HUMORAL CONTROL OF APPETITE: A URINARY ANOREXIGENIC PEPTIDE. CHROMATOGRAPHIC PATTERNS OF URINARY PEPTIDES IN ANOREXIA NERVOSA

By
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ABSTRACTS

A “family” of peptides which produced metabolic or behavioural effects following injections into mice or rabbits had been isolated from the urine from patients with the hypothalamic syndrome congenital, generalized lipodystrophy. Anorexia nervosa is associated with hypothalamic disturbances. Precipitates from urine specimens from 25 patients diagnosed as anorexia nervosa were chromatographed on Sephadex G-25 gel columns, and could be divided into 4 different patterns: One was similar to that for normal controls (4), one similar to that observed for patients with schizophrenia (6), 5 patients with a hysteriform type of neurosis had a third form of pattern, and 10 girls considered to have a primary “hypothalamic” type of anorexia nervosa also had typical chromatograms. Fractions influencing appetite in mice were found in the latter group, only.

Two peptides influencing appetite were purified through several steps of chromatography. The sequence of the anorexigenic peptide was verified by the synthesis of the tripeptide, pyroGlu-His-GlyOH. A total dose of 12 nmole of this peptide injected daily over 20 days induced food refusal in mice with reduction of food consumption from 5.7 to about 3 g per day, and the mean body weight decreased from 35 to a minimum of 24.1 g. Food consumption did not normalize until 6 months later, at the same time body weights increased to the same level as in the controls. An appetite stimulating peptide increased the daily consumption of food to more than 10 g, and the mean body weight increased to 57.2 g. We provide evidence for the existence of a hypothalamic form of anorexia nervosa, and that the appetite may be regulated by humoral factors. Screening of peptides present in the urine from patients with anorexia nervosa may give diagnostic and therapeutic information.
There is evidence for neurohumoral control of the endocrine pancreas and appetite (Martin 1978). Destruction of the ventromedial hypothalamus (Rohner et al. 1977), or stimulation of the lateral hypothalamus (Parameswaran et al. 1977) induced overeating in rats, probably mediated by increased release of insulin. Stimulation of one rat of a parabiotic pair, inhibited food intake in the other to a point where it stopped eating, lost weight and showed aversion to food. These observations suggested that a substance was released in the fat animal which inhibited the intake of food in the thin animal.

We have previously isolated a crude peptide fraction from urine from patients with congenital generalized lipodystrophy. This fraction produced lipoatrophic diabetes when injected into mice or rabbits (Foss & Trygstad 1975). This state in the animal model was completely reversed by treatment with fenfluramine (Trygstad & Foss 1977). The human disease is considered to be the result of deranged hypothalamic function (Seip 1971; Berge et al. 1976). We propose that such disorders are the result of transmitter disturbance with hyperfunction of the hypothalamic “gland” leading to overproduction or release of peptides synthesized in hypothalamic neurosecretory neurones, with an overflow to body humours and urine.

The aim of the present work was to study urinary peptides from patients with anorexia nervosa. The cause of this disease is unknown, but it is generally accepted that patients with anorexia nervosa have a disturbed hypothalamic pituitary function, with a variety of endocrine abnormalities due to irregular secretion of hypophysiotrophic hormones. However, whether these abnormalities are secondary to the undernutrition, or of primary pathogenetic importance, is a matter of dispute. We observed different chromatographic patterns of polypeptides present in the urine from patients diagnosed as having anorexia nervosa. This gave evidence of biochemical disturbances, and indicated the existence of a primary “hypothalamic” form of anorexia nervosa with increased production and release of peptides regulating appetite.

MATERIALS AND METHODS

All chemicals were of reagent grade.

