

## EXPERIMENTAL STUDY

## Endocrine activities of alexamorelin (Ala-His-D-2-methyl-Trp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub>), a synthetic GH secretagogue, in humans

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### Abstract

**Objective:** Peptidyl and non-peptidyl synthetic GH secretagogues (GHS) possess significant GH-, prolactin (PRL)- and ACTH/cortisol-releasing activity after i.v. and even p.o. administration, acting via specific hypothalamo–pituitary receptors in both animals and humans. The hexapeptide hexarelin (HEX) is a paradigmatic GHS whose activities have been widely studied in humans. The heptapeptide Ala-His-D-2-methyl-Trp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub> (alexamorelin, ALEX) is a new synthetic molecule which inhibits GHS binding *in vitro*, but its endocrine activity has never been studied in humans.

**Design:** In six young adults we studied the effects of 1.0 and 2.0 µg/kg i.v. ALEX or HEX on GH, PRL, ACTH, cortisol and aldosterone levels and those of 20 mg p.o. (≈300 µg/kg) on GH levels.

**Results:** Basal GH, PRL, ACTH, cortisol and aldosterone levels in all testing sessions were similar. ALEX and HEX (1.0 and 2.0 µg/kg i.v.) induced the same dose-dependent increase of GH and PRL levels. Both ALEX and HEX induced a dose-dependent increase of ACTH and cortisol levels. The ACTH and cortisol responses to the highest ALEX dose were significantly higher than those after HEX. Aldosterone levels significantly increased after both i.v. ALEX doses, but not after HEX. The GH response to 20 mg p.o. ALEX was higher, though not significantly, than that to the same HEX dose.

**Conclusion:** ALEX, a new GHS, shows the same GH-releasing activity as HEX. On the other hand, ALEX seems endowed with an ACTH-releasing activity more marked than that of HEX; this evidence could explain the significant increase of aldosterone levels after its i.v. administration.

*European Journal of Endocrinology* 143 419–425

### Introduction

Growth hormone secretagogues (GHS) are synthetic, non-natural, peptidyl and non-peptidyl molecules which possess a potent stimulatory effect on somatotroph secretion after i.v., s.c., intranasal and even p.o. administration in both animals and humans (1–4). The activity of GHS is not fully specific; in fact they possess also slight stimulatory effects on prolactin (PRL), adrenocorticotrophin (ACTH) and cortisol secretion (2, 3, 5).

Though synthetic and non-natural, GHS act via specific receptors distributed at the pituitary level as well as within the CNS, particularly at the hypothalamic level (3, 6–8). A human GHS receptor has recently been cloned and shows no significant homology with any other G-protein coupled receptor known so far (3, 6, 9, 10); however, the existence of GHS receptor subtypes

has also been reported (8–11). This evidence pointed towards the existence of an endogenous GHS-like ligand which could be represented by a recently discovered gastric peptide named ghrelin (12).

The mechanisms underlying the endocrine activities of GHS involve actions at the pituitary and, mainly, at the hypothalamic level (1–4). In fact, the endocrine activities of GHS are almost abolished by pituitary stalk lesions in animals as well as in patients with hypothalamo–pituitary disconnection (13, 14).

GH-releasing peptide (GHRP)-6, a hexapeptide, was the first GHS studied in humans (1, 2, 4); more recently, the endocrine activities of other hexapeptides, e.g. GHRP-2 and hexarelin (HEX), two GHRP-6 super-analogues, have also been extensively investigated in humans as well as in animals (1, 4, 15–17). Other penta-, hexa-, hepta- and octapeptides have been synthesized and reported active in humans (1–3, 18,

19). Among non-peptidyl GHRP mimetics, the spiroindoline MK-0677 possesses the most marked bioavailability after p.o. administration and long-lasting effects (3, 20, 21).

Ala-His-D-2-methyl-Trp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub> (alexamorelin, ALEX), is a heptapeptide showing marked inhibitory effect on <sup>125</sup>I-Tyr-Ala-HEX binding in animal and human tissues (22). However, it is still unknown if it possesses the classical endocrine activities of GHS *in vivo* in humans.

Based on the foregoing, the aim of the present study was to characterize the endocrine activities of ALEX in humans. To this goal, we compared the endocrine effects of i.v. and p.o. administration of ALEX with those of HEX in normal young volunteers.

## Subjects and methods

### Peptides and drugs

Vials containing 100 µg lyophilized ALEX or HEX were provided by Europeptides (Argenteuil, France). Lyophilized peptides were dissolved in 2 ml isotonic saline to be injected i.v.

### Study design

Six normal young male volunteers (age (mean ± S.E.M.) 31.3 ± 1.3 years; age range 26.5–35.5 years; body mass index 23.4 ± 0.9 kg/m<sup>2</sup>) were studied. All subjects were within 15% of their ideal body weight.

The study had been approved by the local Ethical Committee and informed consent was obtained from all subjects.

