

## Characterization of GH Pulsatility in Male Shiba Goats: Effects of Postpubertal Castration and KP102

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**Abstract.** The present study was conducted in order to characterize the secretory pattern of GH in the Shiba goat, a native Japanese miniature goat, and to examine the effects of castration and KP102, a GH secretagogue, on this pattern. Blood samples were taken from an indwelling jugular catheter every 15 min for 24 h, and plasma GH was measured by radioimmunoassay. In intact males, GH was secreted in a pulsatile manner with very regular 5-h periodicity, which consisted of a distinctive GH pulse and a trough of virtually no GH secretion. Postpubertal castration increased the height and decreased the width of GH pulses, though it did not affect the interpulse interval and area under the curve. Modification of the shape of each GH pulse by testicular androgen might play a role in the expression of GH action in the male. KP102 (10 µg/kg, i.v.) immediately induced a robust GH pulse, which was followed by a spontaneous GH pulse of normal characteristics at regular intervals, suggesting that the clock generating GH pulses was reset by KP102. From these observations, we concluded that the Shiba goat is a very suitable experimental model for elucidating the mechanisms underlying GH pulse generation, and in particular, the involvement of androgen and GH secretagogues.

**Key words:** GH, Pulsatility, Castration, KP102

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GH is secreted in a pulsatile manner from the pituitary gland, and the pulsatility of GH secretion plays an important role in the regulation of metabolism [1, 2] as well as body growth [3]. Hence, an understanding of the nature of GH pulsatility is of physiological and pathophysiological importance. It is generally believed that GH pulsatility is generated by the interrelationship between two hypothalamic neuropeptides, GH-releasing hormone (GHRH) and somatostatin (SRIF). Studies on rats have suggested that GHRH induces GH pulses, whereas low interpulse GH levels are maintained by SRIF [4–6].

However, GH secretion is also regulated by many other factors including neuropeptides, neurotransmitters, and metabolic substrates [7], and the extent to which these factors contribute to the generation of GH pulsatility is still poorly understood.

GH pulsatility is known to be sexually dimorphic, particularly in the rat [8, 9]. Complete masculinization of GH pulsatility was accomplished by neonatal gonadectomy followed by testosterone therapy in females [9], while neonatal or prepubertal gonadectomy in males changed GH pulsatility to the female pattern [10, 11]. These observations suggest that sexual dimorphism of GH pulsatility depends primarily on androgen during the perinatal period, though it has been suggested that modification of GH pulsatility by gonadal steroids during the postpubertal period occurs as well [12].

GH secretagogues (GHSs) are synthetic non-natu-

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ral peptides or nonpeptides that have potent stimulatory effects on GH secretion [13, 14]. A specific GHS receptor, which has no structural homology with GHRH receptors and is present in both the hypothalamus and pituitary, has been cloned [15]. The natural ligand for the GHS receptor was recently identified as ghrelin, which is found in the stomach [16]. The question of how ghrelin is involved in the GH pulse-generating system has currently attracted attention.

Most studies on the neuroregulation of GH secretion have been performed on male rats, in which GH pulsatility is characterized by a regular ultradian rhythm of large bursts [17]. Mid-sized experimental animals having a similarly distinct GH pulsatility, however, might be more useful for a further understanding of the GH pulse-generating system, because manipulations of the brain under physiological conditions are much easier to perform on such animals than on rats. In the present study, therefore, we examined the characteristics of GH pulses in the male Shiba goat, a Japanese native goat that has been bred as a closed colony for experimental uses [18] and whose brain atlas is available [19], as a new model for the study of mechanisms underlying GH pulse generation. The effects of postpubertal castration and KP102, a kind of GHS, on GH pulsatility were also studied.

## Materials and Methods

### *Animals and blood sampling*

Male Shiba goats maintained for experimental purposes as a closed colony in the experimental farm of the University of Tokyo [18] were used in this study. All animals used were loosely restrained by being tied to a stanchion indoors, where they were allowed free access to food and water. The temperature was kept at 23  $\pm$  2°C and lighting was controlled according to a 12L:12D cycle (lights on at 0900 h). The animals were frequently handled and made to become accustomed to the blood sampling procedures before the experiments were begun. One day before the experiment, a catheter (Argyle Medical Catheter, 18G and 70 cm in length) was inserted into the jugular vein. Blood samples (1 ml each) were drawn via the catheter and placed into tubes containing heparin.

Plasma was separated by centrifugation and stored at  $-20^{\circ}\text{C}$  until the GH assay.

