No proof of anorectic properties of pGlu-His-Gly in rats and mice

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Abstract. Two groups of 7 rats were injected sc with 10 µg/d and 100 µg/d of pGlu-His-GlyOH or the same volume of saline for 20 days. No differences in body weight or food consumption could be detected during two months of observation. The same negative results were obtained when 4 groups of 10 mice were treated with 0.17 µg/d pGlu-His-GlyOH or saline.

A relationship between fat and gonadotrophin secretion is well established (Frisch & Revelle 1970; Knuth et al. 1977; Jeuniewicz et al. 1978). Various mechanisms controlling food intake and body weight have been suggested (Novin et al. 1976). Hypothalamic nuclei play an important part in the regulation of energy balance as well as reproductive changes. As close anatomical and functional relations exist (Kennedy & Mitra 1963) it is not surprising that menstrual dysfunctions are frequently encountered in very thin or obese women (Russell & Beardwood 1970).

The question whether changes in body weight interfere with reproductive cycles due to an unidentified peripheral signal or whether primary central dysfunctions manifest in weight changes as well as amenorrhoea cannot be answered conclusively at the moment. Therefore the report of a tripeptide isolated from urine of anorectic women causing mice to decrease their food intake seemed to offer a chance to investigate underlying mechanisms (Trygstad et al. 1978). The present study was performed in rats in order to reproduce the described effects of pGlu-His-Gly seen in mice.

Materials and Methods

Methods
Female virgin Wistar rats (SPF, Hageman GmbH, D4923 Extertal) weighing 210–220 g served as experimental animals. The rats were housed individually in wire colony cages and maintained on free access of food (Altromin standard chow) and tap water. The colony temperature was 21°C with a humidity of 60%. Lights were on from 7 a.m. to 7 p.m. Vaginal smears were taken daily between 2 and 3 p.m.

For two others experiments two groups of 20 mice (NNR/SPF, Central Institute for Experimental Animals, D3000 Hannover 91) weighing 24–26 g and 28–34 g were housed in groups of five in Macrolon cages under the same conditions as outlined above. No vaginal smears were taken in these animals.

Drugs
For all studies pGlu-His-Gly-OH (Ro 14-61332)1 was used. The peptide was synthesized by classical procedures and showed the following elementary analysis after purification (or correct elementary analysis):

<table>
<thead>
<tr>
<th>C₁₃H₁₇N₅O₅</th>
<th>323.31</th>
</tr>
</thead>
<tbody>
<tr>
<td>calculated</td>
<td>C 48.30 H 5.30 N 21.66%</td>
</tr>
<tr>
<td>found</td>
<td>C 45.28 H 5.56 N 20.10% H₂O 6.05%</td>
</tr>
<tr>
<td>found H₂O free</td>
<td>C 48.20 H 5.20 N 21.39%</td>
</tr>
</tbody>
</table>

1 Kindly supplied by Dr. Gillessen of Hoffmann-LaRoche & Co., Basle.
After standard HCl-hydrolysis the following amino acid ratios were obtained by amino acid analysis taking the glutamic acid value as 1.00: Pyr (1.00); His (0.98); Gly (0.97). The compound was homogeneous as judged from thin layer chromatography in three systems and from paper electrophoresis at pH 2.0 and 6.0 using chlorinetolueene and Pauly staining. The substance was solved in 0.9% saline in concentrations outlined below. For each injection period fresh solutions were prepared and stored at 4°C.

Procedure
The animals were weighed once a week and a defined supply of food was provided. After 7 days the remaining food was weighed again. The mean consumption per day and animal was computed from the difference. No correction was made for spillage.

Experiment I
After a fortnight of regular oestrous cycles groups of 7 rats were formed according to oestrous phase. One group was injected sc with 10 μg in 0.2 ml NaCl for 20 days, the other group served as control receiving only the carrier. Following an interval of 25 days another injection series with 10 times the dose was started applying 100 μg in 0.2 ml NaCl sc. Fourteen days later the animals were sacrificed. The weights of uterus and ovaries were determined after blotting on filter paper.

Experiment II
Ten mice were injected with 0.17 μg in 0.1 ml NaCl sc for 20 days. Another 10 animals served as control.

Weight development and food intake was controlled for 64 days at 5 day intervals.

Experiment III
The same experiment was performed with younger mice.

Statistics
Student's t-tests were used to compare the means at the 5% level.

Results
Increase of body weight in both groups of rats is shown in Fig. 1. Although weights were similar at the beginning of the study, a difference of 6.7 g and 6.6 g was observed during two weeks of control before the experiment started. However, during the course of the injection the profiles of weight development exhibited comparable oscillations without any significant differences. No effect of pyroGlu-His-Gly could be detected.

The differences of body weight observed before the application of the test substance are reflected...
by a higher uptake of food during that period in the control group. During the time pGlu-His-Gly was injected, uptake of food was the same in both groups. Two weeks after the first injection period we observed a slight increase of food consumption during 14 days in the control group without leading to differences of body weight. For the time of observation all rats showed an unaltered 4-day oestrous cycle. The mean weight of the uteri in the experimental group was $0.89 \pm 0.14$ (sd) g vs $0.96 \pm 0.08$ g, an insignificant difference when tested at the 5% level.

Weight development in mice is depicted in Fig. 2. During the injection period body weight increased in a similar manner in both groups. Although a decrease in weight was observed in both groups after stopping the injections, no consistent difference attributable to Glu-His-Gly could be detected. The repetition of this experiment with mice starting at a weight level of 25 g gave the same negative result and is not shown in detail.

**Discussion**

A dose of 1 mg/kg body weight of pyroGlu-His-Gly applied sc had no effect on food consumption and body weight in rats when compared to a control group treated with saline alone. Even an increased dose of 10 mg/kg did not cause a detectable effect.

This is in accordance with reports by Knoll (1979), who reported that the tripeptide did not prevent starved rats from eating when administered in doses as high as 15 mg/kg iv.

As these results contradict the original report by Trygstad et al. (1978) the experiments were repeated under the original conditions using mice as subjects in order to avoid species-dependent differences in sensitivity as reported for goldthio-glucose (Mayer & Marshall 1956). The same absolute amount of the synthesized pyroGlu-His-Gly was injected via the originally reported route. However, the reported refusal of food, which was expected to start 4 days after the application, did not occur. A decrease of body weight from 35 to 21.5 g after one month of treatment, as described, could not be detected either. Although there was a decrease of weight after stopping injections, it appeared in the control groups too and had no significant relevance to the test substance.

There is no apparent reason for the discrepancies in the outcome of the experiments. In contrast to the original study, where an ammonium bicarbonate buffer of pH 8.5 was used, our solvent was saline 0.9% as in the work of Knoll (1979). However it appears unlikely that this might have
effected the activity of the tripeptide as it obviously showed a stable confirmation during the original experiments by its inferred central-nervous action, although injected sc.

Nevertheless, our results demonstrate that pyro-Glu-His-GlyOH given sc does not offer the chance to study the relationship between body weight and oestrous function.

Acknowledgments

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References


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