Cholinergic mediation of growth hormone secretion induced by thyrotropin-releasing hormone in cirrhotic patients

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Abstract. In order to evaluate the possible involvement of muscarinic cholinergic receptors in the GH response to TRH in patients with liver cirrhosis, 8 males with post-hepatitic cirrhosis and 11 males with post-alcoholic cirrhosis were primed with the anticholinergic agent pirenzepine and tested with TRH. In addition, 10 male patients affected by piecemeal necrosis were tested in a similar manner. High basal concentrations of GH were found in all groups. None of the patients with piecemeal necrosis responded to TRH, whereas in patients with post-hepatitic and in post-alcoholic cirrhosis, TRH induced a significant rise in GH levels. The priming with pirenzepine (40 mg given iv 10 min before TRH) completely blocked the TRH-induced GH increase, but did not affect the TRH-induced TSH release. These data suggest that a muscarinic cholinergic pathway is involved in the anomalous response of GH to TRH in patients with liver cirrhosis. The lack of effect of pirenzepine on the TRH-stimulated TSH release suggests that the muscarinic cholinergic mediation is peculiar for the effect of TRH on GH secretion.

The capability of TRH to induce an increase in serum GH levels in man indicates that the regulation of GH secretion is altered.

Like various other diseases such as renal failure, mental depression, anorexia nervosa, cerebellar ataxia, acromegaly, hypothyroidism, β-thalassaemia, and diabetes mellitus (reviewed in Chiodera et al. 1984), liver cirrhosis (Pancerai et al. 1977a; Zanoboni & Zanoboni-Muciaccia 1977) is characterized by this anomalous response. In these pathological conditions, TRH-induced GH increase is not an isolated phenomenon, but is accompanied by other alterations in the secretory pattern of GH. In patients with liver cirrhosis, an abnormal GH response to L-dopa (Borzio et al. 1981), apomorphine (Lal et al. 1982), and arginine (Muggeo et al. 1979) has been observed. Acetylecholine seems to play an important role in the mediation of GH secretion in these experimental situations, since the effect of L-dopa, apomorphine, and arginine is blocked by the treatment with pirenzepine (reviewed in Massara et al. 1986), a specific muscarinic-cholinergic receptor blocker (Hammer et al. 1980; Watson et al. 1982). Muscarinic receptors are involved in the GH response to TRH in patients affected by diabetes mellitus (Chiodera et al. 1984). Therefore, we wondered whether a cholinergic pathway participated in the release of GH induced by TRH in patients with liver cirrhosis. To verify this hypothesis, the effect of TRH on serum GH levels was tested in patients with liver cirrhosis, with or without concomitant administration of pirenzepine. In addition, similar tests were performed in patients with piecemeal necrosis who presented mild alterations of biochemical indices of liver function.

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Patients and Methods

Eight male patients with post-hepatic cirrhosis, 11 male patients with post-alcoholic liver cirrhosis, and 10 male patients with piecemeal necrosis were selected for the study. In all patients the diagnosis was established by liver biopsy and laboratory data (Table 1). All subjects were hospitalized and gave informed consent. This study was carried out in accordance with Helsinki Declaration II. None of them was obese or had a family history of diabetes mellitus. They were all within 10% of their ideal body weight. None of them was encephalopathic as judged by clinical and laboratory criteria, or had clinical evidences of oesophageal bleeding at the time of study. All patients received a low-sodium diet of 2300 Cal, supplemented with oral potassium chloride (20–40 mmol/day). None of them received any pharmacological treatment for at least 3 days before the experimental days.

Two tests were performed in all patients in random order, with a double blind method. The tests were carried out on two different days with an interval of at least 7 days. At 09.00 h of the experimental day, a 19-gauge needle was inserted into an antecubital vein of the patient who had been fasting from the previous evening and was lying in the recumbent position. In one test (control test), the first blood sample (~20 min) was taken 20 min after insertion of the needle, and 8 ml of normal saline (NaCl 0.9%) was injected 10 min before the administration of 200 µg of TRH (time 0). Further blood samples were taken 10, 20, 30, 45, 60, 90 and 120 min later. When the effect of blockade of the muscarinic-cholinergic receptors on the response of GH to TRH was studied, 40 mg of pirenzepine (Gastrozepin®, Boehringer Ingelheim, Italy) was given iv 10 min before the TRH administration. The experimental protocol of this test was the same as previously described.

Serum GH and TSH concentrations were evaluated in all specimens by specific RIA methods (Schalch & Parker 1964; Odell et al. 1967), using commercial kits. All samples from a single patient were analyzed in the same assay. Intra-assay and inter-assay coefficients of variation were, respectively, 3.8% and 8% for GH, and 4.5% and 9.5% for TSH. The lower limit of sensitivity was 0.5 µg/l for GH and 0.5 mIU/l for TSH.

Statistical analyses were performed using analysis of variance and Student's t-test for paired data, as appropriate. All results are expressed as mean ± SE.

Results

The effects of TRH on the serum GH concentration in patients with liver cirrhosis and piecemeal necrosis are shown in Fig. 1.
The basal GH concentration was not significantly different among patients with piecemeal necrosis, post-alcoholic and post-hepatitic cirrhosis. Administration of TRH did not modify the serum GH level in any of the patients with piecemeal necrosis, whereas it significantly increased it in post-alcoholic and post-hepatitic cirrhotic patients. In both groups, the mean peak of GH (10.8 ± 2.5 µg/l in post-alcoholic and 9.4 ± 1.6 µg/l in post-hepatitic cirrhotic patients) was reached at 20 min and was significantly higher than the mean basal value (5.1 ± 0.9 µg/l in post-alcoholic, $P < 0.001$; 3.6 ± 0.3 µg/l in post-hepatitic cirrhosis, $P < 0.001$).

