Verapamil Acute Administration: A New Dynamic Test in Hyperprolactinemic States

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We studied the effect of acute administration of the calcium-channel blocker verapamil (VER) in 27 patients with tumoral hyperprolactinemia ([THPRL] prolactinomas and pseudoprolactinomas). We also studied the effect of VER in seven patients with idiopathic hyperprolactinemia (IHPRL) and a small group of patients with normal prolactin (PRL) levels and minimal incidental anomalies shown by magnetic resonance imaging (MRI). The study was performed on 2 separate days: on the first day, all subjects received VER, and on the second they received placebo. Acute administration of VER evoked a remarkable increase in serum PRL in IHPRL (as in normal healthy subjects used as controls), but no response was shown in THPRL, with no overlap between the two conditions. Acute administration of VER stimulated PRL secretion in patients with minimal incidental lesions shown by MRI; however, this increase was smaller in patients whose PRL level consistently reached the upper-normal limit. Although the meaning of such minimal anomalies shown by MRI is unknown, this could suggest that the test is precociously altered. To further elucidate the action of VER on lactotropes, we investigated the effect of VER given intravenously (IV) and compared different oral formulations in healthy subjects. Our data show that the VER test is effective in distinguishing between THPRL and IHPRL, but unfortunately, like other tests, it is not able to individualize patients in whom THPRL is the result of diminished dopaminergic tone (pseudoprolactinoma). From a pathophysiological point of view, calcium influx would appear less important in PRL regulation in chronic disorders of PRL secretion. VER given IV did not stimulate PRL release in normal subjects. This suggests that IV administration could produce a peak with an inadequate duration or that oral formulations may act also by metabolites formed on first-pass metabolism in the liver.

HYPERPROLACTINEMIA (HPRL) is a common phenomenon and may have many causes.[1-3] Great care should be used in attempting to distinguish between nontumoral HPRL (NTHPRL) and tumoral HPRL (THPRL), which is caused usually by a benign lesion (prolactin [PRL]-secreting pituitary adenoma) and less frequently by a non–PRL-secreting pituitary adenoma dislocating pituitary stalk. Rarely, it can also be caused by infiltrative disease or by a more aggressive tumor. Among NTHPRLs, drug-induced HPRL, hypothyroidism, chest-wall lesions, and renal and kidney failure are easily recognized through the patient's clinical history and/or routine serum tests and screening of thyroid function. In contrast, to distinguish between THPRL and idiopathic HPRL (IHPRL)—in which HPRL is present without any apparent pituitary or central nervous system diseases–it is necessary to use magnetic resonance imaging (MRI).[3]

The extent of basal serum PRL elevations provides valuable information especially when PRL is remarkably elevated, and a number of dynamic tests have been reported. Since PRL is under the tonic inhibitory control of dopamine (DA), while thyrotropin-releasing hormone (TRH) and vasoactive intestinal peptide (VIP) are stimulatory factors, the PRL response to dynamic tests with indirect DA agonists, antagonists of DA receptors and TRH, has been proposed, to distinguish IHPRL from THPRL. Moreover, these have also been proposed to distinguish prolactinomas from pseudoprolactinomas. Unfortunately, in some patients with IHPRL, PRL may not respond. In contrast, in some patients with THPRL, PRL may have a partial response to the above-mentioned agents.[2,4] Calcium (Ca^{2+}) is an important intracellular regulator of PRL secretion, and it probably represents the main second messenger for PRL secretion.[5] Previous reports presented conflicting evidence on the ability of the Ca^{2+}-channel blocker verapamil (VER) to affect PRL secretion in vivo, probably because VER also may act by decreasing central DA generation. Therefore, the PRL response may depend on the dose, form, and time of administration.[6-9] Acute oral administration of VER has been recently reported to help distinguish THPRL from IHPRL.[10,11]

In this study, we have investigated a group of patients with THPRL and IHPRL with the VER test. We also studied the effect of VER given intravenously (IV) and compared different oral formulations in normal subjects.

