Endocrine Journal 2010, **57** (2), 175-180

NOTE

GH-releasing Peptide-2 does not Stimulate Arginine Vasopressin Secretion in Healthy Men

KYUZI KAMOI, SHINICHI MINAGAWA, KEITA KIMURA, MASAHIRO ISHIZAWA, NOBUMASA OHARA, YASUYUKI UEMURA AND JUNPEI TSUCHIYA

The Center of Diabetes and Endocrine & Metabolism Disease, Nagaoka Red Cross Hospital, Niigata 940-2085, Japan

Abstract. Ghrelin has a stimulating effect on arginine vasopressin (AVP). However, it is not known whether GHRP-2, a synthetic ghrelin receptor agonist, also has a stimulating effect on AVP release in men. To determine whether the GHRP-2 test is useful for assessing AVP secretion, blood ACTH, GH, FSH, LH, PRL, TSH and AVP levels, as well as glucose, osmolality, sodium and hematocrit, were measured before and 15, 30, 45 and 60 min after an intravenous bolus of 100 μg GHRP-2 in 10 healthy men with and without fasting. Blood pressure was measured at 15-min intervals. AVP secretion was not stimulated by the GHRP-2 test with and without fasting. There were no significant differences in hematocrit, blood pressure and plasma osmolality before and after GFRP-2 injection, although significant (p<0.001) peak blood GH, and ACTH and PRL levels were observed 30 and 15 min after GHRP-2 injection with and without fasting, respectively, and the maximal peaks were significantly (p<0.05) higher with fasting than without fasting. These results suggest that AVP secretion is not stimulated by the GHRP-2 test both with and without fasting, though GH, ACTH and PRL levels were higher with than without fasting.

Key words: GHRP-2 test, GH, ACTH, PRL, AVP

GH-RELEASING PEPTIDE-2 (GHRP-2) consists of six amino acids with the following structure: D-alanyl-3-(2-naphthyl)-D-alanyl-L-alanyl-L-tryptophyl-D-phenylalanyl-L-lysinamide dihydrochloride; it was synthesized by Bowers *et al*. [1]. The effect of GHRP-2 is similar to that of ghrelin, and it is one of the exogenous ligands that bind the growth hormone secretagogue (GHS) receptor, while ghrelin is an endogenous ligand that binds the GHS receptor [2].

Recently, a diagnostic test for GHRP-2 to stimulate GH in humans [3] has been developed as a simple, safe, and effective alternative to the insulin tolerance test (ITT). This diagnostic test may also be useful to stimulate ACTH secretion in humans [4] and is currently available clinically in Japan. Ghrelin is secreted from the stomach and circulates in the bloodstream under fasting conditions [5], and the GHSs have a stimulating effect not only on ACTH via CRH in animals [6, 7] and humans [4, 8-10], but also on arginine vasopressin (AVP) in animals [6, 11, 12] and humans [9, 10]. Interestingly, ghrelin plays a stimulatory role in AVP release mediated by neuropeptide Y (NPY) neurons in rat [11, 12]. GHRP-2 is known to increase food intake in humans [13] via many neurotransmitters, such as ghrelin [5] and NPY [14, 15]. Accordingly, GHRP-2's stimulatory effect on pituitary hormones may be influenced by feeding via the regulatory factors, which may affect its usefulness as a diagnostic test.

Furthermore, the ITT has a number of disadvantages, such as the potential for hypoglycemia-related adverse reactions [16]. Moreover, the ITT requires close monitoring of patients for several hours in a specialized investigation unit [16].

Thus, the GHRP-2 test could provide a non-osmotic stimulus for AVP secretion as a simple, safe, and effective alternative to the ITT [17-19], because the

Received Jul. 15, 2009 as K09E-203; Accepted Nov. 2, 2009 as K09E-215
Released online in J-STAGE as advance publication Nov. 12, 2009

Correspondence to: Kyuzi KAMOI, M.D., M.Sc., The Center of Diabetes and Endocrine & Metabolism Disease, Nagaoka Red Cross Hospital, Nagaoka, Niigata 940-2085, Japan.
E-mail: kkam-int@echigo.ne.jp

Disclosure Statement: The authors have nothing to disclose.

