{2+}\). Interestingly, we have previously 
shown that repletion of hypocalcemic rats with oral calcium 
also increased the hepatocyte content of the hormone-sensi-
tive Ca\(^{2+}\) pool as well as that of calreticulin (Mailhot et 
al., 2000), a protein known to regulate the functional size of 
IP\(_3\)-sensitive Ca\(^{2+}\) pools (Bastianutto et al., 1995; Mery et 
al., 1996). It is, therefore, possible that the acceleration in 
DNA synthesis observed in calcium-supplemented animals 
may be due to the cellular calcium effect induced by the 
nutritional repletion, an effect that cannot be further in-
creased by CsA exposure.

Our data also indicate that CsA significantly modifies the 
response to Ca\(^{2+}\)-mobilizing agonists and that the presence 
of extracellular Ca\(^{2+}\) is essential for the response to vaso-
pressin and EGF, whereas a blunted response to phenyleph-
rine (54% less than in the presence of Ca\(^{2+}\)) was still 
observed at the highest CsA dose in the absence of extracel-
ular Ca\(^{2+}\). This observation is in accordance with that of 
others where extracellular Ca\(^{2+}\) has been shown to be essen-
tial to the CsA-mediated rise in intracellular Ca\(^{2+}\). Because 
it is known that hepatic mitogens such as EGF/TGF\(_{\beta}\) and 
HGF and comitogens such as vasopressin and \(\alpha_1\)-adrenergic
agonists act at the G0-G1 and/or at the early G1 phase of the cell cycle, a positive CsA-mediated effect on these agents would be expected to translate into an acceleration of the G1 to S phase of the cell cycle and hence by an earlier peak in DNA synthesis. Our observations indicating that DNA synthesis was accelerated by CsA exposure in normal rats in vivo would concur with the in vitro data, indicating a significant CsA-mediated increased resting as well as in stimulated intracellular Ca2+ mobilization, most particularly in the presence of normal extracellular Ca2+. Of interest also is the observation that CsA led to a significantly sustained increase in the circulating calcitriol concentrations in normal animals. This increase in serum calcitriol may also have played a role in the intensity of the response to CsA because the hormone has been shown to increase intracellular Ca2+ concentration as well as to accelerate DNA synthesis and liver weight restitution after partial hepatectomy in the rat (Éthier et al., 1990; Mailhot et al., 2000).

Despite the apparent acceleration effect of CsA on DNA synthesis, liver weight restitution was shown not to be influenced by the drug in any of the groups studied at the early time points studied. These observations indicate that the early recovery of liver mass is not significantly influenced by CsA, an observation also made by others where liver weight restitution was studied for longer periods of time (Grant et al., 1988; Kahn et al., 1990; Kapan et al., 1996). The absence of a sustained effect of CsA on liver mass restitution indicates, contrary to earlier reports, little effect of the drug on the organ recovery, an observation also supported by our previous observations showing that CsA has only a selective effect on the drug metabolism recovery after 2/3 partial hepatectomy (Provencher et al., 1999). Our data, thus, indicate that the extracellular calcium status either directly or indirectly via modulation of intracellular homeostasis plays an essential role as mediator of the cellular calcium response after partial hepatectomy. The data are consistent with CsA accelerating the hepatic compensatory growth process by, in part, increasing the hepatocyte Ca2+ pools and the response to partial hepatectomy.
to growth factors and Ca\(^{2+}\)-mobilizing hormones known to be comitogens for hepatocytes.

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