Complete Inhibition of Hypothalamic Somatostatin Activity Is Only Partially Responsible for the Growth Hormone Response to Strenuous Exercise

Wouter R. de Vries, Salem Ait Abdesselam, Theo J. Schers, Hans C.M. Maas, Mohamoud Osman-Dualeh, Inge Maitimu, and Hans P.F. Koppeschaar

The aim of this study was to investigate whether growth hormone (GH) release during strenuous exercise (EX) is due to complete inhibition of hypothalamic somatostatin (SS) activity. Eight healthy male subjects (age, 22.1 ± 2.2 years; body mass index [BMI], 22.2 ± 2.5 kg/m^2; maximum oxygen consumption [$\dot{V}O_2$max], 52.2 ± 1.5 mL/min/kg [mean ± SD]) were exposed to strenuous EX on a cycle ergometer, with and without administration of pyridostigmine (PD), and to administration of PD alone. PD is an acetylcholine-esterase inhibitor that stimulates GH secretion by suppressing hypothalamic SS secretion and unmasking endogenous GH-releasing hormone (GHRH) tone. Serial blood samples in the fasted state were taken immediately before the start of each trial, and at appropriate intervals over 2 hours. GH responses were calculated as area under the response curve (AUC) by trapezoidal integration. The mean peak serum GH level to PD alone was 18.3 μg/L (range, 0.3 to 40.9), which was significantly lower than to EX alone: 64.1 μg/L (range, 30.5 to 90.5), and to the combined administration of PD and EX (PD+EX): 79.8 μg/L (range, 37.7 to 98.2) ($P < .05$). The arithmetic sum of the individual peak levels of 82.4 μg/L was not different from the mean peak level to PD+EX: 79.8 μg/L. AUC (mean ± SEM) to PD alone (1,721 ± 358 μg/L × 180 min) was not significantly different from that to EX alone (2,472 ± 408 μg/L × 120 min), but was significantly lower than that to PD+EX: 3,526 ± 752 ($P < .05$). Although the latter AUC was 6% smaller than the AUC obtained by arithmetic addition (3,747 ± 706), this difference was not statistically significant. In conclusion, the additive effect between PD and EX indicates that PD and EX act independently in evoking GH responses to strenuous EX. Therefore, GH responses to strenuous EX are only partially due to complete inhibition of hypothalamic SS. Additional potentiating factors, such as activation of endogenous GHRH and ghrelin must be operative.

PITUITARY growth hormone (GH) release is regulated by a final common pathway, consisting of inhibitory control of somatostatin (SS), and stimulatory control of hypothalamic GH-releasing hormone (GHRH). In addition, ghrelin, a peptide of 28 amino acids, is also responsible for the control of GH secretion.[1] In general, increasing serum levels of GH can be reached by inhibition of the effects of SS and/or by increasing the effects of GHRH and ghrelin. Despite this knowledge, the underlying mechanisms for the exercise (EX)-induced increase in serum GH are still poorly understood. Many factors may modulate the EX-induced GH release, such as the nature, duration, or intensity of EX; individual characteristics, such as age, gender, body composition, nutritional status, smoking habits, and training status; as well as pretreatment of drugs.[2] It has already been reported that the GH release to submaximal EX is mainly mediated by an increased central cholinergic tone,[3-7] which reduces the activity of hypothalamic SS. Endurance training has been shown to increase basal GH production, presumably also via inhibition of SS release.[8,9] However, it is not known to what extent inhibition of SS is responsible for the maximal GH release that occurs during strenuous EX.

To assess the significance of complete inhibition of SS to the GH release during strenuous EX, we studied the GH responses to strenuous EX, which evokes a maximal GH response, to pyridostigmine (PD), an acetylcholine-esterase inhibitor that stimulates GH secretion by suppressing hypothalamic SS secretion and unmasking endogenous GHRH tone,[10] as well as to the combined administration of PD and EX (PD+EX). We assume that PD at the doses used (120 mg) suppresses SS completely.[11] Such a design may help define the involvement of SS. For example, if maximally effective doses of PD and EX are given, then tentative conclusions can be drawn: if the GH response to PD+EX is not greater than the maximal response to PD alone, then EX acts by a PD-mediated pathway. If the response to PD+EX is beyond the maximal releasing capacity of PD, additional pathways are likely to be involved. An additive response of PD+EX may indicate that PD and EX act independently in evoking GH responses, whereas a synergistic (potentiating) response may indicate an interaction between the pathways of PD and EX. GH data were evaluated as peak values and as area under the response curve (AUC).