All subjects underwent seven testing sessions in random order and at least 3 days apart. The tests started between 0830 and 0900 h after overnight fasting and 30 min after cannulation of a cubital vein of the forearm, kept patent by the slow infusion of isotonic saline. All subjects kept a supine position from –45 min up to the end of the testing sessions.

All subjects received the following treatments: sessions 1 and 2, 1.0 or 2.0 µg/kg i.v. ALEX at 0 min; sessions 3 and 4, 1.0 or 2.0 µg/kg i.v. HEX at 0 min; sessions 5 and 6, 20 mg (approximately 300 µg/kg) p.o. ALEX or HEX at 0 min; session 7, saline.

Blood samples were taken in basal conditions (–15 and 0 min) and then every 15 min up to 120 min in testing sessions 1–4 and up to 180 min in sessions 5–7.

GH, PRL, ACTH, cortisol and aldosterone levels were assayed in sessions 1–4 and 7, whereas GH levels were assayed in sessions 5 and 6.

All samples from an individual subject were analysed together.

Serum GH levels (µg/l) were measured in duplicate by IRMA (hGH-CTK IRMA; DIASORIN, Saluggia, Italy). The sensitivity of the assay was 0.15 µg/l. The inter- and intra-assay coefficients of variation ranged from 5.1 to 7.5% and from 3.5 to 4.7% respectively.

Serum PRL levels (µg/l) were measured by IRMA (PROL-CTK IRMA; DIASORIN). The sensitivity of the assay was 0.45 µg/l. The inter- and intra-assay coefficients of variation ranged from 7.7 to 23.6% and from 2.3 to 3.4% respectively.

Plasma ACTH levels (pg/ml) were measured by IRMA (Allegro HS-ACTH; Nichols Institute Diagnostic, San Juan Capistrano, CA, USA). The sensitivity of the assay was 1.0 pg/ml. The inter- and intra-assay coefficients of variation ranged from 2.4 to 8.5% and from 3.9 to 9.9% respectively.

Serum cortisol levels (µg/l) were measured by RIA (CORT-CTK 125, IRMA; DIASORIN). The sensitivity of the assay was <0.5 µg/l. The inter- and intra-assay coefficients of variation ranged from 4.3 to 14.6% and from 5.7 to 9.9% respectively.

Serum aldosterone levels (pg/ml) were measured in duplicate by RIA (ALDO-MAIA; Biochem Immuno-system Company, Guidonia, Italy). The sensitivity of the assay was 6.0 pg/ml. The inter- and intra-assay coefficients of variation ranged from 5.8 to 12.5% and from 4.2 to 9.6% respectively.

GH, PRL and aldosterone levels are expressed as absolute hourly areas under curves (hAUC<sup>0'–120'/180'</sup>) whereas ACTH and cortisol responses as absolute delta hAUC<sup>0'–120'</sup> vs baseline (ΔhAUC<sup>0'–120'</sup>). Absolute and ΔAUC were calculated by trapezoidal integration.

The statistical analysis was carried out using a non-parametric ANOVA (Friedman test) and then a Wilcoxon test, when appropriate.

The results are expressed as means ± S.E.M.

**Table 1** Mean (± S.E.M.) GH, PRL and aldosterone AUCs and ACTH and cortisol ΔAUCs after i.v. saline, ALEX and HEX administration.

Drug	Dose (µg/kg)	GH hAUC <sup>0'–120'</sup> (µg/l/h)	PRL hAUC <sup>0'–120'</sup> (µg/l/h)	ACTH ΔhAUC <sup>0'–120'</sup> (µg/l/h)	Cortisol ΔhAUC <sup>0'–120'</sup> (µg/l/h)	Aldosterone hAUC <sup>0'–120'</sup> (pg/ml/h)
Saline	—	3.6 ± 1.0	148.4 ± 18.7	–270.5 ± 63.1	–1500.7 ± 243.5	7012.5 ± 337.7
ALEX	1.0	1180.9 ± 115.4 <sup>a</sup>	324.3 ± 43.1 <sup>a</sup>	–44.1 ± 93.3 <sup>a</sup>	575.3 ± 476.7 <sup>a,d</sup>	9545.7 ± 797.6 <sup>a,d</sup>
	2.0	2428.4 ± 391.9 <sup>a,b</sup>	495.7 ± 60.5 <sup>a,b</sup>	693.1 ± 238.7 <sup>a,b,e</sup>	2224.4 ± 584.3 <sup>a,b,e</sup>	10919.5 ± 1111.2 <sup>a,e</sup>
HEX	1.0	1728.4 ± 406.3 <sup>a</sup>	407.1 ± 78.6 <sup>a</sup>	–124.0 ± 57.4 <sup>a</sup>	–1308.2 ± 577.2 <sup>b</sup>	6857.1 ± 418.5 <sup>b</sup>
	2.0	2244.4 ± 181.2 <sup>a,d</sup>	488.5 ± 78.6 <sup>a,d</sup>	–58.4 ± 67.0 <sup>a,d,c</sup>	175.4 ± 388.1 <sup>a,d,c</sup>	6701.8 ± 646.6 <sup>c</sup>

<sup>a</sup> P < 0.05 vs saline; <sup>b</sup> P < 0.05 vs 1.0 µg/kg ALEX; <sup>c</sup> P < 0.05 vs 2.0 µg/kg ALEX; <sup>d</sup> P < 0.05 vs 1.0 µg/kg HEX; <sup>e</sup> P < 0.05 vs 2.0 µg/kg HEX.

