Effects of New Growth Hormone-Releasing Peptide (KP 102) on the Release of Growth Hormone *in vitro* and *in vivo* in Cattle

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(Received December 13, 1996)

Abstract The effects of KP102 (also named GHRP-2), a new growth hormone-releasing peptide, on the release of bovine growth hormone (GH) were examined *in vitro* and *in vivo*. KP 102 and GH-releasing hormone (GHRH : hGHRH(1-29)-NH₂) significantly ($P < 0.05$) stimulated GH release from cultured bovine anterior pituitary cells at doses as low as 10^{-11} M and 10^{-13} M, respectively. When KP 102 (10^{-8} M) and GHRH (10^{-8} M or 10^{-10} M) were added together, the additive effects of both peptides on the release of GH were observed ($P < 0.05$). Somatostatin (SS, 10^{-7} M) significantly ($P < 0.05$) blunted GH release induced by KP 102 (10^{-8} M) and GHRH (10^{-8} M). Under the presence of SS, the additive effects of KP 102 and GHRH on the release of GH were also observed ($P < 0.05$). Intravenous injection of $20 \mu\text{g/kg}$ BW KP 102 significantly ($P < 0.05$) stimulated GH release in calves. Plasma GH concentrations 10 and 20 min after the iv injection of KP 102 ($20 \mu\text{g/kg}$ BW) plus GHRH ($0.25 \mu\text{g/kg}$ BW) were significantly ($P < 0.05$) greater than those of either KP 102 alone or GHRH alone. The value of the area under the GH response curve for 60 min in GHRH alone and KP 102 plus GHRH was significantly ($P < 0.05$) greater than that of KP 102 alone; however, there were no significant differences between the KP 102 plus GHRH and the GHRH alone groups. These results show that KP 102 stimulates GH release in cattle, and KP 102 interacts with GHRH and SS to release bovine GH.

Anim. Sci. Technol. (Jpn.) 68 (5) : 450-458, 1997

Key words : KP 102, GHRP-2, GHRH, GH, Cattle

Growth hormone-releasing peptides (GHRPs) are a family of synthetic peptides, six to seven amino acids in size, which selectively stimulate GH release^{5,17,18}. The first GHRP identified, His-D-Trp-Ala-Trp-D-Phe-Lys-NH₂ (GHRP-6), was derived from the pentapeptide met-enkephalin through theoretic low-energy conformational calculations, computer modeling, structural modification, and biologic studies¹⁹. GHRP-6 stimulates GH release in many species^{2,17}, and the peptide acts at both the

pituitary and hypothalamic levels to release GH^{11,17}. Recently, newly developed analogues of GHRP-6 have been synthesized and reported to be potent GH secretagogues^{5,17,18,20,22,28,29}. At present KP 102 (also named GHRP-2), D-Ala-D-β-Nal-Ala-Trp-D-Phe-Lys-NH₂, seems to be the most potent one. In cultured ovine pituitary cells, GHRP-6 had 10% of the potency of GH-releasing hormone (GHRH)²⁰; however, KP 102 is equipotent with GHRH as a GH secretagogue for ovine pituitary cells²⁸. The

detailed mechanism of action of KP 102 is not clear, and the effects of KP 102 on the release of GH have not been fully elucidated yet in cattle either *in vitro* and *in vivo*. GHRP-6 has been shown to increase the rate of milk production in cows⁸⁾, and KP 102 has been suggested to be used for enhancing the growth performance in cattle²¹⁾. Infusion of GHRH increase the mean and pulsatile release of GH and increase milk yield in cows^{9,10)}. Moreover, KP 102 and GHRH administered together synergistically stimulates GH release in rats²²⁾ and humans²⁰⁾. It is therefore important to clarify the GH-releasing response to KP 102 and GHRH in cattle for the effective usage of the peptides in milk and meat production.

The present study was designed to examine the effect of KP 102 on the release of GH with the interactive effects of GHRH and somatostatin (SS) using bovine cultured anterior pituitary cells *in vitro*. In addition, the GH responses to the combination of KP 102 plus GHRH were examined complementarily *in vivo* in cattle.