SUBJECTS AND METHODS

Subjects

Eight healthy male subjects, aged 20 to 26 years, were studied after approval of the ethics committee of the University Hospital Utrecht and after giving informed consent. Their habitual activity level ranged from moderately- to well-trained, and all subjects were nonsmokers. None had a history of medical illness, and none was taking any medication or were obese (peak oxygen consumption [$\dot{V}O_2$], 52.2 ± 1.5 mL/kg/min; body weight, 72.6 ± 5.3 kg; height, 1.82 ± 0.05 m; body mass index [BMI], 22.2 ± 2.5 kg/m^2; body fat percentage, 13.4 ± 4.6% [mean ± SD]). Assessment of fat mass (FM) and fat-free mass (FFM) was performed using a tetrapolar bioelectrical impedance analyzer (BIA-101 analyzer, RJL Systems, Detroit, MI) on the basis of resistance and reactance measurements (in Ohm) during application of a alternating electric current of 800 μA at 50 kHz with the electrodes placed as

From the Department of Medical Physiology and Sportsmedicine, University Medical Center Utrecht; and the Department of Endocrinology, University Hospital Utrecht, University Medical Center Utrecht, The Netherlands.

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Address reprint requests to Wouter R. de Vries, MD, PhD, Department of Medical Physiology and Sportsmedicine, University Medical Center Utrecht, PO Box 85060, 3508 AB Utrecht, The Netherlands.

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described by Lukaski et al.[12] FM and FFM were calculated using regression equations, developed by Lukaski and Bolonchuk.[13]

Study Design

At least 1 week before these trials each subject participated in a maximal performance test on a cycle ergometer (Excalibur, Lode, Groningen, The Netherlands) to determine individual levels of peak oxygen uptake (the highest value attained during the test). The test started at a workload of 2 W/kg body weight (25 W=1 kpm) and subsequently every 2 minutes a load of 0.5 W/kg was added until the subject was exhausted. A breath-to-breath gas sampling method was used to measure oxygen uptake by a paramagnetic analyzer (Oxycon Beta, Mijnhardt, Bunnik, The Netherlands).

Each subject participated in 3 different trials, which were performed at least 1 week apart in order to have enough time for recovery. All tests began at 9 AM after an overnight fast, and started with EX alone, followed in random order by PD alone or PD+EX. Thirty minutes before the start of each trial, a catheter was placed in an antecubital vein and was kept patent with a heparin/salt solution. The moment of the start of the trial is called t = 0. Ten blood samples were taken at t = 0, 15, 25, max (immediately after exhaustion), 30, 35, 45, 60, 90, and 120 minutes.

EX consisted of cycling on an ergometer in an upright position, according to an incremental protocol: 5 minutes at 40% of the peak oxygen uptake ($\dot{V}O_2$max), 10 minutes at 60% $\dot{V}O_2$max, 10 minutes at 80% $\dot{V}O_2$max, and finally 100% $\dot{V}O_2$max until exhaustion. Pyridostigmine bromide (Mestinon, Roche Nederland B.V., Mijdrecht, The Netherlands) was administered orally at a dose of 120 mg, 60 minutes before the start of a trial. All subjects reported minor side effects, such as accommodation weakness, involuntary muscle contractions, and gastrointestinal complaints.

Hormonal Assays

Serum GH levels were measured in duplicate by immunoradiometric assay (IRMA; Oris Industry Co, Gif-sur-Yvette, France). GH standards were calibrated according to the World Health Organization reference standard 66/217. There was no cross-reactivity with luteinizing hormone, follicle-stimulating hormone, human chorionic gonadotropin, thyroid-stimulating hormone, or prolactin. Limit of sensitivity was 0.18 μg/L. The mean interassay coeffients of variation (CV) were 6.1%, 5.0%, and 6.0% at GH concentrations of 6.2, 19.6, and 59.8 μg/L, respectively. The intra-assay CV was 7.7% at a GH level of 8.0 μg/L.

Statistics

The AUCs, controlled for differences in baseline, were calculated by trapezoidal integration. To compare mean peak values and AUCs between the different stimuli, nonparametric statistics (Wilcoxon matched-pairs signed-ranks test) were used. The level of statistical significance was set at $P < .05$ (2-tailed).

RESULTS

Mean duration of EX alone was 25 minutes 20 seconds, and this was not significantly different from the duration of EX in the PD+EX trial: 24 minutes 53 seconds.

