Age-Related Modulatory Activity by a Cholinergic Agonist on the Growth Hormone Response to GH-Releasing Hormone in the Rat (43039)

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Abstract. **The involvement of the cholinergic system in growth hormone (GH) secretion has acquired increased importance in the last few years. In rats, pretreatment with muscarinic cholinergic agonists potentiates the GH release induced by GH-releasing hormone (GHRH), via inhibition of somatostatin (SRIF) release from the hypothalamus. The aim of this study was to validate the use of cholinergic agonists to probe the functional activity of the hypothalamic SRIF system. It is known that hypothalamic SRIF displays an age-related increase in its functional activity; therefore, rats from 10 days to 29 months of age were used and challenged with GHRH following acute administration of pilocarpine, a cholinergic muscarinic agonist. Following administration of GHRH alone there was an age-related decline in GH responsiveness. Administration of pilocarpine potentiated the GH response to GHRH during the entire life-span of the rats, the only exception being 10-day-old rats in which the drug was without effect. Pilocarpine, though effective in potentiating the GH response to GHRH, did not restore, in senescent rats, GH stimulation to the level of that present in young (3-month old) or adult rats (8-month old). However, the drug was effective in rejuvenating the GH response to GHRH of the older rats (29- and 18-month old) to the level of 15-month-old rats.**

The present results indicate that modulation of the GH response to GHRH by pilocarpine is consonant with the known changes in the activity of hypothalamic SRIF. Cholinergic drugs may therefore represent a valuable tool to assess SRIF function in physiologic or pathologic conditions of GH secretion, and, in addition, to potentiate GH release during a course of GHRH therapy. [P.S.E.B.M. 1990, Vol 193]

The cholinergic system plays a crucial role in growth hormone (GH) secretion. Antagonists of the muscarinic cholinergic receptors suppress the GH rise elicited by all of the known physiologic or pharmacologic stimuli (1–5), the only exception being insulin-induced hypoglycemia, which is only partially blunted (6). Also, the GH response to GH-releasing hormone (GHRH) is blocked by antimuscarinic drugs and conversely it is potentiated by cholinesterase inhibitors (7).

In the rat, we have provided evidence that the effect of cholinergic drugs on GH secretion takes place through modulation of hypothalamic somatostatin (SRIF) release (8). Depletion of hypothalamic SRIF stores was in fact capable of abolishing the modulatory effect of cholinergic drugs on GHRH-induced GH secretion (8). In addition, studies in the rat (9) and humans (10) have suggested that cholinergic mechanisms are also involved in the autofeedback regulation of GH secretion.

In light of the foregoing, cholinergic agonists may be viewed not only as potential inhibitors of the negative feedback action of GH, but also as probes of the hypothalamic SRIF system in different physiologic or pathologic conditions.

Reportedly, the secretion of GH declines with age (11, 12), an event which has been attributed to different factors. Among them, a progressive increase in hypothalamic SRIF synthesis and/or activity at the pituitary level has to be considered (13, 14). In view of the ability

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of muscarinic cholinergic agonists to modulate the function of hypothalamic SRIF, in this work we decided to use one of these compounds as probe of the age-related changes occurring in the SRIF function. Thus, we evaluated the ability of pilocarpine, an agonist at muscarinic cholinergic receptors, to modulate the GH response to GHRH in rats from 10 days to 29 months of age.

Materials and Methods

Animals. Male Sprague-Dawley rats (Charles River, Calco, Italy) of different ages were used. They were housed in a room under constant conditions of temperature (22 ± 2°C) and illumination (lights on from 0600 to 2000 hr). Standard diet and tap water were available *ad libitum*. At the time of experiments, all adult rats were maintaining stable body weight and were free of observable tumors or diseases.

Experimental Procedure. *Infant rats.* Ten-day-old rats were left with their mothers until 1 hr before the experiments. Pilocarpine hydrochloride (3 mg/kg; Sigma Chemical Co., St. Louis, MO) or saline was injected subcutaneously 20 min before GHRH (1-40) (0.2 μg/kg sc; Bachem, Bubendorf, Switzerland). Pups were killed by decapitation 15 min after GHRH injection, blood was collected into EDTA-containing tubes, and plasma was separated and stored at −20°C until radioimmunoassay.

Weanling rats. Twenty-one-day-old male rats were administered pilocarpine (5 mg/kg ip) or saline and 20 min later they were anesthetized with ketamine-Xylazine (80 and 2 mg/kg ip, respectively). After 10 min, a blood sample (250 μl) was withdrawn from the exposed jugular vein, GHRH (2 μg/kg) was injected intravenously, and blood samples obtained 5 and 10 min later.

Young, Adult, and Aged Rats. Three, 8-, 15-, 18-, and 29-month-old male rats were implanted with atrial cannulas through the right jugular vein under ether anesthesia (15) after a minimum of 2 weeks of adaptation in a single cage.

