Effects of Cholecystokinin Octapeptide on the Release of Growth Hormone from the Perfused Bovine Pituitary *In Vitro*

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Abstract The effect of cholecystokinin octapeptide (CCK-8) was studied on the release of growth hormone (GH) from perfused adenohypophyses of steers. Perfusion with media containing 10^{-7} , 10^{-8} and 10^{-9} M CCK-8 for 90 min significantly increased the mean GH concentrations during the treatments by $\Delta 7.36$ ($P < 0.01$), $\Delta 13.31$ ($P < 0.01$) and $\Delta 5.64$ ($P < 0.05$) ng/ml respectively compared with the control group. There was no significant difference in the rates of increase in GH among the three molar perfusion groups. The present results indicate that CCK-8 acts directly on the adenohypophysis to stimulate release of GH in cattle.

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Key words : cholecystokinin, growth hormone, cattle, pituitary

Growth hormone (GH) secretion is well known to be regulated by two hormones, somatostatin, an inhibitory regulatory factor and growth hormone releasing factor¹⁾. It is reported, however, that some brain peptides also play a role in the control of GH secretion⁶⁾. Cholecystokinin octapeptide (CCK-8) is considered to be one of these peptides in rats^{3-7,10)}. Namely, VIJAYAN *et al.*¹⁰⁾ reported that the plasma level of GH increased within 15 min after injection of CCK-8 into the third ventricle of rats. MATSUMURA *et al.*⁵⁾ also reported that intravenous injection of CCK-8 increased in plasma GH level after 10 and 20 min in rats. It is not certain, however, whether the CCK stimulates release of GH indirectly through the hypothalamus¹⁰⁾ or directly on the adenohypophysis^{5,7)}. And the effect of CCK on GH secretion in domestic animals also has not been elucidated. In our earlier investigation³⁾, we demonstrated that VIP, one of another brain peptides, acts

directly on the adenohypophysis to release GH in cows.

In the present study, we examined the effect of CCK-8 on the secretion of GH from the adenohypophysis of steers, using an *in vitro* perfusion system of anterior pituitary tissues.

Materials and Methods

The adenohypophysis were removed from 9 Holstein steers (age in 17-18 months) at a local slaughter house and were transported to the laboratory in Krebs-Ringer bicarbonate solution to which was added 1.8 mg glucose/ml, 0.5 mg bacitracin/ml, 1.0 mg bovine serum albumin/ml, 0.1 mg streptomycin/ml and 100 IU penicillin/ml (modified KRB solution) at 5°C within 2 h. The hypophysis were sagittally cut into halves before they were immersed into the modified KRB solution. The pituitary tissues used were medial part of the anterior lobe and they were sectioned into small cubes of 1.5 mm dimensions. Five cubes of the

anterior pituitary tissue were loaded into a small plastic chamber of 0.2 ml size and were perfused at 38°C, in a modified KRB solution saturated with 95% O₂ and 5% CO₂. After equilibration for 3 h, the effluent was collected in 3 ml fractions at 10 min intervals at a flow rate of 18 ml/h. Six samples of the effluent were collected over a period of 1 h for determinations of basal GH levels. Then solution containing various concentrations (10⁻⁷, 10⁻⁸ and 10⁻⁹M) of CCK-8 (sulfated form, Peptide Institute Inc., Osaka, Japan) and the medium alone (control) were perfused for the next 1.5 h and nine samples of the effluent were collected during these periods. At the termination of the experiment, a modified solution containing high potassium ion (123.2 mM KCl in KRB solution) was perfused to recognize the viability of the tissues used. Samples were stored at -20°C until assayed.

GH in effluent fractions were measured by radioimmunoassays as described previously⁴¹. In this assay, the intra- and inter-assay coefficients of variation were 6.6 and 17.7%, respectively. Least detectable value of assays was 7.97 ng/ml. Values were expressed as means and standards errors. Each value from nine samples during the 1.5 h-CCK treatment were also expressed as net changes of GH (Δ GH ng/ml) from the mean of six samples obtained prior to CCK-8 infusion. For statistical analysis the mean GH concentrations

during infusion of CCK-8 from 5 to 6 perfusions (nine determinations each) were compared with the mean basal levels and the mean values of the control group. Data were used when the pituitary tissues released GH markedly in response to high potassium ion solution. Statistical comparisons were made using the Student's *t* test after Bartlett's test for uniformity of the variances.

Results

GH release from perfused bovine pituitary tissues in response to different CCK-8 concentrations are shown in Table 1. In the control group there was no significant difference between the mean basal GH levels and the mean GH value, that was obtained from the subsequent perfusions with medium alone, during which period CCK-8 was perfused in other groups. Perfusion with the media containing 10⁻⁷, 10⁻⁸ and 10⁻⁹M CCK for 90 min significantly increased GH concentrations during the treatments compared with the each basal GH levels (*P*<0.05). At the termination of the perfusion all pituitary tissues used in the experiment released GH markedly in response to high potassium ion solution (Table 1).

