Nitric Oxide Modulation of the Growth Hormone–Releasing Activity of Hexarelin in Young and Old Dogs

Antonello E. Rigamonti, Silvano G. Cella, Nicoletta Marazzi, and Eugenio E. Müller

The growth hormone (GH)-releasing activity of Hexarelin, a potent GH-releasing peptide (GHRP) analog, was evaluated in eight young (aged 1 to 6 years) and five old (10 to 16 years) beagle dogs pretreated with erythrityl tetranitrate, a liposoluble nitric oxide (NO) donor, and/or indomethacin, an inhibitor of cyclooxygenase enzymes, and *N*-nitro-L- or *N*-nitro-D-arginine methylester (L-NAME and D-NAME), active and inactive NO synthase (NOS) inhibitors, respectively. Erythrityl tetranitrate (0.3 mg · kg^{-1} oral [PO]) strikingly potentiated Hexarelin-stimulated GH secretion (31.25 μg · kg^{-1} intravenous [IV]) in both young (area under the time-concentration curve at 0 to 90 minutes $AUC_{0\text{-}90}$] 878.50 ± 267.02 *v* 1,994.04 ± 434.20 ng · mL^{-1} · h, $P < .01$) and aged animals (314.82 ± 117.11 *v* 1,314.12 ± 484.75 ng · mL^{-1} · h, $P < .01$). The NO donor alone did not modify baseline GH levels in either young dogs (188.68 ± 85.24 ng · mL^{-1} · h) or old dogs (120.49 ± 22.03 ng · mL^{-1} · h). L-NAME (5 mg · kg^{-1} × 2 IV) suppressed GH release induced by the peptide in young dogs (1,367.68 ± 251.87 *v* 411.12 ± 68.49 ng · mL^{-1} · h, $P < .01$), but potentiated it in old dogs (314.73 ± 117.10 *v* 1,103.97 ± 374.11 ng · mL^{-1} · h, $P < .01$). D-NAME (5 mg · kg^{-1} × 2 IV) did not affect the GH response to Hexarelin in either young (1,328.68 ± 433.54 ng · mL^{-1} · h) or aged (342.32 ± 84.82 ng · mL^{-1} · h) dogs. Indomethacin (1.5 mg · kg^{-1} IM) abolished the NO-donor potentiation of the GH response induced by Hexarelin in both young dogs (1,627.25 ± 260.90 *v* 1,163.37 ± 334.84 ng · mL^{-1} · h, $P < .05$) and old dogs (1,061.47 ± 210.38 *v* 365.69 ± 79.27 ng · mL^{-1} · h, $P < .01$) without affecting the plasma GH peak evoked by the peptide alone (young dogs, 786.04 ± 153.44 *v* 960.04 ± 444.44 ng · mL^{-1} · h, P = NS; old dogs, 474.55 ± 47.30 *v* 490.82 ± 144.86 ng · mL^{-1} · h, P = NS). In conclusion, (1) NO donors are capable to further increase the strong GH-releasing activity of Hexarelin in both young and old dogs, although the site(s) and mechanism(s) of action of NO is still obscure; (2) the different GH response to the peptide after NOS inhibition in young and old dogs signifies in the latter an alteration of the somatotrope function; and (3) prostaglandins are the downstream effectors of the chain of events triggered by activation of the NO-ergic system.

NITRIC OXIDE (NO) is a highly reactive gas that has been recently suggested to function as a neurotransmitter in the neuroendocrine system,[1-4] including the somatotrope axis.[5-9] The enzyme responsible for generation of NO from L-arginine, NO synthase (NOS), exists as three major isoforms: the first two, brain NOS (b-NOS) and endothelial NOS, are constitutive enzymes; the third isoform, macrophage NOS, is an inducible enzyme.[10,11] Interestingly, the prevalent localization of b-NOS in different hypothalamic areas[12,13] but also in the pituitary[1] has raised the intriguing possibility that NO may have a dual role, acting as a neuroendocrine regulator at either the hypothalamic or pituitary sites.

In this connection, Kato[5] reported an inhibitory effect of NO on growth hormone (GH)-releasing hormone (GHRH)-stimulated GH secretion in rat pituitary cells packed in a perifusion column and perifused with a NOS antagonist. In contrast, other in vivo and in vitro experiments have shown that endogenous NO plays a facilitative role at the pituitary level in the control of GHRH- and GH-releasing peptide (GHRP)-stimulated GH release.[8]

With respect to a suprapituitary action, it is currently unclear which modulatory effects NO exerts on somatostatin (SS) and/or GHRH function. Previous studies have shown that GHRH increases SS release and mRNA levels in the rat periventricular nucleus via a NO pathway.[6]

The mechanism(s) underlying the neuroendocrine effects of NO are the activation of guanylate cyclase and/or regulation of the cycloxygenase (COX) pathway.[14,15] In particular, NO activates both COX isoform enzymes, enhancing the production of prostaglandins (PGs),[16] which in turn may modulate neurotransmitter and neuropeptide release[17] or act directly at the pituitary level.[18]

In old mammals, including humans, the spontaneous GH secretion rate is markedly reduced[19,20] and the GH response to GHRH[21,22] or Hexarelin, a potent GHRP analog,[23,24] decreases with aging. The aged-related decline in somatotrope function is likely due to an imbalance between GHRH and SS gene expression and secretion,[25] for a primary defect in central nervous system catecholaminergic and cholinergic functions, without denying the involvement of the other neurotransmitters.[26]

Based on this knowledge, it was of interest to investigate the involvement of NO and the functional link between the NOS and COX pathways in the control of GH secretion in young and old dogs. In particular, we evaluated (1) the ability of a liposoluble NO donor, erythrityl tetranitrate,[27] to modulate GH release induced by Hexarelin, a substance that may act at both the hypothalamic and pituitary level,[28,29] (2) the effects of NOS antagonism[30] on the GH-releasing activity of Hexarelin, and (3) whether indomethacin-induced blockade of PG synthesis affects the GH response to Hexarelin administered alone or together with erythrityl tetranitrate.