A total of 25 patients with anorexia nervosa were referred to the Section of Paediatric Endocrinology, Rikshospitalet, Oslo, because of secondary amenorrhoea (15), delayed puberty (6), or growth disturbances (4). There were 24 females aged 12.5–19 years, and one male, 13 years of age. None of the patients was on drug treatment, and all were in a relatively stable phase of the disease. The patients had been followed for up to 4 years, and from most of the patients urine specimens had been obtained several times, both in periods of positive energy balance and exacerbation. The clinical picture agreed well with the diagnostic criteria given by Feighner et al. (1972) (Table 1).

Urine from each patient was collected in 24 h specimens. Protein present in the specimen was precipitated according to the method described previously (Foss & Tryg-
Table 1.
Clinical features in 24 girls and 1 boy diagnosed as having anorexia nervosa.

1. Age of onset: 11-17 years, mean age 14.4 years
2. Anorexia, with weight loss of 24-50 %
3. Amenorrhea: secondary in 15, primary in 9
4. All with typical psychopathology:
   denial of illness
   enjoyment in losing weight
   distorted body image
   unusual hoarding or handling of food
5. Most of these manifestations:
   overactivity
   cold and cyanotic extremities
   lanugo
   bradycardia
   bulimia and vomiting
6. No known somatic illness
7. No other observed psychiatric disorder

stad 1975). The initial separation of the urinary peptides was performed on a Sephadex G-25 Fine gel column (3 x 130 cm). The precipitate was dissolved in 30 ml 0.1 M ammonium bicarbonate pH 8.5, centrifuged and the supernatant applied on the column which was run with the same buffer at a flow rate of 12 ml/h. The absorbancy was read at 280 nm, and each protein peak was tested biologically. Fractions which produced changes in the consumption of food in mice following subcutaneous injections were processed further by 8 high resolution systems including partition chromatography, gradient elution ion exchange chromatography, and adsorption chromatography (Reichelt et al. subm. for publ.). Sequencing of the purified factor and its synthesis were performed by conventional methods (Johansen et al., subm. for publ.).

Each step of the purification and the synthesis were followed by testing aliquots of each fraction for biological activity in mice. Groups of 5 female albino mice (white lable osloensis), 3 months of age, were caged together and fed chow pellets ad libitum. The consumption of food was calculated by daily weighing the food. The animals were weighed daily during the period of injection, later once a week after an overnight fast. The fractions to be tested, and corresponding fractions from control urines were injected subcutaneously daily for 20 days. The material to be tested consisted of a total dose of 2 mg per mouse of the fraction obtained from the initial Sephadex G-25 gel filtration. The purified or synthetic material was given in a total dose of 12 nmole, i.e. 3.4 μg per mouse.

The consumption of oxygen was determined in the morning after the overnight fast by keeping each animal at constant temperature on a grating in a desiccator containing sodium hydroxide solution in the bottom. The desiccator was connected to a manometer.
and a syringe with oxygen for maintenance of constant pressure for the registration of the oxygen consumption.

The rectal temperature of the animals was taken on alternate days with an Electro-TEMP (Model ET-1, Neptune, New Jersey).

The total body fat content of the mouse was determined, following decapitation, by decomposition of the whole mouse in 40 ml boiling 33 % potassium hydroxide in 160 ml ethanol containing 0.4 % amylalcohol under reflux. Fat was extracted into 200 ml petroleum ether after the addition of 68 ml 25 % hydrochloric acid, and determined gravimetrically after evaporation of an aliquot.

RESULTS

Sephadex G-25 gel filtrations of the urine precipitates obtained from patients diagnosed as having anorexia nervosa gave consistently 4 different patterns of chromatograms, Fig. 1 A-D, which was reproducible for each patient and within each group.