Materials and Methods

Hormones and chemicals

Peptides and chemicals were obtained from the following sources : KP 102 (a generous gift from Kaken Pharmaceutical Co. Ltd., Urayasu, Japan) ; GHRH (hGHRH(1-29)-NH₂, a generous gift from Dr. S. Ohashi, National Institute of Bioscience and Human Technology, Tsukuba, Japan) ; SS (Somatostatin-14, Peptide Institute Inc., Osaka, Japan) ; Dulbecco's modified Eagle Medium (DMEM), horse serum, newborn calf serum, pancreatin 4 X, N-2-hydroxyethyl piperazine-N'-2-ethanesulfonic acid (HEPES) and non-essential amino acids (Gibco, Gland Island, NY, USA) ; collagenase (Wako Pure Chemical Industries LTD., Osaka, Japan) and bovine serum albumin (BSA) (Boehringer Mannheim, Germany).

Adenohypophysial cell dissociation and culture

The pituitary glands were obtained from 12 Holstein steers (age : 17-18 months) at a local slaughterhouse and transported to the laboratory in DMEM medium to which was added 15 mM HEPES, 50 μ g streptomycin/ml, and 100 IU penicillin/ml at 5°C within 2 h. The cell cultures of the anterior pituitary (AP) cells were prepared as previously described by Vale *et al.*²⁶⁾ and modified in our laboratory¹³⁾. In brief, the AP cells were resuspended in DMEM medium containing 10% horse serum, 2.5% newborn calf serum, 1% non-essential amino acids (100 x), 100 IU/ml penicillin, and 50 μ g/ml streptomycin. The cells (7×10^4 cells/ml) were then plated in Costar 24-well dishes and maintained at 37°C in a humidified atmosphere of 5% CO₂. The media were changed 2 days after plating. After 3.5 days culture, the cells were washed twice with DMEM only supplemented with 0.1% BSA, and the media were replaced with the fresh media containing the peptides to be tested ; the cells were then incubated for 2 h except for time-courses of experiments for GH release. At the end of incubation, the media were removed from the cells and stored at -20°C until assayed for GH.

GH responses to a single iv injection of KP 102, GHRH, and KP 102 plus GHRH

The experiments were performed between May and June, using five female Holstein calves (age, 6-9 months ; mean body weight, 134 kg). The calves were housed in pens, and natural light was allowed to enter through windows. The calves were fed grass silage and concentrate at 0930 h and 1600 h daily. Water was available continuously. On the day of the experiment, they were not fed either before or during the experiment ; they were fed only after the experiment. The jugular venous blood of the experimental animals was sampled from the indwelling catheter previously inserted into one of the external jugular veins. The animals were given a single iv injection of KP 102 (20 μ g/kg BW), GHRH (0.25 μ g/kg BW), KP 102 (20 μ g/kg BW) plus GHRH

(0.25 $\mu\text{g/kg}$ BW), and 5 ml saline (as a control). The agents were injected via the indwelling catheter. The animals were repeatedly used for each treatment, and the experiments were carried out at one-week intervals. The blood samples (one ml each) were drawn at -30, -20, -10, 0 (just before injection), 10, 20, 30, 40, 50, 60, 70, 80, 90, 105, 120, 135, 150, 165 and 180 min after injection. The blood samples were collected into centrifuge tubes containing heparin and were immediately chilled with ice. Individual plasma samples were obtained after centrifugation and stored at -20°C until assayed.

Radioimmunoassay (RIA)

Bovine GH concentrations in media and plasma were measured by a validated RIA method as previously described¹⁶. The GH standard preparation and hormone for iodination was bGH-B-1. In these assays, the displacement curve for increasing volumes of the culture medium pool and bovine serum pool was parallel to the standard curve. The least detectable value was 0.1 ng/tube. The intra- and inter-assay coefficients of variation were 7.3% and 13.5%, respectively.

Statistical analysis

In *in vitro* experiments, there were 4 to 6 wells per treatment and the experiments were repeated three to five times with different pituitary glands. All data in the *in vitro* and *in vivo* experiments were presented as mean $\pm$ SE. Statistical significance of the differences in GH concentrations was analysed by one way analyses of variance, and differences between specific groups were determined by subsequent use of Duncan's multiple range test²⁷. Linear regression analyses by SAS were conducted to evaluate changes in media GH release over the concentrations of KP102 and GHRH.