SUBJECTS AND METHODS

Protocol I: Acute VER Administration Per os in THPRL and NTHPRL (VER test)

The study was performed on 34 patients with an elevated PRL level (>30 ng/mL on at least two occasions) after exclusion of drug-induced HPRL, hypothyroidism, chest-wall lesions, and renal or kidney failure. The study was conducted on 2 separate days (nonconsecutive): on the first day, all subjects received VER as described later, and on the second day they received placebo. All patients were subjected to MRI with gadolinium. Following MRI, 27 patients were found to have THPRL. Among them, the distinction between prolactinoma and pseudoprolactinoma was made on one occasion by MRI itself, on two occasions by postsurgical immunochemistry, and on another two occasions by the clinical criterion of an absence of shrinkage after treatment with DA agonist. Seven patients had a negative MRI result, and we concluded that they had IHPRL. Twelve healthy subjects were included as controls with written consent. Six patients with minimal incidental anomalies shown by MRI such as minimal enhancement of gadolinium or indirect signs of adenoma had serum PRL values measured on at least two

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occasions and were enrolled in the study. In conclusion, we studied four groups of patients. Group I included patients with THPRL: Ia, 16 patients with microprolactinoma aged 18 to 42 years (mean, 27.2), 15 females and one male with a PRL level of 35 to 171 ng/mL (mean, 90.7); Ib, six patients with macroprolactinoma aged 28 to 38 years (mean, 34.6), four females and two males with a PRL level of 407 to 1,431 ng/mL (mean, 643.2); and Ic, five patients with pseudoprolactinoma aged 29 to 43 years (mean, 35.3), three females and two males with a PRL level of 32 to 104 ng/mL (mean, 56.2). Group II included seven patients with IHPRL aged 21 to 30 years (mean, 24.2), seven females with a PRL level of 36 to 81 ng/mL (mean, 50.8). Group III included 12 healthy subjects aged 18 to 34 years (mean, 27.6), six females and six males with a PRL level of 7 to 22 ng/mL (mean, 12.1). Group IV included six patients with incidental minimal anomalies as shown by MRI such as minimal enhancement of gadolinium or indirect signs of adenoma, among whom were two female patients aged 23 and 32 years with a constant PRL level between 21 and 25 ng/mL (group IVa) and four patients, three females and one male, aged 19 to 36 years (mean, 31.5) with a PRL level of 7 to 12 ng/mL (mean, 9.1; group IVb). The VER test began in the morning following an overnight fast in all patients and was performed via cubital vein cannulation. Blood samples were taken at the time intervals of −15, −10, 0, +30, +60, +90, +120, and +180 minutes. Short-acting oral VER 160 mg (Isoptin; Knoll, Milan, Italy) was administered at time 0.

Protocol II: Comparison Studies on VER Given IV and as Different Oral Formulations in Healthy Subjects

After receiving placebo and 160 mg short-acting VER in protocol I, the 12 normal subjects received VER in various formulations on nonconsecutive days as follows: VER 5 mg was administered IV over 2 minutes, and blood samples for PRL were taken at −15, −10, 0, +15, +30, +45, and +60 minutes; short-acting VER was administered orally at a dose of 40 and 80 mg, and blood samples for PRL were taken at intervals as in protocol I; and VER in sustained-release form was administered at a dose of 160 mg daily in the morning for 3 days, and blood samples (three samples at intervals of 15 minutes) for PRL were taken every day before administration of VER and on the fourth day.

The serum PRL level was measured by immunometric chemiluminescence (ACS 180; Chiron Diagnostics, Walpole, MA). The normal range was between 6 and 25 ng/mL, and the intraassay and interassay coefficient of variation for PRL was 5.5% and 7.5%, respectively. No side effects were noted apart from a female patient (with IHPRL) who experienced a transient AV block, type Mobitz I. After VER IV, we found a slight decrease in blood pressure and heart rate. However, these changes were not significant and were not associated with clinical disturbances.

The increase of PRL after VER is expressed as a percentage of the basal value. Results are reported as the mean ± SEM. Data analysis was performed using the unpaired or paired Student's test with simultaneous control of type 1 error.

RESULTS

Figure 1 shows that following VER administration, the PRL response showed no overlap between patients with IHPRL and all subgroups with THPRL. No PRL response was present in THPRL patients following VER administration; in fact, the percent variation was 0.2% ± 2.0%. Patients with IHPRL instead showed a clear PRL increase, with a peak percent increase of 245% ± 40.2% ($P = .003$).

However, the increment of PRL in IHPRL was lower than in normal subjects, whose peak percentage reached 685% ± 186.9% ($P = .006$), but there was a wide overlap for the percent stimulation between the two groups and no statistical difference ($P = .8$). VER evoked the secretory peak after 90 to 120 minutes (Fig 2). Figure 3 shows that in patients with minimal incidental anomalies shown by MRI (group IV), VER evoked apparently different responses. Patients with PRL levels always between 7 and 12 ng/mL (group IVb) showed a peak percent stimulation within the range for normal healthy subjects. The two patients with minimal incidental anomalies demonstrated by MRI with PRL levels constantly at the upper-normal limit (group IVa) showed a lower percent increase than group IVb and healthy subjects.