### *Experiment 1*

To obtain normal secretory profiles of the GH of male Shiba goats and to determine whether castration influenced these profiles, five intact and six castrated male Shiba goats approximately 1 year old were used. Castration had been performed at 10 months of age, when puberty had already occurred. Serial blood samples were obtained via the jugular catheter at 15 min intervals for 24 h starting at 1000 h. All the sampling was performed under continuous light conditions.

### *Experiment 2*

To determine the effect of an intravenous injection of KP102 on GH secretory profiles, five castrated male Shiba goats around 2 years old were used. After serial blood samples were collected at 15 min intervals for 6 h starting at 1000 h, KP-102 (10  $\mu\text{g/kg}$ ), which was generously supplied by Kaken Pharmaceutical Co. Ltd., was injected into the jugular vein as a bolus. Blood sampling was continued for an additional 12 h. All the sampling was performed under continuous light conditions.

### *GH assay*

Plasma GH concentrations were measured by double-antibody radioimmunoassay using monkey anti-bovine GH antiserum as previously described [20]. The parallelism between goat plasma and bovine GH has been reported previously [21]. The minimum concentration of GH that could be detected was 0.8 ng/ml, and the maximum assay range was 500 ng/ml. Intra- and interassay coefficients of variation (CV) were 6% and 8%, respectively.

### *Pulse analysis*

Secretory profiles of GH in each animal were plotted against the time after the start of blood sampling. Each potential pulse was first identified by visual inspection, and then the beginning and end of the pulse were defined when the percent change in GH concentration from the previous and subsequent

points, first and last, respectively, was more than two times that of the intraassay CV. For the characterization of GH pulsatility, the interpulse interval, pulse height and pulse width were estimated as shown in Fig. 1. The area under the curve of the pulse was also determined as the sum of all values within the pulse width.

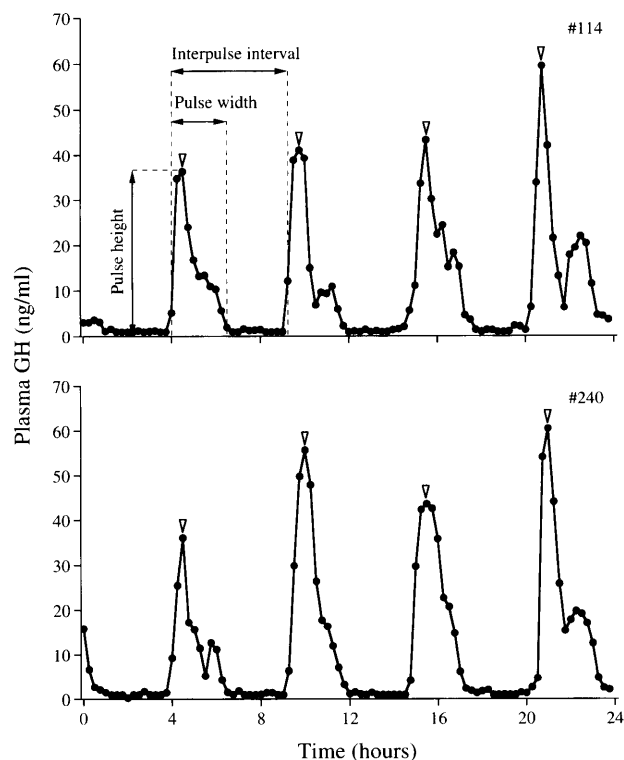
#### Statistical analysis

All the data are presented as the mean  $\pm$  SE. The differences in the characteristics of GH pulsatility in intact and castrated males in experiment 1 were analyzed by ANOVA followed by an unpaired t-test. In experiment 2, the differences were analyzed by ANOVA followed by Fisher's PLSD test. Results were considered significant at  $P < 0.05$ .