Pattern D was similar to that observed for the precipitate from normal control urine. There was an initial peak (I) appearing at the void volume of the column, corresponding to an elution volume of 300–380 ml, a second small

![Graph of Sephadex G-25 Gel Filtration Column](image)

**Fig. 1.**

Gel permeation chromatography of precipitates form urine samples from 25 patients diagnosed as having anorexia nervosa. Absorbancy at 280 nm showed 4 different patterns of chromatograms, A-D.
peak (II) was eluted between 620 and 770 ml, a third peak (III) between 800–940 ml, and a broad final peak (IV–V) between 1170 and 1700 ml. This pattern was observed for 4 girls considered to have a mild anorexia, as their anorexia vanished after a few consultations and did not reappear. This group is regarded as mixed. One of the girls had Turner's syndrome, and another developed rheumatoid arthritis.

In pattern A, the most retarded peak was absent, and that corresponding to peak III was very large and eluted between 650 and 950 ml. The fractions eluting between 800 and 940 ml contained the activity which produced food refusal in mice, whereas the first half of the peak produced overeating and obesity. None of the corresponding regions from pattern B, C or D induced changes in feeding behaviour. Pattern A was observed for 10 patients considered to have the "hypothalamic" type of anorexia nervosa. This pattern, with a large peak III was similar to that found in hypothalamic obesity (to be published). These girls tended to have resistant anorexia. Two improved on treatment with a dopamine accelerator, Trivastal® (Pharmacodex, München, i.e. piribedil), but they refused further treatment. The girls were intellectually very well equipped, with high standards for themselves, phrenetically tense and hyperactive with denial of illness, and a completely distorted body image. In 5 who had regained normal body weight the amenorrhea persisted for years, and 4 of the patients developed hypothyroidism regarded as secondary to a hypothalamic dysfunction.

Pattern B was similar to that observed for the urine precipitate from children with autism, and resembled that observed for adults with schizophrenia (to be published). This pattern was observed in 6 girls. Their weights were more variable. They were silent, withdrawn and demonstrated some emotional aplanation and introversion. Two of these patients were recently treated with pimozide and responded well. Treatment of mice with fractions of peak III of this pattern (B) induced an extrovert aggression against those handling the mice, but there was no fighting in the cages. Injection of material from peak V induced some passivity in the animals with reduced consumption of food, but insignificant loss of weight.

The last group of 5 patients, pattern C, had urinary chromatograms where the most retarded peak was unusually large. These patients had a hysteriform and malevolent behaviour. The only boy belonged to this group; he was referred because of tall stature.

The distribution of chronological age at the onset of the clinical picture of anorexia nervosa was similar for all the four groups (Table 2). However, the mean age of patients with pattern A was lowest, 13.8 years. In addition, this group had delayed biological development. Seven of the girls were referred because of delayed growth and development, only 3 had secondary amenorrhea. Among 13 patients with urinary patterns B, C or D, 12 had secondary
Table 2.
Developmental data in 25 patients with anorexia nervosa with different patterns of their urinary peptide chromatograms.

<table>
<thead>
<tr>
<th>Pattern</th>
<th>n</th>
<th>Age of debut (years)</th>
<th>Menarche</th>
<th>Age at menarche (years)</th>
<th>Height at debut (cm)</th>
<th>Minimum weight (kg)</th>
<th>Loss of weight (%/a)</th>
<th>Referred because of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Amenorrhoea</td>
</tr>
<tr>
<td>Pattern A</td>
<td>10</td>
<td>13.8 ± 1.99</td>
<td>3</td>
<td>13.7 ± 1.15</td>
<td>154.2 ± 9.93</td>
<td>30.2 ± 6.50</td>
<td>36.7 ± 7.68</td>
<td>3</td>
</tr>
<tr>
<td>Pattern B</td>
<td>6</td>
<td>15.5 ± 1.32</td>
<td>6</td>
<td>12.9 ± 0.42</td>
<td>169.8 ± 3.27</td>
<td>38.0 ± 7.07</td>
<td>43.5 ± 9.19</td>
<td></td>
</tr>
<tr>
<td>Pattern C</td>
<td>51</td>
<td>14.1 ± 1.45</td>
<td>4</td>
<td>12.7 ± 0.65</td>
<td>164.3 ± 8.39</td>
<td>37.3 ± 5.51</td>
<td>40.0 ± 5.56</td>
<td></td>
</tr>
<tr>
<td>Pattern D</td>
<td>42</td>
<td>14.8 ± 2.36</td>
<td>2</td>
<td>14.0</td>
<td>164.8 ± 12.42</td>
<td>40.0 ± 10.15</td>
<td>26.7 ± 7.77</td>
<td></td>
</tr>
<tr>
<td>Patterns B, C, D</td>
<td>13</td>
<td>14.4 ± 1.55</td>
<td>12</td>
<td>12.9 ± 0.88</td>
<td>168.7 ± 6.42</td>
<td>39.86 ± 6.12</td>
<td>37.8 ± 6.73</td>
<td></td>
</tr>
</tbody>
</table>