MATERIALS AND METHODS

Animals

Eight young (age, 1 to 6 years; weight, 8 to 14 kg; six female and two male) and five old (age, 10 to 16 years; weight, 11 to 16 kg; one female

From the Department of Medical Pharmacology, University of Milan, Milan, Italy.

Submitted February 12, 1998; accepted July 17, 1998.

Supported by the Centro Nazionale delle Ricerche (CNR) project "Progetto Finalizzato Invecchiamento" (Italy).

Address reprint requests to Eugenio E. Müller, MD, Department of Medical Pharmacology, University of Milan, via Vanvitelli 32, 20129 Milan, Italy.

0026-0495/99/4802-0007$10.00/0

and four male) well-trained beagle dogs were used in this study. They were fed normal dry food (Diete Standard; Charles River, Italy) with water available ad libitum and maintained on a 12-hour light/dark regimen with lights on at 7 AM. The body weight of the dogs was stable, and they had no observable disease. The animals were randomly assigned to each experimental group. All experiments were performed on conscious animals after an overnight fast starting at 9 AM. Before the experiments, animals were kept at rest for at least 1 hour. Blood samples were drawn at regular intervals from the cephalic vein via an indwelling nonthrombogenic catheter. An interval of at least 1 week was used between individual experiments, for which the protocol was previously authorized by the Committee on Animal Care and Use of the University of Milan.

Hexarelin, Erythrityl Tetranitrate, and Hexarelin + Erythrityl Tetranitrate

Hexarelin (31.25 $\mu g \cdot kg^{-1}$ intravenous [IV], EP 23905, Europeptides, Argenteuil, France) or saline (1 $mL \cdot kg^{-1}$ IV) was tested in eight young and five old dogs pretreated 75 minutes prior with erythrityl tetranitrate (0.3 $mg \cdot kg^{-1}$ oral [PO], Cardilate; Wellcome, Milan, Italy) or an oral placebo formulation. Blood was collected beforehand (−75, −30, and 0 minutes) and every 15 minutes until 60 minutes, and at 90 minutes postinjection.

Hexarelin + N-Nitro-L-Arginine Methylester or N-Nitro-D-Arginine Methylester

Six young and five old dogs were administered *N*-nitro-L-arginine methylester ([L-NAME] 5 $mg \cdot kg^{-1}$ IV; Sigma-Tau, Milan, Italy) or *N*-nitro-D-arginine methylester ([D-NAME] 5 $mg \cdot kg^{-1}$ IV; Sigma-Tau), the active or inactive NOS stereoisomer antagonist, respectively, at −75 minutes. Hexarelin (31.25 $\mu g \cdot kg^{-1}$ IV) or saline (0.1 $mL \cdot kg^{-1}$ IV) was administered concomitantly with a second bolus of L-NAME (5 $mg \cdot kg^{-1}$ IV) or D-NAME (5 $mg \cdot kg^{-1}$ IV) delivered at time 0. Blood was collected before (−75, 30, and 0 minutes) and every 15 minutes until 60 minutes, and at 90 minutes postinjection.

Hexarelin + Indomethacin and Hexarelin + Erythrityl Tetranitrate + Indomethacin

Seven young and five old dogs were treated with indomethacin (1.5 $mg \cdot kg^{-1}$ intramuscular [IM], Liometacen; Chiesi Farmaceutici, Parma, Italy) at −120 minutes and then erythrityl tetranitrate (0.3 $mg \cdot kg^{-1}$ PO) or an oral placebo formulation at −75 minutes. Hexarelin (31.25 $\mu g \cdot kg^{-1}$ IV) was administered to the animals at time 0. Blood was collected beforehand (−75, −30, and 0 minutes) and every 15 minutes until 60 minutes, and at 90 minutes postinjection.

GH Radioimmunoassay

Blood was collected into tubes containing EDTA and immediately chilled. Plasma was frozen until assay for GH by a double-antibody radioimmunoassay. Highly purified canine GH (batch AFP 1983b; Pituitary Hormones and Antisera Center, Torrance, CA; courtesy of Dr A.F. Parlow) was used for radioiodination and as a standard. The assay sensitivity was 0.5 $ng \cdot mL^{-1}$. The intraassay coefficient of variation was 3.8% and 4.1% at concentrations of 12.5 and 3.1 $ng \cdot mL^{-1}$, respectively. To avoid possible interassay variation, all samples for a given experiment were assayed in a single radioimmunoassay.

Statistical Analysis

GH values (mean ± SEM) are expressed either as the area under the plasma concentration versus time curve ([$AUC_{0\text{-}90}$] nanograms per milliliter per hour), calculated by trapezoidal integration or as the absolute peak value (nanograms per milliliter). Since no differences in hormonal levels between male and female dogs were observed in the different experimental conditions, the data were pooled.

Statistical comparisons of mean values were performed by the *t* test for unpaired or paired (where necessary) data, preceded by ANOVA. A *P* value less than .05 was considered statistically significant.

