

Original article

Effects of arginine, growth hormone-releasing hormone (GHRH) and neostigmine administered singly or in paired combinations on growth hormone (GH) release in pigs

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(Received 24 December 1996; accepted 30 July 1997)

Summary – In human, arginine (ARG) induces growth hormone (GH) release, probably via a decrease in somatostatinergic tone. To assess the mechanism by which ARG mediates GH release in pigs, the effects on plasma GH release of ARG (1 g/kg body weight, infused between times –15 and –5 min), growth hormone-releasing hormone (GHRH, 2 µg/kg, at time 0 min) and neostigmine, a cholinesterase inhibitor (NEO, 50 µg/kg, at time 4 min), administered intravenously singly or in paired combinations were investigated in piglets between times –30 and 100 min. ARG and GHRH had additive effects on GH release. No potentialization was observed between the two treatments. GH response was higher following the combination of NEO and GHRH treatments than after NEO or GHRH given alone. NEO had no further effect on ARG-induced GH secretion. Therefore, our results suggest that the mechanism by which ARG stimulates GH secretion in pig is the same as in human.

arginine / growth hormone-releasing hormone / neostigmine / growth hormone / pig

Résumé – Effets de l'arginine, du *growth hormone-releasing hormone* (GHRH) et de la néostigmine administrés seuls ou en combinaison deux à deux sur la sécrétion de l'hormone de croissance (GH) chez le porc. Chez l'homme, l'arginine (ARG) induit une sécrétion d'hormone de croissance, probablement via une diminution du tonus somatostatinerigique. Pour déterminer le mécanisme par lequel l'ARG influence la sécrétion de GH chez le porc, les effets sur la GH plasmatique de l'ARG (1 g/kg de poids vif, infusée de –15 à –5 min), du *growth hormone-releasing hormone* (GHRH, 2 µg/kg, à 0 min) et de la néostigmine, un inhibiteur cholinestérasique (NEO, 50 µg/kg, à 4 min), administrés par voie intraveineuse seuls ou en combinaison deux à deux ont été étudiés chez des porcelets entre –30 et 100 min. L'ARG et le GHRH ont des effets additifs sur la sécrétion de GH. Aucune potentialisation n'a été observée entre ces deux traitements. La réponse de la GH était plus

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élevée après le traitement combiné de NEO et de GHRH qu'après les traitements de NEO ou de GHRH seuls. Le NEO n'a pas d'effet supplémentaire sur la sécrétion de GH induite par l'ARG. Nos résultats suggèrent donc que le mécanisme par lequel l'ARG stimule la sécrétion de GH chez le porc est le même que chez l'homme.

arginine / growth hormone-releasing hormone / néostigmine / hormone de croissance / porc

INTRODUCTION

Growth hormone (GH) secretion is regulated by many factors including nutrients. Arginine (ARG) infused intravenously at a dose of 0.5 g/kg body weight is a well-known stimulator of GH release in human (Alba-Roth et al, 1988; Page et al, 1988; Ghigo et al, 1990a) and can be used for the diagnosis of growth impairments. Few studies have been reported in domestic animals. Abomasal, intraduodenal or intravenous infusions of ARG can stimulate GH secretion in lambs (Davenport et al, 1990a, 1995), adult sheep (Hertelendy et al, 1969, 1970; Davis and Borger, 1973; Kuhara et al, 1991; Recabarren et al, 1995) and cattle (Vicini et al, 1988; Davenport et al, 1990b). Little is known about the effect of ARG on GH secretion in pigs. It seems that pigs are less sensitive to ARG than other species (Hertelendy et al, 1970). When infused intravenously at a dose of 0.5 g/kg^{0.75} body weight, ARG induces a small rise in plasma GH in 12-week-old pigs (Atinmo et al, 1978).