1) Includes one boy.

2) Includes one girl with Turner’s syndrome.

3) Excluding the boy, and the girl with Turner’s syndrome.
amenorrhoea (the boy and the girl with Turner's syndrome are excluded in this connection). The girl with primary amenorrhoea belonged to the mixed group, she was 12.3 years old, had a bone age of 14, and was referred because of tall stature. The groups are too small for statistical evaluation.

In vivo studies in duplicate groups of female mice showed refusal of food 4 days after the start of injections of 0.1 mg daily of the heterogenous material in the peak eluting from 600 to 950 ml of pattern A (Fig. 2). The consumption of chow pellets was at a minimum of 2.5 g per day 2 weeks after the period of injections, whereas the control animals consumed an average of 5.7 g per day. During the next 3 months the daily consumption of food increased gradually to a mean of 10.1 g. The mean body weight for the 10 female mice changed

![Graph](image)

**Fig. 2.**

The effect on consumption of food and body weight of daily subcutaneous injections over 20 days into duplicate groups of 5 female albino mice 90 days of age, of total doses of 2 mg crude control peptide, 2 mg of the initial crude fraction (Fig. 1 A), approximately 3.4 µg of the purified appetite stimulating peptide, and 3.4 µg of the synthetic anorexigenic tripeptide, pyroGlu-His-GlyOH.

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Table 3.
The effects of the biological anorexigenic peptide on food consumption, body weight, oxygen consumption and body temperature. A total dose of 12 nmole of peptide, pyroGlu-His-GlyOH, was given by daily subcutaneous injections into duplicate groups of 5 female mice from day 91 to day 110.

<table>
<thead>
<tr>
<th></th>
<th>Age of mice, days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>85</td>
</tr>
<tr>
<td>Consumption of chow pellets, g</td>
<td>5.6 ± 0.3</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>34.5 ± 0.6</td>
</tr>
<tr>
<td>Consumption of oxygen, l/m²/h</td>
<td>14.3 ± 0.8</td>
</tr>
<tr>
<td>Body temperature, °C</td>
<td>37.2 ± 0.1</td>
</tr>
</tbody>
</table>

Mean ± se
correspondingly and decreased from a mean of 35 g to a minimum of 21.5 g one month after ceasing treatment. Three months later the body weights varied between 54 and 59 g. Thereafter food consumption and body weights remained constant.

The purification of the biological anorexigenic factor is to be published (Reichelt et al., subm. for publ.). The pure factor was characterized as a tripeptide with the structure: L-(pyro)glutamyl-L-histidyl-glycine. The potency had increased 500 times as compared to the initial heterogeneous material, and induced refusal of food just after the start of the injections (Fig. 2, Table 3). The daily consumption of the chow pellets was reduced from the normal mean of 5.7 g to 2.9 g one week after the start of injections. At the end of the period of treatment food consumption varied between 2.4 and 2.6 g, and remained at this level for the next 8 months. Body weights decreased from a mean of 34.5 g to 23.3 g at the end of the treatment period, and varied only slightly throughout the next 8 months. The changes in consumption of oxygen and body temperature are given in Table 3. The motor activity of the mice was registered by an Animex data system (Farad Electronics, Stockholm), and was markedly increased during the period of treatment. It decreased below the normal level during the next couple of months, and continued to be low during the rest of the observation period. The changes corresponded well with the transitory increased consumption of oxygen and elevated body temperature, and later the reduction of these below control levels (days 111, 118 and 210, Table 3). The consumption of food was constantly low. On decapitation, atrophy of the genital organs particularly of the ovaries and the uterus was observed. The mean ovarian weight was reduced from 65 mg for controls to 22 mg.

