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Contribution to Characterization of Anorectic Protein Similar to Satietin From Urine

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MALETÍNSKÁ, L. AND J. SLANINOVÁ. *Contribution to characterization of anorectic protein similar to satietin from urine.* PHARMACOL BIOCHEM BEHAV **52**(4) 715–721, 1995.—An anorectic substance similar to satietin was purified from ethanolic-acid precipitate of rat urine by gel filtration and ion exchange chromatography. The obtained glycoprotein (molecular mass 67 kDa) reduced food intake after intraperitoneal and intracisternal administration in mice in a dose-dependent manner (in the range of 5–80 mg/kg). The anorectic effect was specific. In the presence of sodium dodecylsulphate the substance dissociated into small proteins with a molecular mass of 9–11 kDa. Its anorectic effect was long lasting and differed from that of the anorectic peptide cholecystokinin octapeptide. Our finding supports the idea that anorectic protein isolated from plasma, membranes, and urine may be related substances.

Anorectic substance Rat urine Anorexia Satiety Mice Glycoprotein

REGULATION of food intake is a complex process involving central feeding and peripheral satiety systems, and which is affected by the composition and accessibility of food and psychological events (28). The feeding of mammals is a discontinuous process in which periods of food intake alternate with noneating. Appetite involves qualitative aspects of eating and changes in choice of food as well as adjustments of the overall amount (7).

Numerous agents have been shown pharmacologically to affect food intake. These include among others peptides as cholecystokinin, bombesin, and opioids (25,26), monoamines (2,10), and other substances.

During studies on the physiologic basis of hunger, a very potent endogenous satiety agent, satietin, was discovered in human and animal serum (17,18). Similar substances have been studied in feces (17) and red blood cell or liver membranes (13,14) of human or animal origin. Recently, plant membrane proteoglycan, which causes anorexia in rats, was isolated (15).

Another anorectic substance was isolated as ethanolic-benzoic acid precipitate of rabbit (30) or human (9) urine, fat-mobilizing substance (FMS). Properties of the substance were further investigated after purification from rat urine (3,4). It was observed that the substance reduces food intake for periods longer than known peptide hormones (for >24 h) without effect on water intake. The substance was thermostable. The amount of the substance in urine was shown to be dependent on the food composition, temperature, and daily routine of rats from which the urine was collected (3).

Recently, the research on further purification of this substance was reported. Ako et al. (1) used several biochemical methods and obtained partially purified 50-kDa anorexigen protein with long-lasting effects on food intake in rats. However, during isolation, all fractions showed some anorectic activity.

In this study we tried to purify the anorectic agent from urine and to examine its structure and function. The main aim was to compare biological properties of this protein with that of the well-known anorectic peptide hormone cholecystokinin octapeptide in optimized and standardized biological tests.

METHODS

Animals

Male Wistar rats (180–250 g), male NMRI-mice (25–30 g), and male guinea pigs (300–350 g) were obtained from the Institute of Pharmacy and Biochemistry (Konárovice, Czech Republic).

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Chemicals

The following chemicals were obtained as indicated: Sephadex G-75 and a calibration kit for gel filtration from Pharmacia (Uppsala, Sweden); the molecular weight protein standard kit was from Sigma (St. Louis, MO); acrylamide, Bis, bovine serum albumin, sodium dodecyl sulfate (SDS), and *N',N'*-tetramethylethylenediamine (TEMED) were from Serva (Heidelberg, Germany); periodic acid, Schiff's reagent, and 8-anilinonaftalensulphonic acid were from Merck (Darmstadt, Germany); the rest of chemicals were from Lachema (Brno, Czech Republic). Cholecystokinin octapeptide was purchased from Serva.

Purification of Aanorectic Protein

All operations were carried out at 4°C.

Preparation of crude protein fraction. Urine was collected from normally fed rats and stored at −20°C. Crude protein fraction was isolated by the method of Chalmers et al. (9) and Beaton et al. (4) with minor modifications. Proteins were precipitated by ethanol with benzoic acid and diluted in 50 mM $(NH_4)_2CO_3$, and then ultrafiltered through a 10-kDa membrane (Amicon) under N_2.