ARG-induced GH release has been well-documented in human and is thought to be mediated by a decrease in somatostatin release (Locatelli et al, 1986; Alba-Roth et al, 1988; Ghigo et al, 1990a; Volta et al, 1995). In that respect, ARG has been shown to override inhibitory effects on GH secretion that are known to be mediated by an increase in somatostatin release, including glucose administration (Ghigo et al, 1992), obesity (Procopio et al, 1995; Martina et al, 1995), glucocorticoids (Giustina et al, 1992), advanced age (Ghigo et al, 1990b) and GH negative feedback (Massara et al, 1986;

Torsello et al, 1988; Kelijman and Frohman, 1991; Ghigo et al, 1991). Cholinesterase inhibitors, such as neostigmine (NEO) and pyridostigmine, which are known to decrease somatostatin release (Richardson et al, 1980; Muller, 1987) do not further increase GH response to ARG in human (Massara et al, 1986; Ghigo et al, 1990a, 1994; Procopio et al, 1995). On the contrary, the effect of ARG on GH secretion is blunted or abolished by cholinergic antagonists such as atropine (Casanueva et al, 1984) and pirenzepine (Delitala et al, 1982; Maccario et al, 1995), an acute increase in free fatty acids (Maccario et al, 1994) and an administration of a somatostatin analog (Masuda et al, 1990).

ARG probably acting by inhibiting somatostatin release from the hypothalamus is able to enhance GH response to GHRH in human (Alba-Roth et al, 1988; Page et al, 1988; Ghigo et al, 1990a, b, 1991, 1992, 1994). In a preliminary study conducted on eight pigs weighing 30–40 kg, we applied the experimental design used in human studies (intravenous infusion of ARG over 30 min at the dose of 0.5 g/kg) but we were unable to demonstrate any increase in GHRH-induced GH release by ARG. The area under the GH curve (AUC) and the maximum of GH secretion were not significantly different between GHRH treatment and the combination of GHRH and ARG treatments ($P > 0.1$, AUC: 200, 488, 446 min \times ng/mL, maximum of GH secretion: 8, 11, 11 ng/mL for ARG, GHRH and GHRH plus ARG, respectively). These results could be explained either by the existence of a somatostatinergic hypertone in pigs as previously suggested by the poor GH response

to GHRH in this species (Della-Ferra et al, 1986; Etherton et al, 1986; Peticlerc et al, 1987) or by a different mechanism of action of ARG than that proposed in human.

The present study was therefore undertaken in order to determine whether the mechanism by which ARG mediates GH secretion in pigs is the same as in human. For that purpose, the effects of ARG, GHRH and NEO administered singly or in paired combinations were investigated in piglets with a modified experimental design compared to that used in human. The dose of ARG was double that used in human and the period of time over which ARG was infused was shorter.

MATERIALS AND METHODS

Animals and management

Six female crossbred Yorkshire × Pietrain piglets from three litters, two females per litter, were used in the experiment. They were 35 days of age and weighed 9.7 ± 0.9 (SE) kg at the beginning of the study. They were reared in compliance with national regulations for the human care and use of the animals in research. They were housed in individual pens throughout the experiment. They were allowed water ad libitum and fed $70 \text{ g/kg}^{0.75}$ body weight of a standard diet provided in two meals a day. On the days of treat-

ments, the morning meal was delayed until the end of sampling. Animals were weighed the day before each treatment. They gained 6.2 ± 0.5 (SE) kg during the experiment.

Four days before the first injections, a catheter was inserted into one external jugular vein of each animal under general anaesthesia (O_2 : halothane, 92:8 v/v, 2 mL/min). Saline was flushed daily through the catheter to keep it patent throughout the experiment.

Treatments and blood sampling

Six treatments were applied consecutively to each animal at 3-day intervals according to a 6×6 latin square design. The six treatments are described in table I. Arginine (Degussa, Paris, France), GHRH (synthetic human 1–29 amide fragment of GHRH, Sigma, MO, USA) and neostigmine (Prostigmine, Roche, Neuilly-sur-Seine, France) were administered intravenously through the catheter at doses of 1 g/kg, 2 µg/kg and 50 µg/kg body weight, respectively. ARG, 20% in saline, was slowly injected by hand with a syringe at a flow rate of 10 mL per minute. No side-effect was observed during and after the infusion of ARG.

Blood samples were collected in heparinized tubes through the catheter at times -30, -17, -2, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90 and 100 min. Time 0 was 9.00. Blood was cooled on ice then immediately centrifuged. Plasma was stored at -20°C until analysis.

