Effects of propranolol on GH responsiveness to repeated GH-releasing hormone stimulations in normal subjects

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Abstract. The influence of β-adrenergic blockade by oral propranolol on the variability of GH responses to GHRH and on GH responsiveness to repeated GHRH administrations was investigated. Eight normal volunteers underwent three tests on three separate occasions. Each test consisted of two administrations of 80 μg GHRH at 2-h intervals without other medication (test 1) or combined with oral administration of 80 mg propranolol 90 min before the first (test 2) or the second GHRH injection (test 3). In test 1 GH levels increased significantly after the first, but not the second GHRH bolus (net incremental area under the curve [nAUC], mean ± sd: 1453 ± 974 and 178 ± 309 μg·l⁻¹·(120 min)⁻¹, respectively). In test 2 basal GH secretion was not influenced by propranolol administration, whereas the GH response to the first GHRH injection was significantly greater than in test 1 (2327 ± 1814 μg·l⁻¹·(120 min)⁻¹; p<0.05). However, individual subjects showed the same variability of GH response as in test 1. The GH response to the second GHRH bolus remained negligible. In test 3 administration of propranolol 90 min before the second GHRH bolus led to a clear GH increase (690±1002 μg·l⁻¹·(120 min)⁻¹), not significantly different from the GH response to the first bolus (1796±1375 μg·l⁻¹·(120 min)⁻¹). However, only 4 subjects showed a marked restoration of the GH responsiveness to the second GHRH administration. In conclusion, oral administration of propranolol is able to increase GH responsiveness to GHRH without changing the great individual variability. The response to a repeated GHRH stimulation is only partially restored by propranolol.

The usefulness of the GHRH test in clinical practice is limited by the great variability of GH responses to GHRH, observed both in the same subject tested on different occasions, and in different individuals (1-4). It has been suggested that the heterogeneity of the GH response to GHRH is due to fluctuations of the somatostatinergic tone (5-7). Moreover, augmented somatostatinergic tone has been indicated as one of the possible reasons why GH responses are blunted after continuous, or repeated GHRH administration in normal adult subjects (8,9).

Propranolol administration enhances the GH response to several stimuli like hypoglycemia (10), exercise (11), glucagon (12), and GHRH (13,14), probably through inhibition of endogenous SRIH secretion (15-17).

The aim of this study was to verify the influence of β-adrenergic blockade by propranolol on the degree of variability of the GH response to GHRH and to test the effects on the blunting of the GH response to GHRH given repeatedly at short intervals in normal subjects.

Subjects and Methods

Subjects and study protocol
Eight healthy volunteers, 4 women and 4 men, 24 to 36 years old, with a body weight ranging from 55 to 75 kg, gave their informed consent to participate in the study. Three tests were performed on three separate occasions, at least one week apart. After an overnight fast, iv catheter was placed in a forearm vein between 08.00 and
09.00 h. After 90 min GHRH (1-44) NH₄ (kindly provided by Sanofi Recherche, Montpellier, France), 80 μg, was administered as a bolus. A second GHRH bolus was administered 120 min later. The different tests were as follows:

Test 1: no medication but GHRH was given.
Test 2: 80 mg propranolol (Inderal 80, ICI-Pharma) was administered orally 90 min before the first GHRH bolus.
Test 3: propranolol was administered 90 min before the second GHRH bolus (at 30 min).

Blood samples were collected at 45-min intervals for the 90 min before the first injection of GHRH, at 15-min intervals for the first hour, and at 30-min intervals for the second hour after each GHRH administration.

The subjects remained recumbent throughout the test procedures; blood pressure and heart rate were monitored during the tests. Female subjects were tested during the early follicular phase of the menstrual cycle.

