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RAPID COMMUNICATIONS

INHIBITION BY ANTISERUM TO RAT GROWTH HORMONE-RELEASING FACTOR OF GROWTH HORMONE SECRETION INDUCED BY A MET^5-ENKEPHALIN ANALOG, FK33-824, IN RATS[1]

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Abstract. A Met^5-enkephalin analog, FK33-824 (5, 10 and 20 μg/100 g body wt, iv) caused a dose-related increase in plasma growth hormone (GH) in urethane-anesthetized male rats. Pretreatment with cysteamine (30 mg/100 g body wt, sc), a depletor of hypothalamic somatostatin, increased the plasma GH response to FK33-824 (10 μg/100 g body wt, iv). Antiserum specific for rat GH-releasing factor (GRF)(0.5 ml/rat, iv) blunted GH release induced by FK33-824 (10 μg/100 g body wt, iv) in rats with or without cysteamine pretreatment. These results suggest that GH secretion induced by the opioid peptide is mediated, at least in part, by hypothalamic GRF in the rat.

The secretion of growth hormone (GH) from the pituitary is under dual control of the hypothalamus via GH release inhibiting factor (somatostatin, SRIF)(1) and GH releasing factor (GRF)(2,3). Passive immunization with specific antiserum to these factors is known to inhibit the action of endogenous peptides in the hypothalamus. Stress-induced inhibition of GH secretion is blunted by anti-SRIF serum and spontaneous GH bursts are blunted by anti-GRF serum in conscious rats (4,5).

It has been reported that opioid peptides stimulate GH secretion in rats (6-9). Since opioid peptides do not directly act at the pituitary somatotroph (6), the central nervous system is involved in GH release induced by opioid peptides. We have previously reported that icv or iv injection of FK33-824, a Met^5-enkephalin analog, raised plasma GH levels in both conscious and urethane-anesthetized rats (8,9). Alpha-adrenergic mechanisms and GABA in the central nervous system seem to be involved in GH release induced by FK33-824 in conscious rats (9,10). However, the final common pathway for GH secretion induced by FK-33-824 remains to be elucidated.

In the present study, we investigated the possible involvement of hypothalamic GRF in FK33-824-induced GH secretion using a passive immunization technique in urethane-anesthetized rats.

Materials and Methods. Male Wistar strain rats (Japan Animal Co., Osaka, Japan) weighing 250-300 g were used throughout the experiments. The animals were maintained on a constant light-dark cycle (lights on 0600-1800 h) in a temperature (23±1°C)- and humidity (50-60%)-controled room. Laboratory chow (Oriental Yeast Co., Tokyo, Japan) and tap water were given ad libitum.

After overnight fasting, they were anesthetized with urethane (150 mg/100 g body wt, ip). FK33-824 (5, 10 and 20 μg/100 g body wt) or synthetic rat GRF (80 ng/100 g body wt) was injected into a

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jugular vein in a volume of 0.1 ml/100 g body wt as previously described (11). Anti-rat GRF rabbit serum (anti-GRF-RS) or normal rabbit serum (NRS) as a control was injected iv in a volume of 0.5 ml/rat 30 min before the injection of test materials. Blood samples (0.6 ml) were obtained from the jugular vein immediately before, and 5, 10, 20 and 40 min after the iv injection of test substances as previously described (11). Plasma was immediately separated and stored -20°C until assayed.

Anti-GRF-RS was generated in a rabbit against synthetic rat GRF(1-43)(3) conjugated with bovine thyroglobulin. Briefly, 1 mg rat GRF was conjugated with 6 mg bovine thyroglobulin in 1 ml 0.1 M phosphate buffered saline (pH 7.0) by the glutal aldehyde method. The conjugate was dialized to distilled water at 4°C for 2 days and lyophilized. The peptide (50 μg) was reconstituted with physiological saline and a rabbit was immunized by intracutaneous injection with 1 ml complete Freund adjuvant (Difco) at 2-4 week intervals a total of 10 times, followed by three booster injections. Antiserum (KYGS-1) used in this study bound 37% of ^{125}I-rat GRF (82 pg) at a final dilution of 1:500,000 under the conditions used for GRF radioimmunoassay. It had no cross reactivity with SRIF, TRH, LHRH, VIP, porcine PHI, neurotensin, substance P and rat GH. The antiserum had only about 1.6% cross-reactivity to human pancreatic GRF(1-44-NH_2).

Plasma rat GH was measured by specific radioimmunoassay using a kit supplied by the NIADDK, NIH (Bethesda, MD) as described previously (11). Highly purified NIADDK rat GH-RP-2 was used as the standard in the present study. The anti-GRF titer in plasma of the rats treated with anti-GRF-RS was assessed by determining the capacity of ^{125}I-rat GRF to bind to 0.1 ml of rat plasma diluted to 1:250 according to the radioimmunoassay for GRF.

Synthetic rat GRF(1-43) was kindly supplied by Dr. N. Ling (Salk Institute, San Diego, CA). FK33-824 was obtained from Sandoz (Basel, Switzerland). Cysteamine hydrochloride was purchased from Sigma Chemical Co. (St. Louis, MO). The drugs were dissolved in physiological saline.