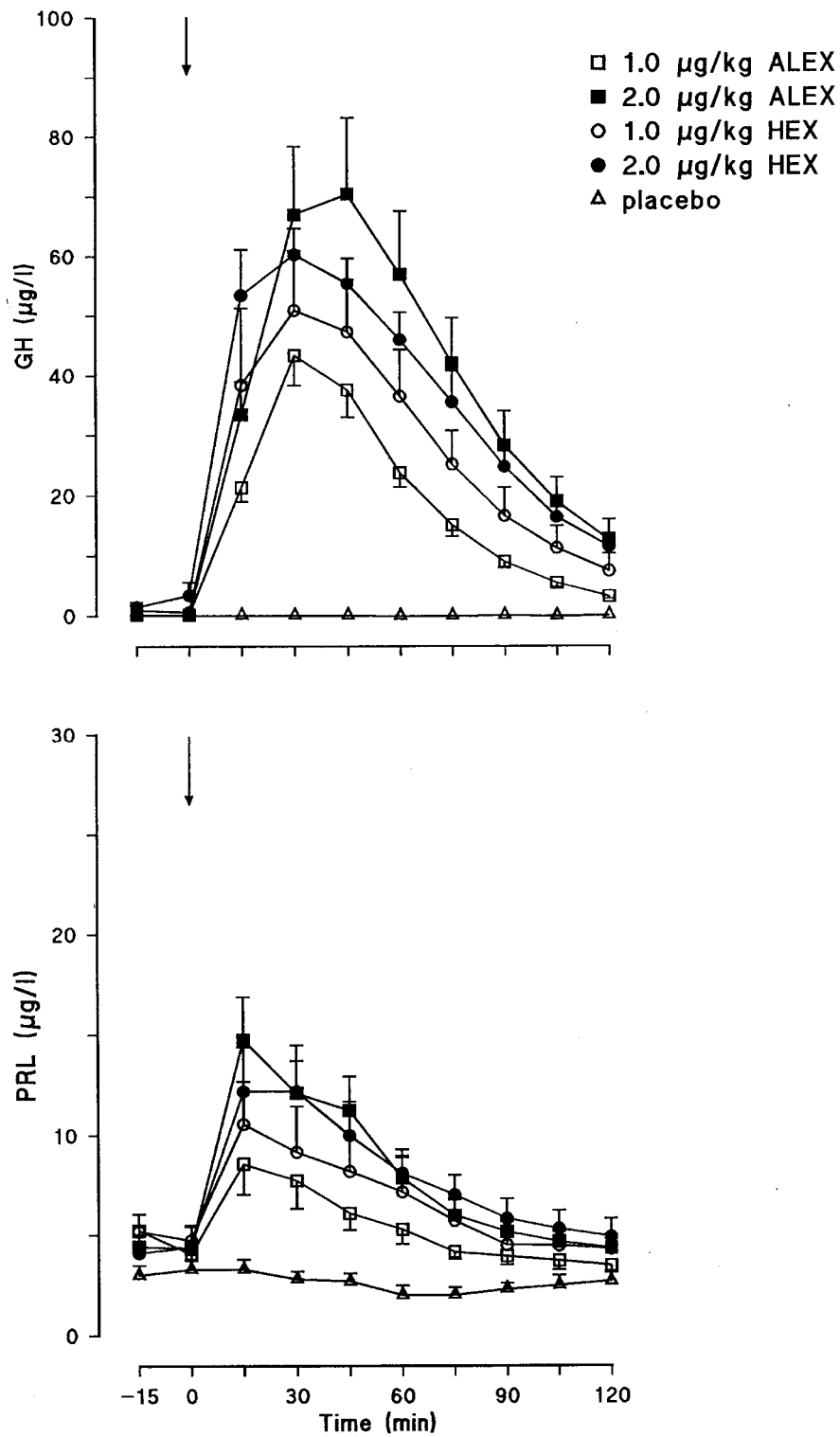


Figure 1 Mean ( $\pm$  S.E.M.) GH and PRL levels after i.v. acute ALEX, HEX or placebo.