Forty-eight hours after surgery, rats underwent experiments between 0900 and 1000 hr, a time at which, in our experience, no major GH pulses are present. Pilocarpine (3 mg/kg) or saline was injected intravenously 20 min before GHRH (2 μg/kg iv). Blood samples (300 μl) were collected immediately before and 5, 10, 15, and 30 min after GHRH.

In all of the experiments, pilocarpine was used at doses capable of inducing release of GH without eliciting nonspecific stress effects (16).

GH Assay. Plasma GH was measured by radioimmunoassay (17) using materials supplied by the NIADDK Bethesda, MD. Values were expressed in terms of NIADDK-rat-GH-RP-2 standard (potency 2 IU/mg), as ng/ml plasma. The minimum detectable value of rat GH was 0.5 ng/ml; intraassay variability was 5%. To avoid interassay variation, samples from each experiment were assayed simultaneously.

Statistical Analysis. Plasma GH concentrations and integrated areas of the response curve (AUC) were compared by Dunnett's *t* test preceded by analysis of variance. A probability of less than 0.05 was considered to be statistically significant.

Results

Infant Rats. In 10-day-old rats, pilocarpine, 20 min after its administration, neither modified plasma GH levels nor the GH response to GHRH (Fig. 1).

Weanling Rats. In 21-day-old rats, pilocarpine significantly increased basal plasma GH levels 30 min after injection, and potentiated the GH response to GHRH, 5 and 10 min post-GHRH administration (Fig. 2).

Young, Adult, and Aged Rats. In 3-, 8-, 15-, and

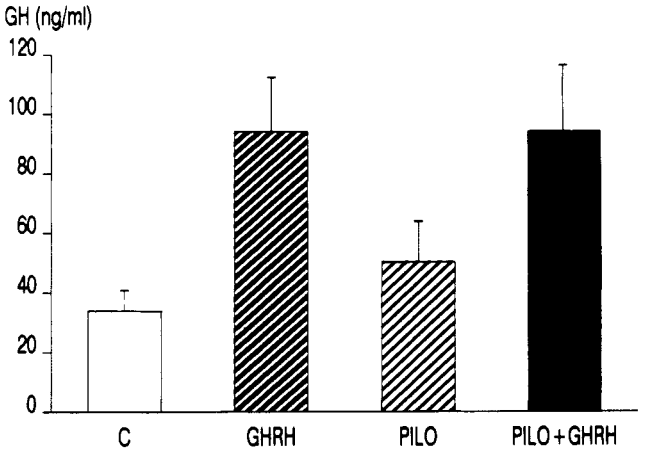

Figure 1. Effect of GHRH (0.2 μg/kg sc) on plasma GH levels in 10-day-old rats pretreated (20 min before) or not with pilocarpine (PILO, 3 mg/kg sc). Values are mean ± SE. Number of rats in parentheses.

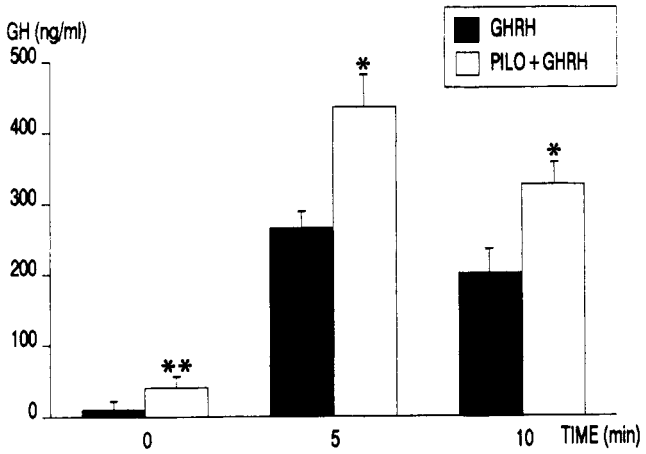

Figure 2. Effect of GHRH (2 μg/kg iv) administered alone at time 0 or preceded 20 min before by pilocarpine (PILO) (5 mg/kg ip) in 21-day-old anesthetized male rats. Values are mean ± SE. Six animals per group. *$P < 0.05$ and **$P < 0.01$ vs GHRH alone (analysis of variance and Dunnett's *t* test).

18-month-old rats, basal GH levels were not modified by pilocarpine (data not shown); in 29-month-old rats the drug induced a slight but significant plasma GH increase (from 0.7±0.4 to 2.8±0.5 ng/ml, $P < 0.05$).

Comparison of the peak plasma GH responses and GH integrated areas after GHRH showed that the GH response was progressively reduced with advancing age. No difference was detectable between 3- and 8-month old rats, but a statistically significant difference in both indices was present between 3- and 15- and 18- and 29-month-old rats. In 29-month-old rats, peak GH response and integrated GH secretion following GHRH were significantly lower than those present in 15-, 8-, and 3-month-old rats (Table I).

Pretreatment with pilocarpine was capable of increasing peak and integrated areas of GH secretion after GHRH in all of the age groups, the only exception being the peak GH response in 15-month-old rats.

