Alpha-Glycerylphosphorylcholine Administration Increases the GH Responses to GHRH of Young and Elderly Subjects

G. P. Ceda, G. Ceresini, L. Denti, G. Marzani, E. Piovani, A. Banchini, E. Tarditi and G. Valenti
Chair of Gerontology and Geriatrics, University of Parma, Parma, Italy

Summary

Growth hormone (GH) secretion is decreased during aging in humans and in rodents. This decrease may be due to increased hypothalamic somatostatin release, which is inhibited by cholinergic agonists, or to decreased secretion of GHRH. Alphaglycerylphosphorylcholine (alpha-GFC) is a putative acetylcholine precursor used in the treatment of cognitive disorders in the elderly. In order to learn what effect alpha-GFC had on GH secretion, GH-release hormone (GHRH) was given to young and old human volunteers, with or without the addition of alpha-GFC. GH secretion was greater in the younger subjects than in the old individuals, and both groups had a greater GH response to the GHRH + alpha-GFC than to GHRH alone. The potentiating effect of alpha-GFC on GH secretion was more pronounced in the elderly subjects. These findings confirm the observation that aged individuals respond less well to GHRH than younger subjects, and provides further evidcence that increased cholinergic tone enhances GH release.

Key words

GHRH – GH – Aging – Alpha-Glycerylphosphorylcholine – Acetylcholine

Introduction

GH secretion is blunted during aging in rodents and humans. A decrease of GH secretory pulses as well as a decline in GHRH-induced GH secretion has been documented in elderly people (*Ho, Evans, Blizzard, Veldhuis, Merriam, Samojlik, Furlanetto, Kaiser* and *Thorner* 1987; *Shibasaki, Shizume, Nakahara, Masuda, Jibiki, Demura, Wakabayashi* and *Ling* 1984). The ability of GHRH to release GH both in vivo and in vitro declines in aged rats (*Ceda, Valenti, Butturini* and *Hoffman* 1986), and this finding is associated with the decreased expression of GHRH receptors in the pituitary (*Abribat, Deslauriers, Brazeau* and *Gaudreau* 1991). Moreover, aged rats have lower levels of hypothalamic GHRH mRNA (*De Gennaro Colonna, Zoli, Cocchi, Maggi, Marrama, Agnati* and *Muller* 1989) and pituitary GH mRNA (*Hoffman, Griffin, Kalinyak, Perkins* and *Ceda* 1988).

Central cholinergic stimuli increase the secretion of GH from the pituitary. Anticholinergic drugs diminish the GH response to a variety of stimuli, while drugs like the cholinesterase inhibitor pyridostigmine induce GH release, potentiate the stimulatory effect of GHRH (*Massara, Ghigo, Demislis, Tangolo, Mazza, Locatelli, Muller, Molinatti* and *Camanni* 1986a) and restore the GH response after intermittent GHRH administration (*Massara, Ghigo, Molinatti, Mazza, Locatelli* and *Camanni* 1986b). These latter actions may be mediated by a decrease of somatostatin release. Since brain acetylcholine (Ach) synthesis declines with aging (*Gibson, Peterson* and *Jenden* 1981), it may be possible to restore normal GH secretion by enhancing cholinergic tone. We have recently shown that the putative Ach precursor CDP-choline is able to increase both basal and GHRH induced GH secretion in elderly human subjects (*Ceda, Ceresini, Denti, Magnani, Marchini, Valenti* and *Hoffman* 1991), lending support to the hypothesis of an increased somatostatin tone during aging.

The putative Ach precursor, alpha-glycerylphosphorylcholine (alpha-GFC), has been used for the treatment of amnestic and cognitive disorders of aging. The aim of this study was to study the effects of alpha-GFC administration on GH secretion in a group of elderly people.