Table I. Treatments.

Treatments	Time ^a		
	-15 to -5 min	0 min	+4 min
ARG	arginine ^b	vehicle	vehicle
GHRH	vehicle	GHRH ^c	vehicle
NEO	vehicle	vehicle	neostigmine ^d
ARG/GHRH	arginine	GHRH	vehicle
ARG/NEO	arginine	vehicle	neostigmine
GHRH/NEO	vehicle	GHRH	neostigmine

^aTime 0 was 9.00; ^barginine: 1 g/kg body weight, 20% solution in saline; ^cGHRH: 2 µg/kg, 20 µg/mL in isotonic glucose; ^dneostigmine: 50 µg/kg, 0.5 mg/mL in saline.

Measurement of plasma GH concentration

Plasma GH concentration was determined by a specific homologous double-antibody radioimmunoassay as previously described (Louveau et al, 1991) using a specific antibody raised in rabbit (UCB Bioproducts, Brussels, Belgium). USDA-GH-I-1 (AFP-6400) was used for iodination and standard curve. Sensitivity was 0.75 ng/mL. Cross-reactivity with porcine prolactin, porcine luteinizing hormone, and porcine follicle-stimulating hormone was < 0.4%. Intra-assay coefficients of variation were 9, 13 and 15% for plasma samples containing 4.9, 15.2 and 32.9 ng/mL, respectively. All samples were measured in duplicate within a single assay.

Data analysis

Area under the GH curve (AUC) was estimated between times -17 and 100 min by calculating the area between the GH curve and basal level extended to 100 min. Basal level was obtained by calculating the mean of GH levels at times -30 and -17 min.

Data were analysed by analysis of variance using the generalized linear model (GLM) procedure of SAS system (SAS, 1989). GH profiles were analysed using the repeated procedure of SAS including the main effects of the day of treatment, animal and treatment. The main effect of time was analysed within treatments. Mean GH levels were compared to basal levels using Dunnett test. The model used for the analysis of areas under the GH curve included the main effects of day of treatment, animal and treatment. Multiple comparison of the means for treatments and AUC was carried out using Duncan test.

Three variables (ARG+GHRH, ARG+NEO and GHRH+NEO) were constructed in order to test respectively the additive effects between ARG and GHRH, between ARG and NEO, and between GHRH and NEO. Plasma GH above basal level measured for ARG and GHRH were summed up at all times studied. The same procedure was applied for ARG and NEO, and for GHRH and NEO. ARG + GHRH, ARG + NEO, and GHRH + NEO were compared to plasma GH above basal level obtained for ARG/GHRH, ARG/NEO and GHRH/NEO, respectively.

RESULTS

GH profiles from time -30 min to time 100 min and AUC are shown in figure 1 and in figure 2, respectively.

Basal plasma GH levels did not differ significantly between treatments ($P > 0.1$). Each treatment induced an increase in plasma GH whose magnitude and time-related pattern differed between treatments ($P < 0.001$). Each piglet responded to all treatments except one for NEO.

Plasma GH responses to ARG, GHRH, or NEO singly administered

GH responses to ARG and GHRH treatments were similar in magnitude (AUC: $P > 0.05$) and much higher than GH response to NEO (AUC: $P < 0.05$). Plasma GH was significantly ($P < 0.05$) higher than basal levels between -2 and 50 min, 10 and 80 min, and 15 and 40 min for ARG, GHRH and NEO treatments, respectively. Maximum GH response occurred earlier with ARG than with GHRH or NEO. Maximum GH responses were observed at 5, 25 and 25 min for ARG, GHRH and NEO, respectively.

Plasma GH response to combined ARG and GHRH treatments

The combined administration of ARG and GHRH elicited a larger GH response compared to ARG or GHRH alone (AUC: $P < 0.05$). GH levels for ARG/GHRH treatment were significantly higher than those induced by ARG alone from -2 to 35 min ($P < 0.05$) and than those induced by GHRH alone from 5 to 80 min ($P < 0.05$). GH profile for ARG/GHRH treatment exhibited two peaks, which rose at the same times as those for the ARG and GHRH treatments. Plasma GH was significantly lower at time 20 min compared to times 10 and 25 min ($P < 0.05$).