Statistical differences were evaluated by one-way analysis of variance in combination with Duncan's new multiple range test and Student's t test. A p value less than 0.05 was considered significant.

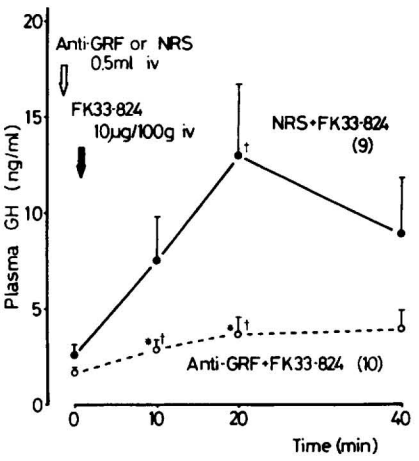

Fig. 1. Effect of anti-rat GRF rabbit serum (anti-GRF) on GH release induced by FK33-824 in urethane-anesthetized rats. All values are the mean±SE of the number of animals indicated in parentheses. *:p<0.05 vs control. †:p<0.05 vs basal.

Results. Intravenous injection of FK33-824 (5, 10 and 20 μg/100 g body wt) resulted in a significant and dose-related increase in plasma GH levels in urethane-anesthetized rats. Peak GH values were obtained 20 min after the injection of FK33-824 (mean±SE peak GH: FK33-824 5 μg 6.8±1.9 ng/ml, 10 μg 12.1 ±2.6 ngml, 20 μg 16.9±3.5 ng/ml vs saline control 1.7±0.6 ng/ml, p<0.05).

As shown in Fig. 1, plasma GH increase induced by FK33-824 (10 μg/100 g body wt, iv) was blunted by pretreatment with anti-GRF-RS which was injected iv at a dose of 0.5 ml per rat 30 min before FK33-824 injection. Plasma GH increases induced by FK33-824 in rats injected by anti-GRF-RS were significantly lower than those in control animals which were pretreated with NRS (peak GH: 13.0±3.8 ng/ml vs 3.6±0.9 ng/ml, p<0.05).

In rats pretreated with cysteamine (30 mg/100 g body wt, sc, 4 h before) and NRS (0.5 ml/rat, iv, 30 min before), basal plasma GH levels were increased and injection of FK33-824 (10 μg/100 g body wt, iv) further raised plasma GH levels (Fig. 2). Anti-GRF-RS administration partially but significantly sup-

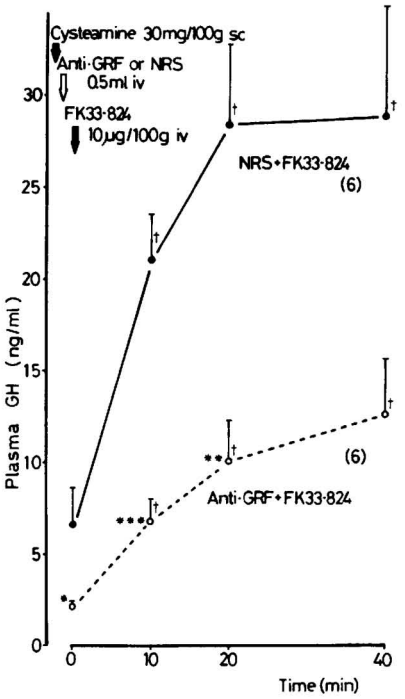

Fig. 2. Effect of anti-rat GRF rabbit serum (anti-GRF) on GH release induced by FK33-824 in the rats pretreated with cysteamine. ***, ** and * indicate P<0.001, p<0.01 and p<0.05 vs control. †:p<0.01 vs basal.

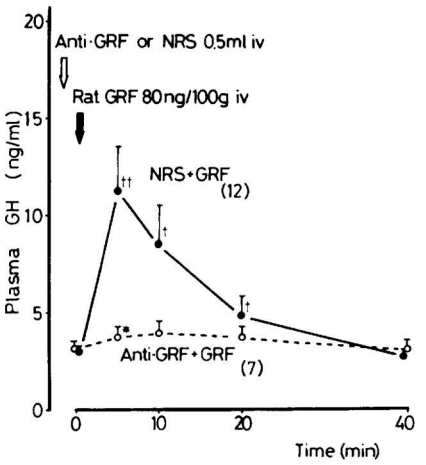

Fig. 3. Effect of anti-rat GRF rabbit serum (anti-GRF) on GH release induced by exogenous rat GRF. *:p<0.05 vs control. †† and † indicate p<0.01 and p<0.05 vs basal.

pressed plasma GH increase induced by FK33-824 (10 μg/100 g body wt, iv) in the animals pretreated with cysteamine (plasma GH increment: anti-GRF-RS 10.6± 2.2 ng/ml vs NRS 25.8±4.7 ng/ml, p<0.05). Basal plasma GH values in the rats pretreated with cysteamine and anti-GRF-RS were lower than those in cysteamine and NRS-treated rats.