As shown in Fig. 1, perfusion with the media containing 10⁻⁷, 10⁻⁸ and 10⁻⁹M CCK-8 for 90 min significantly increased the mean GH concentrations during the treatments by Δ 7.36

Table 1. GH release from perfused bovine pituitary tissues to different CCK-8 concentrations and high potassium ion solution.

CCK-8 conc.	Basal levels	Perfusion of	
		CCK-8	High potassium ion
Controls(6)*	10.26±0.90 ^a	9.36±3.92 ^a	49.30±5.42 ^b
10 ⁻⁷ M (6)	14.82±0.91 ^a	22.07±2.36 ^b	48.55±9.22 ^c
10 ⁻⁸ M (5)	14.20±0.72 ^a	28.16±4.00 ^b	49.88±9.67 ^c
10 ⁻⁹ M (5)	16.12±0.87 ^a	21.19±2.01 ^b	53.00±10.23 ^c

Values represent the mean±SE of GH levels (ng/ml/10 min) before perfusion of CCK-8 (basal levels) and during perfusion of CCK-8 or high potassium ion solution.

*: Numbers in parenthesis represent number of determinations.

^{a, b, c}: Values on the same line with different superscripts are significantly different (*P*<0.05).

Fig. 1. Secretory response of *in vitro* GH release from perifused bovine pituitary tissues to different CCK-8 concentrations. Values represent the mean $\pm$ SE of the total Δ GH levels during perfusion of CCK-8. Numbers at the base of each bar represent number of determinations. GH levels of the CCK-8 perfused groups are significantly greater than that of control group. *: ($P < 0.05$), **: ($P < 0.01$).

($P < 0.01$), Δ 13.31 ($P < 0.01$) and Δ 5.64 ($P < 0.05$) ng/ml respectively compared with the control group. The GH released in the medium in the 10^{-8} M group tended to increase compared with another molar groups, but they were not significantly different each other (Fig. 1).

Discussion

The present study demonstrated that CCK-8 induced GH release directly from the anterior pituitary *in vitro* in cattle. Heretofore, effects of CCK on the release of pituitary GH have never been reported in domestic animals *in vivo*

as well as *in vitro*. VIJAYAN *et al.*¹⁰⁾ suggested that CCK has no direct action on the anterior pituitary, acting indirectly on the hypothalamus to release anterior pituitary hormones. But MORLEY *et al.*⁷⁾ reported that CCK stimulated GH release directly from rat GH₃ cells, and similarly MATSUMURA *et al.*⁵⁾ reported that CCK stimulated GH release from dispersed cells of rat anterior pituitary. The results of the present investigation are consistent with the observations of MORLEY *et al.*⁷⁾ and MATSUMURA *et al.*⁵⁾.

The GH releasing effect of CCK-8 from the anterior pituitary could be seen at a dose as low as 10^{-9} M perfusion group. It is reported, however, that CCK-8 at a concentration of 10^{-11} M could induce GH release from anterior pituitary dispersed cells in rats⁵⁾. Therefore, further studies in consideration of lower doses are also necessary in cattle.

CCK is widely distributed in the hypothalamus, hypophysis and pituitary stalk^{2,8)}. CCK receptors are detected in the brain⁹⁾ and also in the hypophysis¹¹⁾. CCK stimulates GH release *in vivo*^{5,10)} as well as *in vitro*^{5,7)}. These findings may represent an intimate relationship of brain CCK with pituitary GH secretion. It remains to be determined what effects of the CCK would have on GRF and somatostatin.

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牛の下垂体の *in vitro* 灌流系における成長ホルモン放出に 及ぼすコレシストキニンオクタペプチドの効果

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コレシストキニンオクタペプチド (CCK-8) が牛の成長ホルモン (GH) の放出に及ぼす影響について検討した。屠場で採取した去勢雄牛の下垂体前葉組織を *in vitro* 灌流系に置き、 10^{-7} ~ 10^{-9} M の CCK-8 を 90 分間灌流し、灌流液中の GH 濃度の変化を調べた。CCK-8 (10^{-7} ~ 10^{-9} M) を灌流すると灌流液中の平均 GH 濃度は灌流前の基礎濃度に比べ有意に増加した ($P < 0.05$)。また灌流期間中の平均 GH 濃度は 10^{-7} , 10^{-8} および 10^{-9} M において、対照区に比べそれぞれ $\Delta 7.36$ ($P < 0.01$), $\Delta 13.31$ ($P < 0.01$) および $\Delta 5.64$ ($P < 0.05$) ng/ml 有意に増加した。この GH 濃度の増加割合には CCK-8 の添加濃度による差は認められなかった。本研究の結果は CCK-8 は GH の放出機構に促進的に作用すること、またその機能は下垂体に直接作用して起こることを示している。

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