Effects of Intraarterial and Intrahypothalamic Injections of Pituitary Adenylate Cyclase-Activating Polypeptide on the Release of Growth Hormone in Goats

Tsutomu HASHIZUME, Kazuo KOIZUMI, Koichi SASAKI and Hiroshi MASUDA
Faculty of Agriculture, Iwate University, Morioka-shi 020-8550

(Received December 25, 1997)

Abstract The effects of intraarterial and intrahypothalamic injections of pituitary adenylate cyclase-activating polypeptide (PACAP) on the release of growth hormone (GH) were studied in female goats. The intraarterial injection of 0.5 μg/kg body weight (BW) of PACAP failed to stimulate a significant GH release, whereas the dosages of 5 and 15 μg/kg BW of PACAP significantly stimulated the GH release. The concentrations of GH 10 min after the intraarterial injections of 5 and 15 μg/kg BW of PACAP were significantly higher than the pre-PACAP GH concentrations (P<0.05). The intrahypothalamic injection of 0.5 and 5 μg of PACAP significantly stimulated the GH release. The GH levels began to rise after the injections, and the concentrations of GH at 110 and 140 min after the 0.5 μg PACAP injection and 80, 110, 120 and 130 min after the 5 μg PACAP injection were significantly higher than the pre-PACAP concentrations (P<0.05). The area under the GH response curve for 2.5 h after the intrahypothalamic injections of 5 μg of PACAP was significantly greater than that of the control values (P<0.05). These results suggest that PACAP stimulates the GH release in goats by acting not only directly on the adenohypophysis but also indirectly through the hypothalamus.


Key words: PACAP, GH, Hypothalamus, Goat

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide which stimulates adenylate cyclase activity in rat pituitary cells. PACAP stimulates not only growth hormone (GH) release but also luteinizing hormone, adrenocorticotropic hormone and prolactin release from rat pituitary cells in vitro. The effect of PACAP on the release of GH in vivo, in contrast, is still unclear. Serum GH levels increased more than 20-fold in response to an intravenous PACAP infusion in rats, whereas in humans, the serum GH levels did not change during a PACAP infusion. In ewes, the infusion of PACAP into the cervical artery had no effect on GH release but an intracerebroventricular injection of PACAP suppressed GH release. PACAP is widely distributed within neurons of the hypothalamus and anterior pituitary gland, and specific PACAP binding sites are located centrally in regions including the hypothalamus and anterior pituitary gland. We therefore suspected that PACAP can modify GH release in vivo by acting on both the hypothalamus and the adenohypophysis. We have shown that PACAP stimulates the GH release from bovine pituitary cells in vitro. In the present study, considering the controversial in vivo results, we
HASHIZUME, KOIZUMI, SASAKI and MASUDA

investigated whether PACAP stimulates the release of GH in ruminants in vivo. First, we tested whether PACAP has a direct effect on GH release in intact goats by injecting the peptide into the cervical artery. Secondly, we investigated the possible central role of PACAP on GH secretion by injecting it into the hypothalamus in intact goats.

Materials and Methods

Animals
Four female Saanen goats in the luteal phase (age, 2–3 years; mean body weight, 46 kg) were used. Each goat was loosely tied to a stanchion in an animal pen, and natural light entered through the windows. The goats were fed hay and concentrate from 0830 to 0930 h and from 1630 to 1730 h daily. Water was available continuously. On the experimental day, they were not fed before or during the experiment, but were fed after the experiment.

Preparation of pituitary adenylate cyclase-activating polypeptide
Pituitary adenylate cyclase-activating polypeptide with 27 amino acid residues (PACAP) was used in the present study. The peptide was generously supplied by Dr. S. Ohashi, National Institute of Bioscience and Human Technology, Tsukuba, Japan. The PACAP was dissolved in sterilized 0.9% NaCl (saline) before use.

Intraarterial injection experiment
An indwelling catheter for the infusion of PACAP was surgically inserted into one of the cervical arteries in three goats at least 1 week before the experiment. The goats were given a single intraarterial injection of 0.5, 5 or 15 μg/kg body weight (BW) of PACAP dissolved in 2 ml saline. Two ml saline vehicle alone was injected as a control. The injection was given 30 min after the onset of blood collection each day. The experiment was performed at 4- or 5-day intervals in each goat. The order in which each goat received the various dosages was determined at random.

Intrahypothalamic injection experiment
Unilateral guide tubes were stereotactically implanted into the hypothalamus in four goats at least 2 weeks before the first experiment. Anesthesia was initiated by intravenous xylazine hydrochloride (35 mg/head) and maintained by inhalation of halothane through an endotracheal tube. The procedures of surgical implantation of the guide tubes were described in detail. Three of these four goats were used in the intraarterial injection experiment. On the day of the experiment, an infusion cannula was inserted into the implanted guide tube, and 0.5 or 5 μg of PACAP dissolved in 25 μl saline, or 25 μl saline vehicle alone (as a control) was infused over a period of 1 min. An infusion volume of 25 μl was chosen in consideration of previous report. A dosage of 5 μg was chosen because the GH releasing activity of this dosage had already been confirmed in our preliminary study in the goat. The infusion was given 30 min after the onset of blood collection each day. The experiment was performed at the same site in the hypothalamus at 4- or 5-day intervals in each goat. The order in which each goat received the various dosages was determined at random. At the termination of experiments, each goat was anesthetized with intravenous pentobarbital sodium, and the placement of the probe in the brain was histologically confirmed.