Materials and Methods

Eight young (5 males and 3 females) aged 32 ± 1.9 and 7 healthy old subjects (4 males and 3 females) aged 80 ± 2 years, were studied. All subjects were in good health, and none was taking any medication known to affect GH secretion. They were studied on three separate days at > 1 week intervals according to the following protocols. They received in a randomized order: 1) an iv infusion over 30 minutes of 1 g alpha-GFC (Brezal, Sandoz, Italy) dissolved in 100 ml normal saline with blood samples obtained at – 15, 0, 15, 30, 45, 60, 90 and 120 minutes from the start of the infusion; 2) a bolus injection of GHRH(1–44NH2) (Novabiochem, Inalco, Milan, Italy) at a dose of 1 μg/kg with blood samples taken at – 15 and 0 min and at 15, 30, 45, 60, 90 and 120 min after the bolus injection and 3) a 30 min infusion of alpha-GFC followed by a bolus injection of GHRH at the same dosage.

GH levels were measured using commercial kits obtained from Ares-Serono (Milan, Italy). Serum GH was measured by a specific double antibody RIA with a sensitivity of 0.25 μg/l and intra- and interassay coefficient of variations of 4 and 8% respectively.

Statistical evaluation was performed using analysis of variance for repeated measures and Wilcoxon's test (due to the relatively low numbers of subjects) using a Statview II program. The area under the curve (AUC, $\mu g \cdot l^{-1} \cdot h^{-1}$) of GH secretion was calculated by a trapezoidal method.

Horm. metab. Res. 24 (1992) 119–121

Received: 19 June 1991 Accepted: 11 July 1991

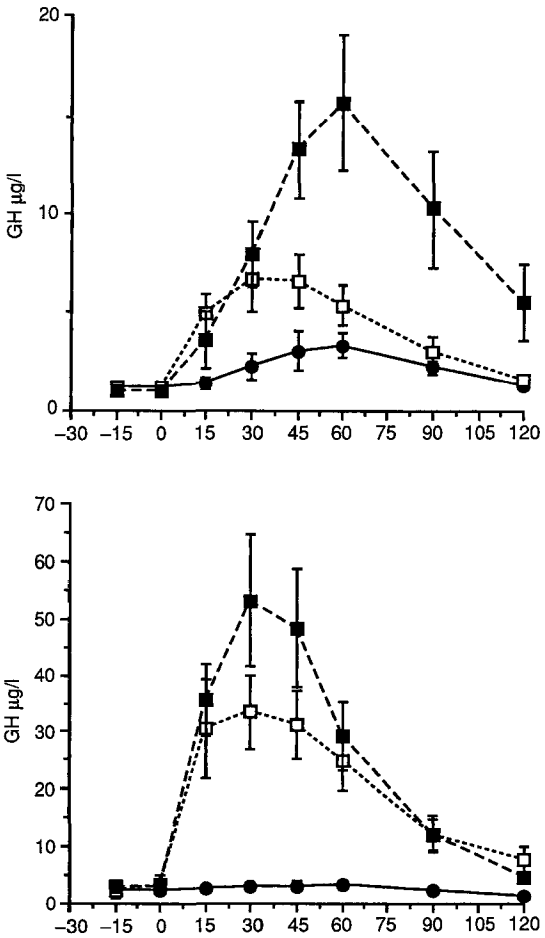

Fig. 1 GH responses to alpha-GFC (closed circles), GHRH (open squares), and GHRH + alpha-GFC (closed squares) in a group of aged (upper panel) and young (lower panel) normal subjects. The data are expressed as mean ± SEM.

Results

Alpha-GFC infusion induced a small increase of GH basal values in both young and old subjects (Fig. 1). In the aged subjects, the GHRH infusion caused a blunted GH response when compared with that seen in the young subjects (186.9 ± 74.6 vs 1028.6 ± 227.8 $\mu g \cdot l^{-1} \cdot h^{-1}$; $P < 0.001$). The administration of alpha-GFC together with GHRH induced GH responses significantly higher than those found after GHRH alone both in young ($P < 0.05$) and old subjects ($P < 0.005$) (Fig. 1). In the aged subjects, there was a 3 fold higher value of GH AUC after the combined administration of alpha-GFC + GHRH than after GHRH alone (554.8 ± 103.2 vs 186.9 ± 74.6 $\mu g \cdot l^{-1} \cdot h^{-1}$; $P < 0.01$), while the potentiating effect of alpha-GFC on GHRH stimulation was less in the young subjects (1397.14 ± 297.7 vs 1028.6 ± 227.8 $\mu g \cdot l^{-1} \cdot h^{-1}$). However, the GH response to the combined administration of alpha-GFC and GHRH was lower in the aged subjects as compared to the young subjects (554.8 ± 103.2 vs 1397.1 ± 297.7 $\mu g \cdot l^{-1} \cdot h^{-1}$, $P < 0.05$). In the young subjects, the GH response to GHRH alone was greater than the response to GHRH + alpha-GFC in the aged subjects (1028.6 ± 227.8 vs. 554.8 ± 103.2 $\mu g \cdot l^{-1} \cdot h^{-1}$) (Fig. 2).