Table 1 shows the mean GH responses (with ranges) of the 3 trials as a function of time. The mean peak (range) GH response to PD alone was significantly lower than to EX and to PD+EX: 18.3 (0.3 to 40.9) μg/L versus 64.1 (30.5 to 90.5) versus 79.8 ([37.7 to 98.2), respectively ($P < .05$). Individual peak values to PD were reached between t = 0 and t = 90 minutes, whereas in the trials with EX alone and with PD+EX peak values were reached at or shortly after the moment of exhaustion, ie, between t = 25 and t = 27 minutes. The arithmetic sum of the separate peak levels (82.4 μg/L) was not different from the mean peak level to PD+EX: 79.8 μg/L. Figure 1 shows the responses of mean serum GH levels as a function of time, as well as the summated response, obtained by arithmetical addition.

Table 1. GH Responses to PD Alone, EX Alone, and PD+EX as a Function of Time

	Mean (range) GH Response (μg/L)		
t(min)	PD	EX	PD+EX
−60	2.1 (0.1−8.6)	—*	3.5 (0.1−14.1)
0	14.2 (0.2−31.8)	0.9 (0.2−7.7)	9.3 (0.2−22.7)
15	15.8 (0.4−35.5)	19.3 (1.8−42.3)	24.0 (8.6−34.1)
25	18.0 (0.3−38.6)	58.9 (22.7−89.5)	71.6 (53.2−85.5)
max	18.2 (0.3−40.9)	64.1 (30.5−90.5)	79.2 (61.8−98.2)
30	17.9 (0.3−40.0)	59.5 (31.8−88.6)	69.7 (53.2−86.8)
35	16.6 (0.4−38.2)	54.5 (31.4−90.0)	61.6 (45.5−73.2)
45	14.4 (1.8−32.7)	39.2 (25.9−51.8)	48.4 (36.4−67.7)
60	8.6 (1.0−27.7)	18.2 (3.6−26.4)	28.7 (22.7−35.9)
90	5.5 (0.5−15.9)	5.2 (0.0−17.3)	14.9 (8.2−22.3)
120	2.7 (0.2−9.5)	1.0 (0.0−2.3)	6.6 (3.3−11.4)

*Not measured.

AUCs were calculated over a period of 120 minutes. However, because PD was administered 60 minutes before the start of a trial, the AUC after PD alone was calculated over a period of 180 minutes. AUCs (mean ± SEM) after PD alone and EX alone were not significantly different: 1,721 ± 358 μg/L × 180 min versus 2,472 ± 408 μg/L × 120 min. In addition, the AUC after PD+EX (3,526 ± 752) was greater than those after both stimuli separately ($P < .05$), but similar to the arithmetic sum of both stimuli (3,747 ± 706).

DISCUSSION

The responses to the combined administration of PD+EX are significantly greater than those to a maximally effective dose of PD alone. Furthermore, this combined effect of PD and strenuous EX is additive and not synergistic, which makes an interaction between the central pathways used by PD and strenuous EX unlikely.

Reduction of hypothalamic SS by an increased central cholinergic tone has been reported to occur under submaximal EX conditions.[3-7] However, during high-intensity EX, involvement of a further reduction of SS as potentiating factor is only possible when one assumes that SS activity could be further reduced, ie, when this mechanism is not saturated.

During submaximal exercise at lactate threshold, Nooitgedagt et al[6] showed that administration of PD gave an additive effect of the GH responses, indicating that PD and EX may act independently. In contrast, Thompson et al[7] found no alterations in peak GH concentrations by administration of PD during moderate-intensity exercise, indicating that the GH response to EX was solely due to an increase in central cholinergic tone. Recently, Marcell et al[14] reported that GH secretion during PD+EX was greater than during PD or EX alone, suggesting that some additional pathway is operative. Based on the purported dual action of PD (suppressing SS secretion and unmasking endogenous GHRH tone[10]) and on the findings of