Gel-filtration chromatography. The crude protein fraction (solution containing 400–500 mg protein, maximal vol. 40 ml) was applied to the Sephadex G-75 column (4.5 × 65 cm) equilibrated with 50 mM $(NH_4)_2CO_3$. The column was eluted with the same solution at a flow rate of 0.5 ml/min. Fractions obtained were pooled, ultrafiltered through a 10-kDa membrane, and freeze-dried.

High-performance liquid chromatography (HPLC) ion-exchange chromatography. The fraction obtained from the gel-filtration with anorectic activity was further purified using anion exchange Mono-Q column (Pharmacia, 5 × 50 mm) in an HPLC system (Waters, Millipore Corp., Milford, MA). The column was equilibrated with 20 mM Tris-HCl buffer, pH 8.5. The sample (1 mg protein) was diluted in the same buffer. Elution was performed with a linear gradient of 0–0.5 M NaCl in an equilibrating buffer at a flow rate of 2 ml/min. Fractions of major protein peaks were collected, ultrafiltered as previously described, and freeze-dried.

Gel filtration in the presence of SDS. The active fraction obtained from gel filtration was purified by gel filtration on a Sephadex G-75 column (4 × 50 cm) in the presence of 1% SDS. The same buffer was used both for equilibration and elution (0.02 M buffer Tris-HCl, pH 8.5, with 0.1 M NaCl and 1% SDS), at a flow rate of 0.5 ml/min. Before use, the samples were incubated with SDS [100 mg protein in 30 ml of 15% SDS (wt./vol.)] overnight at 4°C. Two fractions were collected. SDS was removed by acetone precipitation (12) and the resulting fractions were freeze-dried.

Analytical Assay

Fractions of anorectic protein were analyzed using following methods:

SDS-polyacrylamide gel electrophoresis (PAGE). SDS-PAGE was carried out in the discontinuous buffer system of Laemmli (20) in the presence of 0.1% SDS, using 10–20% acrylamide gradient gel. Molecular mass of protein components was determined by comparison with the protein calibration kit. The gels were stained with Coomassie brilliant blue and scanned using a Beckman DU 65 spectrophotometer (Fullerton, CA) and corresponding software.

PAGE without SDS. The electrophoretic mobility of substances was studied also in the system described earlier, without SDS. The sample buffer contained 0.1 M Tris-HCl (pH 6.8), 20% saccharose, and dissociating agent (8 M urea or 10% mercaptoethanol). Electrophoresis was performed in the presence of 4 M urea or 5% mercaptoethanol in gels.

HPLC gel filtration. Gel-filtration chromatography on Superdex 75 column (Pharmacia, 10 × 100 mm) in an HPLC system (Waters) was used as a second method for molecular mass determination. Column was equilibrated and eluted using 20 mM Tris-HCl buffer (pH 8.5) with 0.1 M NaCl at a flow rate of 0.5 ml/min. Sample volumes of 25 μl contained 25 μg protein. For molecular mass determination, we constructed a calibration curve of calibration gel filtration proteins. The peak area was evaluated by corresponding software.

Detection of glycoproteins. For the detection of carbohydrates, the gels after SDS-PAGE were Schiff-stained by the method of Glossmann and Neville (11). Ovalbumin was used as a standard.

Protein determination. Total protein was determined according to Lowry et al. (23) using bovine serum albumin as a standard.

Amino acid composition. Amino acid analyses were performed on a D-500 analyzer (Durrum, Dionex Corp., Sunnyvale, CA) after the hydrolysis of samples at 110°C for 20 h in the presence of 6 M HCl.

Statistical analysis. The results are expressed as mean ± SD. Comparison was made with appropriate controls employing Student's *t*-test. Differences were considered significant at $p < 0.05$.