Blood sampling method
The jugular venous blood of the experimental animals was taken from the indwelling catheter previously inserted into one of the external jugular veins. Blood samples (one ml each) were collected into centrifuge tubes containing heparin and immediately chilled with ice. Blood samples were drawn at 10-min intervals for 180 min. Individual plasma samples were obtained after centrifugation and stored at −20°C until assayed.

Radioimmunoassay (RIA)
The plasma GH concentrations were
measured by a double-antibody RIA\(^\text{[13]}\). The GH standard preparation and hormone for iodination were USDA-bGH-B-1. Antiserum to bGH prepared in monkeys was supplied by Dr. T. Johke (National Institute of Animal Industry, Tsukuba, Japan) and goat anti-monkey IgG serum (2nd antibody) was supplied by Dr. K. Wakabayashi (Institute of Endocrinology, Gunma University, Maebashi, Japan). The parallelism between goat plasma GH and bGH was described previously\(^\text{[6]}\). All samples were assayed in a single assay. The intra assay coefficient of variation was 7.4\%. The least detectable value was 1 ng/tube.

**Statistical analysis**

All data are presented as the mean±SE. The area under the GH response curve (AUC) for 2.5 h (150 min) after the injection of PACAP was calculated. The significance of differences between the values pre-injection and post-injection of PACAP and the AUCs in each group was determined by Student’s \(t\)-test or the Cochran–Cox test after analysis of the uniformity of the variances by Bartlett’s test\(^\text{[26]}\). Results were considered significant at the P<0.05 level.

**Results**

A single intra arterial injection of PACAP increased the respiration rate and heart rate two- or threefold after the injection, and these responses continued for about 20 min. Skin flushing was also observed after the intra arterial injection. These responses seemed to occur in a dose-dependent manner, and the responses were not observed when 0.5\(\mu\)g/kg BW of PACAP was injected. In contrast to the results of intra arterial injection, the intrahypothalamic injection of PACAP had no effect on the respiration rate, heart rate and skin flushing.

The responses of plasma GH in goats to a single intra arterial injection of 0.5, 5, or 15\(\mu\)g/kg BW of PACAP and that of saline are shown in Fig. 1. The mean plasma GH concentra-

![Fig. 1. Plasma growth hormone (GH) responses to the intra arterial injections of 0.5, 5, or 15\(\mu\)g/kg body weight (BW) of pituitary adenylate cyclase-activating polypeptide (PACAP), and saline in goats. An arrow indicates the time of PACAP or saline injection. Each value represents the mean ± SE for three animals. Significant differences from the pre-PACAP concentration of GH are denoted with * P<0.05.](image-url)
Plasma growth hormone (GH) responses to the intrahypothalamic injections of 0.5 or 5 μg of pituitary adenylate cyclase-activating polypeptide (PACAP), and saline in goats. An arrow indicates the time of PACAP or saline injection. Each value represents the mean ± SE for four animals. Significant differences from the pre-PACAP concentration of GH are denoted with * P<0.05.

of 3.2 to 6.6 ng/ml, and no significant changes in the plasma GH levels were observed throughout the experiment. The intrahypothalamic injections of 0.5 μg and 5 μg of PACAP significantly stimulated the GH release. The GH responses to PACAP in a goat (goat #4) occurred promptly when compared with the other 3 goats (not shown). The average GH levels in goats began to rise after the injections, and the concentrations of GH at 110 and 140 min after the 0.5 μg PACAP injection and 80, 110, 120 and 130 min after the 5 μg PACAP injection were significantly higher than the pre-PACAP concentrations (P<0.05). The AUCs for 2.5 h after the intrahypothalamic injections of saline, 0.5 and 5 μg of PACAP were 558±188, 1,453±393 and 1,741±383 ng min m⁻¹, respectively, and the AUC after the injection of 5 μg of PACAP was significantly greater than that of saline (P<0.05) (Fig. 3).

Each site of infusion of PACAP in the hypothalamus was examined histologically in serial sections of the brain (Fig. 4). The areas into which we infused the test agent in 3 (#1, #2, #3) of 4 goats were 1 to 2 mm lateral to the midline of the third ventricle, and 1 to 2 mm above the optic chiasm. These sites were in the preoptic
area. The area into which we infused the test agent in a goat (#4) was 0.5mm lateral to the midline of the third ventricle in the region of the mediobasal hypothalamus.

**Discussion**

The results described above showed that PACAP stimulated the release of GH in goats in vivo. To our knowledge, the present study is the first demonstration of the effect of PACAP in stimulating GH release in goats.