Results

GH-releasing effects of KP102 and GHRH from cultured bovine adenohypophysial cells

As shown in Figs. 1 and 2, KP102 and GHRH

Fig. 1. Effects of KP102 (10^{-14} to 10^{-8} M) on the release of GH from cultured bovine anterior pituitary cells over a 2-h incubation period. There were four wells per treatment and the experiment was repeated three times with three different pituitary glands. Each value represents the mean of twelve wells $\pm$ SE. Significant differences from controls are indicated by asterisks (* $P < 0.05$).

Fig. 2. Effects of GHRH (10^{-14} to 10^{-8} M) on the release of GH from cultured bovine anterior pituitary cells over a 2-h incubation period. Other explanations are shown in Fig. 1.

significantly increased bovine GH concentrations in cultured media after 2 h incubation at doses as low as 10^{-11} M and 10^{-13} M, respectively ($P < 0.05$). Based on linear regression analysis, KP 102 (10^{-8} to 10^{-13} M) and GHRH (10^{-8} to 10^{-14} M) stimulated the release of GH in a dose-dependent manner ($P < 0.05$). Effect of incubation time on KP 102 (10^{-8} M)- and GHRH (10^{-8} M)-stimulated bovine GH release was shown in Fig. 3. Both KP 102 and GHRH significantly ($P < 0.05$) increased GH release for 15, 60, and 120 min incubation. GH concentrations after treatment with GHRH were significantly ($P < 0.05$) greater than those of KP 102 both for 60 and 120 min incubation. The maximum responses of GH release to KP 102 and GHRH were observed after 60 min and 120 min incubation, respectively.

Combined effects of KP102 and GHRH on GH release from cultured bovine adenohypophyseal cells

As shown in Fig. 4, KP102 (10^{-8} M) and GHRH (10^{-8} or 10^{-10} M) and combined treatment with KP102 plus GHRH induced significant increases in the GH concentration in

Fig. 3. Effects of incubation time on KP102- and GHRH-stimulated bovine GH release. There were eight wells per treatment and the experiment was repeated three times with three different pituitary glands. Each value represents the mean of twenty four wells $\pm$ SE. The different letters on the bar in each incubation time denote significant differences ($P < 0.05$).

media compared with the controls ($P < 0.05$). When KP102 and 10^{-8} M GHRH were added together, the release of GH induced by both peptides was significantly greater than those by either KP102 or GHRH alone ($P < 0.05$). The percent increase in GH released with KP102 and 10^{-8} M GHRH together was 102% and 36% greater than those by either KP102 or GHRH alone, respectively. When KP102 and 10^{-10} M GHRH were added together, the release of GH induced by both peptides was significantly greater than those by either KP102 or GHRH alone ($P < 0.05$). The percent increase in GH released with KP102 and 10^{-10} M GHRH together was 102% and 51% greater than those by either KP102 or GHRH alone, respectively.

Effects of SS on KP102- and GHRH-induced GH release from cultured bovine adenohypophyseal cells

Fig. 5 shows the combined effect of KP102 (10^{-8} M), GHRH (10^{-8} M), and SS (10^{-7} M) on GH

Fig. 4. The combined effects of KP102 (10^{-8} M) and GHRH (10^{-8} M or 10^{-10} M) on the release of GH from cultured bovine anterior pituitary cells over a 2-h incubation period. There were four wells per treatment and the experiment was repeated three times with three different pituitary glands. Each value represents the mean of twelve wells $\pm$ SE. - : Absence of a substance(s) in a media. + : Presence of a substance(s) in a media. The different letters on the bar denote significant differences ($P < 0.05$).

Fig. 5. The combined effects of KP102 (10^{-8} M), GHRH (10^{-8} M), and somatostatin (SS, 10^{-7} M) on the release of GH from cultured bovine anterior pituitary cells over a 2-h incubation period. There were six wells per treatment and the experiment was repeated five times with five different pituitary glands. Each value represents the mean of thirty wells $\pm$ SE. Other explanations are shown in Fig. 4.

release. The SS significantly blunted KP102- or GHRH-induced increase in bovine GH when compared with the KP102 alone or with the GHRH alone, respectively ($P < 0.05$). GH concentrations after combined treatments SS with KP102 plus GHRH were significantly suppressed when compared with the KP102 plus GHRH ($P < 0.05$), but they were significantly greater than those of the SS with the KP102 alone or with the GHRH alone ($P < 0.05$). Under the presence of SS, the percent increase in GH released with KP102 plus GHRH was 121% and 60% greater than those by either KP102 or GHRH alone, respectively.

