

Subcutaneously Administered Prolactin and 20K hGH, but not rGH or 22K hGH, Prevent Restraint Stress-Induced Gastric Ulcers in Rats

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Abstract. Stress causes gastric ulcer in vertebrates. In humans, growth hormone (hGH) and prolactin (hRPL) are promptly released into the circulation under the stress conditions, while in rats exposed to stress, the circulating levels of GH (rGH) are decreased and the circulating PRL (rPRL) levels are rapidly increased as in humans during stress. However, the roles of the circulating rGH and rPRL during stress are still unclear. Here we analyzed whether 22K hGH, 20K hGH or rGH, when compared to rPRL, can affect restraint stress in water (RSW)-induced gastric ulcers. Pretreatments of rats with subcutaneously (sc) administered rPRL or 20K hGH clearly prevented the development of the gastric injuries in rats subjected to 7 h RSW. The sc pretreatment with 22K hGH resulted in little cytoprotection in the rats exposed to RSW, while sc pretreatment with rGH showed no such protective effect against RSW-induced gastric injuries. Results suggested that rPRL and 20K hGH were acting on PRL receptor, but not on GH receptor, to prevent RSW-induced gastric injuries.

Key words: PRL, 20K hGH, 22K hGH, Subcutaneous administration, Stress-induced gastric ulcer

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HUMAN 20 kDa growth hormone (20K hGH) is produced by alternative splicing of the same mRNA precursor as for pituitary 22 kDa hGH (22K hGH), but lacks 15 amino acid residues from Glu-32 to Gln-46 of 22K hGH. Several reports have demonstrated that 20K hGH has a weaker affinity than 22K hGH for human liver cells, which contain receptors for both GH and prolactin (GH-R and PRL-R) [1], IM-9 cells, which contain only GH-R [2], and GH-binding protein (GH-BP) [3]. Recently, Wada *et al.* [4] have documented that 20K hGH poorly forms a 1 : 1 complex with GH-BP prevailing in human plasma, while 22K hGH forms the 1 : 1 complex efficiently. They have also shown that 20K hGH has a unique property to form a 1 : 2 complex with GH-

BP to the same extent as 22K hGH but has difficulty in forming a 1 : 1 complex, ascribing this to a conformational change in the site 1 region of 20K hGH.

In human, GH and PRL are well-known to be released from the anterior pituitary in response to a number of physical and psychological stresses, and in rats subjected to stress, the circulating levels of GH are decreased and the circulating levels of PRL are transiently increased as in humans exposed to stress [5, 6]. These hormones are not only involved in a variety of different endocrine processes [6, 7], but also acting as a neuromodulator on the central nervous system (CNS) [6–8]. Hyperprolactinemia in lactating rats or in rats with pituitary homografts under the kidney capsule has been shown to be accompanied by a marked resistance to stress-induced changes in body temperature [9] and gastric damage, and, furthermore, the peripheral treatment of rats with PRL appears to be followed by gastro-

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cytoprotection [10]. Whereas pretreatment with 22K hGH was followed by a raised percent incidence and severity of gastric injuries induced by cysteamine [11], which when injected would cause a depletion of somatostatin (SS) in the gastro-intestinal tract resulting in ulcerogenic effects [12]. It is not clear yet, however, whether 22K hGH, 20K hGH or rat GH (rGH), comparative to rat PRL (rPRL), can affect the development of gastric injuries in the rats subjected to restraint stress in water (RSW).

Herein, we report that the peripheral treatments of rats by rPRL or 20K hGH clearly prevented the occurrence of RSW-induced gastric injuries while 22K hGH or rGH did not.

Materials and Methods

Adult male rats of the Sprague-Dawley strain, 8 weeks old, were kept in a room maintained at 23–25°C and controlled to a humidity of 50–60% under a 12-h light / 12-h dark lighting cycle (lights on at 07:10). Osmotic minipumps (ALZA Corp., Palo Alto, CA) containing rPRL, rGH, 22K hGH or 20K hGH of 84 µg/ 200 µl in 0.9% NaCl were subcutaneously (sc) implanted on the back of rats for 6 days. Other osmotic minipumps containing only 0.9% NaCl were implanted for 6 days in the control rats. After 6 days, the animals with the osmotic minipumps were subjected to RSW by putting into individual restraining wire net cages, and immersing in water (23±0.5°C) to the chest. After 7 h of RSW, the animals were taken out from the restraining cages and decapitated as described previously [13]. Blood from each rat was used for the measurement of serum rPRL and rGH. The serum hormone concentrations were assayed by specific enzyme-linked immunoassay (EIA) or by double-antibody radioimmunoassay (RIA). The rats' stomachs were removed and fixed with 1% formalin for 30 min. The index of gastric mucosal erosions was calculated by measuring the length of the erosions [14]. The mean and SEM of the data were determined and are presented in the Tables. The significance of difference between the values was analyzed by Dunnett's *post-hoc* procedure test after performing a Bartlett test (more than two groups). *P* values of less than 0.05 were considered to be significant. The RSW-experiments were initiated in the middle of the night (within a 01:00–