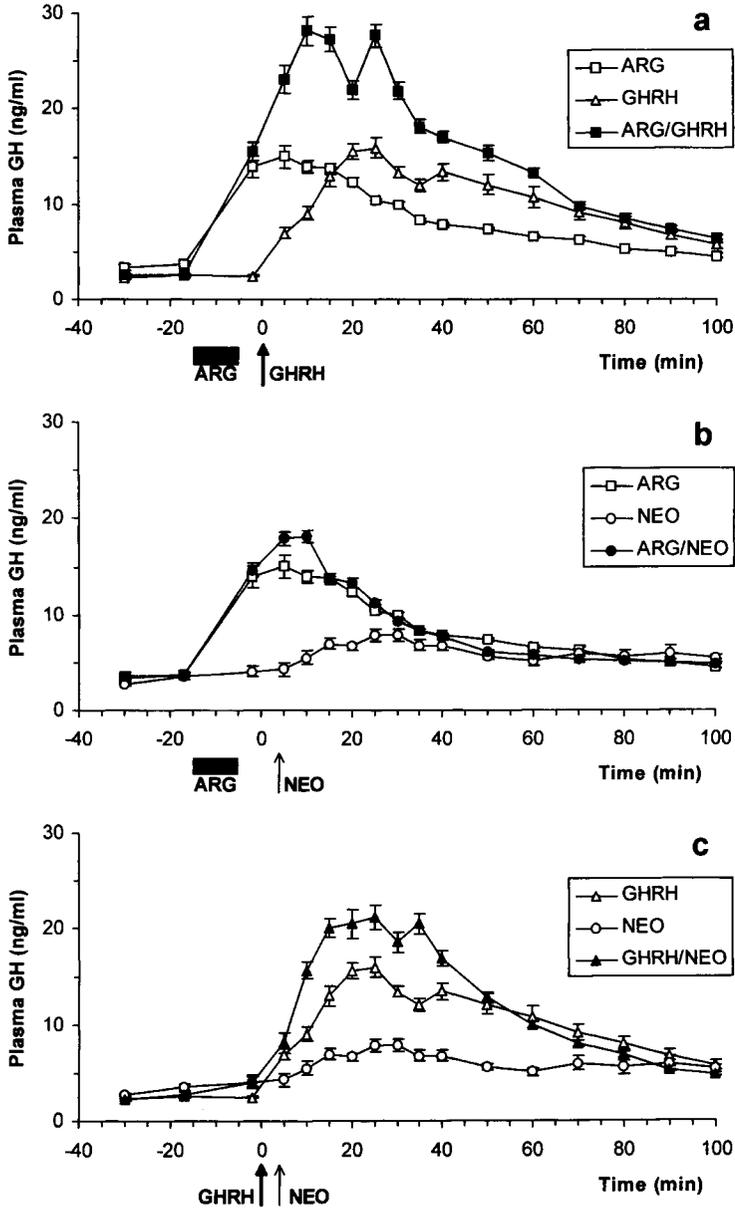


Fig 1. Plasma GH responses (means \pm SEM). Comparison of paired combination treatments with treatments alone. (a) Plasma GH responses to arginine (ARG), to growth hormone-releasing hormone (GHRH), and to ARG plus GHRH (ARG/GHRH). (b) Plasma GH responses to ARG, to neostigmine (NEO), and to ARG plus NEO (ARG/NEO). (c) Plasma GH responses to GHRH, to NEO, and to GHRH plus NEO (GHRH/NEO). Plasma GH concentrations were determined as indicated in *Material and methods*.