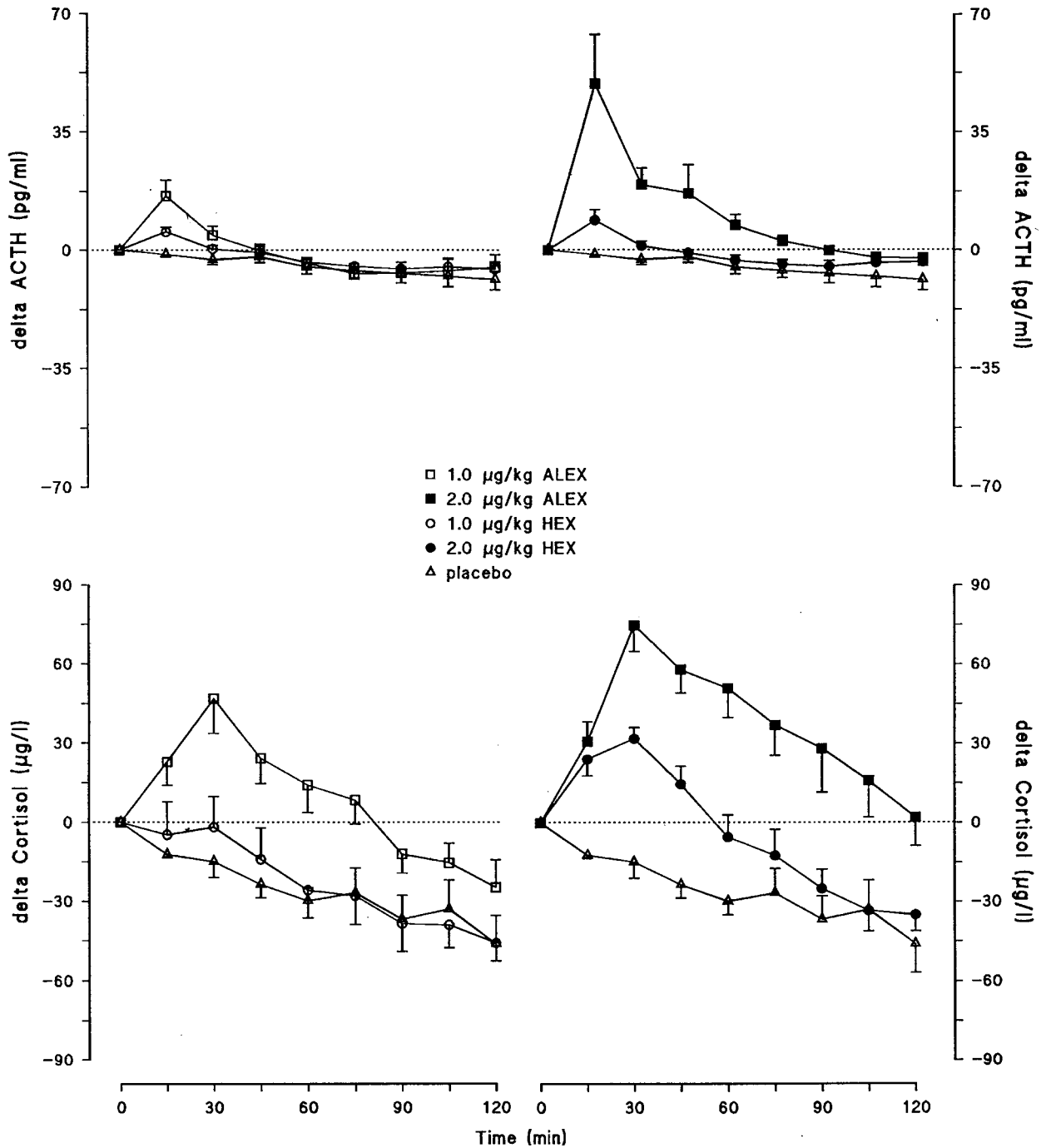


Figure 2 Mean ( $\pm$  S.E.M.)  $\Delta$ ACTH and cortisol levels after i.v. acute ALEX, HEX or placebo.

## Results

Basal GH, PRL, ACTH, cortisol and aldosterone levels in the various testing sessions were similar.

GH, PRL and aldosterone levels were not modified by saline whereas ACTH and cortisol levels showed a significant decrease ( $P < 0.05$ ).

The administration of 1.0 and 2.0  $\mu\text{g}/\text{kg}$  i.v. ALEX and HEX induced the same dose-dependent and marked increase of GH secretion (Table 1 and Fig. 1).

The administration of 1.0 and 2.0  $\mu\text{g}/\text{kg}$  i.v. ALEX and HEX induced the same dose-dependent increase of PRL secretion (Table 1 and Fig. 1).