The synthetic tripeptide pyroGlu-His-GlyOH was observed to produce changes quite similar to those produced by the purified native peptide, although an analogous dose gave a less pronounced refusal of food and decrease in body weight (Fig. 2). This difference probably is a matter of dosage, due to inaccuracy in the calculation of the dose of the native peptide. After the period of treatment, the consumption of food varied from 2.9 to 3.9 g per day, and the body weights varied between 24.1 and 26.0 g. However, 7 months after start of treatment the consumption of food and the body weight increased to the normal level. The synthetic peptide produced similar changes in the consumption of oxygen, body temperature and motor activity as observed for the native peptide. These features normalized when the body weight increased.

A peptide producing overeating and development of obesity was obtained from the original crude "anorexigenic" peak (Fig. 2). The structure of this factor has not yet been determined. A total dose in the range of 10–12 nmole induced a small transitory decrease in food consumption from 5.8 to 4.6 g per day during the period of treatment, and a corresponding decrease in the body weight from 34.8 to 31.1 g. After this period, consumption of chow pellets in-
creased and varied between 10.1 to 10.8 g per day 4 months later, compared to 5.7 g for the controls. The mean body weight increased to 57.2 g 7 months after the end of treatment. At decapitation the total body fat content was 49.8 °/o, for control mice it was about 17 °/o, and 6–7 °/o for mice showing food refusal and body weights of 22 g.

Rabbits and pigmented mice were also treated with the anorexigenic peptide and developed food refusal and reduction in weight.

**DISCUSSION**

A peptide fraction producing lipodystrophy or lipoatrophic diabetes on injection into mice and rabbits, has previously been isolated from urine from patients with congenital generalized lipodystrophy (Foss & Trygstad 1975). We recently isolated and characterized three small peptides from the same urine inducing aggression in a stable mouse hierarchy, hyperinsulinaemia in mice (Reichelt et al. 1977), and an increase in serum testosterone in mice (in press). We have now isolated two peptides from urine from patients considered to have a "hypothalamic" type of anorexia nervosa. The tripeptide pyroGlu-His-GlyOH which produced food refusal in mice was synthesized and the structure confirmed. The other factor produced overeating and obesity in mice, and is not yet fully characterized. Thus a new system of short chain peptides, which control metabolic as well as behavioural processes, has been exposed. These peptides seem to be related structurally to hypophysiotrophic factors produced in the hypothalamus. It is probable that the central nervous system controls major processes in a living organism. This control can be maintained by stimulatory or inhibitory signals, neurogenic as well as humoral. The factors isolated from the urine are assumed to be humoral messengers, synthesized in the central nervous system, probably in the hypothalamus, and seem to be different from the peptides originating from the lipotrophins, the endorphin or enkephalin group. The factors are like releasing hormones, very potent.

We believe that in a normal organism, the humoral positive and negative control mechanisms are in a state of balance at a low level, and the factors are excreted into the urine in concentrations too low to be detected by common *in vivo* assay systems. An increased peptide production, and the release of one humoral signal from neurosecretory cells seems to increase the production/release of other signals related to a particular control mechanism. It is generally accepted that the hypothalamus is the link between the brain and the body, and controls several vital functions including the appetite. Mayer (1969) showed that cerebrospinal fluid withdrawn from the third ventricle of hungry monkeys increased eating on infusion into the lateral hypothalamus of satiated monkeys. Anand (1972) suggested the presence of hypothalamic substances which stimu-
lated the feeding centre and eating behaviour in hungry animals, and a similar mechanism which activated the satiety centre in animals full of food. Abnormal changes in the production or degradation of these substances could lead to failure to recognize nutritional needs.