**Hormone measurements and statistical analysis**

Serum samples for GH and TSH determinations were stored at −20°C until assayed. Serum GH was assayed by double-antibody RIA (hGH Lisophage, Sclavo, Milan, Italy), and TSH by immunofluorometric assay (IFMA) (Pharmacia SpA, Milan, Italy). The intra- and inter-assay variation coefficients were less than 10% for both methods; the sensitivity was 0.3 μg/l (GH), and 0.02-0.05 mU/l (TSH), respectively.

The results are expressed as the mean ± sd. Statistical analysis was performed using non-parametric methods: Friedman test (followed by the Nemenyi procedure) or Wilcoxon signed-ranks, as appropriate. The net incremental area under the curve (nAUC) was calculated by the trapezoidal integration. Hormonal levels at 0 and 120 min were taken as baseline for the calculation of nAUC after the first and the second GHRH administration, respectively. Statistical significance was defined as p<0.05.

**Results**

In the control study, without propranolol pretreatment (test 1) (Fig. 1), mean GH levels increased significantly from 1.1±0.7 at 0 min to 18.1±10 μg/l at 45 min after the first GHRH administration, with a mean nAUC of 1453±974 μg/l·min⁻¹·(120 min)⁻¹. After the second GHRH administration the increase in serum GH concentrations was negligible (from 6.9±6.3 at 120 min to 7.9±4.1 μg/l at 135 min); moreover, the nAUC after the second GHRH administration (178±309 μg/l·min⁻¹·(120 min)⁻¹) was significantly smaller than after the first one (p<0.05). All subjects but one showed the same pattern of blunted GH secretion to the second GHRH stimulation.

In test 2 (Fig. 1), propranolol pretreatment at −90 min did not influence basal GH secretion (from 1.7±2 at −90 min to 1.4±0.7 μg/l at 0 min). The first GHRH administration stimulated GH secretion to 25.5±19 μg/l at 60 min, with a mean nAUC of 2327±1814 μg/l·min⁻¹·(120 min)⁻¹, which was significantly greater than in the control test (p<0.05) (Fig. 2). The variability between the subjects was not modified (Table 1). The second GHRH administration did not increase GH levels significantly (from 17.2±16 at 120 min to 18.6±15 μg/l at 135 min). The nAUC (209±384 μg/l·min⁻¹·(120 min)⁻¹) was significantly smaller than after the first GHRH injection (p=0.01).

When propranolol was administered at 30 min
(test 3) (Fig. 1), the GH response to the first GHRH administration (from 0.9±0.5 at 0 min to 19.6±12 μg/l at 60 min) was quite similar to that obtained in the control study, except for a more prolonged duration of GH secretion. The second GHRH administration led to a minor but clear GH increase, from 12.8±9 at 120 min to 20±16 μg/l at 150 min. The nAUC of the second GH response (690±1002 μg·l⁻¹·(120 min)⁻¹) was not significantly different from the first one (1796±1375 μg·l⁻¹·(120 min)⁻¹); however, because of the great heterogeneity of individual responses, it was also not significantly different from the GH responses to the second GHRH administration during test 1 and test 2 (Fig. 2). In fact, 4 subjects had a clear GH response to the second GHRH administration, whereas the other 4 showed the usual pattern of GH unresponsiveness to repeated GHRH stimulation (Table 1).

TSH secretion was not affected by GHRH administration, with mean levels ranging from 1.3±0.6 at 0 min to 1.0±0.5 mU/l at 90-120 min. Pretreatment with oral propranolol at −90 and 30 min did not change the pattern of TSH secretion.

No serious adverse reactions were recorded during or after any test. A minor complaint was transient facial flushing, which subsided within 3-5 min after GHRH administration. Following propranolol administration heart rate decreased by 18-29%, whereas a decrease of 5-17% was observed during test 1.