ITT for AVP secretion is useful in patients who have lost the capacity to respond selectively to osmotic, hemodynamic, or emetic stimuli [20]. However, it is unclear whether AVP secretion is stimulated by the GHRP-2 test in men.

In this study, we evaluated the usefulness of GHRP-2 test for assessing AVP secretion in healthy men.

Materials and Methods

Ten healthy men, aged 24 to 34 years (mean age, 30 ± 0.9 years), with BMIs ranging from 20.0 to 26.7 (mean, 22.0 ± 0.7) kg/m^2, were studied based on the method of Coiro *et al*. [10]. They had received no drugs. Written informed consent was obtained from all subjects. This study was performed in accordance with the Declaration of Helsinki and with the approval of relevant ethics committee.

On two different occasions in a random order with and without more than 8 h of overnight fasting with intervals of more than 1 week, a single dose (100 μg) of GHRP-2 (KP-102, Kaken Pharmaceuticals, Tokyo, Japan) from a diagnostic test using a GHRP-2 stimulus to GH [3] was administered to each of the subjects as an intravenous bolus injection at 0900 h. The subjects remained supine for more than 30 min before the injection of GHRP-2 and throughout the study period.

Blood samples were collected into tubes from a catheter inserted into an antecubital vein immediately before and 15, 30, 45 and 60 min after the injection of GHRP-2 [3, 4], and then the samples were separated by centrifugation at 4°C for later determination of ACTH, AVP, FSH, GH, LH, PRL and TSH levels based on the study by Kageyama *et al*. [4], as well as glucose and sodium concentrations, osmolality and hematocrit (Hct). Blood pressure was measured at 15-min intervals throughout the study.

Blood samples for ACTH, AVP and Hct were collected in vacutainers containing EDTA, and blood samples for glucose were collected in vacutainers containing NaF. A portion of the freshly separated plasma was taken immediately for determination of glucose by an oxidase method (N-assay Glu-UL, Nittobo Medical Co., Tokyo, Japan), Hct by an electrical impedance method (Automat hematology analyzer XT-2000i, Tokyo, Japan), and osmolality by a freezing point depression method (OSM STATION OM-6050, ARKRAY Inc. Kyoto, Japan). Serum sodium was measured by an ion selective electrode method (JCA-BM 6050, CA-ELA 04, JEOL Co., Tokyo, Japan).

Serum FSH, GH, LH, PRL and TSH levels were measured by immunoenzymetric assay methods (ST AIA-PACKs, Tosho Co., Tokyo, Japan). Remaining plasma was stored at -20°C for 2 days to 1 week before being assayed for AVP by RIA [19, 21] and ACTH by RIA [19], as previously described. Briefly, the intra- and inter-assay coefficients of variation for ST AIA-PACKs were <3.0 % and <5.0 %, respectively, and the intra- and inter-assay coefficients of variation for ACTH-RIA were 8.4% and 10.8 %, respectively. For AVP, the lower limit of sensitivity of the assay was 0.06 fmol/tube. The intra- and inter-assay coefficients of variation were 7.4 % and 7.8 %, respectively. The hormones were measured in duplicate samples.

The results are expressed as means ± SEM. Differences between the means were evaluated statistically by paired or unpaired *t*-tests. To compare every pair of means, Tukey's multiple comparison test was used following one-way ANOVA with repeated measures. The change in each value after GHRP-2 injection was expressed as the percentage of its difference from the baseline value. To determine how the response was affected by changes in two factors (with and without fasting), two-way ANOVA was used. Following two-way ANOVA, Bonferroni's test was used as a post hoc test to compare the variables in the two groups. Analysis was performed using GraphPad Prism version 5.02 software (GraphPad Software, La Jolla, CA, USA). Two-tailed values of $p<0.05$ were defined as statistically significant.