The pattern of the GH response to GHRH alone was not duplicated by coadministration of GHRH and pilocarpine. With coadministration of GHRH and pilocarpine, rats of 15, 18, and 29 months could not be differentiated in their GH responses, in terms of both GH peaks and integrated areas, and the age-related dependency of the GH response was evident only in 3- and 8-month-old rats, in which higher responses were present.

Administration of pilocarpine induced in all rats behavioral signs typical of cholinergic activation.

Discussion

The differences in the modulatory action of pilocarpine on the GHRH-induced GH rise observed at various ages of the rats are in fairly good agreement with the reported age-related increase of hypothalamic SRIF function (13, 14).

Pilocarpine, in keeping with the notion that SRIF function is defective at the level of the somatotrophs in the immediate postnatal period (18), failed to increase basal and GHRH-stimulated GH secretion in 10-day-old rats. On the other hand, the drug was fairly active in 21-day-old rats, in which the striking decline in baseline GH levels has been related to development and increased effect of SRIF on the somatotrophs (19).

Pilocarpine potentiated the GH response to GHRH in 3- and 8-month-old rats, and, though to a lesser extent, in the older age groups. It is remarkable, however, that pilocarpine prevented the decline of the GH response to GHRH alone observed in older rats, allowing GHRH to stimulate GH secretion to a similar extent in 15-, 18-, 29-month-old rats. No ready explanation is apparent for the lesser ability of pilocarpine alone to stimulate baseline GH levels in older rats, except that endogenous GHRH tone is strikingly reduced in older rats (20).

Decline of GH secretion with aging may be related in rodents to increasingly defective GHRH function (20, 21) and/or to a primary or secondary reduction of pituitary somatotrophs (21). Either factor may account for the clearcut age-related decline of the GH response to GHRH alone observed in our study. However, the observation that there was no difference in the GH response to combined GHRH-pilocarpine administration in rats of 15,18, and 29 months, which showed only an age-related decline of the GH response to GHRH alone, indicates that this reduction is in part due to increased secretion of SRIF from the hypothalamus or enhanced sensitivity of the somatotrophs to the latter. In this context, recent studies have shown that in 24-month-old rats SRIF immunoreactivity is unchanged in the median eminence (23), a finding which does not rule out, however, a change in the SRIF

Table I. Peak Plasma GH Levels and GH-Integrated Areas following GHRH (2 μg/kg iv) Administered Alone or Preceded 15 Min before by Pilocarpine (3 mg/kg iv) in Male Rats of Different Ages[a]

Treatment	Age (months)				
	3	8	15	18	29
	Peak plasma GH (ng/ml)				
GHRH	174 ± 11[b] (8)	222 ± 16[c] (8)	78 ± 16[d] (11)	19 ± 6[d] (7)	15 ± 7[d] (5)
Pilocarpine + GHRH	416 ± 42[c] (8)	325 ± 43[c] (8)	140 ± 28[d] (10)	91 ± 7[d] (6)	134 ± 24[d] (5)
	$P^e < 0.01$	0.05	NS	0.01	0.01
	GH integrated areas (ng/ml 30 min)				
GHRH	2691 ± 228[c] (8)	2348 ± 171[c] (8)	770 ± 92[b,d] (11)	422 ± 75[d] (7)	148 ± 67[d] (5)
Pilocarpine + GHRH	5860 ± 702[c] (8)	3898 ± 471[c,d] (8)	1457 ± 197[d] (10)	1199 ± 73[d] (6)	1295 ± 233[d] (5)
	$P^e < 0.01$	0.01	0.01	0.01	0.01

[a] Values are mean ± SE; number of rats in parentheses; NS, not significant.
[b] $P < 0.05$ vs 29-month-old group (analysis of variance and Dunnett's t test).
[c] $P < 0.01$ vs 29-month-old group (analysis of variance and Dunnett's t test).
[d] $P < 0.01$ vs 3-month-old group (analysis of variance and Dunett's t test).
[e] Probability versus GHRH alone in the same age group.

synthesis and release. Even if SRIF secretion from the hypothalamus of aged rats is not actually increased, there could be, however, a relative predominance of SRIF tone, in view of the drastic reduction of GHRH biosynthesis in the hypothalamus of older rats (20). Moreover, an increased sensitivity of pituitaries to the inhibitory action of SRIF *in vitro* has been observed with increasing age, although pituitaries of older rats were not tested in these experiments (18).

As shown by this study acute administration of pilocarpine rejuvenated the GH response to GHRH in rats of 18 and 29 months of age. Previous data have shown that treatment with clonidine, an α_2-adrenergic agonist, increases GH pulse amplitude and frequency in old rats (25) and dogs (26), probably by stimulating release of hypothalamic GHRH (27). It is tempting to speculate that combined administration of α-adrenergic and cholinergic compounds, when which coadministered display an additive effect on GH secretion (28), may provide an effective means to reestablish GH function in aged rats. In addition to aging, there are pathologic conditions of humans which may benefit from a neuropharmacologic approach with α-adrenergic and cholinergic compounds, e.g., children with growth disorders (29) or obesity (30), who exhibit fairly good tolerability to these compounds.

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