The administration of 1.0  $\mu\text{g}/\text{kg}$  i.v. ALEX and HEX

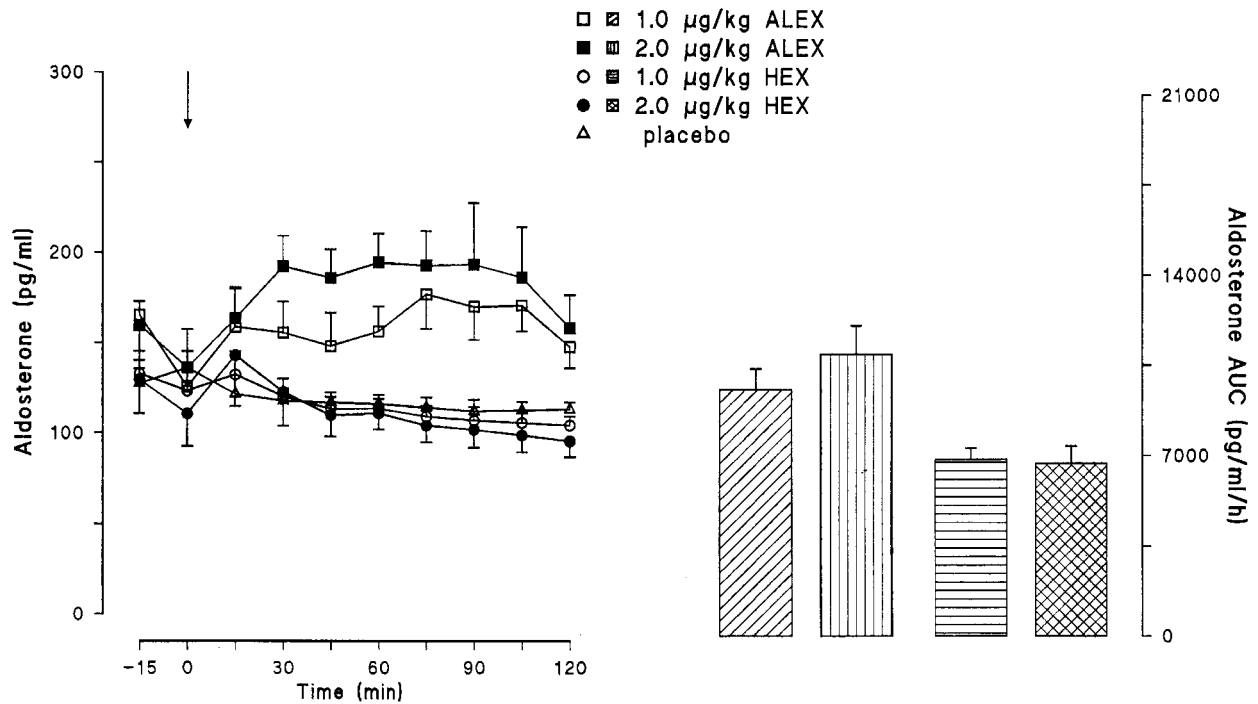


Figure 3 Mean ( $\pm$  S.E.M.) aldosterone levels after i. v. acute ALEX, HEX or placebo.

induced a similar, dose-dependent increase of ACTH secretion (Table 1). The ACTH response to 2.0 µg/kg i.v. ALEX was significantly higher than that after the same HEX dose ( $P < 0.05$ ) (Table 1 and Fig. 2).

The administration of 1.0 µg/kg i.v. ALEX but not that of HEX induced significant cortisol increase (Table 1). On the other hand, the administration of both 2.0 µg/kg i.v. ALEX and HEX induced a significant increase in cortisol secretion (Table 1); again the

cortisol response to ALEX was higher than that after HEX ( $P < 0.05$ ) (Table 1 and Fig. 2).

The administration of 1.0 and 2.0 µg/kg i.v. ALEX, but not that of either HEX dose, significantly stimulated aldosterone levels (Table 1 and Fig. 3). A positive correlation was observed between the  $\Delta$ ACTH hAUC and the  $\Delta$ aldosterone hAUC after ALEX administration ( $r = 0.64$ ;  $P < 0.05$ ) when considering both doses as a whole group. On the other hand no association was

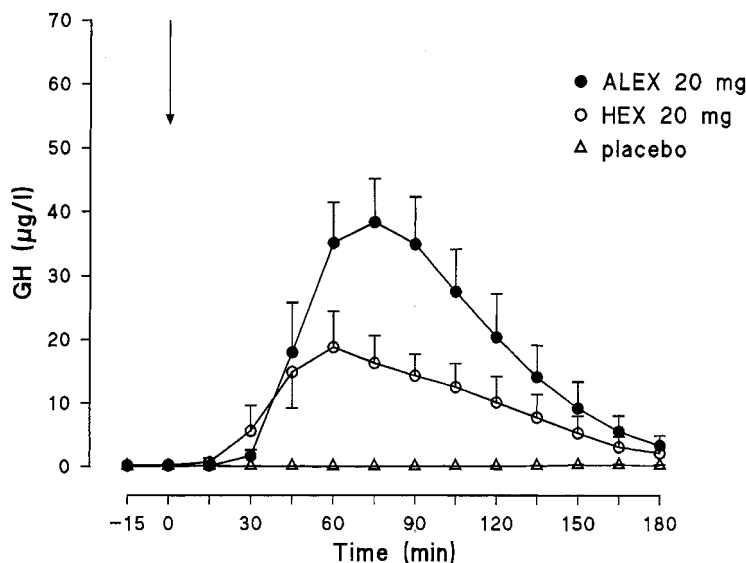


Figure 4 Mean ( $\pm$  S.E.M.) GH levels after p.o. ALEX, HEX or placebo.

found between peak plasma ACTH and aldosterone concentrations.