Figure 4 shows the PRL response after administration of different formulations of VER. VER given IV did not elicit a significant increase in PRL (peak percent increase, 15.1% ± 13.6%, $P = .2$). In contrast, administration of a sustained-release form evoked an increase in PRL, with a peak percent increase of 180% ± 43.2% ($P = .004$). This peak was lower than that evoked by the same dose given in short-acting form ($P = .01$). The peak evoked by the sustained-release form was also lower, albeit without a statistical difference ($P = .2$), than the peak evoked by half the dose given as a short-acting formulation, whose peak percent increase in PRL was 260% ± 45.3% ($P = .003$). The short-acting formulation caused a PRL increase even at a dose of 40 mg, with a peak percent increase of 41% ± 14.3% ($P = .04$).

DISCUSSION

The use of dynamic tests in patients with HPRL has always been frustrating for the endocrinologist. PRL is under tonic inhibition by DA, while TRH, VIP, and a number of other peptides have been shown to have stimulatory activity. The use of DA antagonist or agonist and TRH for diagnostic purposes has failed, since the absence of a response of PRL to such substances usually characterizes THPRL patients. However, sometimes, in IHPRL patients, such a response is absent, and some THPRL patients may have a "partial" or normal increase.[1-4] Ca^{2+} rather than cyclic adenosine monophosphate probably represents the main second messenger for PRL release.[5] Previous studies in vitro with VER and Ca^{2+} ionophores have shown that both basal and stimulated PRL secretion and the inhibitory action of DA are dependent on Ca^{2+} influx.[12-15] Moreover, Ca^{2+} influx and the pattern of Ca^{2+} can stimulate PRL gene expression.[16-18] In vivo, the effects of VER appear more intricate, since VER could affect PRL secretion also by decreasing central DA generation.[9]

Moreover, following oral administration, there is first-pass metabolism in the liver and some metabolites may contribute to the VER action.[19,20] For these reasons, the effect of VER may depend on the dose, form, and time of administration, and some reports show no effect of VER on basal and TRH-stimulated PRL values while others demonstrate PRL secretion after VER administration.[6-8] When VER is administered acutely orally, it stimulates PRL secretion, and preliminary results have shown that acute VER administration orally can stimulate PRL secretion in IHPRL as in healthy subjects, but not in THPRL.[10,11] Thus, acute administration of VER could be used for diagnostic purposes, and it would represent a dynamic test that, contrary to other tests, uses an antagonist of the second messenger.

