NOTE

Effects of Muscarinic and Adrenergic Blockade on Growth Hormone Secretion Induced by Growth Hormone-Releasing Peptide-2 (GHRP-2) in Ovariectomized Ewes

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Abstract. This study was performed to investigate whether the blockade of cholinergic muscarinic and adrenergic pathways was involved in the GH-releasing effect of GH-releasing peptide-2 (GHRP-2) in ovariectomized ewes. Cholinergic muscarinic antagonist, atropine (0.2 mg/kg, iv, 15 min before GHRP-2 administration) blunted the GH secretion caused by GHRP-2 (12.5 µg/kgBW). α -Adrenergic antagonist phentolamine (15 µg/kgBW·min, infusion from –15 to 30 min) did not affect the GH response to GHRP-2, and β -adrenergic antagonist propranolol (0.25 mg/kgBW, iv, 15 min before GHRP-2 administration) did not suppress the GHRP-2-induced GH release. These results showed that cholinergic muscarinic antagonist agent, atropine, exerts an inhibitory effect on GHRP-2-induced GH secretion in ovariectomized ewes.

Key words: GH-releasing peptide-2 (GHRP-2), GH, Muscarinic receptor, Adrenergic receptor
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THE synthetic growth hormone (GH)-releasing peptide-2 (D-Ala-D- β Nal-Ala-Trp-D-Phe-Lys-NH₂, GHRP-2) releases GH in many species [1–3]. It is widely recognized that GHRPs for releasing GH act not only directly at the level of anterior pituitary receptor [4, 5], but also have a hypothalamic site of action through the intervention of the central nervous system [6, 7]. GHRP-6 (His-D-Trp-Ala-Trp-D-Phe-Lys-NH₂) infusion in the third ventricle has a minor effect on the pulsatile release of GH-releasing factor (GRF) and no effect on somatostatin (SS) secretion in conscious ewes [8]. GHRP-2 and GHRP-6 have been shown to induce *c-fos* activity in cells within the arcuate nucleus of the rat,

suggesting that they may activate GRF neurons [6, 7]. GHRP-6 was not unable to modify the changes in somatostatinergic tone induced by cholinergic muscarinic pathways in man and beagle [9, 10]. Although α_2 -adrenergic receptor activation with clonidine did not alter the GH responses to GHRP-6, it was decreased by the α_1 -adrenergic receptor antagonist prazosin [9]. It could be thought that GHRPs induce the GH secretion via a hypophysiotropic pathway. In addition, unlike GHRP-6 and GHRP-1 (D-Ala-His-D- β Nal-Ala-Trp-D-Phe-Lys-NH₂), there are species differences in the response of pituitary somatotrophs to GHRP-2 in sheep and rat pituitary cells [2]. This study was therefore conducted to determine whether the mechanism by which GHRP-2 acts is regulated by the blockade of the classical neurotransmitter systems in ovariectomized ewes.

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Materials and Methods

Animals

Six adult ovariectomized ewes were used (58–63 kg). The ewes were housed in metabolic cages and fed alfalfa pellets at 2% of body weight in a single meal at 1600 h. Water was available continuously. At least a week before the experiments, the polyethylene catheter for GHRP-2 injection and blood sampling was inserted into a jugular vein, and was kept patent by daily flushing with a sterile solution of 3.8% trisodium citrate.

Experimental procedures

Experiments were performed in random order, and had a time interval of at least 1 week. Ewes were intravenously injected through the catheter with GHRP-2 dissolved in sterile saline at a dose of 12.5 $\mu\text{g/kgBW}$. Serial blood samples were collected at -30, -20, -10, 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105 and 120 min after the injection of GHRP-2 or saline. Drugs were dissolved in 0.9% NaCl and injected via the catheter before the injection of GHRP-2. Atropine sulfate monohydrate (Wako Pure Chemical, Japan), a cholinergic muscarinic antagonist, was administered as a bolus injection at a dose of 0.2 mg/kg iv 15 min before GHRP-2. To investigate the possible participation of an α -adrenergic antagonist, phentolamine (Regitin, Ciba-Geigy, Japan) was infused at a dose of 15 $\mu\text{g/ml-min}$ for 45 min from -15 to 30 min. Blockade of β -adrenergic receptors was achieved by iv injection of propranolol-HCl (Wako Pure Chemical, Japan) at a dose of 0.25 mg/kg 15 min before GHRP-2. The doses of atropine, phentolamine and propranolol were based on the previous report [11].