The p.o. administration of 20 mg ALEX and HEX had the same stimulatory effect on GH secretion (saline  $\text{hAUC}^{0'-180'}$ :  $7.1 \pm 1.4 \mu\text{g/l/h}$ ; ALEX:  $1035.3 \pm 254.7 \mu\text{g/l/h}$ ,  $P < 0.05$  vs saline; HEX:  $554.4 \pm 138.0 \mu\text{g/l/h}$ ,  $P < 0.05$  vs saline) (Fig. 4).

The timing of the endocrine responses to ALEX and HEX after i.v. or p.o. administration was similar.

### Side-effects

The administration of ALEX as well as of HEX induced transient facial flushing in four subjects. No medication was required and no test had to be stopped.

### Discussion

The present study demonstrates that Ala-His-D-2-methyl-Trp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub> (ALEX), a heptapeptide belonging to the GHS family, possesses the same strong, dose-dependent stimulatory effect as HEX on GH secretion. ALEX increases PRL secretion to the same extent as does HEX. On the other hand, ALEX releases more ACTH and cortisol than HEX and possesses significant aldosterone-releasing activity.

The strong, dose-dependent and reproducible GH-releasing activity of several p.o. active molecules belonging to the GHS family has been reported by many authors (1–4). Among peptidyl GHS, GHRP-6 and its superanalogues GHRP-1, GHRP-2 and HEX are paradigmatic compounds the activity of which has been widely investigated in humans (1, 2, 4, 16). The activity of other peptidyl molecules including Tyr-Ala-HEX and ipamorelin, a pentapeptide, has been also studied (17, 19, 23); ipamorelin has been reported to be endowed with strictly selective GH-releasing activity but to be inactive after p.o. administration (19, 24).

With respect to non-peptidyl GHS such as MK-0677, peptidyl GHS have markedly shorter-lasting effects and bioavailability after p.o. administration (3, 20, 21). On the other hand, generally, the activity of both peptidyl and non-peptidyl GHS is not fully specific for GH, notable exceptions being ipamorelin and NN-703 (2, 3, 5, 18, 19).

ALEX is a heptapeptide which strongly inhibits <sup>125</sup>I-Tyr-Ala-HEX binding *in vitro* in animal and human tissues (22). Our present data show that it has GH-releasing activity as potent as that of HEX. Also the activity of ALEX is not, however, specific for GH and it has short-lasting effects. Actually, after i.v. administration, ALEX seems even more potent than HEX as ACTH and cortisol secretagogues.

Based on the foregoing, the mechanisms underlying the endocrine activities of ALEX should be assumed the same hypothesized for GHS: (i) action at the pituitary and, mainly, at the hypothalamic level to release GH; at

these levels GHS probably act as functional somatostatin antagonists and meantime enhance the activity of GHRH-secreting neurons (1–3), although an action mediated by the GHS-like ligand cannot be ruled out (1–3, 12); (ii) direct action at the pituitary levels to release PRL (1–3); (iii) central action to release ACTH and, in turn, cortisol (1–3). In fact, there is evidence that the stimulatory effect of GHS on the hypothalamo–pituitary axis is totally abolished by hypothalamo–pituitary disconnection (14). Although specific binding for GHS has been shown also in human adrenal (25), there is no evidence for direct action of GHS at the adrenal level; in fact, HEX does not possess stimulatory effects on glucocorticoid and catecholamine release from bovine adrenal (H Ong, personal communication).

The peculiar, stimulatory effect of ALEX on aldosterone secretion we have found in the present study probably reflects its enhanced ACTH-releasing activity as suggested by the positive correlation between the  $\Delta\text{ACTH}$  and the  $\Delta\text{aldosterone}$  responses. In fact, in humans aldosterone increases have been also reported after corticotrophin-releasing hormone administration as a function of the ACTH response (26, 27); moreover, it has been shown that aldosterone levels are increased even by extremely low ACTH–1–24 doses (27–29).

However, taking into account that peripheral activities of peptidyl GHS have been already shown and are probably mediated by specific GHRP receptor subtypes (30–32), the possibility that ALEX has peculiar, direct activity on the glomerularis zone of the adrenal remains to be verified. This is of particular interest because direct cardiovascular activities have been reported for both aldosterone (33) and GHRPs (30–32, 34).

In conclusion, this study demonstrates that the heptapeptide Ala-His-D-2-methyl-Trp-Ala-Trp-D-Phe-Lys-NH<sub>2</sub>, ALEX, is a new molecule belonging to the GHS family and shows the same GH-releasing activity as HEX. However, like the majority of GHS, its activity is not specific for GH, showing an even more marked ACTH-releasing effect which could explain the peculiar increase in aldosterone levels.