RESULTS

Hexarelin, Erythrityl Tetranitrate, and Hexarelin + Erythrityl Tetranitrate

In young dogs, the mean peak plasma GH concentration after erythrityl tetranitrate alone was 3.05 ± 1.19 $ng \cdot mL^{-1}$ ($AUC_{0\text{-}90}$, 188.68 ± 85.24 $ng \cdot mL^{-1} \cdot h$), not significantly different versus saline treatment (mean peak plasma GH, 2.60 ± 0.30 $ng \cdot mL^{-1}$; $AUC_{0\text{-}90}$, 122.00 ± 8.02 $ng \cdot mL^{-1} \cdot h$; data not shown). The GH response to Hexarelin alone (mean peak plasma GH, 25.15 ± 3.95 $ng \cdot mL^{-1}$) increased significantly after erythrityl tetranitrate pretreatment (mean peak plasma GH, 63.27 ± 7.59 $ng \cdot mL^{-1}$; $AUC_{0\text{-}90}$, 878.50 ± 267.02 *v* 1,994.04 ± 434.20 $ng \cdot mL^{-1} \cdot h$; $P < .01$) (Fig 1).

In old dogs, the mean peak plasma GH concentration was 2.84 ± 0.89 $ng \cdot mL^{-1}$ after erythrityl tetranitrate alone ($AUC_{0\text{-}90}$, 120.49 ± 22.03 $ng \cdot mL^{-1} \cdot h$), not significantly different versus saline treatment (mean peak plasma GH, 2.40 ± 0.10 $ng \cdot mL^{-1}$; $AUC_{0\text{-}90}$, 122.00 ± 2.01 $ng \cdot mL^{-1} \cdot h$; data not shown). The GH response to Hexarelin alone (mean peak plasma GH, 10.48 ± 4.08 $ng \cdot mL^{-1}$) increased significantly after erythrityl tetranitrate pretreatment (mean peak plasma GH, 42.50 ± 15.23 $ng \cdot mL^{-1}$; $AUC_{0\text{-}90}$, 314.82 ± 117.11 *v* 1,314.12 ± 484.75 $ng \cdot mL^{-1} \cdot h$, $P < .01$) (Fig 2).

The mean plasma GH response was significantly higher in young versus aged dogs after both Hexarelin alone or Hexarelin + erythrityl tetranitrate ($P < .01$).

Hexarelin + L-NAME or D-NAME

In young dogs, L-NAME significantly suppressed the mean plasma GH peak value evoked by Hexarelin (mean peak plasma GH, 33.49 ± 8.28 *v* 9.86 ± 1.88 $ng \cdot mL^{-1}$, $AUC_{0\text{-}90}$, 1,367.68 ± 251.87 *v* 411.12 ± 68.49 $ng \cdot mL^{-1} \cdot h$, $P < .01$) without affecting the baseline value (mean peak plasma GH, 2.59 ± 0.51 $ng \cdot mL^{-1}$; $AUC_{0\text{-}90}$, 114.00 ± 42.90 $ng \cdot mL^{-1} \cdot h$; data not shown). The GH response to Hexarelin was not affected by the biologically inactive stereoisomer D-NAME (mean peak plasma GH, 34.10 ± 1.65 $ng \cdot mL^{-1}$; $AUC_{0\text{-}90}$, 1,328.68 ± 433.54 $ng \cdot mL^{-1} \cdot h$ *v* Hexarelin-induced GH release, P = NS) (Fig 3).

In old dogs, L-NAME significantly increased the mean plasma GH peak evoked by Hexarelin (mean peak plasma GH, 10.48 ± 4.08 *v* 25.94 ± 10.08 $ng \cdot mL^{-1}$; $AUC_{0\text{-}90}$, 314.73 ± 117.10 *v* 1,103.97 ± 374.11 $ng \cdot mL^{-1} \cdot h$; $P < .01$) without affecting the baseline value (mean peak plasma GH, 3.34 ± 0.21 $ng \cdot mL^{-1}$; $AUC_{0\text{-}90}$, 117.23 ± 3.85; data not shown). The GH response to Hexarelin was not affected by D-NAME (mean peak plasma GH, 9.22 ± 2.46 $ng \cdot mL^{-1}$; $AUC_{0\text{-}90}$, 342.32 ± 84.82 $ng \cdot mL^{-1} \cdot h$ *v* Hexarelin-induced GH release, P = NS) (Fig 4).