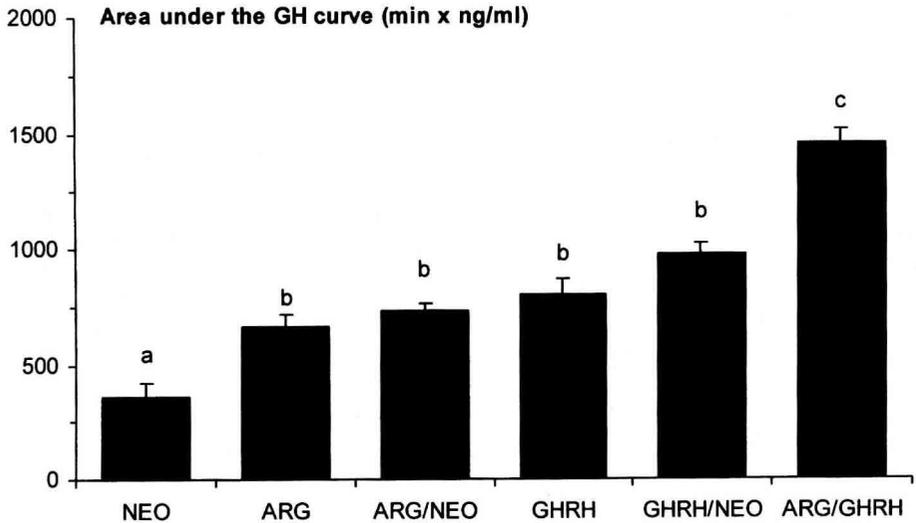


Fig 2. Plasma GH responses (AUC \pm SEM) to arginine (ARG), growth hormone-releasing hormone (GHRH), neostigmine (NEO), and to the combined administrations of ARG and GHRH (ARG/GHRH), of ARG and NEO (ARG/NEO), and of GHRH and NEO (GHRH/NEO). AUC were estimated as indicated in *Materials and methods*. Columns with different letters differ ($P < 0.05$).

The sums of GH values above basal level for ARG and GHRH treatments were not statistically different from values above basal level for ARG/GHRH ($P > 0.05$) demonstrating that the effects of ARG and GHRH were additive. The additivity of those effects is further demonstrated by AUC values.

Plasma GH response to combined ARG and NEO treatments

Plasma GH levels did not differ between ARG and ARG/NEO treatments (AUC: $P > 0.05$). Plasma GH concentrations were higher after ARG/NEO treatment than after NEO treatment between -2 and 10 min ($P < 0.05$, AUC: $P < 0.05$). The peak of GH secretion after ARG/NEO was observed at 10 min.

Plasma GH response to combined GHRH and NEO treatments

Plasma GH levels were higher after GHRH/NEO than after GHRH treatment at 10 min ($P < 0.10$), 15, 30 and 35 min ($P < 0.05$). AUC was higher for GHRH/NEO than for GHRH; however, the difference was not significant ($P > 0.05$). Plasma GH differed significantly ($P < 0.05$) between the GHRH/NEO and NEO treatments from 10 to 60 min. The AUC calculated for NEO and GHRH/NEO differed significantly ($P < 0.001$). Maximal secretion for GHRH/NEO was observed between 15 and 35 min. The sums of GH levels above basal level for GHRH and NEO treatments were not statistically different from values above basal level for GHRH/NEO ($P > 0.05$) suggesting that the effects of NEO and GHRH could be additive.

DISCUSSION

ARG has been shown to induce GH release in human as well as in domestic animals including pigs. In human, ARG effect is probably mediated by a decrease in somatostatinergic tone. To determine if the same mechanism occurs in pigs, we investigated the effects of ARG, GHRH and NEO administered singly or in paired combinations on GH release in piglets. In a preliminary study, we applied the experimental design used in human (ARG infused over 30 min at a dose of 0.5 g/kg body weight) on pigs weighing 30–40 kg but we were unable to observe any increase in GHRH-induced GH release by ARG. Owing to a hypothesized somatostatinergic hypertone in pigs, we therefore infused ARG on a shorter period (10 min) and we doubled the dose of ARG (1 g/kg) in the present study.

