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Insulin-like growth factor-I restores the reduced somatostatinergic tone controlling growth hormone secretion in cirrhotic rats

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Abstract: *Background/Aims:* An altered growth hormone/insulin-like growth factor-I (GH/IGF-I) axis occurs in advanced liver cirrhosis, characterised by diminished serum levels of IGF-I and increased concentrations of GH. Under normal conditions, GH release is mediated by somatostatin (SS) inhibition. However, the influence of SS on GH release in cirrhosis is not well known. IGF-I supplementation has beneficial effects in experimental cirrhosis, and – under physiological conditions – IGF-I increases SS, inhibiting GH. The aims of this work were to study SS tone in cirrhotic animals and to evaluate whether IGF-I treatment influences SS tone, controlling GH secretion in cirrhosis. *Methods:* We studied the influence of SS on GH secretion by assessing GH response to pyridostigmine (PD) in cirrhotic rats treated and untreated with IGF-I. Liver cirrhosis was induced with CCl_4-inhalation for 11 weeks in male Wistar rats. The animals were randomly divided into two groups: CI+IGF (n=12), which received IGF-I treatment for 12 days (2 μg/100 g body $wt^{-1}\times d^{-1}$) and CI (n=12), which received saline. Healthy controls (CO, n=12) were studied at the same time. On day 13, animals from each group were subdivided into two groups (n=6) in order to explore the effect of a PD intrajugular bolus (10 μg×100 gbw^{-1}) on serum GH levels (at 0,10,20,30 and 60 min), which were assessed by RIA. *Results:* PD bolus did not exert any effect on GH serum levels in the CI group, suggesting a low SS tone in cirrhotic rats. However, PD induced an increase in GH levels into CO and CI+IGF groups. In conclusion, as occurs under normal conditions, the cholinergic system is a significant modulator of GH secretion in *experimental* liver cirrhosis. *Conclusion:* Cirrhotic rats have a reduced somatostatinergic tone which can be restored by IGF-I supplementation, suggesting that somatostatin is the main factor involved in the feed-back regulation between GH and IGF-I in cirrhosis.

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Patients with advanced liver cirrhosis show elevated growth hormone (GH) levels and reduced insulin-like growth factor-I (IGF-I) values (1–4). GH levels result from the interplay between GHRH and somatostatin (SS) (5, 6). The cholinergic system has been shown to play a significant role in the control of GH secretion. In fact, the GH release induced by cholinomimetic drugs is mediated by SS inhibition (6, 7).

The enhancement of central nervous system cholinergic activity by pyridostigmine (PD), an indirect cholinergic agonist, has been shown to be an effective tool to explore GH regulation in man (8). Compelling indirect and direct evidence led to the view that PD is able to reduce the release of somatostatin from the hypothalamus, increasing basal GH release and, notably, potentiating GHRH-induced GH release. For such reasons, in the last few years, PD administered alone or in combination with several GH stimuli, has been used by many groups to investigate the mechanisms involved in the neuroregulation of GH secre-

tion (5–10). In fact, the somatostatinergic tone can be assessed by estimating the GH response to the cholinergic agonist PD (7, 8).

The liver is the major source of circulating IGF-I, and it has been demonstrated that the failing liver produces reduced amounts of IGF-I, resulting in increased GH levels because of loss of negative feedback (1). Diminished serum levels of IGF-I and increased concentrations of GH characterise the altered GH/IGF-I axis occurring in advanced liver cirrhosis (1, 3). In the early stage of hepatic disease, serum levels of IGF-I are normal, but treatment with IGF-I has beneficial effects in experimental liver cirrhosis, suggesting that this is a tissular condition of 'IGF-I deficient state'. In fact, IGF-I administration to rats with early liver cirrhosis improves intestinal absorption of nutrients, food efficiency, liver function, osteopenia and hypogonadism (11–16).

The aims of this study were to evaluate the somatostatinergic tone controlling GH secretion in experimental cirrhosis; and to study whether IGF-I treatment influences this response in cirrhosis. We therefore tested the inhibitory action of PD in cirrhotic rats under basal conditions and following treatment with low doses of IGF-I.

Materials and Methods

Animals and experimental design

Liver cirrhosis was induced with CCl_4-inhalation for 11 weeks in male Wistar rats, and phenobarbital (Luminal®, Bayer, Leverkusen, Germany) in drinking water (400 mg/l), for 11 weeks, as previously described (11–13). The animals were then divided into two groups: CI+IGF (n=12) which received IGF-I treatment for 12 days (sc, 2 μg×100 g body $wt^{-1}\times d^{-1}$) and CI (n=12) which received saline sc. Healthy controls (CO, n=12) were studied at the same time and received saline. On day 13, animals from each group were subdivided into two groups (n=6) in order to explore the effect of a PD intrajugular bolus (10 μg×100 g pc^{-1}, Mestinon® Roche, Madrid, Spain) (17) or saline on GH serum levels. There were then six experimental groups: CO, CO+PD, CI, CI+PD, CI+IGF, and CI+IGF+PD.