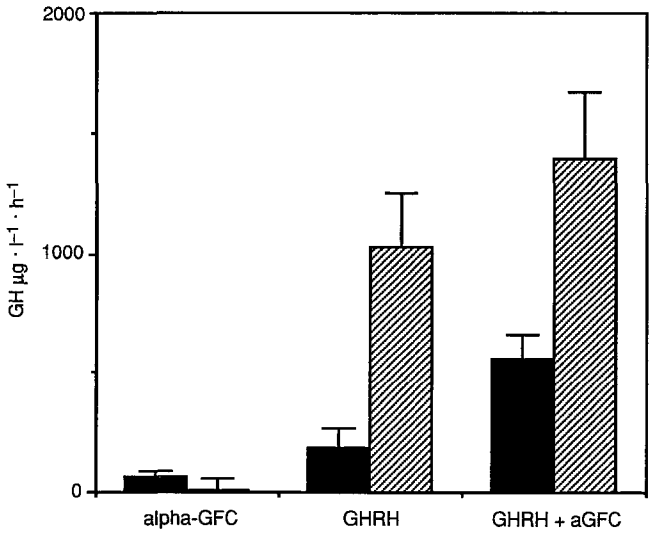

Fig. 2 Area under the curve (Auc) of GH ($\mu g \cdot l^{-1} \cdot h^{-1}$) after different stimuli in old (black bars) and young (striped bars) subjects.

Discussion

Our results confirm the previous finding that GH secretion is blunted in aged subjects (*Hoffman* et al. 1988). The mechanism for this diminished somatotroph activity is probably multifactorial. In the aged rat, somatostatin tone is enhanced (*Sonntag, Forman, Miki, Steger, Ramos, Arimura* and *Meites* 1981). Hypothalamic somatostatin release is inhibited by cholinergic agonists, and cholinergic activity may also be decreased during aging. In addition to this increased GH inhibitory tone, GHRH hormone action also declines with age. In the aged rat, there are reduced concentrations of immunoreactive hypothalamic GHRH (*Morimoto, Kawakami, Makino, Chihara, Masegawa* and *Ibata* 1988) and fewer pituitary GHRH receptors (*Abribat* et al. 1991). Decreased pituitary responsiveness to GHRH in vivo and vitro have also been shown (*Ceda* et al. 1986; *Robberecht, Gillard, Waelbroeck, Camus, De Neef* and *Christophe* 1986).

This study has demonstrated that alpha-GFC potentiates the effect of GHRH on the somatotroph in both young and in old human subjects. The enhancing effect of alpha-GFC on GH release is greater in the normal elderly subjects even if the absolute amount of GH released after both GHRH and GHRH + alpha-GFC is much lower than that in the young subjects. The mechanism for this effect on GH secretion has not been proven, but a cholinergic mechanism of action for alpha-GFC has been suggested since the drug may serve as an Ach precursor. Intraperitoneal administration of alpha-GFC to rats increases Ach release from specific brain areas in a dose-dependent manner (*Imperato, De Mei, Scrocco* and *Angelucci* 1990). Thus, the mechanism by which alpha-GFC increases GHRH-stimulated GH responses is probably due to an inhibition of somatostatin release caused by increased hypothalamic cholinergic tone. Furthermore, alpha-GFC is also a precursor for the synthesis of phosphatidylcholine, one of the major structural components of cell membranes (*Kennedy* 1957). One may therefore speculate that alpha-GFC administration changes the fluidity of pituitary

cell membranes, resulting in greater GHRH signal transduction at the pituitary level.