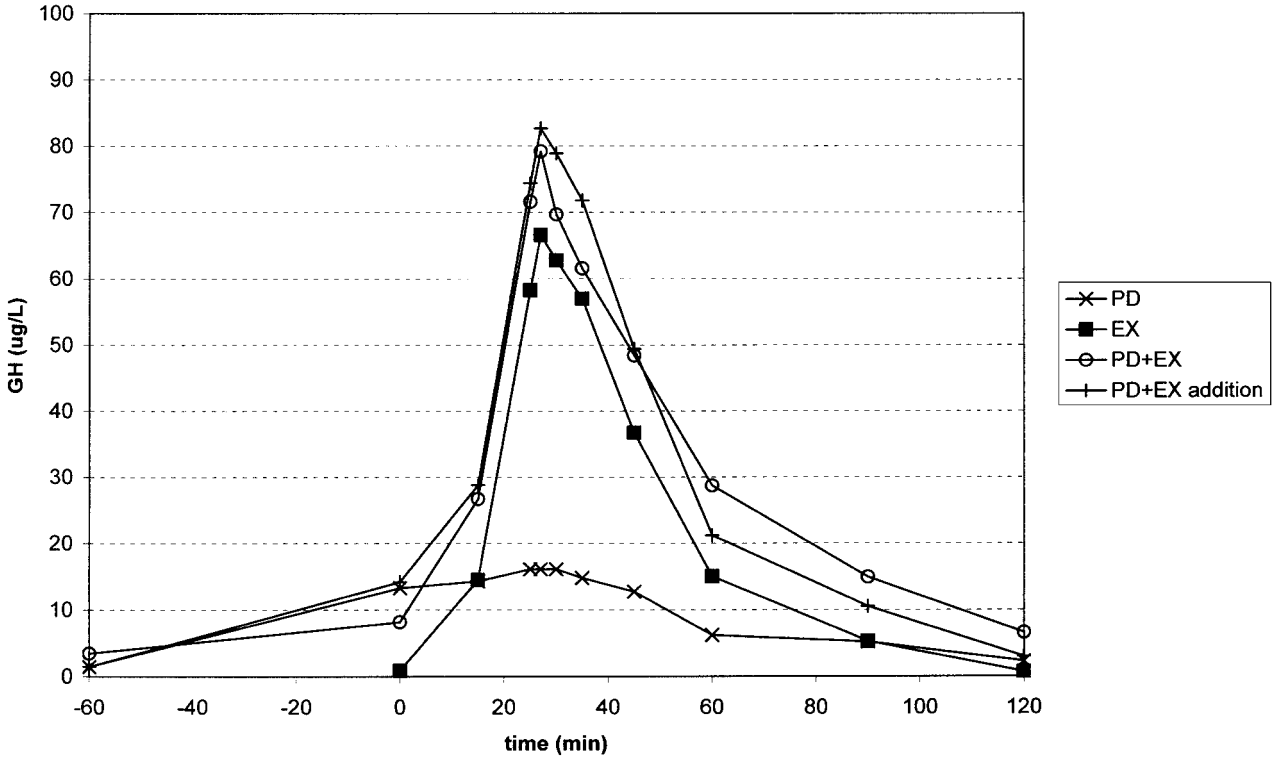

Fig 1. Responses of mean serum GH (in μg/L) as a function of time. EX performance between t = 0 and t = 25 min 20 s. PD administration: 60 min before t = 0 (t = -60). For clarity, no SEM data are depicted. Peak GH to EX and peak GH to PD+EX > peak GH to PD ($P < .05$). Peak GH to EX = peak GH to PD+EX = peak GH to PD+EX add.

our study, we postulate that during high-intensity exercise, activity of SS is maximally inhibited, and that GH release must be enhanced by further activation of a GHRH-dependent[10] and/or ghrelin-dependent mechanism.[1,15,16]

We used a dose of 120 mg PD, which has been reported to suppress the majority of the somatostatinergic tone.[11,17] The observed mean peak GH levels of about 16 μg/L were reached during a wide range in time: between t = 0 and t = 90 minutes, ie, 60 to 150 minutes after oral administration, which is in accordance with clinical practice. Therefore, despite the intention to reach peak values synchronously, there is a clear difference in time course of the responses to PD and EX. As already mentioned before, the combined administration of PD+EX evoked additive responses, indicating that the pathways used by EX act independently from those used by PD.

We used an incremental exercise protocol on a cycle ergometer, which maximally stimulates the GH release.[15] Mean peak values of approximately 67 μg/L were observed, mainly at, or shortly after the time of exhaustion, ie, about 26 to 27 minutes after the start of EX. Such a response is at the level observed in elite rowers after a competition race,[18] and also in line with data in trained subjects, which range from 10 to 60 μg/L.[2,19-22]

In conclusion, GH responses to strenuous EX are only partially due to complete inhibition of hypothalamic SS activity. Additional potentiating factors, such as activation of endogenous GHRH and ghrelin must be operative.