Biological Assay

Biological activities in the course of protein isolation were determined by the following tests:

Anorexia. Anorectic activity was assayed in the test used routinely for determination of cholecystokinin analog effect as previously described (24). For 5 days, mice (in a collective cage) or rats (in individual metabolic cages) had free access to food from 0800–1600 h and were fasted overnight. They had free access to water during the whole 24-h period. On the 6th day, animals were injected (IP) with the compound to be tested (0.2 ml/animal) and placed in individual cages. After 10 min following the injection, a weighed amount of food (pellets) was given to the animals. Then, the food was withdrawn at 30-min intervals, weighed, and again added into the cage. Rats had their cages specially arranged for measuring the consumed quantity of food by the decrease of pellets in the container. The amount of food consumed was monitored for 8 h. The results were compared with those of control group (saline-treated, 100% of consumed food). In addition to IP administration, mice were also injected intracisternally (29) using specially arranged needles on Hamilton syringes (vol. of 10 μl/mouse).

Other biological activities. Selected protein fractions were tested for sedative activity in mice [antirearing activity (31)], ability to contract guinea pig gall bladder or ileum in vitro (22), and ability to influence rat blood pressure (8).

RESULTS

Purification and Biochemical Characterization of Protein.

After repeated precipitation using ethanol with benzoic acid, the pooled fractions were dissolved in 50 mM $(NH_4)_2CO_3$. Because it was necessary to remove the salts and concentrate the samples for in vivo tests, ultrafiltration

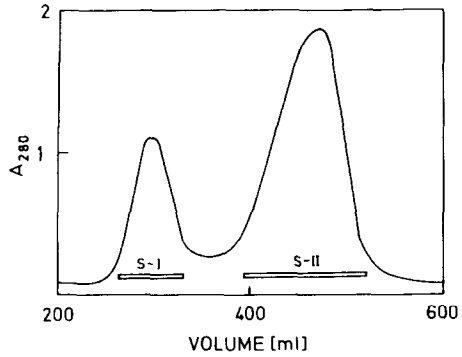

FIG. 1. Sephadex G-75 gel filtration of crude protein fraction. Column (900 ml) was eluted as indicated in Methods. Fractions S-I and S-II were collected for further analyses.

through a 10-kDa membrane was employed. The protein loss amounted to <3%. The yield of protein in the crude protein fraction constituted 1-1.5 g/1 l urine per batch.

Crude protein fraction was chromatographed on a Sephadex G-75 column and two main fractions (S-I and S-II) (Fig. 1) were obtained. Table 1 shows the yields of proteins of different molecular masses in the two fractions analyzed by two different methods (i.e., SDS-PAGE and gel filtration on a Superdex column in an HPLC system). Figure 2 demonstrates a densitometric evaluation of SDS-PAGE. In an SDS-PAGE system, i.e., under dissociating conditions (presence of SDS, mercaptoethanol), fractions were dissociated to smaller fragments in comparison with the Superdex gel filtration method.

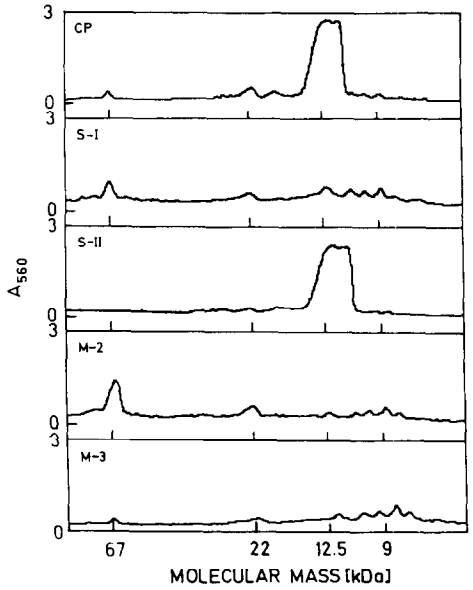

FIG. 2. Denzitometric scanning of SDS-PAGE gels. Isolated fractions were analyzed by SDS-PAGE in the Laemmli system (18) (see also Table 1).