As shown in Fig. 3, iv injection of synthetic rat GRF (80 ng/100 g body wt) caused a prompt increase in plasma GH with a peak value at 5 min in the rats pretreated with NRS. Anti-GRF-RS blunted the GH response to rat GRF (peak GH: anti-GRF-RS 3.7±0.5 ng/ml vs NRS 11.0 ±2.4 ng/ml, p<0.05).

Rat plasma obtained 40 min after iv injection of anti-GRF-RS was proved to bind 46.7±1.4% of ^{125}I-rat GRF (82 pg) added in vitro at the final dilution of 1:250.

Discussion. In line with previous studies (8,9), iv injection of FK33-824, a Met^5-enkephalin analog, stimulated GH release in the rat. Since opioid peptides have no direct action on the pituitary somatotroph (6), the opiate-induced GH release could be mediated by the hypothalamus via GRF or SRIF. Our findings that FK33-824 raised plasma GH levels in the rats pretreated with cysteamine, which reduces SRIF concentrations in the hypothalamus (12), may indicate the possible involvement of hypothalamic GRF in GH release induced by the opioid peptides.

This speculation was confirmed by the fact that specific anti-rat GRF-RS suppressed plasma GH increase induced by FK33-824 in rats with or without cysteamine pretreatment. Anti-GRF-RS also blunted the plasma GH response to exogenous rat GRF, indicating the antiserum which we used was capable of inhibiting the stimulating effect of rat GRF on GH secretion in vivo.

We also found that plasma GH release induced by FK33-824 was exaggerated by cysteamine. Exaggerated plasma GH respose to FK33-824 may be explained by the activation of endogenous GRF due to reduced hypothalamic SRIF. We have previously reported that exogenous GRF stimulates rat GH secretion by acting at the pituitary level and that the hypothalamic SRIF interacts with the action of GRF since inhibition of endogenous SRIF action by cysteamine or anti-SRIF

serum results in exaggerated plasma GH response to GRF in urethane-anesthetized rats (11). We cannot rule out the possibility that endogenous GRF release may be increased by cysteamine in these experiments since anti-GRF-RS suppressed basal plasma GH levels in rats pretreated with cysteamine but not in control animals.

Adrenergic mechanisms and GABA in the central nervous system are also involved in the regulation of GH secretion in close connection with opioid peptides in the rat (9,10). The precise interrelation among them requires further study.

1. Brazeau P, Vale W, Burgus R, Ling N, Butcher M, Rivier J, Guillemin R, Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science 179:77-79, 1973.
2. Guillemin R, Brazeau P, Böhren P, Esch G, Ling N, Wehrenberg WB, Growth hormone-releasing factor from a human pancreatic tumor that caused acromegaly. Science 218:585-587, 1982.
3. Spiess J, Rivier J, Vale W, Characterization of rat hypothalamic growth hormone-releasing factor. Nature 303: 532-535, 1983.
4. Terry LC, Willoughby JO, Brazeau P, Martin JB, Antiserum to somatostatin prevents stress-induced inhibition of growth hormone secretion in the rat. Science 192:565-567, 1976.
5. Wehrenberg WB, Brazeau P, Luben R, Böhlen P, Guillemin R, Inhibition of the pulsatile secretion of growth hormone by monoclonal antibodies to the hypothalamic growth hormone releasing factor (GRF). Endocrinology 111:2147-2148, 1982.
6. Rivier C, Vale W, Ling N, Brown M, Guillemin R, Stimulation in vivo of the secretion of prolactin and growth hormone by β-endorphin. Endocrinology 100:238-241, 1977.
7. Kato Y, Iwasaki Y, Abe H, Ohgo S, Imura H, Effects of endorphins on prolactin and growth hormone secretion in rats. Proc Soc Exp Biol Med 158:431-436, 1978.
8. Kato Y, Katakami H, Imura H, Role of neuropeptides in the control of growth hormone secretion in humans and rats. In: Shizume K, Takano K eds. Growth and Growth Factors. Tokyo:Univ Tokyo Press, p159, 1980.
9. Katakami H, Kato Y, Matsushita N, Hiroto S, Shimatsu A, Imura H, Involvement of alpha-adrenergic mechanisms in growth hormone release induced by opioid peptides in conscious rats. Neuroendocrinology 33: 129-135, 1981.
10. Katakami H, Kato Y, Matsushita N, Shimatsu A, Imura H, Possible involvement of γ-aminobutyric acid in growth hormone release induced by a Met^5-enkephalin analog in conscious rats. Endocrinology 109:1033-1036, 1981.
11. Kabayama Y, Kato Y, Shimatsu A, Ohta H, Yanaihara N, Imura H, Inhibition by gastrin-releasing peptide of growth hormone secretion induced by human pancreatic GH-releasing factor in rats. Endocrinology 115:649-653, 1984.
12. Szabo S, Reichlin S, Somatostatin in rat tissues is depleted by cysteamine administration. Endocrinology 109:2255-2257, 1981.

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