Analysis

The plasma concentrations of GH were assayed by RIA as previously reported [1]. The sensitivity was 0.5 ng/ml, and inter- and intra-assay coefficients of variation were less than 10%.

Statistical analysis

For each study, plasma GH concentrations (mean $\pm$ SEM) were plotted against time for each ewe. The GH AUC (ng/ml·min) was calculated by a trapezoidal method from -30 to 120 min in each ewe. Significant differences between AUCs were analyzed by analysis of variance (ANOVA) followed by Duncan's multiple range test.

Results

GHRP-2 (12.5 $\mu\text{g/kgBW}$) elicited a striking rise in the plasma GH concentration with peak levels occurring at 15–25 min in ewes (Fig. 1). Compared to the saline control, iv injection of GHRP-2 increased GH concentrations with an 8-fold increase in the peak GH concentration (16.3 ± 2.5 ng/ml) at the 12.5 $\mu\text{g/kgBW}$ dose. GH AUC at the injection of saline after the injection of GHRP-2 was significantly greater than that obtained on the treatment of saline and saline as a control (990.8 ± 136.3 vs. 390.1 ± 29.3 ng/ml·min $\pm$ SEM; $P < 0.01$) (Table 1). The injection of saline after pretreatment with atropine did not change the resting GH

Fig. 1. Effect of iv administration of GHRP-2 (12.5 $\mu\text{g/kg BW}$, iv, 0 min) after pretreatment with atropine (0.2 mg/kg BW, at -15 min) on plasma GH levels (mean $\pm$ SEM) in six ewes.

Table 1. GH AUC (mean $\pm$ SEM) to saline or GHRP-2 injection after pretreatment with all the different drugs tested in six ewes*

Drug		GH AUC
Saline	Saline	390.1 $\pm$ 29.3
Atropine	Saline	401.0 $\pm$ 70.0
Phentolamine	Saline	365.8 $\pm$ 61.6
Propranolol	Saline	398.5 $\pm$ 116.9
Saline	GHRP-2	990.8 $\pm$ 136.3 ^a
Atropine	GHRP-2	502.0 $\pm$ 79.5 ^b
Phentolamine	GHRP-2	970.6 $\pm$ 133.4 ^a
Propranolol	GHRP-2	1032.3 $\pm$ 144.2 ^a

*GH AUCs were calculated from -30 to 120 min. GHRP-2 $12.5 \mu\text{g/kg}$ iv; atropine 0.2 mg/kg iv; phentolamine $15 \mu\text{g/kg}$ iv; propranolol 0.25 mg/kg iv. ^a Means significant difference between saline and GHRP-2 on same drug treatment ($P < 0.01$). ^b Means significant difference among the GHRP-2 injections on all the different drugs in the lower part ($P < 0.01$).

concentration, but atropine suppressed the GH release caused by GHRP-2. The mean peak concentration was lower in the pretreatment of atropine after the injection of GHRP-2 ($10.5 \pm 3.4 \text{ ng/ml}$) than in saline plus GHRP-2 ($16.3 \pm 2.5 \text{ ng/ml}$). GH AUC on atropine plus GHRP-2 was significantly lower than on saline plus GHRP-2 ($P < 0.01$) (Table 1).

Figure 2 indicates the effects of phentolamine and propranolol on the GH secretion caused by GHRP-2. Infusion of phentolamine did not change the GH secretion elicited by GHRP-2 (Fig. 2a). The GH AUC after the treatment with saline plus GHRP-2 was similar to that on phentolamine plus GHRP-2 (990.8 ± 136.3 vs. 970.6 ± 133.4) (Table 1). Propranolol after the injection of saline did not change the basal concentration of GH, and pretreatment of propranolol did not attenuate the GH release caused by GHRP-2 (Fig. 2b). There was no significant difference between propranolol + GHRP-2 and saline + GHRP-2 in GH AUC.