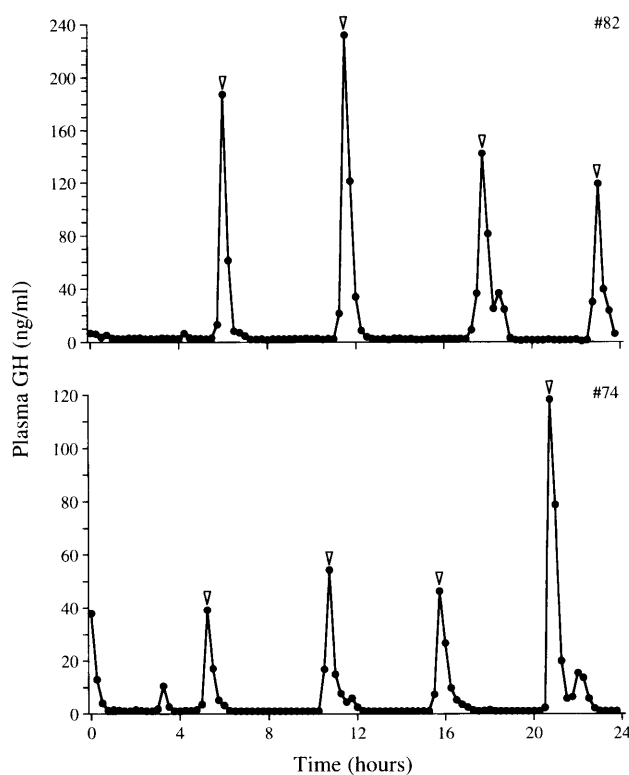
## Results

### *Exp. 1: Plasma GH profiles in intact and castrated male Shiba goats*

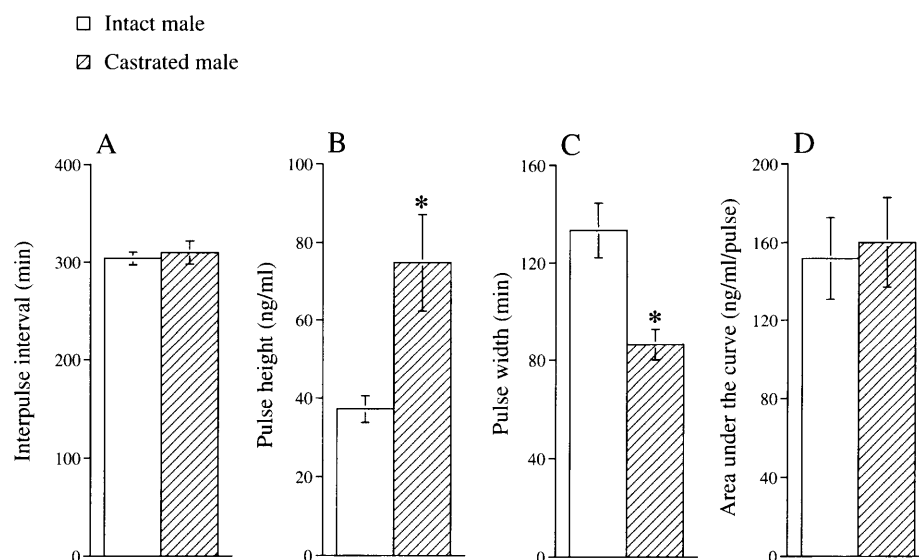
GH secretory profiles in intact and castrated male Shiba goats throughout a 24 h period are shown in Figs. 1 and 2, respectively. In both intact and castrated males, GH was secreted in a pulsatile manner with great regularity, and the pattern of this secretion involved a recurrence of a distinctive pulse followed by a trough of virtually no GH secretion. No circadian variation in the periodicity was observed. Although some pulses had a shoulder in the descending phase or consisted of two peaks, especially in intact males, they were regarded as one pulse in the present study. The characteristics of the pulsatile pattern of GH secretion are summarized in Fig. 3. GH pulses occurred with a constant interpulse interval of around 5 h in both intact and castrated males (Fig. 3A). Although the interpulse interval was not different, the shape of each pulse was different for intact



**Fig. 1** Plasma GH profiles in two individual intact male Shiba goats. Open triangles indicate GH pulses. The criteria for interpulse interval, pulse height and width are also shown.