Table 2 shows the results of an anorectic assay in mice after IP administration of samples. Fraction S-I, containing high-

TABLE 1

MOLECULAR MASS OF PROTEINS IN PURIFIED FRACTIONS DETERMINED BY SDS-PAGE (SEE ALSO FIG. 2) AND SUPERDEX 75 IN AN HPLC SYSTEM

	SDS-PAGE		Gel Filtration	
Method of Determination (Fraction)	M_r (kDa)	Quantity (%)	M_r (kDa)	Quantity (%)
Crude protein extract	67-70	1.4	67	15-20
	22.5	6.2	58	5-6
	18-16	5.9	47	0-4
	12.5	60.4	32.5	9-11
	11.5	20.5	25	50-60
	11-9	5.7	10	3-5
S-I	67-70	17.3	70	0-4
	22.5	19.1	67	75-87
	12.5	24.8	58	9-25
	11-9	38.8		
S-II	22.5	1.1	32.5	14-20
	18-6	4.0	25	78-80
	12.5	63.1		
	11.5	31.6		
	11-9	0.3		
M-2	67-70	35.9	67	27
	22.5	17.5	65	26
	12.5	9.5	58	31
	11-9	37.1	47	16
M-3	67-70	4.5	67	100
	22.5	20.5		
	12.5	18.2		
	11-9	56.9		

TABLE 2

ANORECTIC ACTIVITY OF ISOLATED PROTEIN FRACTIONS: EFFECT IN MICE AFTER IP ADMINISTRATION

Fraction	Dose (mg/kg)	Food Intake (40 min After Administration)
Crude protein fraction	40	73.4 ± 33.6
	160	25.3 ± 19.2
	400	2.7 ± 3.9
S-I	10	44.2 ± 4.3
	40	33.9 ± 20.4
	160	5.3 ± 5.3
S-II	20	96.9 ± 22.2
	40	81.0 ± 28.5
	160	52.0 ± 32.8
M-2	2.5	87.0 ± 38.2
	10	48.1 ± 28.7
	40	17.0 ± 11.7
M-3	2.5	100 ± 26.1
	10	67.2 ± 14.7
	40	29.5 ± 21.6

Mean ± SD of at least three separate experiments are given. Food intake is calculated as a percentage of control group food intake (saline-treated).

er molecular mass components (Table 1), significantly suppressed food intake. The effect was dose dependent and long lasting. This fraction was then used for further purification. However, fraction S-II (i.e., the lower molecular weight component) also showed some anorectic activity.

Fraction S-I was purified on an anion exchange Mono-Q column in an HPLC system (using a cation exchange column, no protein was bound). In the pH range of 7.5-9.0 (20 mM Tris-HCl buffer) the elution profile of proteins was identical. Thus, for the following isolation we used 20 mM Tris-HCl buffer, pH 8.5, and a linear gradient of 0-0.5 M NaCl. A typical elution profile is shown in Fig. 3. Collected fractions were designated M-1, M-2, M-3, and M-4. The molecular mass of their protein components is given in Table 1. All fractions contained high amounts of the 67-70 kDa component when analyzed by gel filtration on a Superdex column. Fractions M-2 and M-3 (eluted by 0.2-0.35 and 0.35-0.5 M NaCl, respectively) had different compositions according to SDS-PAGE. With the Laemmli system, M-2 was partially disintegrated, and M-3, completely. The possible dissociation of these fractions in the presence of 6 M urea or 5% mercaptoethanol alone was attempted and the fractions were then analyzed by gel filtration on Superdex or in a PAGE system without SDS. No dissociation of samples was observed.

All fractions obtained from ion exchange chromatography showed anorectic activity. Fractions M-1 and M-4 were present in small quantities. For further analyses, fractions M-2 and M-3 were used. Table 2 shows a dose-dependent decrease of food intake after IP administration of fractions in mice. As can be seen, in the course of purification, the specific anorectic activity increased (Fig. 4).

The anorectic activities of fractions M-2 and M-3 were not significantly different. The dose of protein required to suppress food intake by 50% in comparison with the saline-treated group (ED_{50}) was about 10 mg/kg. Purified fraction of anorectic protein M-2 constituted about 5% of the protein in the crude protein fraction.