The left carotid artery and the right jugular vein were catheterised using Abbocath®-T Venisystems™ 26 G×19 mm 3/4 G944 (Abbot Ireland, Sligo, Ireland) catheters. Blood (approx. 1 ml, each time) was collected from the artery at 0, 10, 20, 30 and 60 min and stored at −20°C until used. A saline bolus (in animals from groups CO, CI and CI+IGF) or a PD bolus (in rats from CO+PD, CI+PD, CI+IGF+PD) was administered into the jugular vein (0.5 ml) immediately after extraction at time 10.

This design was used to eliminate endogenous pulsate GH peaks, a potentially confounding factor. All procedures were performed in conformity with *The Guiding Principles for Research Involving Animals.*

GH and IGF-I determinations

Serum GH and IGF-I levels were assessed by RIA, using kits from Amersham Life Science (Biotrak™) and from Nichols Institute Diagnostics (S. Juan Capistrano, CA, USA), respectively.

Statistical study

Data were expressed as mean±SEM. To analyse the homogeneity among groups, the Kruskall–Wallis test was used, followed by multiple *post-hoc* comparisons using Mann–Witney U-tests with the Bonferroni adjustment. The Wilcoxon signed rank test was used to compare GH values before and after PD administration in the same animals. Any p-value <0.05 was considered statistically significant. Calculations were performed with the SPSS program, version 6.0 (SPSS Inc., Chicago, IL, USA).

Results

Confirmation of liver cirrhosis

Liver cirrhosis was histologically proven in all animals treated with CCl_4. Cirrhotic animals showed splenomegaly (spleen weight, g, CI=1.4±0.2; CI+IGF=1.4±0.1; both groups $p<0.001$ *vs* controls, CO=0.8±0.0), but no ascites.

Assessment of SS tone

In the first step, the response to PD was evaluated in animals from the CO+PD, CI+PD and CI+IGF+PD groups, comparing GH levels at baseline and GH concentrations after PD bolus. GH baseline values were determined from the mean of two samples obtained before PD bolus. Pyridostigmine induced a peak of GH levels in control rats and in cirrhotic animals previously treated with IGF-I. However, failure of PD to modulate the GH response was observed in the untreated cirrhotic rats (CI group). On day 13, IGF-I levels were similar in the three experimental groups, as is usual in this early stage of cirrhosis (CO=1040±60; CI=1100±55; CI+IGF=1042±40 ng/mL, p=ns). However, as we have previously reported in this condition, the bioavailability of IGF-I in cirrhotic rats was reduced (13).

Table 1. Growth hormone (GH) values at baseline and GH increment after pyridostigmine (PD) bolus in the three experimental groups

	Control group (CO+PD, n=6)	Untreated cirrhotic rats (CI+PD, n=6)	Cirrhotic rats treated with IGF-I (CI+IGF+PD, n=6)
GH basal levels (ng/ml)	0.31±0.03	1.22±0.46	0.38± 0.04
GH increment after PD stimulus			
Absolute increment (ng/ml)	18.95±5.32[&]	−0.17±0.92*	11.69±7.23[&]
Relative increment (%)	7305±2500[&]	−312±253*	2904±1371[&]
Maximum increment (ng/ml)	19.26±5.29[&]	1.05±0.58*	12.08±7.26[&]

[&] $p<0.05$ between baseline values and peak and increments in the same group; * $p<0.05$ *vs* CO group.

Table 1 shows GH values at baseline and GH peaks and GH absolute and relative increments after PD. In CO and CI+IGF groups, significant differences were found between baseline values and GH peaks as well as between baseline levels and absolute and relative increments (Wilcoxon Signed Rank test). However, no response was observed in the untreated cirrhotic group.

On the other hand, in order to eliminate endogenous pulsatile GH peaks, the means of all determinations of GH at different times were assessed (in a similar way to the area under the curve). The means from groups with or without PD bolus were compared (Fig. 1). Controls, which received a saline bolus (CO), showed values not significantly different from those found in the cirrhotic groups (CO: 1.69±0.93; CI: 1.55±0.55; CI+IGF: 1.17±0.26, ng/ml). Controls, which received a bolus with PD, exhibited higher levels of GH than untreated controls (CO+PD: 4.24±2.74 ng/ml, $p<0.001$ *vs* CO), suggesting a normal SS tone. However, no response to PD was found in untreated cirrhotic rats (CI+PD: 1.29±0.38, p=ns *vs* CI), showing that these animals have a low SS tone because PD had no effect on GH release, although its major mechanism is SS inhibition. Interestingly, IGF-I-treated cirrhotic rats which received PD, showed a significant GH response (CI+IGF+PD: 2.77±1.60). This finding suggested that IGF-I treatment induces recovery of SS tone in cirrhotic animals. These results showed that the CI+IGF group had a GH response similar to that of the CO group and quite different from that exhibited by the CI group.