**Fig. 2.** Plasma GH profiles in two individual castrated male Shiba goats. Open triangles indicate GH pulses.

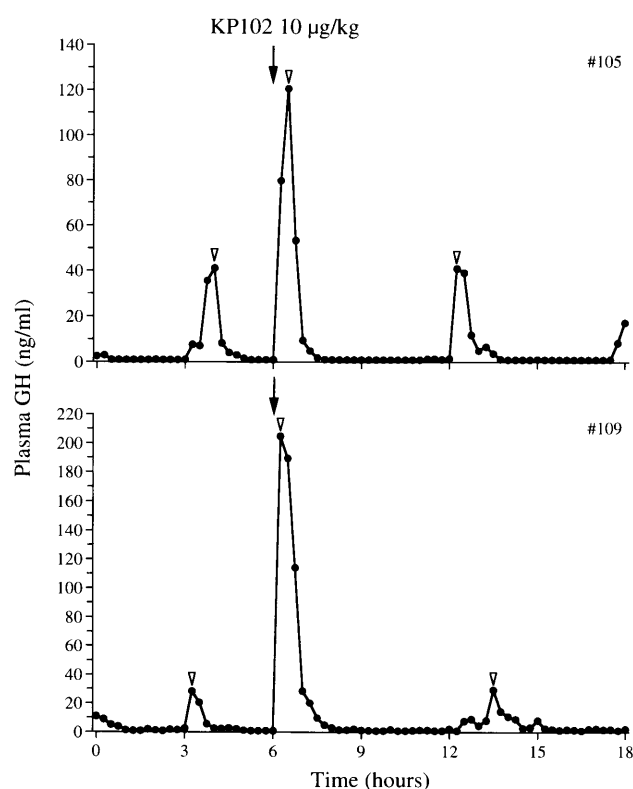


**Fig. 3.** Comparison of interpulse interval (A), pulse height (B), pulse width (C) and area under the curve (D) of GH pulses between intact ( $n=5$ ) and castrated ( $n=6$ ) male Shiba goats. Values are mean  $\pm$  SE. \*,  $P<0.05$  vs. Intact male.

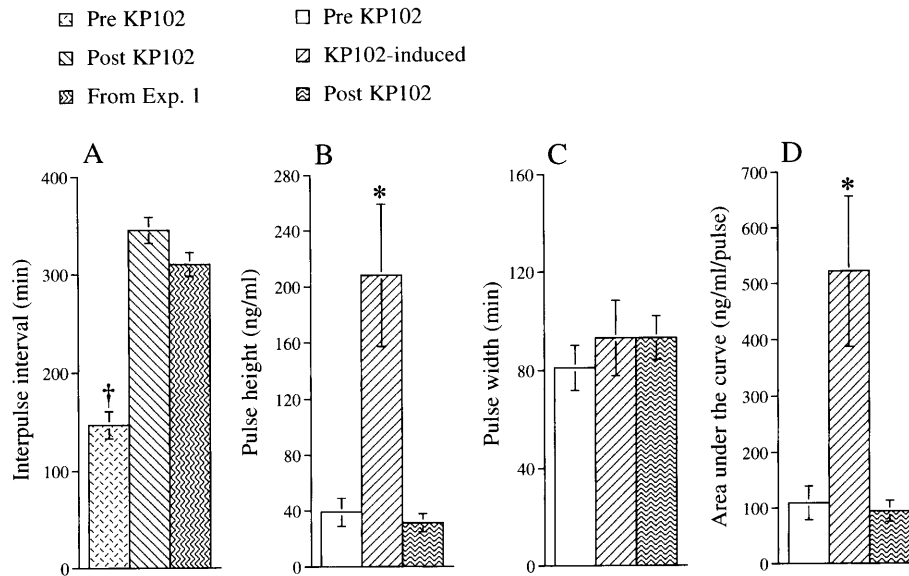
and castrated goats, i.e., the pulse height was higher and the pulse width was smaller in castrated than in intact goats (Fig. 3B and C). Interestingly, however, the area under the curve of the pulses was not different in the two (Fig. 3D).

*Exp. 2: Effect of intravenous injection of KP102 on plasma GH profiles*

Treatment with KP102 immediately induced a robust GH pulse in castrated goats (Fig. 4). KP102 was injected during the trough period in all the goats studied, and the range of the interval between the previous spontaneous pulse and the KP102-induced pulse was 120–180 min with a mean of 147 min. As shown in Fig. 5A, the interval between the KP102-induced and the next spontaneous pulse was 345 min, which was not different from the normal interpulse interval observed in Exp. 1. The pulse height and area under the curve of GH pulses induced by KP102 were approximately 5 times greater than those of spontaneous GH pulses, but the pulse width was not different for KP102-induced and spontaneous pulses (Fig. 5B, C and D). In addition, the characteristics of the spontaneous GH pulse next to the KP102-induced one were not different from those of the spontaneous pulse prior to the KP102 injection.



**Fig. 4.** Effects of intravenous injection of KP102 on plasma GH profiles in two individual castrated male Shiba goats. Open triangles indicate GH pulses.



**Fig. 5.** Effects of intravenous injection of KP102 on interpulse interval (A), pulse height (B), pulse width (C) and area under the curve (D) of GH pulses in castrated male Shiba goats ( $n=5$ ). Values are mean  $\pm$  SE. \*,  $P < 0.05$  vs. other groups.