GH responses to a single iv injection of KP102, GHRH, and KP102 plus GHRH

The responses to plasma GH in calves to a single iv injection of KP102, GHRH or KP102 plus GHRH, and saline are shown in Fig. 6. Saline injections did not alter the basal GH concentrations. On the other hand, plasma GH increased immediately following the injection of $20\mu\text{g/kg}$ BW of KP102, $0.25\mu\text{g/kg}$ BW

Fig. 6. Plasma GH responses to the iv injections of KP102 ($20\mu\text{g/kg}$ BW), GHRH ($0.25\mu\text{g/kg}$ BW), KP102 ($20\mu\text{g/kg}$ BW) plus GHRH ($0.25\mu\text{g/kg}$ BW) or saline in calves. An arrow indicates the time of peptide or saline injection. Each value represents the mean $\pm$ SE of 5 animals. * $P < 0.05$ compared with the corresponding values of controls. The different letters denote significant differences ($P < 0.05$).

of GHRH, and 20 $\mu\text{g/kg}$ BW of KP102 plus 0.25 $\mu\text{g/kg}$ BW of GHRH ($P < 0.05$), and reached maximum mean values of 42 ± 11 (mean $\pm$ SE), 78 ± 12 , and 108 ± 4 ng/ml, respectively, from 14–15 ng/ml just before the injection. After the peak levels appeared, GH concentrations began to decrease, and the GH concentrations in KP102, GHRH, and KP102 plus GHRH returned to the same levels as those of the controls at 60, 180, and 150 min, respectively, after each injection. GH concentrations 10 min after the injection of KP102 plus GHRH were 157% and 38% greater than those by either KP102 or GHRH alone, respectively ($P < 0.05$), and GH concentrations 20 min after the injection of KP102 plus GHRH were 236% and 22% greater than those by either KP102 or GHRH alone, respectively ($P < 0.05$). The areas under the GH response curve (AUC) for 60 min after the injection of KP102, GHRH, and KP102 plus GHRH were $1,340 \pm 266$, $3,488 \pm 412$ and $4,037 \pm 163$ ng $\cdot$ min $\cdot$ ml $^{-1}$, respectively. The values of the AUC in GHRH alone and KP102 plus GHRH were significantly ($P < 0.05$) greater than that of KP102 alone; however, there were no significant differences between the KP102 plus GHRH and the GHRH alone groups.

Discussion

The present study demonstrated that KP102 stimulates GH release in cattle. As far as we know, the present study is the first investigation to evaluate the direct action of KP102 on GH secretion from cultured adenohypophysial cells in cattle. The potency of GHRH for GH secretion was greater than that of KP102 in bovine pituitary cells, and the potency of KP102 seemed to be similar to that of GHRP-6 when compared with the results tested in our previous study²³. Wu *et al.*²⁸, however, reported that KP102 was equipotent with GHRH as a GH secretagogue for ovine pituitary cells *in vitro*. One possible explanation for this discrepancy is a species difference. However, this discrepancy might also be

caused by the difference in the experimental procedure. We used a static culture system of bovine anterior pituitary cells and the cells were incubated for 2 h with the peptide. In contrast to our method, they used a perfusion system of cultured ovine anterior pituitary cells, and the peptides were added for only 8 min during the experiment. The higher potency of the GHRH-stimulated GH releasing effect over KP102 was also observed in our time-courses of experiments for GH release.

An additive effect of KP102 and GHRH on GH release was observed when both peptides were added together in the *in vitro* experiment. Our results in cattle are in agreement with the previous reports in sheep²⁸. Cheng *et al.*⁷ reported that GHRP-6 and GHRH produced a synergistic effect on GH release in rat pituitary cell cultures. Bowers *et al.*⁴ demonstrated that a synergistic effect of GHRP-6 and GHRH on GH release *in vitro* is only small at best or nonexistent in rats. The mechanism of the action of KP102 is not clear. KP102, however, has been suggested to act on a novel non-GHRH receptor and use signal transduction systems different from those employed by GHRP-6²⁸.

SS blunted GH release induced by not only GHRH but also KP102 *in vitro*. Signal transductions for GH release in GHRH and GHRPs are considered to be different²⁸. However, SS blunted GH release induced by both peptides. Therefore, the signal transductions for GH release in GHRH and KP102 are considered to be the same in some part in cattle. The additive effect of GH release was also observed under the presence of SS. Similar results were reported when GHRP-6²³, angiotensin II²⁵ or PACAP²⁴ were added together with GHRH and SS.