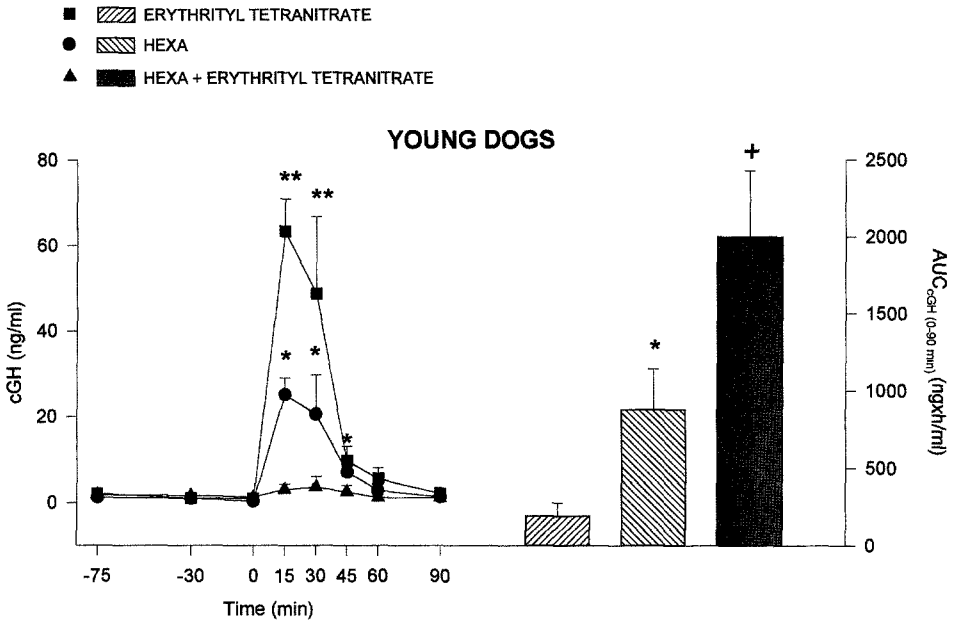

Fig 1. GH response (mean ± SEM and AUC_{0-90}) after acute challenge with Hexarelin (31.25 μg · kg^{-1} IV), Hexarelin + erythrityl tetranitrate (0.3 mg · kg^{-1} PO), or erythrityl tetranitrate in 8 young dogs. *$P < .01$ v erythrityl tetranitrate; **$P < .01$ v Hexarelin; †$P < .01$ v Hexarelin.

Hexarelin + Indomethacin and Hexarelin + Erythrityl Tetranitrate + Indomethacin

In young dogs, the mean plasma GH peak evoked by Hexarelin was not affected by indomethacin (mean peak plasma GH, 31.59 ± 8.02 *v* 29.46 ± 14.91 ng · mL^{-1}; AUC_{0-90}, 786.04 ± 153.44 *v* 960.04 ± 444.44 ng · mL^{-1} · h; P = NS). However, this drug abolished the erythrityl tetranitrate–induced potentiation of the GH response to Hexarelin (mean peak plasma GH, 57.65 ± 11.89 *v* 31.32 ± 15.89 ng · mL^{-1}; AUC_{0-90}, 1,627.25 ± 260.90 *v* 1,163.37 ± 334.84 ng · mL^{-1} · h; $P < .05$) (Fig 5).

A similar pattern was present in old dogs, in which the mean peak plasma GH level evoked by Hexarelin was not affected by indomethacin (mean peak plasma GH, 19.37 ± 1.47 *v* 17.20 ± 6.11 ng · mL^{-1}; AUC_{0-90}, 474.55 ± 47.30 *v* 490.82 ± 144.86 ng · mL^{-1} · h; P = NS), although the drug abolished erythrityl tetranitrate potentiation of the GH response to Hexarelin (mean peak plasma GH, 29.91 ± 11.51 *v* 9.57 ± 4.48 ng · mL^{-1}; AUC_{0-90}, 1,061.47 ± 210.38 *v* 365.69 ± 79.27 ng · mL^{-1} · h, $P < .01$) (Fig 6).

DISCUSSION

Pretreatment with the liposoluble NO donor erythrityl tetranitrate potentiated the GH response to Hexarelin in both young and old dogs. In considering the potential hypothalamic and/or pituitary site(s) of the Hexarelin-NO interaction, it must be recalled that NOS activity has been detected in rat follicular-stellate cells, a pituitary cell population closely related to

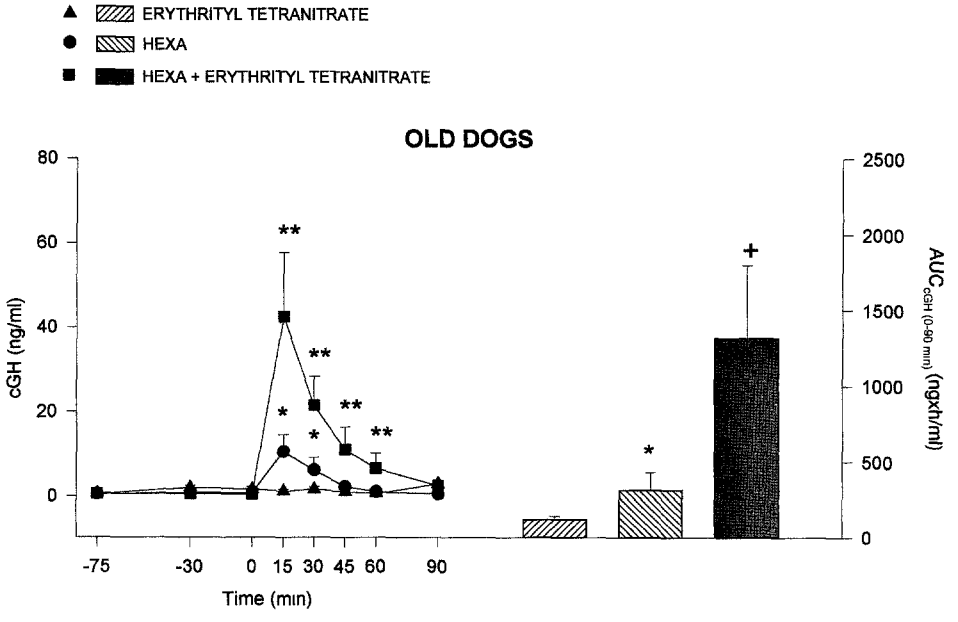

Fig 2. GH response (mean ± SEM and AUC_{0-90}) after acute challenge with Hexarelin (31.25 μg · kg^{-1} IV), Hexarelin + erythrityl tetranitrate (0.3 mg · kg^{-1} PO), or erythrityl tetranitrate in 5 old dogs. *$P < .01$ v erythrityl tetranitrate; **$P < .01$ v Hexarelin; †$P < .01$ v Hexarelin.