Discussion

This study shows that β-adrenergic receptor blockade, with a single oral administration of propranolol, significantly increases GHRH-induced GH release in normal subjects, without affecting basal GH levels. These data are in keeping with those observed by other authors (13,14) despite the different route of administration of the β-blocking drug. The variability of the GH responses to GHRH was not modified by pretreatment with the drug. As it is not clear whether the β-adrenergic receptors involved in GH inhibition are of the β₁ or β₂ subtype (14,18), we chose to use a nonspecific β-selective

Table 1.
Individual GH responses to repetitive GHRH administration without (test 1) and with propranolol (test 2 and 3). Data are expressed as area under the curve in μg·l⁻¹·(120 min)⁻¹

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>1st GHRH bolus</th>
<th>2nd GHRH bolus</th>
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<tbody>
<tr>
<td></td>
<td>Test 1</td>
<td>Test 2</td>
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<tr>
<td>1</td>
<td>473</td>
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<td>2</td>
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<td>1677</td>
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<tr>
<td>8</td>
<td>3485</td>
<td>5131</td>
</tr>
<tr>
<td>mean ± sd</td>
<td>1453±974</td>
<td>2527±1814</td>
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agent, whose action on GH secretion has been largely documented (10-14).

Two main mechanisms may be involved in the adrenergic modulation of the GH response to GHRH: direct action at the pituitary level and inhibition of endogenous SRIH secretion. However, β-adrenergic receptors present at the pituitary level have been shown to be stimulatory in in vitro studies (19-21), making the former hypothesis unlikely. On the other hand, several experimental approaches in rats suggest that β-adrenergic blockade diminishes hypothalamic SRIH secretion (15-17). Since the ultradian rhythm of GH secretion is thought to derive from the interplay of GHRH and SRIH secretion (6,22), the administration of exogenous GHRH during a period of predominant hypothalamic SRIH secretion would induce a low GH response and vice versa (6,7). Consequently, pretreatment with propranolol should be expected to enhance the GH response to exogenous GHRH, and to make it more homogeneous. Our results do not seem to support this hypothesis, since a marked variability among subjects is still present. Devesa et al. (7), using a different approach to inhibit endogenous somatostatin secretion, also did not succeed in making the GH response to GHRH more homogeneous. According to the hypothesis that the intrinsic rhythm of the hypothalamic-somatotrope axis can not be easily disrupted (7), it is conceivable that the functional status of the somatotropes at the moment of testing was the primary factor influencing the degree of GH responsiveness to GHRH in our subjects.

Alternatively, since the lack of TSH modifications during all studies might suggest that no significant variation of hypothalamic SRIH release occurred, it is also possible that propranolol administration increases GH responsiveness to GHRH by different central mechanisms, for example by inducing the secretion of other hypothalamic peptides able to potentiate stimulated GH release (23-25).

When propranolol was given 90 min before the second GHRH administration, there was a tendency towards a restoration of the GH responsiveness to the second GHRH stimulation, even if such an effect was highly variable.

It has been suggested that an increase in endogenous somatostatinergic tone reactive to the first GHRH injection may cause the blunting of the GH response to subsequent GHRH challenges (8). In keeping with this hypothesis, Massara et al. (9) were able to restore the GH response to a second GHRH bolus by pretreatment with pyridostigmine, which acts by inhibiting endogenous SRIH secretion. Our data could also be explained by β-blockade-induced decrease of somatostatinergic tone, even though the magnitude of the response is clearly lower than that reported with pyridostigmine. On the other hand, the loss of responsiveness to repeated GHRH stimulations is a multifactorial phenomenon in which cellular mechanisms also play an important role, as demonstrated by in vitro studies showing both receptor desensitization and depletion of a GHRH-sensitive releasable pool (26-28).

In conclusion, oral administration of propranolol 90 min before GHRH injection is able to enhance the GH response, without changing the great heterogeneity of individual responses, and the response to repeated GHRH stimulation is only partially restored by β-adrenergic blockade. Thus, it seems unlikely that a combined propranolol-GHRH test with these modalities could improve the diagnostic usefulness of GHRH testing in clinical practice.

References
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Received December 22nd, 1989.
Accepted March 14th, 1990.

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