Intravenous injection of KP102 stimulated GH release in cattle. However, the potency of KP102 for GH secretion was weaker than that of GHRH. The dose of KP102 used in the present study was chosen in consideration of previ-

ous results in cattle²¹⁾ and goats¹⁴⁾. In cattle, the effective dose of iv injection of KP 102 has been reported more than 12.5 µg/kg BW²¹⁾. In the goat, iv injection of KP 102 failed to stimulate GH release at the doses of 5 or 10 µg/kg BW, and the GH release was stimulated by the dose of 20 µg/kg BW. In human, 1 µg/kg BW of sc or iv injection of KP 102 stimulates GH release^{5,20)}, and in rats, 0.5 µg/kg BW of iv injection of KP 102 also stimulates GH release²²⁾. Therefore, the responsiveness to KP 102 for GH release in ruminant animals may be weaker than that in human^{5,20)} and rats²²⁾. GHRPs act on both the pituitary and hypothalamus to release GH^{17,18)}. Therefore, the mechanism by which KP 102 stimulated GH release is complicated, although the KP 102 stimulated GH release by acting directly on bovine anterior pituitary cells in our *in vitro* experiment.

GHRP-6^{3,4)} or GHRP-1⁵⁾ administered together with GHRH synergistically stimulate GH release in rats⁴⁾ and humans^{3,5)}. KP 102 and GHRH administered together also synergistically stimulate GH release in rats²²⁾ and humans²⁰⁾. In the present study, GH concentrations 10 and 20 min after the injection of KP 102 plus GHRH were significantly greater than those of either KP 102 alone or GHRH alone; however, in the AUC, there were no significant differences between the KP 102 plus GHRH and the GHRH alone groups. Synergistic effects for GH release by KP 102 and GHRH were also not clear in the goat in our previous study¹⁴⁾. Therefore, in ruminant animals, typical synergistic effects as shown in rats²²⁾ and humans²⁰⁾ may not appear. This difference might be due to the difference in GH secretory patterns in the species. GH secretory patterns in ruminant animals¹²⁾ are different from those in rats¹²⁾ and human⁶⁾. The effect of GHRPs on GH secretion is apparently attenuated by the endogenous GHRH^{1,15)}. Therefore, these endocrine backgrounds might affect the sensitivity for KP 102 in GH release, and consequently might affect the synergistic effects.

In conclusion, our results demonstrated that KP 102 stimulated GH release in cattle, and suggested that KP 102 interacted with GHRH and SS to release bovine GH.

Acknowledgments

The authors wish to thank Dr. T. Johke, National Institute of Animal Industry, Japan, for providing GH kits, Dr. K. Wakabayashi, the Institute of Endocrinology, Gunma University, Japan, for providing goat anti-monkey IgG serum to bGH and monkey serum, and Dr. S. Ohashi, National Institute of Bioscience and Human Technology, Japan, for providing hGHRH(1-29)-NH₂. The authors also thank the USDA Animal Hormone Program, USA for USDA-bGH-B-1 and Kaken Pharmaceutical Co. Ltd., Japan for providing KP 102. Finally, we would like to thank Dr. Paul Langman, Iwate Medical University, for assistance with English usage.

References

- 1) Bercu BB, Yang S-W, Masuda R, Walker RF. Role of selected endogenous peptides in growth hormone-releasing hexapeptide activity: analysis of growth hormone-releasing hormone, thyroid hormone-releasing hormone, and gonadotropin-releasing hormone. *Endocrinology*, 130 : 2579-2586. 1992.
- 2) Bowers CY, Momany FA, Reynolds GA, Hong A. On the *in vitro* and *in vivo* activity of a new synthetic hexapeptide that acts on the pituitary to specifically release growth hormone. *Endocrinology*, 114 : 1537-1545. 1984.
- 3) Bowers CY, Reynolds GA, Durham D, Barrera CM, Pezzoli SS, Thorner MO. Growth hormone (GH)-releasing peptide stimulates GH release in normal men and acts synergistically with GH-releasing hormone. *J. Clin. Endocrinol. Metab.*, 70 : 975-982. 1990.
- 4) Bowers CY, Sartor AO, Reynolds GA, Badger TM. On the actions of the growth hormone-releasing hexapeptide, GHRP. *Endocrinology*, 128 : 2027-2035. 1991.
- 5) Bowers CY. GH releasing peptides - structure and kinetics. *J. Pediatr. Endocrinol.*, 6 : 21-31. 1993.
- 6) Casanueva FF. Physiology of growth hormone