We determined the amino acid composition of the M-2 and M-3 fractions. The representation of amino acids in both samples was almost identical. Fractions contained a higher amount of acid amino acids (20%) and a smaller amount of basic amino acids (8%), 30% of nonpolar, and 20% of polar amino acids.

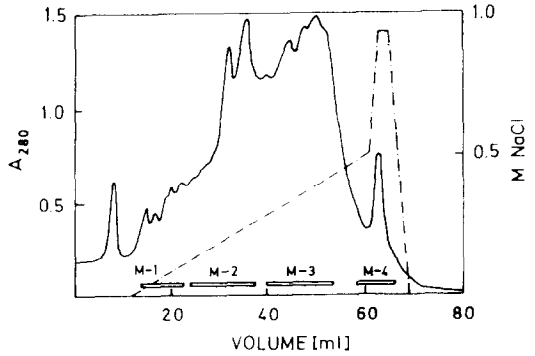

FIG. 3. Anion exchange chromatography of fraction S-I (from gel filtration). A Mono-Q HR 5/5 column was used in an HPLC system and eluted employing a linear gradient of NaCl (— · — · —) as described in Methods. Fractions from eluted peaks were collected for further analyses.

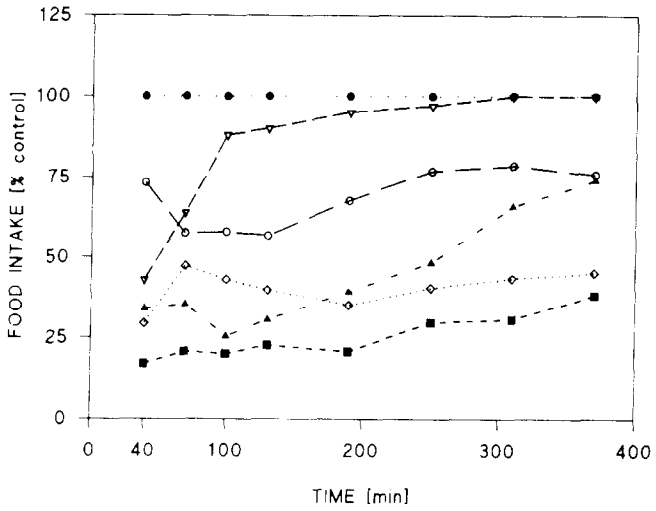

FIG. 4. Anorectic effect of crude protein fraction (○) and fractions S-I (▲), M-2 (■), M-3 (□), and CCK-8 (▽) in mice; cumulative food intake curves. Each fraction was administered IP in doses of 40 mg/kg, CCK-8 in doses of 20 μg/kg. Food intake is expressed as a percentage of corresponding intake of the saline-treated group (●).

The detection of carbohydrates was performed to determine whether glycoproteins were present in different fractions. Schiff's staining after SDS-PAGE showed the existence of carbohydrates in the protein components of fractions M-2 and M-3 with molecular masses of 67 and 9-11 kDa.

Screening of Biological Effects

For further characterization, the M-2 fraction containing a higher amount of nondissociable 67 kDa component was used. Figure 4 compares the cumulative food intake curves of classic

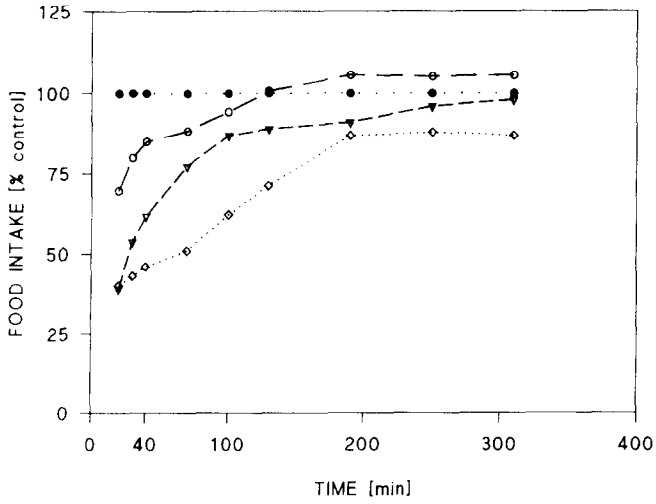

FIG. 5. Anorectic effect of fraction M-2 administered intracisternally in doses of 5 mg/kg (○) and 20 mg/kg (◇) and CCK-8 in doses of 20 μg/kg (▽) in mice; cumulative food intake curves. Food intake is expressed as a percentage of corresponding intake of the saline-treated group (●).