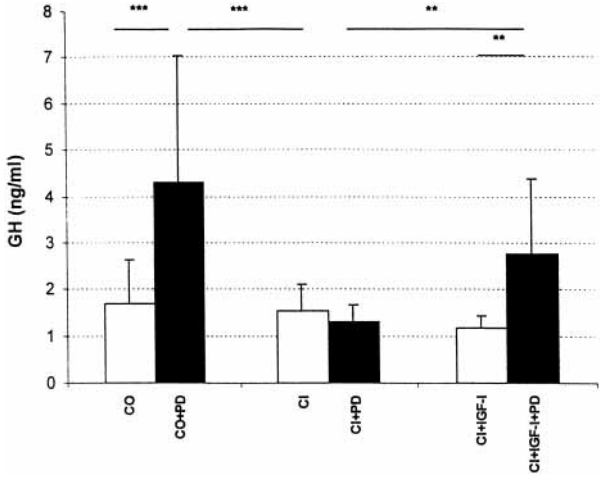

Fig. 1. Growth hormone (GH) response to pyridostigmine or saline bolus in the three experimental groups. Increased GH levels were observed after pyridostigmine (PD) stimulus in healthy controls (CO+PD, n=6) and in cirrhotic animals treated with insulin-like growth factor-I (IGF-I) (CI+IGF+PD, n=6) as compared to the control group (CO, n=6) and the cirrhotic group treated with IGF-I (CI+IGF, n=6), which received a bolus of saline. However, PD did not influence GH levels in the untreated cirrhotic group (CI+PD, n=6), showing similar values to those found in untreated cirrhotic rats (CI, n=6) which received a bolus of saline instead of PD. Each diagram represents the mean±SEM of GH determinations at six different points (basal and after bolus – saline or PD) in a similar way to the area under the curve. ** $p<0.01$, *** $p<0.001$.

Discusion

In physiological conditions, IGF-I is produced in the liver by GH stimulus. In turn, IGF-I inhibits GH secretion acting on the hypothalamus by two feedback mechanisms: the first by inhibiting GH gene expression (17) and the second by stimulating the secretion of SS (18, 19), that inhibits GH production. This normal GH/IGF-I axis is altered in cirrhosis (1, 3), but the mechanisms involved are not well understood. This study provides evidence that reduced control of GH secretion by SS is the main factor involved in this altered axis. In addition, it shows that IGF-I administration restores the normal influence of SS on GH production.

According to previous studies, PD administration to normal rats was followed by significant increases in GH levels (6, 7, 20). Assuming that cholinergic agonists stimulate GH secretion through inhibition of SS release (6), the GH response to PD can be considered as an indirect estimate of the hypothalamic somatostatinergic tone

controlling GH secretion. Therefore, the lack of GH response to PD seen in cirrhotic rats suggests a reduced influence of SS on GH secretion. This finding has also been described in diabetes, another condition characterised by low GH-IGF-I axis activity (21). The administration of low-dose IGF-I to cirrhotic rats was effective in normalising the GH response to PD, demonstrating that the reduced somatostatinergic tone was restored to values similar to those found in control rats.

There is much evidence indicating that GH synthesis and release are modulated by GH secretion and by IGF-I levels acting at the hypothalamic and pituitary level (19–21). Previous studies suggested that IGF-I effects are mainly mediated by interaction with SS rather than with GHRH (22). Although a contribution of GHRH cannot be ruled out, our results also argue in favour of mediation of the somatostatinergic tone in the effects of IGF-I on the somatotropic axis in cirrhotic rats.

As mentioned above, an altered GH/IGF-I axis occurs in advanced liver cirrhosis with reduced serum concentrations of IGF-I and increased levels of GH. Animals included in the present work had compensated cirrhosis. In this early stage of the hepatic disease, it has been proved that serum levels of IGF-I are normal (13). However, IGF-I availability seems to be reduced, as we have previously reported (13). In fact, basal levels of GH are moderately increased in untreated cirrhotic animals (Table 1), suggesting a loss of negative feedback. Indeed, treatment with IGF-I induces recovery of SS tone, suggesting again that this is a tissular condition of 'IGF-I deficient state'. Interestingly in previous works, it has been demonstrated that IGF-I administration to rats with early liver cirrhosis (with normal serum levels of IGF-I) improved intestinal absorption of nutrients, food efficiency, liver function, osteopenia and hypogonadism (11–16).

In conclusion, as it occurs in normal conditions, the cholinergic system is a fundamental modulator of GH secretion in liver cirrhosis. Since rats with early cirrhosis display a reduced somatostatinergic tone, which can be restored to the normal state by IGF-I supplementation, it is suggested that IGF-I is necessary to maintain the normal influence of SS on GH secretion in this clinical condition. This evidence points to SS as the main factor involved in the feedback regulation between GH and IGF-I in liver cirrhosis.

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