LACK OF GROWTH HORMONE-RELEASING ACTIVITY OF (PYRO)GLU-SER-GLY-NH_2

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SUMMARY

Youdaev et al. (1) reported that (pyro)Glu-Ser-Gly-NH_2 isolated from bovine hypothalami or made synthetically stimulates the release of growth hormone (GH). Therefore, we synthesized the tripeptide and tested it in several in vivo and in vitro assay systems for GH releasing activity. Our results demonstrate that (pyro)Glu-Ser-Gly-NH_2 does not stimulate the release of immunoreactive GH from rat pituitaries in vitro in doses of 0.1-1000 nanog/ml. Similarly, (pyro)Glu-Ser-Gly-NH_2, injected intravenously into rats in doses of 1μg and 10μg/rat or infused into a hypophysial portal vessel in doses of 0.01 μg and 0.1 μg/rat did not increase serum GH levels as measured by radioimmunoassay. When this tripeptide was injected intravenously in doses of 500 μg into sheep it did not raise plasma GH levels. These results demonstrate that under the conditions of this investigation (pyro)Glu-Ser-Gly-NH_2 does not display any GH-releasing activity.

Much physiological evidence, summarized by Schally et al. (2), indicates that hypothalamus regulates the release of growth hormone (GH) from the anterior pituitary gland. The stimulation of GH-release in vitro by hypothalamic extracts of rats (3) and of cattle, sheep and pigs (4) was demonstrated almost a decade ago. However, the isolation of the GH-releasing hormone (GH-RH) has been hampered by the inconsistency of the assay methods used for its detection. A decapeptide isolated by us from porcine hypothalami, structurally characterized and synthesized, stimulated in certain tests the release of bioassayable GH (2,5). However, since that decapeptide did not increase plasma levels of immunoreactive GH in rats, sheep, pigs, monkeys and humans (2,5), it is unlikely that it is the physiological GH-RH. Recently Youdaev et al. (1,6), reported the isolation from bovine hypothalami of a tripeptide which they structurally characterized as (pyro)Glu-Ser-Gly-NH_2 and synthesized. They claimed that the

natural as well as synthetic (pyro)Glu-Ser-Gly-NH_2 stimulated the release of growth hormone from rat pituitaries *in vitro*. Acting on this information we synthesized (pyro)Glu-Ser-Gly-NH_2 and tested it in several *in vivo* and *in vitro* assays. This paper reports our findings.

MATERIALS AND METHODS

A protected tripeptide, (pyro)Glu-Ser(Bzl)-Gly-NH_2, was prepared by a version (7,8) of the Merrifield solid-phase method (9) and was cleaved from the resin support by treatment with ammonia in methanol. The benzyl protecting group on the serine residue was then removed by hydrogenation over 10% palladium on charcoal. The resulting peptide, (pyro)Glu-Ser-Gly-NH_2, was purified by recrystallization from ethanol and appeared to be homogeneous by thin layer chromatography in several solvent systems and by amino acid analysis. (Pyro)Glu-Ser-Val-NH_2 was synthesized by Dr. R. Geiger (Hoechst) by classical methods.

The effect of (pyro)Glu-Ser-Gly-NH_2 was tested in the following assay systems:

(a). GH release *in vitro* from isolated rat pituitary halves during two successive one-hour incubation periods (10). The results are expressed as percentage ratio of medium GH in the second hour to medium GH in the first hour (10).

(b). GH release *in vivo* in estrogen and reserpine treated rats after intravenous administration of samples. Male Sprague-Dawley rats, weighing about 270 g. were injected with estradiol benzoate in oil (50 μg/rat intramuscularly) and with reserpine (0.5 mg/rat subcutaneously) 18 hours before the experiment. The samples were injected intravenously under urethane anesthesia (150 mg./100 g. body weight). Blood was collected before and 15 and 30 minutes after the injection.

(c). GH release *in vivo* after infusion of samples into a hypophysial portal vessel of male rats. The technique described by Porter *et al.* (11) and Sandow *et al.* (12) for perfusion of anterior pituitary *via* a microcannula inserted into a hypophyseal portal vessel was used.