References

Abribat, T., N. Deslauriers, P. Brazeau, P. Gaudreau: Alterations of pituitary GHRH binding sites in aging rats. Endocrinology 128: 633–635 (1991)

Ceda, G. P., G. Valenti, U. Butturini, A. R. Hoffman: Diminished pituitary responsiveness to growth-hormone releasing factor in aging male rats. Endocrinology 118: 2109–2114 (1986)

Ceda, G. P., G. Ceresini, L. Denti, D. Magnani, L. Marchini, G. Valenti, A. R. Hoffman: Effects of cytidine 5'-diphosphocholine administration on basal and growth hormone-releasing hormone-induced growth hormone secretion in elderly subjects. Acta Endocrinologica 124: 516–520 (1991)

De Gennaro Colonna, V., M. Zoli, D. Cocchi, A. Maggi, P. Marrama, L. F. Agnati, E. E. Muller: Reduced growth hormone-releasing factor (GHRF)-like immunoreactivity and GHRF gene expression in hypothalamus of aged rats. Peptides 10: 705–709 (1989)

Gibson, G. E., C. Peterson, D. J. Jenden: Brain acetylcholine synthesis declines with senescence. Science 213: 674–676 (1981)

Ho, K. Y., W. S. Evans, R. M. Blizzard, J. D. Veldhuis, G. R. Merriam, E. Samojlik, R. Furlanetto, D. L. Kaiser, M. O. Thorner: Effects of sex and age on the 24-hour profile of growth hormone secretion in man: importance of endogenous estradiol concentrations. J. Clin. Endocrinol. Metab. 64: 51–58 (1987)

Hoffman, A. R., C. Griffin, J. Kalinyak, S. Perkins, G. P. Ceda: The hypothalamic-somatotroph-somatomedin axis and aging. In: Psychoneuroendocrinology of aging: basic and clinical aspects, Ed. G. Valenti (Springer Verlag), pp. 43–60 (1988)

Kennedy, E. P.: Biosynthesis of phospholipids. Fed. Proc. 16: 847–853 (1957)

Imperato, A., C. De Mei, M. G. Scrocco, L. Angelucci: Attività colinergica di alfa-GFC a livello ippocampale e striatale. Studio "in vivo" mediante microdialisi cerebrale. Le Basi Razionali della Terapia 20 (1): 17–22 (1990)

Massara, F., E. Ghigo, K. Demislis, D. Tangolo, E. Mazza, V. Locatelli, E. E. Muller, G. M. Molinatti, F. Camanni: Cholinergic involvement in the growth hormone releasing factor-induced growth hormone release: studies in normal and acromegalic subjects. Neuroendocrinology 43: 670–675 (1986a)

Massara, F., E. Ghigo, P. Molinatti, E. Mazza, V. Locatelli, F. Camanni: Potentiation 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. 113: 12–16 (1986b)

Morimoto, N., F. Kawakami, S. Makino, K. Chihara, M. Masegawa, Y. Ibata: Age-related changes in growth hormone releasing factor and somatostatin in the rat hypothalamus. Neuroendocrinology 47: 459–464 (1988)

Robberecht, P., M. Gillard, M. Waelbroeck, J. C. Camus, P. De Neef, J. Christophe: Decreased stimulation of adenylate cyclase by growth hormone releasing factor in the anterior pituitary of old rats. Neuroendocrinology 44: 429–432 (1986)

Shibasaki, T., K. Shizume, M. Nakahara, A. Masuda, K. Jibiki, H. Demura, T. Wakabayashi, N. Ling: Age-related changes in plasma growth hormone response to growth hormone releasing factor in man. J. Clin. Endocrinol. Metab. 58: 212–214 (1984)

Sonntag, W. E., L. J. Forman, N. Miki, R. W. Steger, T. Ramos, A. Arimura, J. Meites: Effects of CNS active drugs and somatostatin antiserum on GH release in young and old male rats. Neuroendocrinology 33: 73–78 (1981)

Requests for reprints should be addressed to:

Gian Paolo Ceda

Cattedra di Gerontologia e Geriatria
Università di Parma
Ospedale "G. Stuard"
Via Don Bosco 2
43100 Parma (Italy)