Results

In the 10 healthy men, the means of urinary volume per day, osmolality and AVP concentration were 1544 ± 156 mL/day, 690 ± 56 mOsm/kg and 29.1 ± 4.5 pg/mgCr, respectively. The means of basal and simultaneously measured blood GH, ACTH, PRL,TSH, FSH, LH and AVP levels at each time point after GHRP-2 injection in the subjects with or without fasting are shown in Table 1. Significant peak blood GH, and ACTH and PRL levels were observed 30 min and 15 min after GHRP-2 injection in the two groups with and without fasting, respectively. The peak GH and ACTH levels were significantly higher in the fasting group than in the non-fasting group. The significant ($p<0.05$) maxima in the ACTH and PRL levels in the fasting and non-fasting groups were 4.2 ± 0.9- and 1.9 ± 0.2-fold,

Table 1. Changes in pituitary hormones levels after GHRP-2 injection in 10 healthy men

	Time (min)				
	0	15	30	45	60
GH (μg/L)					
Without fasting	0.1 ± 0.0	23.3 ± 3.0***	40.0 ± 4.9***	27.6 ± 4.3***	25.0 ± 3.4***
With fasting	0.1 ± 0.0	39.1 ± 5.0***	64.7 ± 9.4$^{***, \#\#}$	47.8 ±6.4$^{***, \#}$	40.6 ± 5.5***
ACTH (pmol/L)					
Without fasting	6.3 ± 0.6	11.3 ± 1.5***	8.5 ± 1.2	6.0 ± 0.9	5.9 ± 0.9
With fasting	6.5 ± 1.1	25.0 ± 5.0$^{***, \#\#\#}$	19.5 ± 2.9$^{***, \#\#\#}$	14.1 ± 2.4$^{*, \#}$	12.1 ± 2.0*
PRL (μg/L)					
Without fasting	8.3 ± 1.1	17.3 ± 3.3***	15.3 ± 2.7***	12.7 ± 2.3	10.2 ± 1.6
With fasting	7.6 ± 1.0	26.2 ± 6.3***	23.8 ± 6.0***	19.9 ± 4.9**	16.9 ± 4.6*
TSH (mU/L)					
Without fasting	1.18 ± 0.12	1.21 ± 0.12	1.14 ± 0.11	1.07 ± 0.10	1.07 ± 0.12
With fasting	1.29 ± 0.18	1.32 ± 0.19	1.24 ± 0.21	1.15 ± 0.17	1.11 ± 0.18
FSH (IU/L)					
Without fasting	4.30 ± 0.68	4.34 ± 0.70	4.14 ± 0.67	4.02 ± 0.68	4.06 ± 0.67
With fasting	4.34 ± 0.71	4.40 ± 0.71	4.19 ± 0.69	4.18 ± 0.67	4.14 ± 0.64
LH (IU/L)					
Without fasting	2.21 ± 0.24	2.07 ± 0.19	1.80 ± 0.15	1.65 ± 0.15	1.60 ± 0.12
With fasting	2.81 ± 0.42	2.71 ± 0.46	2.44 ± 0.38	2.23 ± 0.35	2.12 ± 0.27
AVP (pmol/L)					
Without fasting	3.77 ± 0.69	4.16 ± 0.79	3.46 ± 0.69	3.76 ± 0.71	3.64 ± 0.66
With fasting	3.57 ± 0.87	4.01 ± 0.64	3.92 ± 0.69	4.19 ± 0.85	3.76 ± 0.69

The results are expressed as means ± SEM. Differences between the means in each group were evaluated by Tukey's multiple comparison test following one-way ANOVA with repeated measures ($^{***}p<0.001$, $^{**}p<0.01$ and $^{*}p<0.05$ *vs.* basal level). To compare the difference in the two groups with and without fasting, Bonferroni's test was used as a post hoc test after two-way ANOVA ($^{\#\#\#}p<0.001$, $^{\#\#}p<0.01$ and $^{\#}p<0.05$ *vs.* group without fasting).

and 3.2 ± 0.4- and 2.0 ± 0.2-fold, respectively. The values were approximately two-fold higher in the fasting group than in the non-fasting group. Basal plasma AVP levels were slightly lower in the fasting group than in the non-fasting group; however, there were no significant differences in the AVP levels before and after GHRP2 injection between the two groups.