The present study shows that the VER test can distinguish

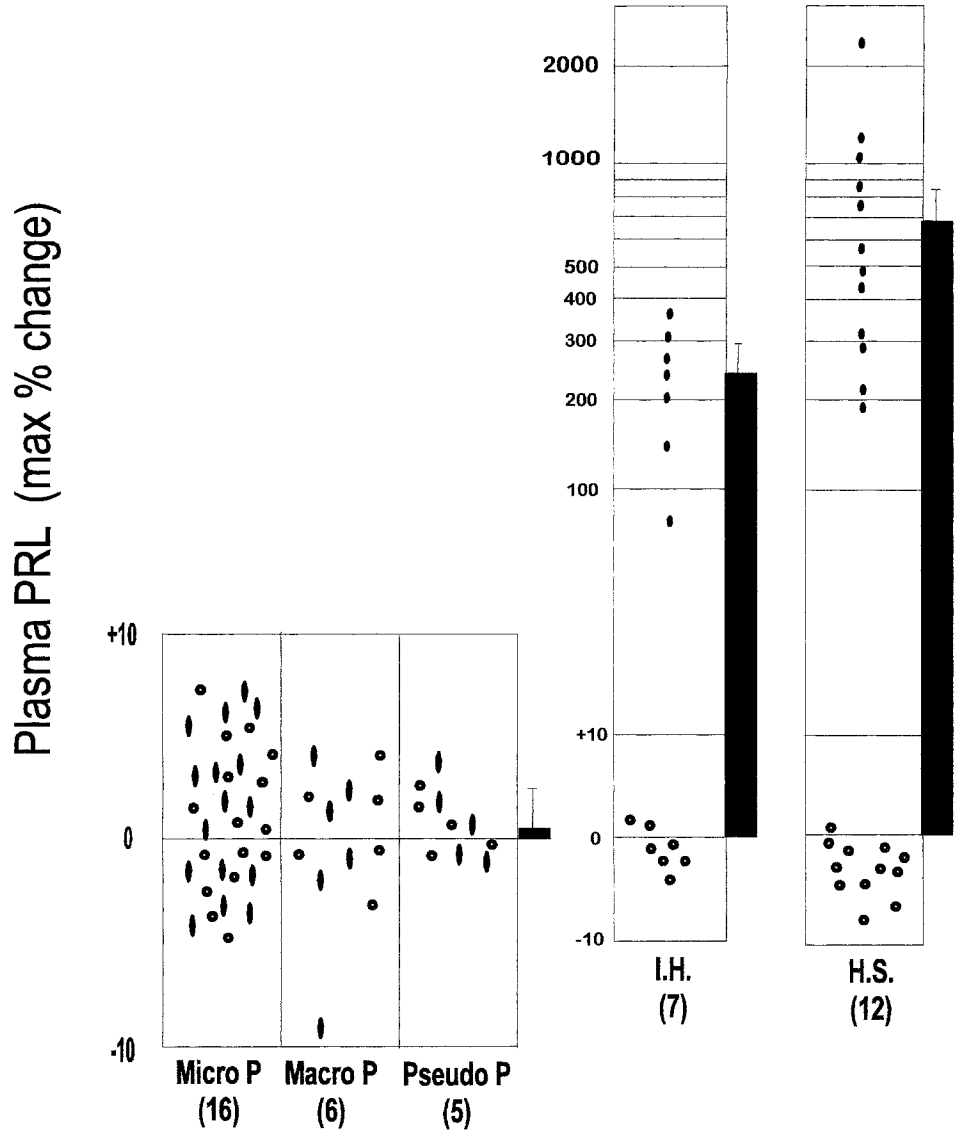

Fig 1. Changes in plasma PRL (maximum % change) after administration of VER short-acting form 160 mg orally (●) and placebo (○) in patients with microprolactinoma (Micro P), macroprolactinoma (Macro P), pseudoprolactinoma (Pseudo P), or IHPRL (I.H.) and healthy subjects (H.S.). The number of subjects is shown in parentheses. (■) Mean ± SEM for max % change of PRL after VER.

between IHPRL and THPRL. All of these IHPRL patients have always shown an increase in PRL levels, and there has never been any overlap in the percent stimulation with THPRL patients, in whom we did not observe any response even when PRL was only slightly increased. Moreover, the percent stimulation was so remarkable as to allow a clear separation between the two groups. Therefore, the VER test appears to have two major advantages with respect to the aforementioned dynamic tests: first, we have always shown a positive PRL response to the test in IHPRL patients; and second, the PRL response to VER was absent in all THPRL patients (independent of the PRL level), and thus, there is not a fixed percent stimulation above which the response should be considered positive.

The result of the test in patients showing minimal incidental anomalies (after MRI) was interesting. In this group, on the basis of basal serum PRL values, we could distinguish two subgroups of patients: patients with PRL levels within the normal range (but always at the upper limit), who had a clearly lower response than the healthy group of patients with minimal anomalies shown by MRI but with completely normal PRL values. Although it was impossible to determine the nature of such minimal lesions, patients with the highest serum PRL value could represent a subset of patients who probably had prolactinomas. Although further studies will be necessary to confirm the data, the VER test appears precociously altered. Pituitary adenomas are the most common pituitary incidental lesions,[21] and for this reason, it is logical to evaluate patients endocrinologically regardless of the size of the incidental mass. These results suggest the possibility of recognizing very small prolactinomas before PRL is clearly elevated.

Like other dynamic tests for PRL secretion, the VER test cannot differentiate between prolactinomas and pseudoprolactinomas, which increase PRL secretion through pituitary stalk compression. However, the possibility of distinguishing patients with THPRL with a simple dynamic test may represent an important first diagnostic step when PRL is slightly or moderately elevated, and this represents a common clinical finding.