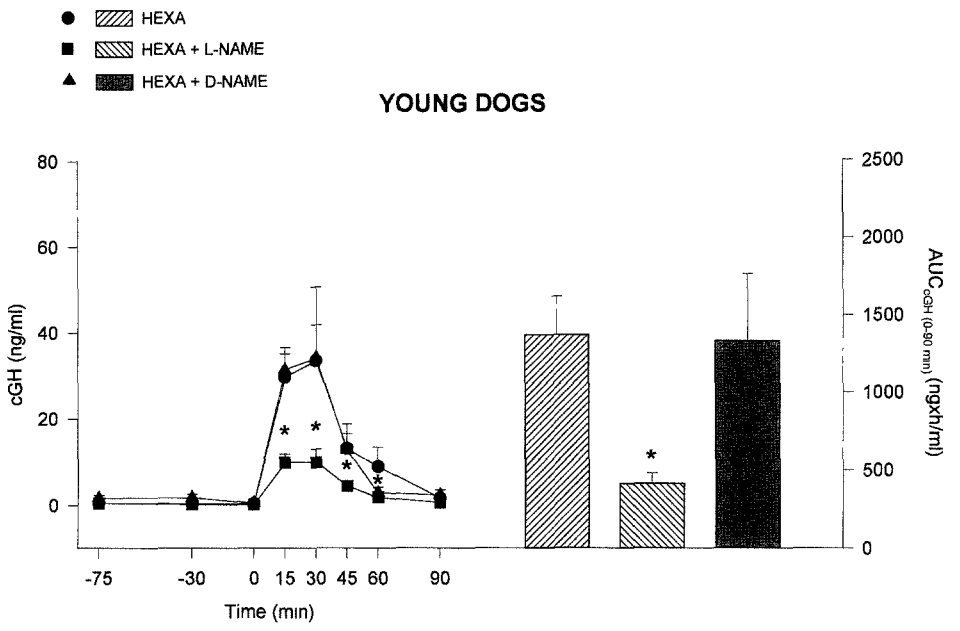

Fig 3. GH response (mean ± SEM and $AUC_{0\text{-}90}$) after acute challenge with Hexarelin (31.25 μg · kg^{-1} IV), Hexarelin + L-NAME (5 mg · kg^{-1} × 2 IV), or Hexarelin + D-NAME (5 mg · kg^{-1} × 2 IV) in 6 young dogs. *$P < .01$ *v* Hexarelin.

somatotropes,[1] and moderately dense b-NOS immunostaining has been evidenced in the rat arcuate nucleus and median eminence, where GHRH neurons and fibers, respectively, are particularly abundant.[13]

A primary defect in the functional GHRH/SS balance in the hypothalamus is the likely cause of the poor GH responsiveness to GHRH and, to a lesser extent, GHRP in old mammals.[25] In contrast, pituitary mechanisms subserving GH secretion would be better preserved during aging such that, rather than a state of pituitary hypofunction, the reduced GH secretion of old mammals would be based on inadequate hypothalamic stimulation.[24,26] Thus, the finding in our study that enhancement of NO activity by erythrityl tetranitrate induced a comparable potentiation of Hexarelin-induced GH secretion in young and old dogs could indicate that NO acts at the pituitary level.

However, it is noteworthy that at both age periods, the enhancement of NO function per se did not affect baseline GH secretion, implying that irrespective of the site(s) of action of the gaseous transmitter, it acts downstream of a series of events that induce the primary activation of the hypothalamic (GHRH, SS, and/or GHRP receptors) or pituitary (somatotrope cell) target.

Our data showing that Hexarelin-induced GH release was reduced in young dogs after blockade of NOS by the active, but not the inactive, stereoisomer antagonist support the view that an adequate NO supply is required for a complete GH secretory response after stimulation by a GH secretagogue,[8] although NOS antagonism per se did not alter basal GH secretion.

An unexpected finding of our study in the old dogs is the enhancement of stimulated GH release by NOS antagonism.