Figure 1 shows the change in AVP secretion after GHRP-2 injection, as well as osmotic- and hemodynamic-related factors. For plasma osmolality, the levels measured simultaneously before and after GHRP-2 injection were significantly lower in the fasting group (basal level, 282 ± 1 mOsm/kg) than in the non-fasting group (basal level, 288 ± 1 mOsm/kg), and they were accompanied by decreased plasma glucose levels (basal level, 4.87 ± 0.10 mmol/L) in the fasting group compared to the non-fasting group (basal level, 6.33 ± 0.39 mmol/L). Serum sodium levels before and after GHRP-2 injection tended to be lower in the fasting group than in the non-fasting group, although there was no significant difference between the two groups. With respect to the basal levels, the resultant increase in plasma AVP was found to be directly proportional to the level of plasma osmolality (data not shown). There were also no significant differences in Hct and mean blood pressure measured simultaneously before and after GHRP-2 injection between the two groups.

There were no serious adverse effects (including nausea) of GHRP-2 injection throughout the study, while transient hot flushes and/or borborygmi were the major adverse reactions within 15 min after GHRP-2 injection, as previously described [3].

Discussion

AVP secretion is primarily regulated by plasma osmolality [18]. In this study, basal plasma AVP levels in healthy subjects who had normal renal concentration ability with AVP secretion as demonstrated by Kondo *et al*. [22] were slightly lower in the fasting than in the non-fasting, while plasma osmolality was more decreased in the fasting than in the non-fasting. Generally, blood osmolality is estimated from serum sodium, serum BUN, and blood glucose by the equa-