Our results could have pathophysiological implications. In prolactinoma, PRL is secreted autonomously by the tumor cells, and normal lactotropes are blunted by the increased dopaminer-

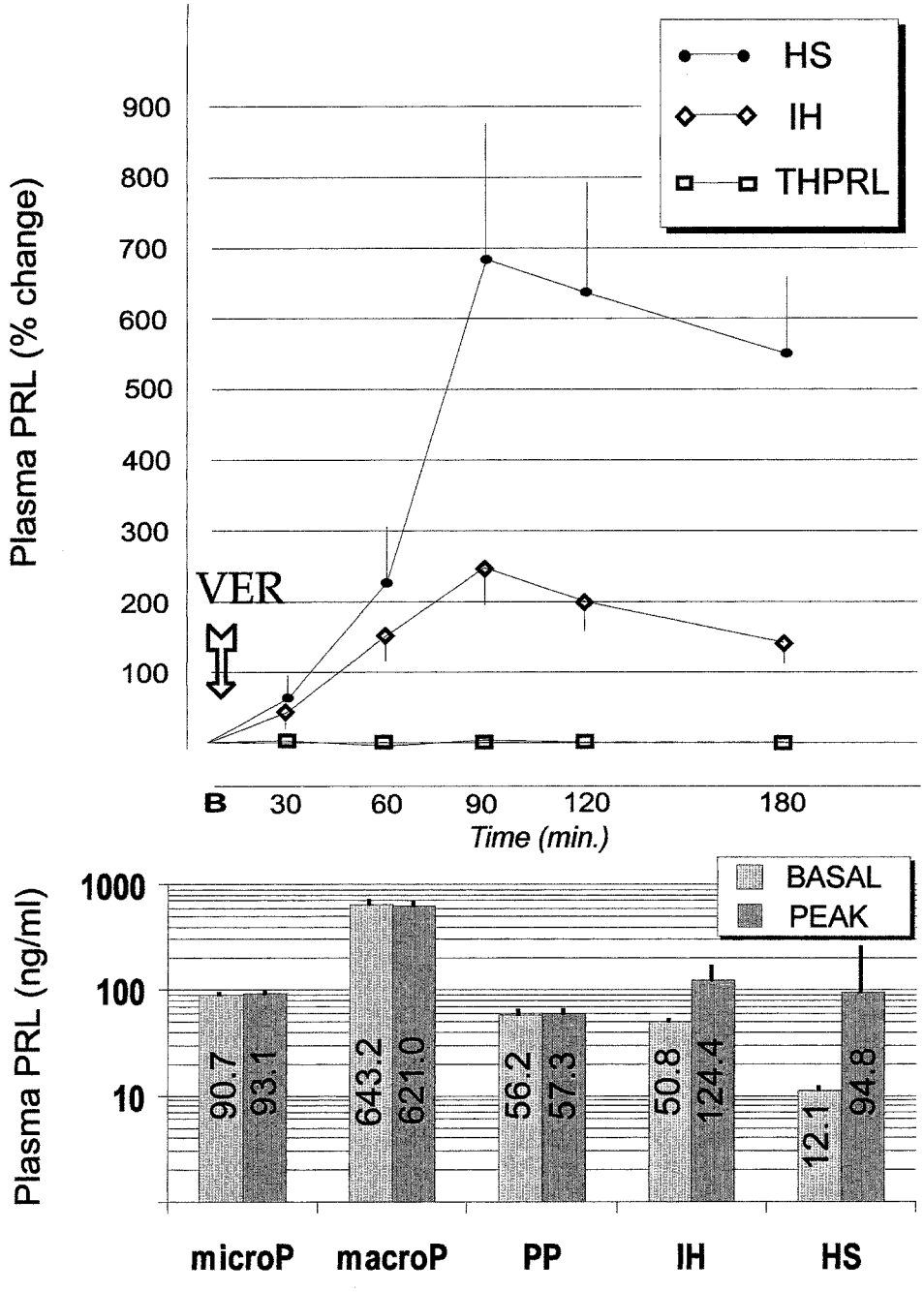

Fig 2. Increase in plasma PRL after VER short-acting form expressed as a percent increase (mean ± SEM) in 12 healthy subjects (HS), 7 patients with IHPRL (IH), and 27 patients with THPRL. Basal value (B) is the mean of −15, −10, and 0 minutes (top). Basal and peak PRL values (mean ± SEM) are expressed as serum levels in the same groups (bottom).

gic tone. In pseudoprolactinoma, HPRL is due to the diminished dopaminergic tone, but lactotropes are probably also subject to a decrease of the physiological PRL-releasing factors. Chronic hypersecretion by normal lactotropes, due to such deprivation or an imbalance of the normal physiological regulators, would appear at least partially independent from the influx of extracellular Ca^{2+}. According to this hypothesis, IHPRL could be the result of an altered set point for PRL regulation.