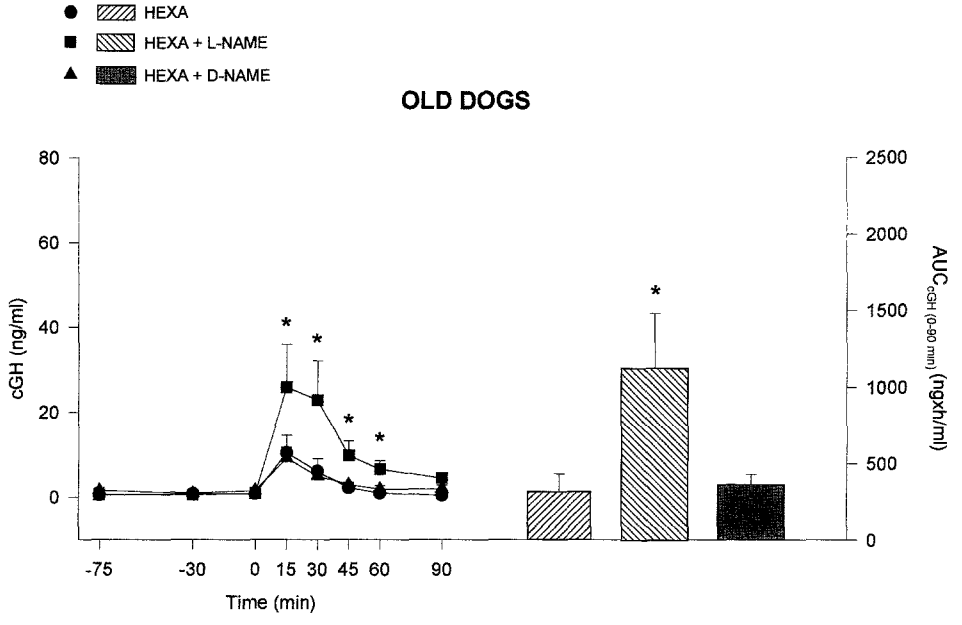

Fig 4. GH response (mean ± SEM and $AUC_{0\text{-}90}$) after acute challenge with Hexarelin (31.25 μg · kg^{-1} IV), Hexarelin + L-NAME (5 mg · kg^{-1} × 2 IV), or Hexarelin + D-NAME (5 mg · kg^{-1} × 2 IV) in 5 old dogs. *$P < .01$ *v* Hexarelin.

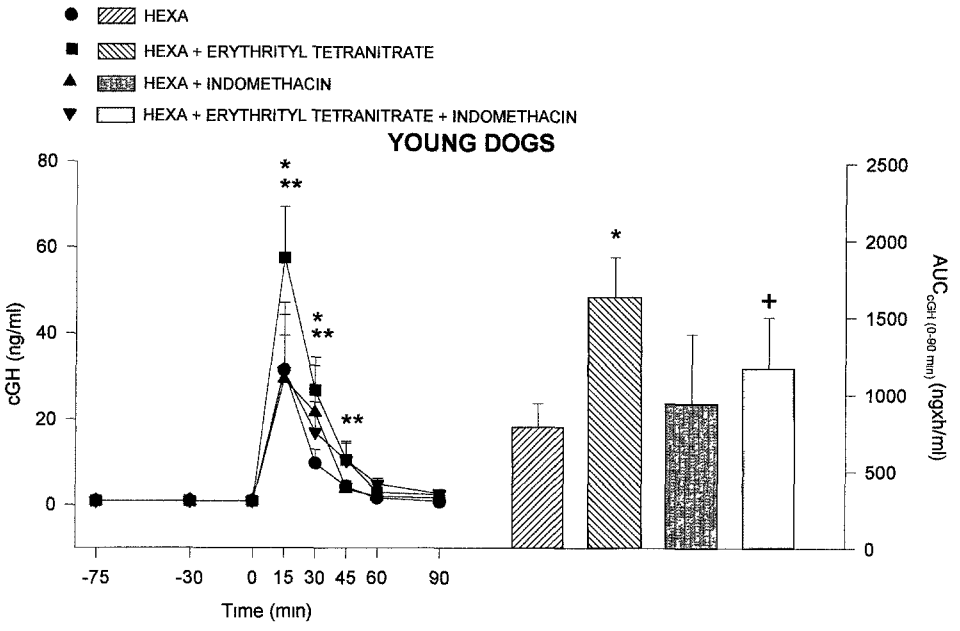

Fig 5. GH response (mean ± SEM and AUC_{0-90}) after acute challenge with Hexarelin (31.25 μg · kg^{-1} IV), Hexarelin + erythrityl tetranitrate (0.3 mg · kg^{-1} PO), Hexarelin + indomethacin (1.5 mg · kg^{-1} IM), or Hexarelin + erythrityl tetranitrate + indomethacin in 7 young dogs. *$P < .01$ *v* Hexarelin; **$P < .01$ *v* Hexarelin + erythrityl tetranitrate + indomethacin; $^{+}P < .05$ *v* Hexarelin + erythrityl tetranitrate.

That this was not a casual event is proved by the inability shown in this context by the inactive stereoisomer antagonist.

We have no valid explanation for this "paradoxical" result, which nevertheless compounds the interpretation of a possible pituitary site of action for NO function. Whatever the precise mechanism(s) of action, it seems more likely that the functional balance between hypothalamic GHRH and SS function[26] and/or alterations in postreceptor mechanisms may underlie the occurrence of this event in old dogs.

Downstream, NO may activate a dual second-messenger system: the branches of the soluble guanylate cyclase and COX pathways.[15,16] This functional link between the NOS and COX pathways is operational in the regulation of several physiological actions such as antiplatelet activity, vasodilation, and cytoprotection.[15] This may also be extended to include the neuroendocrine system. In fact, in the rat anterior pituitary, arachidonic acid metabolites[31] and cyclic guanosine monophosphate (cGMP)[8] are involved in GHRH-induced GH release. Moreover, the release of cGMP and PG has been associated with luteinizing hormone–releasing hormone release induced by norepinephrine in rat hypothalamic explants.[14]

Interestingly, a functional interaction between the NO and COX pathways could be envisioned in our experimental setting, too. Our results showing that indomethacin abolished the NO-donor potentiation of the GH response to Hexarelin suggest that NO modulation of stimulated GH secretion is ultimately mediated by the COX pathway.