03:00 time frame), when rats are known to be highly active corresponding to daytime in human. The experiments described in this report were conducted according to the principles set forth in the 'Guides to the Care and Use of Laboratory Animals', Institute of Laboratory Animal Resources, National Research Council, DHEW Publ. (NIH) 85–23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD and Mie University School of Medicine. Both rPRL and rGH used in this experiment were donated by NIDDK.

Results

Thirty minutes of RSW caused a 265.8% increase in the circulating levels of PRL and a 52.8% decrease in those of GH (Table 1). The elevated serum PRL level quickly decreased and returned to the initial level after 7 h of RSW. Serum levels of GH were lowered to 12.8% of the control by 7 h RSW. The stomachs of the rats subjected to 7 h of RSW without hormone treatments developed obvious severe gastric mucosal erosions. Pretreatments of rats with sc rPRL or 20K hGH for 6 days more clearly prevented the incidence of RSW-induced gastric mucosal injuries than 22K hGH or rGH (Table 2). The sc-implantation of 22K hGH showed a 37.9% cytoprotection against RSW, however rGH did not show any protective effect on the rats against the RSW-induced gastric injuries (Table. 2).

Discussion

In the present study, we demonstrated that RSW causes a sharp elevation in the circulating level of

Table 1. Changes in the circulating levels of rPRL and rGH in the rats exposed to RSW

	Number of animal	Serum concentrations	
		rPRL (ng/ml)	rGH (ng/ml)
Non-RSW	9	11.4±2.2	21.8±4.2
0.5 h-RSW	7	41.7±1.3***	10.3±3.4*
2 h-RSW	7	26.0±0.7*	2.8±1.0*
7 h-RSW	9	9.4±3.9	2.1±0.4*

*, *P* < 0.05; ***, *P* < 0.0005 vs. Non-RSW.

Table 2. Effects of rPRL, 20K hGH, 22K hGH and rGH on the development of RSW-induced gastric injuries

Subcutaneous hormone treatments (84 µg/200 µl)	Number of animal	Index of gastric erosions (mm)	
		Mean ± SEM	% Inhibition by hormone treatment
0.9% NaCl	8	57 ± 1.1	
rPRL	8	14.3 ± 1.3****	75.1
rGH	7	53.4 ± 3.8	7.1
20K hGH	7	16.5 ± 1.6****	71.3
22K hGH	7	35.7 ± 1.3	37.9

****, $P < 0.0001$ vs. 0.9% NaCl.

PRL and a reduction in that of GH at an early stage. The elevated level of PRL had decreased, and also the GH level, at 7 h of RSW. Pretreatment of rats with sc administered rPRL or 20K hGH clearly prevented the occurrence of gastric mucosal injuries in the rats subjected to 7 h RSW. Several previous investigations have shown that hyperprolactinemia in lactating rats, or rats transplanted with pituitary homografts under the kidney capsule, is followed by a marked resistance to stress-induced gastric damage or decrease in body temperature [9, 10], whereas, higher circulating levels of GH, as in the exogenous 22K hGH-administered rats, potentiate the ulcerogenic activity of cysteamine [11]. Contrary to the latter report [11], our present study showed that sc pretreatment with 22K hGH resulted in little gastric

cytoprotection in the rats exposed to RSW. The rGH treatment, however, showed no such protective effect against RSW-induced gastric injuries. 22K hGH is known to bind to both GH-R and PRL-R, and it forms a 1 : 1 complex efficiently with GH-BP in human plasma. 20K hGH, on the other hand, has a weaker affinity than 22K hGH to those receptors, and it forms a 1 : 1 complex very poorly [1–4]. These findings may indicate that rPRL and 20K hGH are acting on PRL-R, but not on GH-R, to prevent RSW-induced gastric injuries, and, furthermore, the contradictory results of 20K hGH and 22K hGH suggest that the circulating GH-BP may have neutralized and lowered the effect of subcutaneously administered 22K hGH, but not of 20K hGH.

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