To elucidate the stimulatory effect of VER on PRL release, we studied different routes of administration. We also made a comparison between a short-acting and a slow-release oral formulation in normal subjects. To compare these effects, we chose the VER dose of 5 mg IV, which produces approximately the same peak in plasma VER as 160 mg of the short-acting oral administration.[19,20] We also chose to administer the same amount (160 mg) of VER in the sustained-release oral formulation. Our results, according to other published studies, show that VER given IV does not stimulate PRL release in normal subjects. The possible explanations could be that the peak has an insufficient duration or that some metabolites of VER may be more active Ca^{2+}-channel blockers in pituitary (or hypothalamic) cells. Using the oral slow-release formulation, we observed a stimulation of PRL, but the increment was lower than for the half-dose given as the short-acting form. This also suggests that an overly flat peak would be less effective for PRL release.

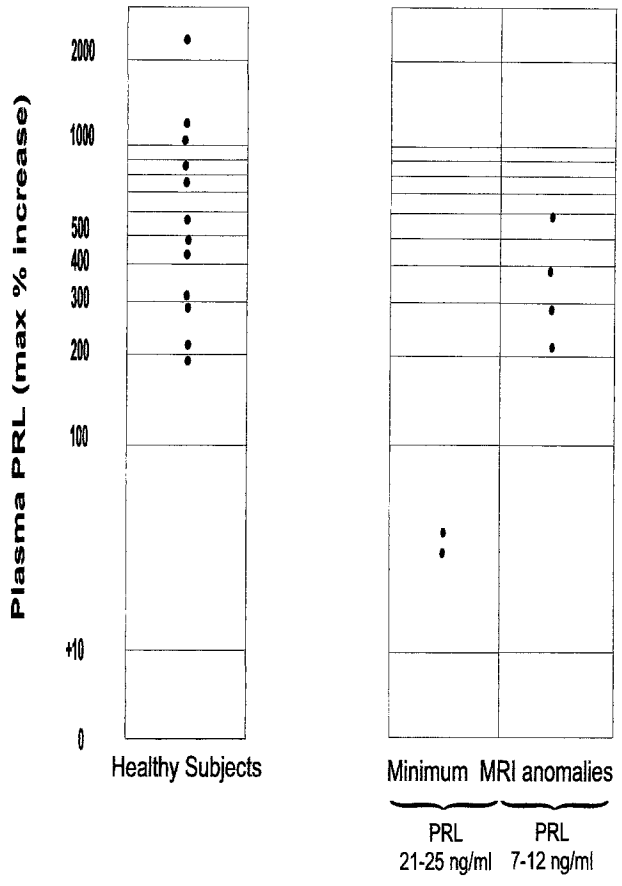

Fig 3. Changes in plasma PRL (maximum % increase) after VER short-acting form 160 mg orally in patients with minimum MRI anomalies and healthy subjects (control).

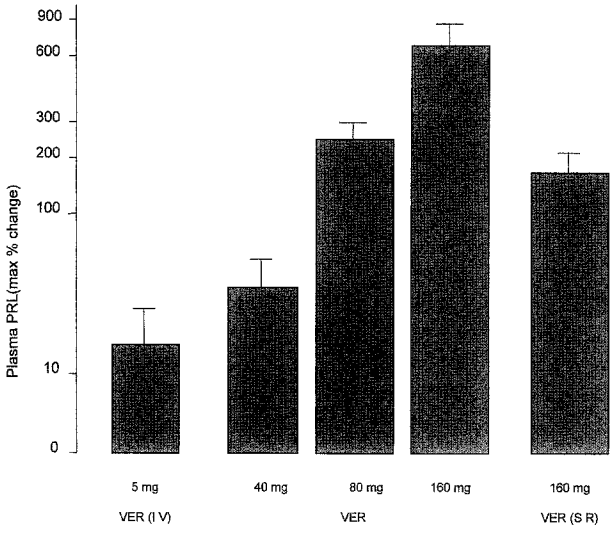

Fig 4. Peak percent increase in plasma PRL (mean ± SEM) after VER administered in various formulations and doses in normal subjects (IV, oral short-acting [VER], or oral sustained-release [SR]).

In conclusion, our results show that the VER test can distinguish IHPRL from THPRL also when PRL does not appear maximally secreted and PRL serum levels are slightly increased. Moreover, when PRL is still in the normal range, it appears possible to distinguish patients with prolactinomas. Our studies in normal subjects show that the peak duration of VER could be of crucial importance in PRL release. Furthermore, a possible explanation for the divergent effects of VER given IV and orally is that active metabolites formed upon first-pass metabolism in the liver may have important actions.

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