TABLE 1. Lack of effect of (pyro)Glu-Ser-Gly-NH_2 on the GH release in vitro from rat pituitaries.

SAMPLE	DOSE (ng./ml.)	% of CONTROL ± SE* (hr. 2/ hr. 1 x 100)	p.
Control	-----	98.2 ± 6.4	---
(pyro)Glu-Ser-Gly-NH_2	0.1	85.8 ± 3.5	N.S.
	1.0	90.5 ± 4.0	N.S.
(pyro)Glu-Ser-Val-NH_2	10.0	78.4 ± 4.2	N.S.
	100.0	82.7 ± 3.4	N.S.
Control	-----	100.2 ± 5.9	---
(pyro)Glu-Ser-Gly-NH_2	10.0	90.8 ± 4.8	N.S.
(pyro)Glu-Ser-Gly-NH_2	100.0	94.2 ±12.4	N.S.
(pyro)Glu-Ser-Gly-NH_2	1000.0	89.9 ± 8.0	N.S.
Control	-----	98.9 ± 3.3	---
Pig GH-RH**	38,000.	183. ±16	0.01

* Four incubation beakers per group containing 4 pituitary halves each.
** Partially purified

TABLE 2. Lack of increase in Serum GH levels in male rats* after intravenous injection of (pyro)Glu-Ser-Gly-NH_2 as compared with pre-injection levels.

SAMPLE	DOSE (per rat)	Mean Serum GH increase (ng./ml. ± SE **)	p
Saline	---	2.8 ± 2.4	---
(pyro)Glu-Ser-Gly-NH_2	1 μg	1.8 ± 0.9	N.S.
(pyro)Glu-Ser-Gly-NH_2	10 μg	3.8 ± 1.9	N.S.
Rat hypothalamic extract	3.5 fragments	38.1 ±10.7	<0.01

* pretreated with estrogen and reserpine
** preinjection GH levels in serum ranged from 0 to 6.2 ng./ml.

The levels of GH in the incubation medium (system a) and in serum (systems b and c) were measured by radioimmunoassay (RIA) for rat GH (13). The results were compared statistically to controls using Duncan's new multiple range test.

(d). GH release in sheep; (pyro)Glu-Ser-Gly-NH_2 in saline was administered intravenously into Nembutal anesthetized ewes. Blood was collected from the

TABLE 3. Lack of increase in serum GH levels after infusion of (pyro)Glu-Ser-Gly-NH_2 into a hypophysial portal vessel of rats.

SAMPLE	DOSE (per rat)	Mean increase in GH (ng./ml. ± SE)	p.
Saline	---	11.1 ± 4.2	---
(pyro)Glu-Ser-Gly-NH_2	10 ng.	8.1 ± 5.9	N.S.
(pyro)Glu-Ser-Gly-NH_2	100 ng.	12.1 ± 9.2	N.S.
Rat hypothalamic extracts	0.6 fragments	201. ±29.4	< 0.01

The samples were dissolved in 60 µl. saline and infused over a 30 minute period into a hypophysial portal vessel of male rats under urethane anesthesia. Blood was collected from jugular vein, before and 15, 30, and 60 minutes after the onset of infusion. Maximum increase in serum GH levels as compared to pre-infusion level in each animal was used as an index of activity. Five to twelve rats were used per group.

jugular cannula 60, 45, 30, and 15 minutes before injection and every 15 minutes for three hours after the injection. Serum GH we measured by a RIA for sheep GH (14).