However, in our study, a major functional difference between

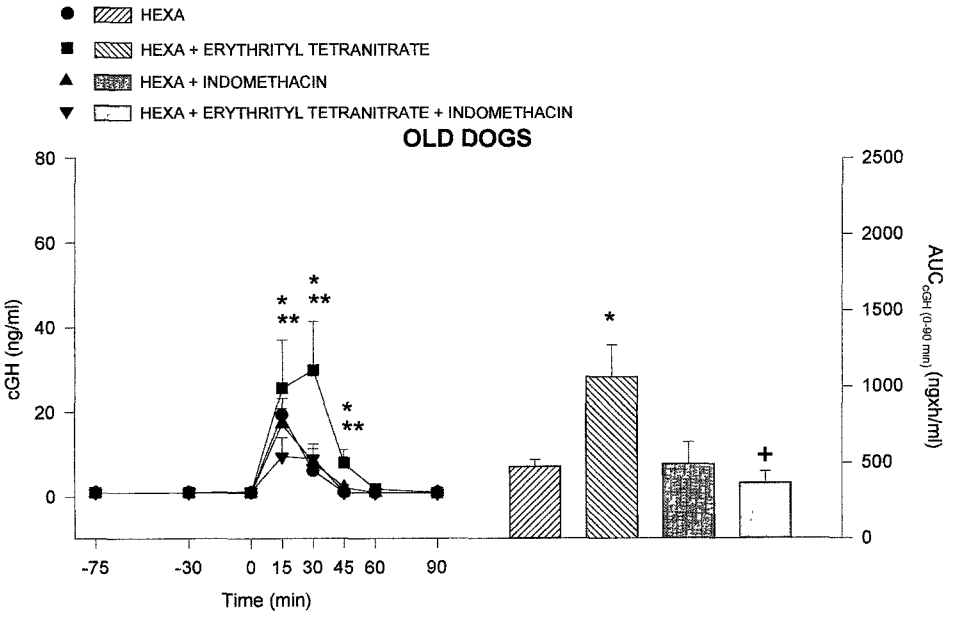

Fig 6. GH response (mean ± SEM and AUC_{0-90}) after acute challenge with Hexarelin (31.25 μg · kg^{-1} IV), Hexarelin + erythrityl tetranitrate (0.3 mg · kg^{-1} PO), Hexarelin + indomethacin (1.5 mg · kg^{-1} IM), or Hexarelin + erythrityl tetranitrate + indomethacin in 5 old dogs. *$P < .01$ *v* Hexarelin; **$P < .01$ *v* Hexarelin + erythrityl tetranitrate + indomethacin; $^{+}P < .01$ *v* Hexarelin + erythrityl tetranitrate.

COX (indomethacin) and NOS (NAME) inhibition was the inability of the former to alter the GH response to Hexarelin alone, while the latter inhibited or enhanced the action of Hexarelin in young or old dogs, respectively.

Irrespective of the site(s) of action of NO and of the NO-COX interaction, our data support the view alluded to previously that NO-induced activation of PG synthesis is a downstream event in stimulated GH secretion.

In contrast to our findings, in rats, pretreatment with indomethacin reduced GHRP-6–induced GH release.[32] Differences in the experimental protocol (dose, timing, and route of administration) and/or species may explain the discrepancy.

Unlike the paradoxical potentiation of the GH response to Hexarelin in old dogs pretreated with L-NAME, the inhibition of GH release induced by the peptide plus erythrityl tetranitrate was also present after COX inhibition in aged animals. This apparent discrepancy may result from a preservation in aged animals of the functional interaction between the NOS and COX pathways.

Interestingly, recent data obtained in a rat model of GH deficiency and experimentally induced coronary ischemia have shown that the myocardial protective role exerted by Hexarelin against different vasoconstrictor agents is related to NO endothelial production.[33] The functional relatedness of these findings with our present results is apparent.

In conclusion, the recent availability of potent peptidyl[34] and nonpeptidyl[35] GHRP compounds for the stimulation of GH secretion in old mammals, including humans,[23,24] and the present findings of further enhancement of GHRP-induced GH release by a liposoluble NO donor may provide a valid therapeutic approach to restore somatotrope function in adults with GH deficiency or in elderly subjects. However, careful preclinical and clinical studies are required before recommending the use of NO donors as adjunctive therapy for humans.