ARG when infused at a dose of 1 g/kg induced a clear GH response in all pigs of the present study, whereas a much smaller response was obtained with smaller doses in previous studies with pigs. A dose of 0.5 g/kg^{0.75} body weight induced a limited increase in plasma GH (Atinmo et al, 1978). A dose of 0.5 g/kg body weight, the same as that used in human studies, resulted in no or little GH response (Hertelendy et al, 1970; our own preliminary results). Together with the observation that GH response to NEO alone was small, these data are consistent with an elevated somatostatinergic tone in the pig as previously suggested by inconsistent GH responses to GHRH and the need for higher GHRH doses in pigs compared to other species (Della-Ferra et al, 1986; Etherton et al, 1986; Peticlerc et al, 1987). The dose of GHRH used in our study was optimal for the secretion of GH in pigs weighing 10–20 kg as determined previously (our unpublished results) and is smaller than the one necessary to elicit maximal GH response in older and heavier pigs (Peticlerc et al, 1987).

ARG and GHRH had additive effects on plasma GH in the pigs of the present study. The combination of NEO and GHRH treatments also resulted in a higher GH response than NEO or GHRH given alone although one could have expected a larger difference as previously observed in GHRH pigs immunized against somatostatin (Dubreuil et al, 1989). This is probably related to the fact that the NEO dose used in the present experiment was not optimal so that the additivity of the effects of NEO and GHRH was not clearly demonstrated particularly with the AUC. The combined administration of ARG and NEO had no further effect compared to ARG alone. These results are consistent with a common mechanism of action for NEO and ARG. Otherwise, we would have had a higher GH response after the combination of ARG and NEO treatments than after ARG given alone. This common mechanism of action involves somatostatin pathway as described for NEO (Richardson et al, 1980) and not GHRH pathway. The present results are also consistent with different mechanisms of action for ARG and GHRH since the actions of ARG and GHRH were additive. It may therefore be hypothesized that ARG induces a decrease in the somatostatinergic tone in pigs as described in human (Locatelli et al, 1986; Alba-Roth et al, 1988; Ghigo et al, 1990a, Volta et al, 1995). The mechanisms by which ARG could inhibit somatostatin release in the hypothalamus remain to be investigated. ARG could act directly or via its metabolites. ARG can be metabolized in the brain to ornithine by brain arginase (Carjaval et al, 1995) and then to glutamic acid via ornithine aminotransferase (Wroblewsky et al, 1985). Both metabolites have been shown to stimulate GH secretion. Ornithine elicits GH secretion in sheep (Davenport et al, 1990a, 1995; Recabarren et al, 1995). Glutamic acid is able to stimulate GH release in pigs (Barb et al, 1996). ARG is also the precursor of nitric oxide (NO), which is synthesized by NO synthase (NOS) (Palmer et al,

1988). NOS has been localized in various hypothalamic areas and in the pituitary. NO, which is involved in a wide range of physiological events (Moncada et al, 1991), seems to play a permissive role in the control of stimulated GH release. Endogenous NO deprivation by the use of NOS inhibitors attenuates glutamic acid-induced GH release (Tenasempere et al, 1995) and GHRH-induced GH secretion in prepubertal rats (Tenasempere et al, 1996) but not basal GH. Further studies are needed to determine whether ARG could stimulate NOergic neurons in the hypothalamus.

A potentiating effect between ARG and GHRH on GH release has been described in human (Alba-Roth et al, 1988; Page et al, 1988; Ghigo et al, 1990b, 1991, 1992, 1994) except with children of short stature (Ghigo et al, 1990a). In the pigs of the present study, the effects of ARG and GHRH were only additive and no potentialization could be demonstrated. Further studies are needed to establish if this potentialization only occurs in adult animals and human or if there are species differences.

In conclusion, the present results suggest that a higher dose of ARG in pigs compared to human is required to obtain a significant GH response owing to a high somatostatin-ergic tone. The mechanism by which ARG stimulates GH secretion in pigs seems to be the same as the one proposed in human. In human, ARG-induced GH release is probably mediated by a decrease in somatostatin release although this mechanism was demonstrated indirectly by the use of a cholinesterase inhibitor as in the present study. Direct measurements of somatostatin and GHRH in the hypothalamo-pituitary portal vein in pigs are therefore needed to verify definitively these hypotheses.

ACKNOWLEDGMENTS

The authors express their thanks to Y Lebreton, F Giovanni, M Formal and M Lesage for their

contribution to the experiment. The GH preparation was a gift from the National Hormone and Pituitary Program (University of Maryland School of Medicine) and the National Institute of Diabetes and Kidney diseases.

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