RESULTS

Addition of (pyro)Glu-Ser-Gly-NH_2 in doses of 0.1, 1, 10, 100, and 1000 ng./ml. to incubation medium did not stimulate the release of immunoreactive GH from isolated rat pituitary halves (Table 1). (Pyro)Glu-Ser-Val-NH_2 was also inactive. The levels of prolactin in the medium were not affected by either peptide. Intravenous administration of (pyro)Glu-Ser-Gly-NH_2 in doses of 1 and 10 µg. did not raise serum GH level in estrogen and reserpine-treated male rats, 15 and 30 minutes after the injection as compared with controls (Table 2). Infusion of (pyro)Glu-Ser-Gly-NH_2 into a hypophysial portal vessel in doses of 10 ng. and 100 ng./rat was equally ineffective in raising serum GH levels in rats (Table 3). Partially purified porcine GH-RH or crude rat hypothalamic extracts were active in all these tests (Tables 1-3).

The administration of (pyro)Glu-Ser-Gly-NH_2 tripeptide in doses of 500 µg. into sheep did not raise serum GH levels, in agreement with the lack of effect of this tripeptide in rats. The results are shown in Table 4.

TABLE 4. Serum GH levels (ng./ml.) before and after i.v. injection of 500 μg. (pyro)Glu-Ser-Gly-NH_2 into ewe under Nembutal anesthesia.

	(MINUTES)												
TIME:	-60	-45	-30	-15	0*	15	30	45	60	75	90	105	120**
Ewe #1	6.7	6.3	5.8	4.3	3.4	3.4	6.4	4.4	3.4	5.6	5.0	5.2	4.0
Ewe #2	11.8	20.7	13.4	17.9	12.6	12.8	12.0	11.5	12.0	10.9	11.5	12.0	11.3
Ewe #3	10.3	9.8	8.6	8.2	10.0	11.0	9.8	10.3	8.0	8.8	8.3	8.5	5.8

* The peptide was injected at 0 time.

** The GH levels between 120 and 180 minutes were similarly unchanged.

DISCUSSION

There is good evidence of the existence of a growth hormone-releasing hormone (GH-RH) (2,3,4), but progress in its isolation has been hampered by lack of satisfactory methods for its detection. A decapeptide isolated from porcine hypothalami on the basis of tests utilizing the in vivo depletion of bioassayable GH in the rat pituitary or the release of bioassayable GH from rat pituitaries in vitro, did not stimulate the release of immunoreactive GH in 5 mammalian species including the pig (2,5). The claims of Youdaev et al. (1,6) that (pyro)Glu-Ser-Gly-NH_2, isolated from bovine hypothalami or made by synthesis, is GH-RH, are convincingly nullified by the findings described in this paper. In our hands, synthetic (pyro)Glu-Ser-Gly-NH_2 did not stimulate the release of immunoreactive GH in vivo or in vitro in rats and in sheep, in dosages that were previously shown to be highly effective in the case of other hypothalamic hormones (1) in releasing the respective pituitary hormones (1). Although the presence of GH release-inhibiting factor (GIF) (10,15) in hypothalamic fractions complicates the detection of GH-RH, this consideration cannot hold for a pure natural or synthetic substance proposed as GH-RH. The testswe utilized for the measurement of GH-RH activity of (pyro)Glu-Ser-Gly-NH_2 were previously shown to be suitable for the detection of GH-RH in crude hypothalamic extracts or purified materials (1,12). GH-RH may be rapidly inactivated in the blood stream after a systemic injection so that

only a small fraction of this substance may reach the pituitary. However the use of infusion into a hypophysial portal vessel obviates these complications and offers an effective system for the detection of GH-RH (12,16). The results of Youdaev et al. (1,6) can be probably attributed in part to the nonspecific method used for the detection of GH and which was based on electrophoretic separation and measurement of the so-called "GH"-bands, although in some tests they used the RIA for GH also. Although the adequacy of radioimmunoassay for detecting growth hormone in rat plasma has been questioned, it should be at least valid for measuring the GH released into the incubation medium. Moreover, the absence of effect of (pyro)Glu-Ser-Gly-NH_2 on the release of GH in sheep confirms the lack of GH-RH activity of this substance. It is, therefore, likely that a different substance secreted by the hypothalamus is responsible for the GH-RH activity. In view of the probable clinical and veterinary importance of GH-RH, the search for it must continue.

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