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REFERENCES

1. Dieguez C, Casanueva FF: Ghrelin: A step forward in the understanding of somatotroph cell function and growth regulation. Eur J Endocrinol 142:413-417, 2000

2. Cuneo RC, Wallace JD: Growth hormone, insulin-like growth factors and sport. Endocrinol Metab 1:3-13, 1994

3. Casanueva FF, Villanueva L, Cabranes JA, et al: Cholinergic mediation of growth hormone secretion elicited by arginine, clonidine and physical exercise in man. J Clin Endocrinol Metab 59:526-530, 1984

4. Few JD, Davies CT: The inhibiting effect of atropine on growth hormone release during exercise. Eur J Appl Physiol 43:221-228, 1980

5. Jaffe CA, DeMott-Friberg R, Barkan AL: Endogenous growth hormone (GH)-releasing hormone is required for GH responses to pharmacological stimuli. J Clin Invest 97:934-940, 1996

6. Nooitgedagt A, Koppeschaar HPF, de Vries WR, et al: Influence of endogenous cholinergic tone and growth hormone-releasing peptide-6 on exercise induced growth hormone release. Clin Endocrinol 46:195-202, 1997

7. Thompson DL, Weltman JY, Rogol AD, et al: Cholinergic and opioid involvement in release of growth hormone during exercise and recovery. J Appl Physiol 75:870-878, 1993

8. Rogol AD, Weltman JY, Evans WS, et al: Long-term endurance training alters the hypothalamic-pituitary axes for gonadotropins and growth hormone. Endocrinol Metab 21:817-832, 1992

9. Weltman A, Weltman JY, Schurrer R, et al: Endurance training amplifies the pulsatile release of growth hormone: Effects of training intensity. J Appl Physiol 72:2188-2196, 1992

10. Müller EE: Cholinergic function and neural control of GH secretion. A critical re-appraisal. Eur J Endocrinol 137:338-342, 1997

11. Massara F, Ghigo E, Molinatti P, et al: Potentiating of cholinergic tone by pyridostigmine bromide reinstates and potentiates the growth hormone responsiveness to intermittent administration of growth hormone-releasing factor in man. Acta Endocrinol (Copenh) 113:12-16, 1986

12. Lukaski HC, Johnson PE, Bolonchuk WW, et al: Assessment of fat-free mass using bioelectrical impedance measurements of the human body. Am J Clin Nutr 41:810-817, 1985

13. Lukaski HC, Bolonchuk WW: Theory and validation of the tetrapolar bioelectrical impedance method to assess human body composition, in Ellis KJ, Yasumura S, Morgan WD (eds): In Vivo Body Composition Studies. London, UK, Institute of Physical Science in Medicine, 1987, pp 410-414

14. Marcell TJ, Wiswell RA, Hawkins SA, et al: Age-related blunting of growth hormone secretion during exercise may not be solely due to increased somatostatin tone. Metabolism 48:665-670, 1999

15. Maas HCM, de Vries WR, Maitimu I, et al: Growth hormone (GH) responses during strenuous exercise: The role of GH-releasing hormone (GHRH) and GH-releasing peptide-2 (GHRP-2). Med Sci Sports Exerc 32:1226-1232, 2000

16. Kojima M, Hosoda H, Date Y, et al: Ghrelin is a growth hormone-releasing acylated peptide from stomach. Nature 402:656-660, 1999

17. Locatelli V, Torsello A, Redaelli M, et al: Cholinergic agonist and antagonist drugs modulate the growth hormone response to growth hormone-releasing hormone in the rat: Evidence for mediation by somatostatin. J Endocrinol 111:271-278, 1986

18. Snegovskaya V, Viru A: Elevation of cortisol and growth hormone levels in the course of further improvement of performance capacity in trained rowers. Int J Sports Med 14:202-206, 1993

19. De Vries JH, Noorda RJP, Voetberg GA, et al: Growth hormone release after the sequential use of growth hormone releasing factor and exercise. Horm Metab Res 23:397-398, 1991

20. Friedmann B, Kindermann W: Energy metabolism and regulatory hormones in women and men during endurance exercise. Eur J Appl Physiol 59:1-9, 1989

21. Luger A, Watschinger B, Deuster P, et al: Plasma growth hormone and prolactin responses to graded levels of acute exercise and to a lactate infusion. Neuroendocrinology 56:112-117, 1992

22. Sutton JR, Jones NL, Toews CJ: Growth hormone secretion in acid-base alternations at rest and during exercise. Clin Sci Mol Med 50:241-247, 1976