REFERENCES

1. Ceccatelli S, Hulting A, Zhang X, et al: Nitric oxide synthase in the rat anterior pituitary gland and the role of nitric oxide in regulation of luteinizing hormone secretion. Proc Natl Acad Sci USA 90:11292-11296, 1993
2. Costa A, Trainer P, Besser M, et al: Nitric oxide modulates the release of corticotropin-releasing hormone from the rat hypothalamus in vitro. Brain Res 605:187-192, 1993
3. Chiodera P, Volpi R, Coiro V: Inhibitory control of nitric oxide on the arginine-vasopressin and oxytocin response to hypoglycaemia in normal men. Neuroreport 5:1822-1824, 1994
4. Duvilanski BH, Zambruno C, Seilicovich A, et al: Role of nitric oxide in control of prolactin release by the adenohypophysis. Proc Natl Acad Sci USA 92:170-174, 1995
5. Kato M: Involvement of nitric oxide in growth hormone (GH)-releasing hormone–induced GH secretion in rat pituitary cells. Endocrinology 131:2133-2138, 1992
6. Aguila MC: Growth hormone–releasing factor increases somatostatin release and mRNA levels in the rat periventricular nucleus via nitric oxide by activation of guanylate cyclase. Proc Natl Acad Sci USA 91:782-786, 1994
7. Korbonits M, Trainer PJ, Fanciulli G, et al: L-Arginine is unlikely to exert neuroendocrine effects in humans via the generation of nitric oxide. Eur J Endocrinol 135:543-547, 1996
8. Tena-Sempere M, Leonor P, Gonzalez D, et al: Involvement of endogenous nitric oxide in the control of pituitary responsiveness to different elicitors of growth hormone release in prepubertal rats. Neuroendocrinology 64:146-152, 1996
9. Volpi R, Chiodera P, Caffarri G, et al: Influence of nitric oxide on hypoglycemia- or angiotensin II–stimulated ACTH and GH secretion in normal men. Neuropeptides 30:528-532, 1996
10. Moncada S, Higgs FA The L-arginine–nitric oxide pathway. N Engl J Med 329:2002-2012, 1993
11. Knowles RG, Moncada S: Nitric oxide synthases in mammals. Biochem J 258:249-258, 1994
12. Bredt DS, Hwang PM, Snyder SH: Localization of nitric oxide synthase indicating a neuronal role for nitric oxide. Nature 347:768-770, 1990
13. Bhat G, Mahesh VB, Aguan K, et al: Evidence that brain nitric oxide synthase is the major nitric oxide synthase isoform in the hypothalamus of the adult female rat and that nitric oxide potently regulates hypothalamic cGMP levels. Neuroendocrinology 64:93-102, 1996
14 Rettori V, Gimeno M, Lyson K, et al: Nitric oxide mediates norepinephrine-induced prostaglandin E2 release from the hypothalamus. Proc Natl Acad Sci USA 89 11543-11546, 1992
15. Salvemini D: Regulation of cyclooxygenase enzymes by nitric oxide. Cell Mol Life Sci 53:576-582, 1997
16. Salvemini D, Misko TP, Masferrrer JL, et al: Nitric oxide activates cyclooxygenase enzymes. Proc Natl Acad Sci USA 90:7240-7244, 1993
17. Ojeda SR, Negro-Vilar A, McCann SM, et al. Release of prostaglandins by hypothalamic tissue: Evidence for their involvement in catecholamine-induced luteinizing hormone–releasing hormone release. Endocrinology 104:617-624, 1979
18. Sobel DO: Characterization of PGE2 inhibition of corticotropin releasing factor–mediated ACTH release. Brain Res 411:102-107, 1987
19. Sonntag WE, Steger RW, Forman LJ, et al: Decreased pulsatile release of growth hormone in old rats. Endocrinology 107:1875-1879, 1977
20. Zadik Z, Chalew SA, McCarter RJ, et al: The influence of age on the twenty-four-hour integrated concentration of growth hormone in normal individuals. J Clin Endocrinol Metab 60:513-516, 1985
21. Shibasaki T, Shizume K, Makahara M, et al: Age-related changes in plasma growth hormone response to growth hormone–releasing factor in man. J Clin Endocrinol Metab 58:212-214, 1984
22. Cocchi D, Calderini G, Ganzetti I, et al: Aspects of the control of prolactin and growth hormone secretion in aging, in Müller EE, MacLeod RM (eds): Neuroendocrine Perspectives, vol 5 Amsterdam, The Netherlands, Elsevier, 1986, pp 191-204
23. Arvat E, Gianotti L, Grottoli S, et al: Arginine and growth hormone–releasing hormone restore the blunted growth hormone–releasing activity of Hexarelin in elderly subjects. J Clin Endocrinol Metab 79.1440-1443, 1994
24. Cella SG, Locatelli V, Poratelli M, et al: Hexarelin, a potent GHRP analogue: Interactions with GHRH and clonidine in young and aged dogs. Peptides 16:81-86, 1995
25. Müller EE, Cella SG, De Gennaro Colonna V, et al: Aspects of the neuroendocrine control of growth hormone secretion in ageing mammals. J Reprod Fertil Suppl 43:99-114, 1993
26. Müller EE, Cella SG, Parenti M, et al: Somatotropic dysregulation in old mammals. Horm Res 43:39-45, 1995
27. Robertson RM, Robertson D. Drugs used for the treatment of myocardial ischemia, in Hardman JG, Limbird LE, Molinoff PB, et al (eds): Goodman & Gilman's The Pharmacological Basis of Therapeutics (ed 9). New York, NY, McGraw-Hill, 1996, pp 759-767
28. Korbonits M, Grossman AB: Growth hormone–releasing peptide and its analogues. Trends Endocrinol Metab 6:43-46, 1995

29. Torsello A, Grilli R, Luoni M, et al: Mechanism of action of Hexarelin. Growth hormone–releasing activity in the rat. Eur J Endocrinol 135:481-488, 1996

30. Rees DD, Palmer RMJ, Sculz R, et al: Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. Br J Pharmacol 101:746-752, 1990

31. Roudbaraki MM, Drouhault R, Bacquart T, et al: Arachidonic acid–induced release in somatotropes: Involvement of calcium. Neuroendocrinology 63:244-256, 1996

32. Anhnfelt-Ronne I. Agerholm PC, Bistrup HN: Indomethacin pretreatment reduces GHRP-6–induced GH release in rats. Second International Symposium on Growth Hormone Secretagogues, Tampa, FL, February 13-17, 1997

33. DeGennaro Colonna V, Rossoni G, Bernareggi M, et al: Cardiac ischemia and impairment of vascular endothelium function in hearts from growth hormone–deficient rats. Protection by Hexarelin. Eur J Pharmacol 334:201-207, 1997

34. Ghigo E, Arvat E, Muccioli G, et al: Growth hormone–releasing peptides. Eur J Endocrinol 136:445-460, 1997

35. Smith RG, Cheng K, Schoen WR, et al: A nonpeptidyl growth hormone secretagogue. Science 260:1640-1643, 1993