### Acknowledgements

This study was supported by grants from Comitato Nazionale della Ricerca (CNR, grant no. 98.03040.CT04, Rome, Italy), Fondazione per lo Studio delle Malattie Endocrino-Metaboliche (FSMEM) and Europeptides. The authors wish to thank Prof. F Camanni for his support and Dr L Di Vito for her participation in the study, and Dr A Bertagna, Mrs A Barberis and M Taliano for their technical assistance.

### References

- 1 Bowers CY, Veeraragavan K & Sethumadhavan K. Atypical growth hormone releasing peptides. In *Growth Hormone II, Basic*

- and *Clinical Aspects*, pp 203–222. Eds BB Bercu & RF Walker. New York: Springer-Verlag, 1993.
- 2 Ghigo E, Arvat E, Muccioli G & Camanni F. Growth hormone-releasing peptides. *European Journal of Endocrinology* 1997 **136** 445–460.
  - 3 Smith RG, Van der Ploeg LHT, Howard AD, Fleighner SD, Cheng K, Hickey GY *et al.* Peptidomimetic regulation of growth hormone secretion. *Endocrine Reviews* 1997 **18** 621–645.
  - 4 Korbonits M & Grossman AB. Growth hormone-releasing peptide and its analogues. Novel stimuli to growth hormone release. *Trends in Endocrinology and Metabolism* 1996 **6** 43–49.
  - 5 Ghigo E, Arvat E & Camanni F. Growth hormone secretagogue as corticotrophin-releasing factors. *Growth Hormone and IGF Research* 1998 **8** 145–148.
  - 6 Howard AD, Feighner SD, Cully DF, Arena JP, Liberato PL, Rosenblum CI *et al.* A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science* 1996 **273** 974–977.
  - 7 Muccioli G, Ghè C, Ghigo MC, Papotti M, Arvat E, Boghen MF *et al.* Specific receptors for synthetic GH secretagogues in the human brain and pituitary gland. *Journal of Endocrinology* 1998 **157** 99–106.
  - 8 Ong H, McNicoll N, Escher E, Collu R, Deghenghi R, Locatelli V *et al.* Identification of a pituitary GHRP receptor subtype by the photoaffinity labeling approach using <sup>125</sup>I-p-benzoyl-phenylalanine hexarelin derivative. *Endocrinology* 1998 **139** 432–435.
  - 9 McKee KK, Tan CP, Palyha OC, Liu J, Feighner SD, Hreniuk DL *et al.* Cloning and characterization of two human G-protein-coupled receptor genes (GPR38 and GPR39) related to the growth hormone secretagogues and neurotensin receptors. *Genomics* 1997 **46** 426–434.
  - 10 Tan CP, McKee KK, Qingyun L, Palyha OC, Feighner SD, Hreniuk DL *et al.* Cloning and characterization of a human and murine T-cell orphan G-protein-coupled receptor similar to the growth hormone secretagogue and neurotensin receptors. *Genomics* 1998 **52** 223–229.
  - 11 Feighner SD, Tan CP, McKee KK, Palyha OC, Hreniuk DL, Pong SS *et al.* Receptor for motilin identified in the human gastrointestinal system. *Science* 1999 **284** 2184–2188.
  - 12 Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H & Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999 **402** 656–660.
  - 13 Popovic V, Damjanovic S, Micic D, Djurovic M, Dieguez C & Casanueva FF. Blocked growth hormone-releasing peptide (GHRP-6)-induced GH secretion and absence of the synergic action of GHRP-6 plus GH-releasing hormone in patients with hypothalamopituitary disconnection: evidence that GHRP-6 main action is exerted at the hypothalamic level. *Journal of Clinical Endocrinology and Metabolism* 1995 **80** 942–947.
  - 14 Hickey GJ, Drisko J, Faidley T, Chang C, Anderson LL, Nicolich S *et al.* Mediation by the central nervous system is critical to the *in vivo* activity of the GH secretagogue L-692,585. *Journal of Endocrinology* 1996 **148** 371–380.
  - 15 Deghenghi R, Bontignon F, Luoni M, Grilli R, Guidi M & Locatelli V. Small peptides as potent releasers of growth hormone. *Journal of Pediatric Endocrinology and Metabolism* 1995 **8** 311–333.
  - 16 Arvat E, Di Vito L, Maccagno B, Broglio F, Boghen MF, Deghenghi R *et al.* Effects of GHRP-2 and hexarelin, two synthetic GH-releasing peptides, on GH, prolactin, ACTH and cortisol levels in man. Comparison with the effects of GHRH, TRH and hCRH. *Peptides* 1997 **18** 885–891.
  - 17 Arvat E, Di Vito L, Lanfranco F, Broglio F, Giordano R, Benso A *et al.* Tyr-alexarelin, a synthetic octapeptide, possesses the same endocrine activities of hexarelin and GHRP-6 in humans. *Journal of Endocrinological Investigation* 1999 **21** 92–97.