### Discussion

In the present study, we found that GH secretion in the intact male Shiba goat was distinctly pulsatile, consisting of pulses of regular 5-h periodicity with trough periods in between. No circadian variation in the periodicity was discernible, at least under continuous light conditions. In sheep, it has been reported that GH is also secreted in an episodic fashion but with rather irregular interpulse intervals ranging from 3–6 h [22], indicating that there are species differences in the GH pulsatility of the goat and sheep. In the present study, each GH pulse was found to have a shoulder or additional small peak in the descending phase, suggesting that each pulse consisted of two components. Variations in periodicity (in other words, those other than 5 h) might be involved in the GH pulse-generating system in the Shiba goat. Similar features of pulsatility in GH secretion have also been reported in the male rat, in which a typical GH secretory profile shows large GH bursts every 3–4 h that are subdivided into two peaks [17].

The comparison of GH pulsatility between intact and castrated males revealed that postpubertal gonadectomy affected the shape of each GH pulse, i.e., it increased the height and decreased the width of the pulse. This suggests that testicular androgen may

affect the secretory pattern of GHRH and SRIF from the hypothalamus. There is a possibility that castration might lead to an increase in SRIF tone with a resulting elimination of the second component of each GH pulse, since the shoulder or second peak of the pulse was seldom observed in castrated males. The effects of androgen on the shape of each GH pulse might induce changes in the responsiveness of the pituitary to GHRH and SRIF as suggested before [23]. An altered pulse shape also suggests androgen modification of the metabolic clearance rate of GH, but it has previously been shown that testosterone does not influence the metabolic clearance rate of GH in the rat [24]. Further studies are needed to clarify the mechanisms underlying the effects of androgen on GH pulse shape.

Despite the changes in the pulse shape, the area under the curve of the GH pulses was not affected by castration. In addition, the interval of GH pulses was not changed by castration, suggesting that androgen does not affect the clock mechanisms generating GH pulses. Although the quantity and frequency of GH pulses are known to be of great importance in the expression of GH bioactivity [3], the physiological significance of the pulse shape to GH bioactivity has not been proven. Modification of GH pulse shape might be involved in changes in body growth and metabolism, as well as the lack of ana-

bolic action of testicular androgen after castration.

Intravenous injection of KP102 induced a robust GH pulse in castrated male Shiba goats. Both the height and area under the curve of KP102-induced pulses were around 5 times greater than those of the spontaneous GH pulses, though pulse width did not differ between the two. This indicates that only a part of the releasable pool of GH in the somatotroph is released at each episode. GHS receptors have been discovered both in the pituitary and hypothalamus [15], and GHS actions on GH secretion are believed to involve both direct action on the pituitary somatotroph and indirect action through GHRH [25] and SRIF neurons [26]. Consistently, both intravenous injection [27] and intrahypothalamic infusion of KP102 [28] have been shown to be effective in inducing GH secretion in domestic animals in studies using calves and Saanen goats, respectively. We have also previously shown that third ventricular injection of ghrelin increased GH secretion in Shiba goats [29]. These results, together with those of the present study, provide evidence that KP102/ghrelin exerts both central and peripheral actions involved in inducing GH secretion.

The next spontaneous GH pulse after the KP102-induced one occurred with an interval equal to the normal interpulse interval mentioned above. The pulse characteristics, i.e., height, width and area under the curve, were also the same as those of the spontaneous GH pulse prior to the KP102-induced one. It seems that the endogenous rhythm of GH pulsatility was reset by the treatment. In the rat, although endogenous GH pulsatility can be entrained by serial 3 h GH injections [30], a single injection of

GH is ineffective in modifying GH pulsatility [30, 31]. There might be a species difference in the GH pulse-generating systems of rats and goats. Alternately, since the activation of either  $\gamma$ -aminobutyric acid [31] or opioid receptors [32] can reset the GH rhythm, the possibility that KP102 directly stimulates hypothalamic neurons containing such receptors cannot be ruled out. Ghrelin, the endogenous ligand for GHS receptors [16], may play an important role in the clock mechanism generating GH pulsatility.

In summary, GH pulsatility in male Shiba goats was found to be characterized by strictly regular 5-h periodicity. Postpubertal castration increased the height and decreased the width of each GH pulse but did not affect the interpulse interval nor the area under the curve. Intravenous injection of KP102 caused a robust GH pulse and reset GH pulsatility in castrated animals. Based on the results of the present study, we propose that the Shiba goat is a very suitable experimental model for the study of mechanisms generating GH pulses, particularly in terms of sex steroids and GHSs actions on pulse-generating systems.

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