- secretion and action. *Acromegaly*, 21 : 483-517. 1992.
- 7) Cheng K, Chan WW-S, Barreto A Jr, Convey EM, Smith RG. The synergistic effects of His-D-Trp-Ala-Trp-D-Phe-Lys-NH₂ on growth hormone (GH)-releasing factor-stimulated GH release and intracellular adenosine 3', 5'-monophosphate accumulation in rat primary pituitary cell culture. *Endocrinology*, 124 : 2791-2798. 1989.
 - 8) Croom Jr WJ, Leonard ES, Baker PK, Kraft LA, Ricks CA. The effects of synthetic growth hormone releasing hexapeptide BI 679 on serum growth hormone levels and production in lactating dairy cattle. *J. Dairy Sci. [Suppl. 1]*, 67 : 109 (Abstract). 1984.
 - 9) Enright WJ, Chapin LT, Moseley WM, Zinn SA, Tucker HA. Growth hormone-releasing factor stimulates milk production and sustains growth hormone release in Holstein cows. *J. Dairy Sci.*, 69 : 344-351. 1986.
 - 10) Enright WJ, Chapin LT, Moseley WM, Tucker HA. Effects of infusions of various doses of bovine growth hormone-releasing factor on growth hormone and lactation in Holstein cows. *J. Dairy Sci.*, 71 : 99-108. 1988.
 - 11) Frohman LA, Downs TR, Chomczynski P. Regulation of growth hormone secretion. *Frontiers in Neuroendocrinology*, 13 : 344-405. 1992.
 - 12) Gluckman PD, Breier BH, Davis SR. Physiology of the somatotrophic axis with particular reference to the ruminant. *J. Dairy Sci.*, 70 : 442-466. 1987.
 - 13) Hashizume T, Soliman EB, Kanematsu S. Effects of pituitary adenylate cyclase-activating polypeptide (PACAP), prostaglandin E₂ (PGE₂) and growth hormone releasing factor (GRF) on the release of growth hormone from cultured bovine anterior pituitary cells *in vitro*. *Domest. Anim. Endocrinol.*, 11 : 331-337. 1994.
 - 14) Hashizume T, Sasaki K, Sakai M, Tauchi S, Masuda H. The effect of new growth hormone-releasing peptide (KP 102) on the release of growth hormone (GH) in goats. *Anim. Sci. Technol. (Jpn.)*, 68 : 247-256. 1997.
 - 15) Jansson J-O, Downs TR, Beamer WG, Frohman LA. The dwarf "little" (LIT/LIT) mouse is resistant to growth hormone releasing peptide (GHRP-6) as well as to GH-releasing hormone (GRH). *Endocrinology*, 118 (Suppl.) : 397. 1986.
 - 16) Johke T. Effects of TRH on circulating growth hormone, prolactin and triiodothyronine levels in the bovine. *Endocrinol. Jpn.*, 25 : 19-26. 1978.
 - 17) Korbonits M, Grossman AB. Growth hormone-releasing peptide and its analogues. Novel stimuli to growth hormone release. *TEM.*, 6 : 43-49. 1993.
 - 18) Laron Z. Growth hormone secretagogues. Clinical experience and therapeutic potential. *Drugs*, 50 : 595-601. 1995.
 - 19) Momany FA, Bowers CY, Reynold GA, Hong A, Newlander K. Conformational energy studies and *in vitro* and *in vivo* activity data on growth hormone-releasing peptides. *Endocrinology*, 114 : 1531-1536. 1984.
 - 20) Pihoker C, Middleton R, Reynolds GA, Bowers CY, Badger TM. Diagnostic studies with intravenous and intranasal growth hormone-releasing peptide-2 in children of short stature. *J. Clin. Endocrinol. Metab.*, 80 : 2987-2992. 1995.
 - 21) Roh S-G, Matsunaga N, Hidaka S, Hidari H. Characteristics of growth hormone secretion responsiveness to growth hormone-releasing peptide-2 (GHRP-2 or KP 102) in calves. *Endocrine J.*, 43 : 291-298. 1996.
 - 22) Sawada H. Effect of newly developed analogue of growth hormone releasing peptide [D-Ala-D-β Nal-Ala-Trp-D-Phe-Lys-NH₂ (KP-102)] on growth hormone secretion in adult male rats. *J. Nippon Med. Sch.*, 62 : 142-149. 1995 (in Japanese).
 - 23) Soliman EB, Hashizume T, Kanematsu S. Effect of growth hormone (GH)-releasing peptide (GHRP) on the release of GH from cultured anterior pituitary cells in cattle. *Endocrine J.*, 41 : 585-591. 1994.
 - 24) Soliman EB, Hashizume T, Kanematsu S. The combined effect of PACAP, GHRH, and SRIF on bovine GH release *in vitro*. *Anim. Sci. Technol. (Jpn.)*, 66 : 130-133. 1995.
 - 25) Soliman EB, Hashizume T, Kanematsu S. Effect of angiotensin II on the release of growth hormone from cultured anterior pituitary cells in cattle. *Anim. Sci. Technol. (Jpn.)*, 66 : 823-829. 1995.
 - 26) Vale W, Grant G, Amoss M, Blackwell R, Guillemin R. Culture of enzymatically dispersed anterior pituitary cells: Functional validation of a method. *Endocrinology*, 91 : 562-572. 1972.
 - 27) Wakabayashi K. Life Science Personal Computer Series 5, Arrangement of Experimental Data (PC-8801, 9801). 44-57, 90-133. Baihukan.