  - 18 Sehested Hansen B, Raun K, Kramer Nielsen K, Bygballe Johansen P, Kruse Hansen T, Peschke B *et al.* Pharmacological characterization of a new oral GH secretagogue, NN703. *European Journal of Endocrinology* 1999 **141** 180–189.
  - 19 Raun K, Hansen BS, Johansen NL, Thogersen H, Madsen K, Ankensen M *et al.* Ipamorelin, the first selective growth hormone secretagogue. *European Journal of Endocrinology* 1998 **139** 552–561.
  - 20 Copinschi G, Van Onderbergen A, L'Hermite-Baleriaux M, Mendel CM, Caufriez A, Leproult R *et al.* Effects of 7-day treatment with a novel orally active, growth hormone (GH) secretagogue, MK-677, on 24-h GH profiles, insulin-like growth factor I, and adrenocortical function in normal young men. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 2776–2782.
  - 21 Chapman IM, Bach MA, Van Cauter E, Farmer M, Krupa D, Taylor AM *et al.* Stimulation of the growth hormone (GH)-insulin-like growth factor I axis by daily oral administration of a GH secretagogue (MK-677) in healthy elderly subjects. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 4249–4257.
  - 22 Deghenghi R, Boutignon F, Luoni M, Rigamonti A, Cella SG, Broglio F *et al.* Small impervious peptides as GH secretagogues. In *Second International Symposium on Growth Hormone Secretagogues*, 13–16 February 1997, Tampa, FL, USA.
  - 23 Johansen PB, Nowak J, Skjaerbaek C, Flyvbjerg A, Andreassen TT, Wilken M *et al.* Ipamorelin, a new growth-hormone-releasing peptide, induces longitudinal bone growth in rats. *Growth Hormone and IGF Research* 1999 **9** 106–113.
  - 24 Ankensen M, Johansen NL, Madsen K, Hansen BS, Raun K, Nielsen KK *et al.* A new series of highly potent growth hormone-releasing peptides derived from ipamorelin. *Journal of Medicinal Chemistry* 1998 **41** 3699–3704.
  - 25 Muccioli G, Ghè C, Papotti M, Boghen MF, Nilsson M, Deghenghi R *et al.* Tissue distribution of GHRP receptors in humans. In *Fourth European Congress of Endocrinology*, Seville, Spain 9–13 May, 1998.
  - 26 Hermus AR, Pieters GF, Smals AG, Benraad TJ & Kloppenborg PW. Plasma adrenocorticotropin, cortisol and aldosterone responses to corticotropin-releasing factor: modulatory effect of basal cortisol levels. *Journal of Clinical Endocrinology and Metabolism* 1984 **58** 187–191.
  - 27 Olkers W, Boelke T, Bahr V, Exner P, Faust B & Harendt H. Dose-response relationships between plasma adrenocorticotropin (ACTH), cortisol, aldosterone and 18-hydroxycorticosterone after injection of ACTH-(1–39) or human corticotropin-releasing hormone in man. *Journal of Clinical Endocrinology and Metabolism* 1988 **66** 181–186.
  - 28 Arvat E, Di Vito L, Lanfranco F, Maccario M, Baffoni C, Rossetto R *et al.* Stimulatory effect of ACTH on cortisol, aldosterone and dehydroepiandrosterone secretion in normal humans. *Journal of Clinical Endocrinology and Metabolism* 2000 (In Press).
  - 29 Daidoh H, Morita H, Mune T, Murayama M, Hanafusa J, Ni H *et al.* Responses of plasma adrenocortical steroids to low dose ACTH in normal subjects. *Clinical Endocrinology* 1995 **43** 311–315.
  - 30 Muccioli G, Broglio F, Valetto MR, Ghè C, Catapano F, Graziani A *et al.* Growth hormone-releasing peptides and the cardiovascular system. *Annals d'Endocrinologie* 2000 **61** 27–31.
  - 31 Tivesten A, Bollano E, Caidhal K, Kujacic V, Sun XY, Hedner T *et al.* The growth hormone secretagogue hexarelin improves cardiac function in rats after experimental myocardial infarction. *Endocrinology* 2000 **141** 60–66.
  - 32 Locatelli V, Rossoni G, Schweiger F, Torsello A, De Gennaro Colonna V, Bernareggi M *et al.* Growth hormone-independent cardioprotective effects of hexarelin in the rat. *Endocrinology* 1999 **140** 4024–4031.
  - 33 Wehling M, Spes CH, Win N, Janson CP, Schmidt BM, Theisen K *et al.* Rapid cardiovascular action of aldosterone in man. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 3517–3522.
  - 34 Berti F, Muller E, De Gennaro Colonna V & Rossoni G. Hexarelin exhibits protective activity against cardiac ischaemia in hearts from growth hormone-deficient rats. *Growth Hormone and IGF Research* 1998 **8** 149–152.

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Received 17 February 2000