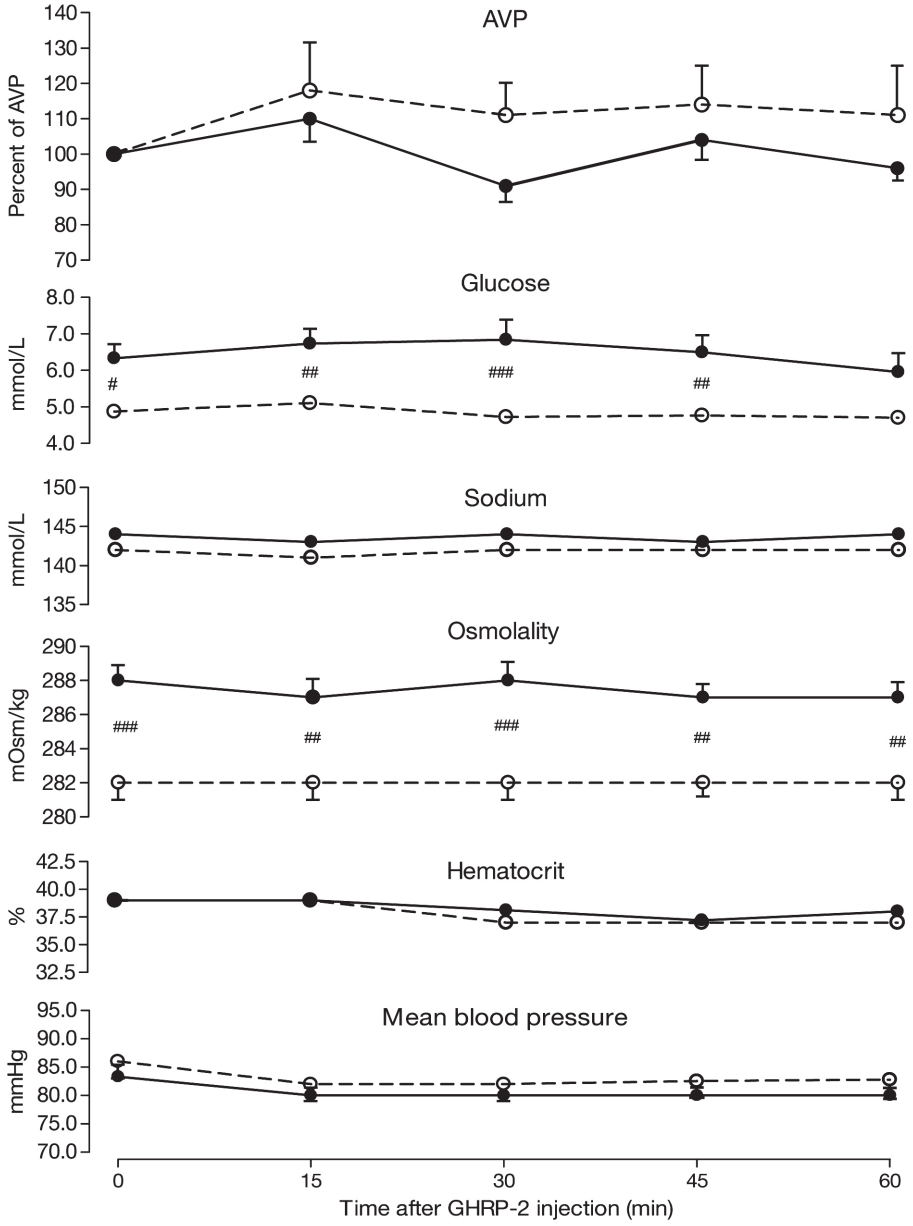

Fig. 1. Percent change from basal level in plasma arginine vasopressin (AVP), blood concentrations of glucose and sodium, plasma osmolality, hematocrit, and mean blood pressure before and 15, 30, 45, and 60 min after an injection of a single dose (100 μg) of GH-releasing peptide-2 (GHRP-2) in two groups with (○- - -○) and without (●—●) overnight fasting (n = 10 healthy men). The data are expressed as means ± SEM. Differences between the means of each group were evaluated by Tukey's multiple comparison tests following one-way ANOVA with repeated measures. There were no significant differences in the variables. To compare the difference in the two groups, Bonferroni's test was used as a post hoc test after two-way ANOVA ($^{\#\#\#}p<0.01$ and $^{\#\#}p<0.01$ *vs.* group without fasting).

tion 2Na (mmol/L) + BUN (mg/dL)/3 + glucose (mg/dL)/18. Although serum sodium levels before and after GHRP–2 injection between the two groups were not statistically significant, the values by 2 x Na (mmol/L) in the fasting (basal level: 283 ± 1 mmol/L) were significantly lower ($p<0.03$) than in the non-fasting (basal level: 287 ± 2 mmol/L), while the differences of plasma glucose values (1.5 ± 0.3 mg/dL) by glucose (mg/dl)/18 between the two groups (basal level: 4.9 ± 0.1 mg/dL *vs*. 6.3 ± 0.4 mg/dL) were significant but small for blood osmolality. Accordingly, the different values of osmolality may be mainly due to the different values

by the sodium owing to fasting and non-fasting [18]. The resultant increase in plasma AVP was found to be directly proportional to the level of plasma osmolality. Basal hemodynamic factors that affect plasma AVP secretion [18] were not significantly changed, because there were no significant differences in Hct and blood pressure between the two groups. Therefore, plasma AVP secretion in healthy men is normally regulated by plasma osmolality.