Anorexia may be a symptom related to an organic disease, and Theologides (1976) proposed that low molecular weight metabolites produced by cancer cells (ectopically?) induced anorexia through a direct effect on the hypothalamus. Moreover anorexia may be secondary to mental disorders such as schizophreniform or psychopathic states, primary depressions, phobic anxiety, hysterical neurosis, or even mild neurosis. According to our experience the chromatographic pattern of the urinary peptides may be helpful in diagnosing anorexia nervosa, and consequently of therapeutical importance.

Anorexia nervosa is usually observed in adolescent girls, and our sex distribution of 24:1 is fairly common. The main symptom was progressive self-imposed starvation, resulting in loss of body weight, often of more than 30%. Amenorrhoea was always present, often primary, and hypotrophy or atrophy of the genital tract, as observed in the mice, was present. In “hypothalamic” anorexia nervosa, the amenorrhoea could precede the refusal of food, and a functional anterior hypothalamic defect has been discussed (Katz et al. 1977). Evidence for a hypothalamic disease has been given by a secondary hypothyroidism (Croxon & Ibbertson 1977; Miyai et al. 1975), a transitory growth hormone deficiency (Trygstad 1977), occasionally elevated growth hormone and prolactin levels (Mecklenburg et al. 1974), increased concentrations of serum cortisol (Boyar et al. 1977), an abnormal feedback on ACTH secretion (Takahara et al. 1976), partial diabetes insipidus, and an increased sensitivity to insulin with hypoglycaemia (Mecklenburg et al. 1974). A hypothalamic disturbance was also indicated by abnormalities in cholesterol metabolism, thermoregulation, and bradycardia (Lupton et al. 1976). The symptoms suggest a disturbance of neurotransmitter regulation, in which dopamine appears to play a central role in the pathophysiology of most of the major symptoms of primary anorexia nervosa. Therapeutically, L-DOPA has been used successfully in some patients (Johanson & Knorr 1974). It may be of interest that the dopamine blocker, pimozide, was also effective in two of our patients. The origin of an anterior hypothalamic defect remains obscure, and it is unknown whether it precedes, coincides with, or is a consequence of the illness. It is even possible that the manifest illness and the functional defect are a consequence of a third as yet undetermined variable. In “hypothalamic” anorexia nervosa we propose a genetic predisposition, and that the clinical features are provoked by a variety of means, at different ages depending on the degree of the biochemical disturbance.

The peptide isolated from the urine from patients with “hypothalamic” anorexia nervosa produced refusal of food which persisted for one year in mice,
whereas the mice treated with the synthetic peptide reversed to an increase in food consumption and normalization of body weight and temperature 6 months after the period of treatment. It is obvious that the food refusal peptide of human origin suggests itself as a biological anorexigenic agent, and will be further evaluated for its potential use in the treatment of overeating and obesity.

The mechanism of action of the anorexigenic peptide is not established. The atrophy of the genital organs is probably secondary to the refusal to eat. The changes observed in body temperature and activity may suggest a direct effect on the hypothalamic transmitters or on cell receptors with prolonged – chronic inhibition of the lateral feeding centre or a lasting stimulation of the ventromedial satiety area. It has been demonstrated that these areas of the hypothalamus control insulin and glucagon secretion from the endocrine pancreas (Martin 1978). However, we have observed no changes in serum insulin or glucagon. A pancreatic polypeptide which has been observed to reduce food intake and to initiate reduction of body weight in the hyperphagic obese mouse has been isolated (Malaisse-Lagae et al. 1977). It cannot be excluded that our tripeptide may act through release of this peptide from the pancreatic endocrine cells.

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