Discussion

Although the mechanisms of action of GHRP-2 have not been fully established, there is probably a dual site of action on the pituitary and hypothalamus, possibly involving regulatory

Fig. 2. Effect of iv administration of GHRP-2 ($12.5 \mu\text{g/kg}$ BW, iv, 0 min) after pretreatments with phentolamine and propranolol on plasma GH levels (mean $\pm$ SEM) in six ewes. Phentolamine ($15 \mu\text{g/kg}$ BW-min) was continuously infused from -15 to 30 min. Propranolol (0.25 mg/kg BW) was injected at -15 min before GHRP-2.

factors in addition to GRF and SS. The early studies on the GHRPs suggested a major mode of action at the level of the pituitary, but in more recent years it has been suggested that GHRPs also have, at least in part, a direct effect on the hypothalamus [2, 12]. The secretion of GRF and SS are governed by a host of neuropeptides and neurotransmitters that provide a functional link between higher central nervous system and hypophysiotropic neurons. Factors that have been implicated include the biogenic amines, e.g. acetylcholine, adrenaline, dopamine, serotonin, histamine, etc. [13, 14]. Indeed, the fact that GHRPs are much more potent *in vivo* than *in vitro* suggested that GHRPs are closely related to these factors [2,4].

It is now widely accepted that a cholinergic muscarinic pathway plays a major role in GH secretion [15]. Atropine ($100 \mu\text{g}$, iv), which acts by

increasing hypothalamic SS release, completely abolished the stimulatory action of GHRP-6 in adult beagles [9]. Administration of atropine (1 mg, im) completely prevented the GH responses to GHRP-6 in man [10], but in an experimental setting of low somatostatinergic tone due to pyridostigmine, GHRP-6 is capable of increasing the GH response [9, 10]. An increase and decrease in GH responses to GHRP-6 following administration of cholinergic agonist and antagonist respectively indicated that GHRP-6 is unable to modify the changes in somatostatinergic tone induced by cholinergic muscarinic pathways. In our results the administration of atropine blunted the GH responses to GHRP-2 in ewes. These results suggested that the inhibitory effect of atropine on GHRP-2-induced GH release is mediated by the increase in hypothalamic SS release, but the GH response to GHRP-2 was not completely inhibited by atropine in contrast to GHRP-6 in Beagle [9]. It seems to be that besides a cholinergic muscarinic pathway there may possibly be GH secretion to GHRP-2 via another neural pathway.

Adrenergic mechanisms participate in the regulation of GH secretion, α - and β -adrenergic receptors having, respectively, stimulatory or inhibitory effects [15], but in ovine pituitary cells α_2 -adrenergic receptors induce the inhibitory actions of adrenergic agonists, while α_1 - and β -adrenergic receptors appear to mediate the stimulatory actions of adrenergic agonists on GH release [16]. Evidence has accumulated during the

last few years indicating that adrenergic neurons participate in the regulation of the normal pulsatile GH secretion [14]. In view of these facts, α - and β -adrenergic receptors, located either in the hypothalamus or in the pituitary, are an indispensable prerequisite for GH secretion, but our results showed that the GH secretion caused by GHRP-2 occurs independent of α - and β -adrenergic receptors. Although administration of the α_1 -adrenergic receptor antagonist prazosin (20 mg, iv) reduced GHRP-6-induced GH secretion, α_2 -adrenergic agonist clonidine did not influence the GHRP-6-induced GH secretion [9]. These results indicated that although the adrenergic system participates in physiological regulation of GH secretion, GHRP-2 does not have a direct action via this pathway.

In conclusion, this study showed that the effect of GHRP-2 on GH secretion was blunted by a cholinergic muscarinic blockade with atropine, but the blockade of adrenergic receptor did not attenuate the GHRP-2-induced GH release in ovariectomized ewes.

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