- Tokyo. 1984.
- 28) Wu D, Chen C, Katoh K, Zhang J, Clarke IJ. The effect of GH-releasing peptide-2 (GHRP-2 or KP 102) on GH secretion from primary cultured ovine pituitary cells can be abolished by a specific GH-releasing factor (GRF) receptor antagonist. J. Endocrinology, 140 : R9-R13. 1994.
- 29) Wu D, Chen C, Zhang J, Katoh K, Clarke I. Effects *in vitro* of new growth hormone releasing peptide (GHRP-1) on growth hormone secretion from ovine pituitary cells in primary culture. J. Neuroendocrinology, 6 : 185-190. 1994.

新しい成長ホルモン放出ペプチド (KP 102) の *in vitro* および *in vivo* におけるウシ成長ホルモン放出効果

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第二世代目の新しい成長ホルモン (GH) 放出ペプチド, KP 102 (D-Ala-D- β -Nal-Ala-Trp-D-Phe-Lys-NH₂, 別名 GHRP-2) のウシ GH 放出効果を *in vitro* および *in vivo* で検討した. KP 102 と GH 放出ホルモン (GHRH) は培養したウシ下垂体前葉細胞からそれぞれ 10^{-11} M および 10^{-13} M まで GH を放出させた ($P < 0.05$). KP 102 (10^{-8} M) と GHRH (10^{-8} M あるいは 10^{-10} M) を同時添加すると, 両ペプチドによる GH 放出の付加効果が見られた ($P < 0.05$). ソマトスタチン (SS, 10^{-7} M) は KP 102 (10^{-8} M) と GHRH (10^{-8} M) による GH 放出を抑制させた ($P < 0.05$). また KP 102 と GHRH による GH 放出の付加効果は SS 存在下でも認められた ($P < 0.05$). $20 \mu\text{g}/\text{kg BW}$ の KP 102 の静脈内投与は子ウシの末梢血中 GH 濃度を有意に増加させた ($P < 0.05$). KP 102 ($20 \mu\text{g}/\text{kg BW}$) と GHRH ($0.25 \mu\text{g}/\text{kg BW}$) を同時投与すると, 10 分および 20 分後の血中 GH 濃度はそれぞれの単独投与時の値に比べ, 有意に高い値を示した ($P < 0.05$). KP 102 と GHRH 同時投与時の投与 60 分間における GH 放出反応曲線下の面積 (AUC) は KP 102 単独投与時に比べ有意に高い値を示したが ($P < 0.05$), GHRH 単独投与時と比べると有意差は認められなかった. 本研究の結果は KP 102 はウシの GH 放出を刺激すること, また KP 102 は GHRH や SS と相互的に作用して GH を放出させることを示唆している.

日畜会報, 68 (5) : 450-458, 1997