In such subjects, one must consider why there is no significant difference in AVP secretion before and after GHRP-2 injection between the fasting and non-fasting states. Since GHRP-2 has a role as a ghrelin receptor agonist, the GHRP-2 test could provide a non-osmotic stimulus for AVP secretion. Coiro *et al*. demonstrated that ghrelin stimulated AVP secretion in healthy men with fasting [10]. There were no significant differences in sex, age, and BMI between the two studies. However, the doses of GHRP-2 appear to have been larger than those of ghrelin, although they used ghrelin 1 μg/kg [10]. Furthermore, the time and the maximum increment of the peak blood ACTH level after an injection of GHRP-2 were shorter and higher, respectively, than after an injection of ghrelin [10], showing that an injection of GHRP-2 100 μg may stimulate ACTH secretion more than an injection of ghrelin 1 μg/kg. *In vivo*, ghrelin plays a stimulatory role not only for GH and ACTH, but also for AVP release mediated by NPY neurons [5, 11, 12]. NPY neurons are increased in fasting [14, 15], indicating that fasting may amplify the effect of GHRP-2 injection via NPY neurons action on pituitary hormone secretion, as for ghrelin. In fact, fasting enhanced the increase in GH, ACTH and PRL levels following GHRP-2 injection, although we did not measure plasma NPY levels because of the difficulty in doing so. Nevertheless, there was no significant AVP secretion following GHRP-2 administration both with and without fasting. The reason for this may be that GHRP-2 might have a different effect on AVP secretion independent of osmolality and hemodynamic factors, unlike ghrelin, probably because GHRP-2 is an exogenous ligand that binds the GHS receptor, while ghrelin is an endogenous ligand that binds the GHS receptor [2]. This difference supports the notion that, for ACTH secretion, GHRP-2 acts via CRF [7], while ghrelin acts via AVP [12], and ghrelin stimulates secretion of gastric juice with gastrin, whereas GHRP-2 has no such effects [23].

Moreover, AVP levels increase significantly with the ITT [17-19]. However, there was no significant change in AVP secretion after a GHRP-2 injection. This differs from the diagnostic test by GHRP-2 to stimulate GH [3] and ACTH [4], which is an alternative to the ITT. Although the exact reason for this discrepancy is unclear, the GHRP-2 test cannot be an alternative for the ITT, probably because of different mechanisms of action.

Acknowledgments

We are grateful to the nursing and laboratory staff for their valuable technical assistance and Ogata Yuji (Kaken Pharmaceutical Co., Ltd, Kyoto, Japan) for his valuable comments.

References

1. Bowers CY (1993) GH-releasing peptides structure and kinetics. *J Padiatr Endocrinol.* 6: 21-31.
2. Doi N, Hirotani C, Ukai K, Simada O, Okano T, Kurasaki S, Kiyofuji T, lkegami R, Futamata M, Nakagawa T, Ase K, Chihara K (2004) Pharmacological characteristics of KP-102 [GHRP-2], a potent growth hormone-releasing peptide. *Arznemittelorschung* 54: 857-867.
3. Chihara K, Shimatsu A, Hizuka N, Tanaka T, Seino Y, Kato Y; KP-102 Study Group (2007) A simple diagnostic test using GH-releasing peptide-2 in adult GH deficiency. *Eur J Endocrinol* 157: 29-30.
4. Kageyama K, Nigawara T, Sakihara S, Takayasu S, Terui K, Moriyama T, Suda T (2008) Diagnostic usefulness of the growth hormone-releasing peptide-2 test as a substitute for the insulin tolerance test in hypopituitarism. *Endocr J* 55: 777-783.
5. Kojima M, Kangawa K (2005) Ghrelin: structure and function. *Physiol Rev* 85: 495-522.
6. Mozid AM, Tringali G, Forsling ML, Hendricks MS, Ajodha S, Edwards R, Navarra P, Grossman AB, Korbonits M (2003) Ghrelin is released from rat hypothalamic explants and stimulates corticotrophin-releasing hormone and arginine-vasopressin. *Horm Metab Res* 35: 455-459.
7. Hirotani C, Oki Y, Ukai K, Okuno T, Kurasaki S, Ohyama T, Doi N, Sasaki K, Ase K (2005) ACTH releasing activity of KP-102 (GHRP-2) in rats is medi-