Effect of pirenzepine on the TRH-induced GH release (mean ± SE) in 11 patients with post-alcoholic cirrhosis, 8 patients with post-hepatitic cirrhosis, and 10 patients with piecemeal necrosis. TRH (200 µg iv) was administered at time 0. Pirenzepine (40 mg iv) was given 10 min before TRH administration.
The pre-injection of pirenzepine did not modify the basal concentration of GH as tested 10 min after drug administration. In contrast, pirenzepine completely blocked the TRH-induced GH increase in patients with post-alcoholic (F = 23.47, \( P < 0.001 \)) and post-hepatitic cirrhosis (F = 51.66, \( P < 0.001 \)). The order by which the NaCl and pirenzepine tests were carried out was of no importance, since in 6 out of the 11 patients with post-alcoholic and in 4 out of the 8 with post-hepatitic cirrhosis, the pirenzepine test was the first to be performed.

The basal TSH levels (post-alcoholic cirrhosis: 3.2 ± 0.4 mU/l; post-hepatitic cirrhosis: 3.1 ± 0.2 mU/l) and the TRH-induced TSH increments (mean peak in post-alcoholic cirrhosis 14.4 ± 2.2 mU/l, \( P < 0.01 \) vs basal value; in post-hepatitic cirrhosis 14.8 ± 1.1 mU/l, \( P < 0.005 \) vs basal value) were similar in all cirrhotic patients. In both groups, the mean peak of TSH was observed at 20 min. Pirenzepine did not modify the TRH-induced TSH release in any subject. Pirenzepine produced a transient impairment of visual accommodation in all patients, whereas TRH did not produce any untoward reaction.

Blood pressure and heart rate remained constant in all subjects during the tests.

Discussion

The data presented here demonstrate that TRH stimulates GH release in patients with liver cirrhosis, but not in those with piecemeal necrosis, suggesting that only profound alterations in the liver function induce the development of this anomalous response. Furthermore, our results indicate the involvement of a cholinergic pathway in the mechanism of action of TRH, since the muscarinic-cholinergic blocker pirenzepine completely abolished the TRH-induced GH increase. Thus, besides intervening in many normally active GH-releasing mechanisms (Evans et al. 1985; Massara et al. 1984, 1986), the cholinergic system appears to mediate also the GH response to an anomalous stimulus such as TRH. Our data agree with recent findings of Zanoboni et al. (1986). The ability of pirenzepine to antagonize the effect of exogenously administered TRH strongly suggests that the cholinergic mediation takes place outside the blood-brain barrier (BBB), at the level of the pituitary gland and/or the median eminence. This is also supported by the fact that pirenzepine crosses the BBB very poorly (Hammer & Koss 1979). However, an altered permeability of the BBB cannot be excluded in patients affected by liver cirrhosis. A simple explanation of the development of the acetylcholine involvement in the GH response to TRH includes the possibility that the cholinergic system controlling the GH secretion becomes sensitive to TRH in cirrhotic patients.

This acquired capacity is not a surprising phenomenon, since in other structures within the nervous system TRH influences the cholinergic activity. Thus, TRH potentiates excitatory actions of acetylcholine on cerebral cortical neurones (Yarbrough 1976), and is retained to induce chewing, teeth chattering, piloerection, tremor, and salivation through cholinergic stimulation (Ervin et al. 1981; Ushijma et al. 1984).

The mechanism by which the cholinergic system may develop its sensitivity to TRH in patients with cirrhosis is unknown. However, this process may not be causally related to modifications of acetylcholine receptors, since studies carried out in rabbits with hepatic encephalopathy showed no appreciable changes in the density or affinity of acetylcholine receptors in cerebral synaptic membranes (Ferenci et al. 1984). A more complex explanation suggests that the cholinergic involvement in the anomalous response of GH to TRH might be the expression of a general disorder of releasing and/or inhibiting factors present in patients with liver cirrhosis. The GH response to TRH has been interpreted by Panerai and co-workers as being consequent to the removal of a negative hypothalamic influence hindering, in normal conditions, the potential GH-releasing effect of TRH (Muller et al. 1976; Udeschini et al. 1976). Somatostatin is a likely mediator of this hypothalamic inhibition, since the administration of somatostatin antiserum in normal rats allows the GH response to TRH to become apparent (Panerai et al. 1977b). Furthermore, Salerno et al. (1982) have demonstrated that infusion of somatostatin in cirrhotic patients suppresses the TRH-induced GH rise. On the other hand, it has been reported that acetylcholine inhibits the release of somatostatin from the hypothalamus in vitro through a muscarinic mechanism. This suggests that acetylcholine might have a regulatory role.
modulating somatostatin and consequently the GH secretion (Richardson et al. 1980).

In light of this hypothesis, we wondered whether administration of the anticholinergic agent pirenzepine in cirrhotic patients enhances somatostatin release from the hypothalamus, thus inhibiting the GH response to TRH. In this case, pirenzepine should also be capable of inhibiting the effect of TRH on TSH secretion, since somatostatin is known to reduce this response (Siler et al. 1974). In order to verify this hypothesis we tested the effect of pirenzepine on the TRH-induced TSH secretion in our patients. The results failed to show changes in the TRH-induced TSH increase, speaking against the possibility that somatostatin mediates the inhibitory effect of pirenzepine on the response of GH to TRH. The possibility that the dose of pirenzepine used here (40 mg) may induce an increase in somatostatin sufficient to suppress GH release without suppressing TSH secretion must be considered. For ethical reasons, however, higher doses of pirenzepine cannot be used in this experimental model.

In conclusion, this study demonstrates that in patients with liver cirrhosis, the muscarinic-cholinergic system is involved in the TRH-induced GH secretion.

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