TABLE 3

ANORECTIC ACTIVITY OF ISOLATED PROTEIN FRACTIONS. EFFECT IN RATS AFTER IP ADMINISTRATION

Fraction	Dose (mg/kg)	Food Intake (40 min After Administration)
S-I	10	33.5 ± 3.3
S-II	10	78.8 ± 13.0
M-2	0.625	88.4 ± 16.1
	2.5	53.5 ± 33.4
	10	21.7 ± 29.8

Mean ± SD (five rats per dose) is given. Food intake is calculated as a percentage of control group food intake (saline-treated).

TABLE 5

ANORECTIC ACTIVITY OF FRACTIONS ISOLATED BY GEL FILTRATION IN THE PRESENCE OF SDS IN MICE. DOSES WERE INJECTED IP

Fraction	Dose (mg/kg)	Food Intake (40 min After Administration)
SDS-1	2.5	74.8 ± 16.9
	10	51.4 ± 23.2
	40	20.5 ± 5.3
SDS-2	10	57.8 ± 20.2
	40	22.9 ± 15.3

Means ± SD are expressed as a percentage of food intake of control group (saline treated).

anorectic peptide cholecystokinin octapeptide and protein fractions from urine. The effect of CCK-8 began 5–10 min after IP injection and lasted about 100 min. The effect of the purified protein fractions started slightly later (about 10–20 min after administration of the sample). The food intake of mice did not differ when the first food was given either 10 or 120 min after injection of the sample. In comparison with the course of food intake after the cholecystokinin octapeptide injection, after anorectic protein administration, mice consumed a small amount of food at the beginning and then almost stopped eating for a long time period (8 h and more).

Food intake after the central administration of fraction M-2 was also tested (Fig. 5). Whereas intracisternally administered cholecystokinin octapeptide affected the food intake of mice only slightly, the fraction M-2 from urine following central administration had [on a dose basis (mg/kg)] similar activity as that after IP administration.

Table 3 demonstrates that in rats the anorectic effect of crude protein fraction and fractions S-I, S-II, and M-2 is almost the same as in mice.

Specificity of the anorectic effect of the protein was studied. Fraction M-2 was tested in several biological assays typical for biologically active peptides: for smooth muscle contractions in vitro (guinea pig gall bladder and ileum), blood pressure (rat), and sedative and analgesic activity in mice. All tests were negative in doses of protein that cause anorexia, with the exception of the sedative test. Fraction M-2 caused a significant and dose-dependent sedation in mice after IP administration (Table 4).

TABLE 4

SEDATIVE ACTIVITY OF FRACTION M-2 IN MICE. DOSES WERE INJECTED IP

Fraction	Dose (mg/kg)	Activity (10 min After Administration)
M-2	5	65.3 ± 21.2
	20	39.1 ± 13.4

Means ± SD are expressed as a percentage of rearing of control group (saline-treated).

Gel Filtration in the Presence of SDS.

Fraction S-I was chromatographed on a Sephadex G-75 column in the presence of 1% SDS (Fig. 6). From eluted fractions (SDS-1 and SDS-2) SDS was removed by precipitation in acetone. The protein composition of both SDS-1 and SDS-2 fractions is shown in Fig. 7. A determination of proteins by SDS-PAGE demonstrated differences between the two fractions. Whereas SDS-1 contained only a 67-kDa component, the SDS-2 fraction was dissociated into small proteins with a molecular mass in the range of 9–11 kDa. However, gel filtration on Superdex 75 in the absence of SDS showed, in the case of both fractions, only one peak corresponding to 67 kDa molecular mass protein.