ated mainly by release of CRF. *Naunyn Schmiedebergs Arch Pharmacol* 371: 54-60.
8. Arvat E, di Vito L, Maccagno B, Broglio F, Boghen MF, Deghenghi R, Camanni F, Ghigo E (1997) 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* 18: 885-891.
9. Korbonits M, Kaltsas G, Perry LA, Putignano P, Grossman AB, Besser GM, Trainer PJ (1999) The growth hormone secretagogue hexarelin stimulates the hypothalamo-pituitary-adrenal axis via arginine vasopressin. *J Clin Endocrinol Metab* 84: 2489-2495.
10. Coiro V, Saccani-Jotti G, Minelli R, Melani A, Milli B, Manfredi G, Volpi R, Chiodera P (2005) Adrenocorticotropin/cortisol and arginine-vasopressin secretory patterns in response to ghrelin in normal men. *Neuroendocrinology* 81: 103-106.
11. Korbonits M, Little JA, Forsling ML, Tringali G, Costa A, Navarra P, Trainer PJ, Grossman AB (1999) The effect of growth hormone secretagogues and neuropeptide Y on hypothalamic hormone release from acute rat hypothalamic explants. *J Neuroendocrinol* 11: 521-528.
12. Ishizaki S, Murase T, Sugimura Y, Kakiya S, Yokoi H, Tachikawa K, Arima H, Miura Y, Oiso Y (2002) Role of ghrelin in the regulation of vasopressin release in conscious rats. *Endocrinology* 143: 1589-1593.
13. Laferrère B, Abraham C, Russell CD, Bowers CY (2005) Growth hormone releasing peptide-2 [GHRP-2], like ghrelin, increases food intake in healthy men. *J Clin Endocrinol Metab* 90: 611-614.
14. Sahu A, Kalra PS, Kalra SP (1988) Food deprivation and ingestion induce reciprocal changes in neuropeptide Y concentrations in the paraventricular nucleus. *Peptides* 9: 83-86.
15. Zhou W, Murakami M, Hasegawa S, Yoshizawa F, Sugahara K (2005) Neuropeptide Y content in the hypothalamic paraventricular nucleus responds to fasting and refeeding in broiler chickens. *Comp Biochem Physiol A Mol Integr Physiol* 141: 146-152.
16. Jones SL, Trainer PJ, Perry L, Wass JA, Bessser GM, Grossman A (1994) An audit of the insulin tolerance test in adult subjects in an acute investigation unit over one year. *Clin Endocrinol* 41: 123-128.
17. Baylis PH, Heath DA (1977) Plasma arginine-vasopressin response to insulin-induced hypoglycaemia. *Lancet* 27; 2(8035): 428-430.
18. Baylis PH, Zerbe RL, Robertson GL (1981) Arginine vasopressin response to insulin-induced hypoglycemia in man. *J Clin Endocrinol Metab* 53: 935-940.
19. Watabe T, Tanaka K, Kumagae M, Itoh S, Takeda F, Morio K, Hasegawa M, Horiuchi T, Miyabe S, Shimizu N (1987) Hormonal responses to insulin-induced hypoglycemia in man. *J Clin Endocrinol Metab* 65: 1187-1191.
20. Robertson GL (2001) Neurohypophysial system. In: Becker KL (ed) Principles and Practice of Endocrinology and Metabolism 3rd ed. Lippincott Williams & Wilkins, Philadelphia: 276-305.
21. Kamoi K, Ebe T, Kobayashi O, Ishida M, Sato F, Arai O, Tamura T, Takagi A, Yamada A, Ishibashi M, Yamaji T (1990) Atrial natriuretic peptide in patients with the syndrome of inappropriate antidiuretic hormone secretion and with diabetes insipidus. *J Clin Endocrinol Metab* 70: 1385-1390.
22. Kondo K, Otake K, Iwasaki Y, Oiso Y, Takatsuki K (1989) Radioimmunoassay of vasopressin in unextracted random urine; clinical application for screening of central diabetes insipidus. *Folia Endocrinol* 66: 537-548.
23. Furuta S, Shimada O, Doi N, Ukai K, Nakagawa T, Watanabe J, Imaizumi M (2004) General pharmacology of KP-102 (GHRP-2), a potent growth hormone-releasing peptide. *Arznemittelorschung* 54: 868-880.