The stimulatory effect of PACAP on the release of GH following an intraarterial injection was consistent with the report of Jarry et al.\(^\text{12}\), who showed that serum GH levels increased more than 20-fold in response to an intravenous PACAP infusion in rats. However, Chiodyera et al.\(^\text{1}\) and Sawangjaroen and Curlewis\(^\text{21}\) found no stimulatory effect of PACAP on the release of GH after the intravenous or intraarterial PACAP infusion in humans and ewes. The reason for the discrepancy between their results and ours could be due to the differences in the dosages of PACAP used in each study. The maximum dosages infused in their experiments were 8pmol/kg/min for 20min\(^\text{1}\) and 1.0nmol/min for 10min\(^\text{21}\). Those dosages might have been too small to stimulate GH release in vivo. PACAP injected into the cervical artery is thought to act mainly at the anterior pituitary level. Our results obtained with intraarterial injections therefore suggest that PACAP is one of the hypophysiotropic factors in hormone secretions of the goat.

Sawangjaroen and Curlewis\(^\text{21}\) reported that the intracerebroventricular (ICV) injection of PACAP suppressed GH secretion in ewes. In our present microinjection study, the intrahypothalamic injection of PACAP stimulated the GH release. The target site of the brain in the ICV injection method is not known, even if the administered substances modify pituitary hormones by acting indirectly on the pituitary gland. The reason for the discrepancy between their results and ours might be due to the differences in the brain sites affected by PACAP.

The GH-releasing responses after intrahypothalamic injection of PACAP were delayed when compared with intraarterial injections of the peptide. Intraarterial PACAP was thought to be able to easily reach the adenohypophysis by the bloodstream, and promptly stimulate somatotrophs to release GH. In contrast, injected PACAP into the hypothalamus was thought to be necessary to stimulate growth hormone-releasing hormone (GHRH) secretion, reduce somatostatin (SS) or both to release GH. Furthermore, there is a need of intrahypothalamic PACAP to have access to some neurons to increase GHRH and reduce SS or both. The injected sites of PACAP in the present study might not be adjacent to these neurons, and GH–releasing responses after intrahypothalamic injection of the peptide might be delayed. There is also another possibility that intrahypothalamic PACAP directly stimulated the somatotroph to release GH after the traversing of the hypothalamic tissues.

The single intraarterial injection of PACAP in the present study produced physical changes such as skin flushing, increased respiration rate and heart rate. Similar effects were reported when PACAP was infused into the cervical artery in sheep\(^\text{20}\). These effects did not seem to be a central action, since these changes did not occur when PACAP was injected into the hypothalamus. Specific PACAP binding sites are located not only in the hypothalamus and pituitary gland\(^\text{4,16,20}\) but also in various tissues including the lung and blood vessels\(^\text{4,10}\). Therefore, PACAP appears to have functions in various tissues, including a role in pituitary hormone secretions.

In summary, the present results suggest that PACAP stimulates the GH release in goats by acting not only directly on the adenohypophysis but also indirectly through the
HASHIZUME, KOIZUMI, SASAKI and MASUDA

hypothalamus. However, the detailed mechanism by which PACAP stimulated GH release through the hypothalamus remains unclear.

Acknowledgments

The authors wish to thank Dr. T. Johke, National Institute of Animal Industry, Japan, for providing monkey anti-bGH serum, 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 PACAP. The authors also thank the USDA Animal Hormone Program, Beltsville, MD, USA, for providing USDA-bGH-B-1.

References


16) Masuo Y, Ohtaki T, Masuda Y, Nagai Y, Suno
GH Release by PACAP in Goats


Pituitary adenylate cyclase-activating polypeptide (PACAP) の
顎動脈および視床下部内投与がヤギの成長ホルモン (GH)
放出に及ぼす影響

橋爪 力・小泉和生・佐々木浩一・植田博司
岩手大学農学部, 盛岡市 020-8550

PACAP を雄ヤギの顎動脈および視床下部内に投与して in vivo での PACAP の GH 放出効果を検討した。PACAP を体重 1kg 当たり 0.5μg 顎動脈内に投与しても、GH の放出効果は見られなかったが、5 および 15μg 投与すると投与 10 分後にそれぞれ GH の有意な放出が見られた（P<0.05）。PACAP を
視床下部内に投与した場合は、1 頭当たり 0.5μg の投与では投与 110 分および 140 分後に投与前の値に
比べ有意に高い値を示した（P<0.05）。また 5μg の投与では投与 80 分、110 分、120 分および 130 分後
に投与前の値に比べ有意に高い値を示した（P<0.05）。視床下部内に PACAP を 5μg 投与した時の 150
分間における GH 放出反応曲線下の面積 (AUC) は対照区に比べ有意に高かった (P<0.05)。本研究の
結果は PACAP は in vivo でもヤギの GH を放出させること、またその放出機構には下垂体への直接作
用のほか、視床下部を介した作用もあることを示唆している。

日畜会報, 69 (6): 563-570, 1998