The anorectic activity of SDS-1 and SDS-2 fractions in mice (Table 5) was not significantly different. The ED_{50} value for both fractions was about 10 mg/kg (i.e., the same as in the case of the M-2 fraction).

DISCUSSION

The possibilities that specific anorectic substances exist in organisms and that satietin from plasma and anorectic protein from urine are identical compounds are still open.

Knoll (17) concentrated on discovering the substance supporting his hypothesis that a signal exists in blood which ter-

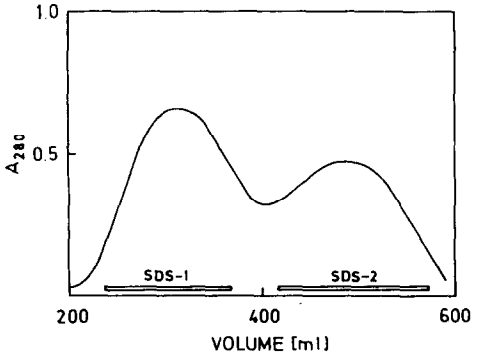

FIG. 6. Sephadex G-75 gel filtration of fraction S-I in the presence of 1% SDS. Column (800 ml) was eluted as described in Methods. Fractions SDS-1 and SDS-2 were collected and analyzed.

TABLE 6

BIOCHEMICAL PROPERTIES OF ANORECTIC PROTEIN FROM URINE

Property	Result
Molecular mass	67 kDa
Glycoprotein	Yes
pH stability	7.0–9.5
Temperature stability	Stable at room temperature
Effect of chaotropic agents	8 M urea or 10% mercaptoethanol – no influence alone
Effect of detergent	In the presence of SDS dissociation to subunits 9–11 kDa (after removal of SDS association)

Molecular mass was determined by gel filtration on Superdex 75 as described in Methods.

minates the hunger drive. His satietin is a substance that is strong candidate for such an anorectic substance: It is present in the blood of different species and is active after both peripheral and intracerebroventricular administration (19). According to Knoll, the satiety center in hypothalamus contains highly specific satietin receptors that, after interaction with satietin, cause the inhibition of food intake and lead to a subjective feeling of fullness (18).

Kidwai and Upreti attempted to determine satietin origin (13). It is known that cell destruction or enhanced cell membrane turnover is common in chronic diseases as cancer of lung, liver, and gastrointestinal tract and leukemia, which are accompanied by the loss of appetite. Their findings suggest that anorectic substances (glycoproteins of membranous origin) circulate in the blood and could be identical to satietin.

Ako et al. (1) suggested the possibility that satietin could be similar to the anorectic protein from urine. We tried further to purify and characterize the protein from urine and to investigate the idea of uniform anorectic protein in organism.

After obtaining the crude protein fraction by ethanolic-acid precipitation, we used gel filtration and ion exchange chromatography for further separation. We started from the presumption that anorectic activity is combined with the high molecular mass component of the crude protein fraction. This hypothesis was confirmed, as shown in Tables 1 and 2 (high molecular mass fractions are designated S-I, M-2, and M-3). However, we also confirmed the observation (1) that anorectic activity is distributed among all fractions during different steps of purification, although to various extents.

The specific anorectic activity of protein fractions increased significantly only after the first isolation step (i.e., comparing crude protein and S-I fraction). However, further purification on the ion exchange column did not enhance activity. Active fractions obtained by gel filtration (S-I) and ion exchange chromatography (M-2) affected food intake comparably (Table 2). Fractions obtained by ion exchange chromatography in contrast to the initial fraction S-I were easily water soluble. The higher content of acidic amino acids in these fractions explained the binding of all of the substances to the anion exchange column.

Fractions M-2 and M-3 were not homogenous. Ion exchange chromatography did not separate fraction S-I into individual peaks (see Fig. 3). Molecular mass determination by gel filtration on Superdex column was different from that obtained by SDS-PAGE (Table 1 and Fig. 2). Whereas analytical gel filtration showed fractions S-I, M-2, and M-3 as proteins with molecular masses in the range 50–67 kDa, SDS-PAGE showed in every fraction the presence of smaller proteins with a molecular mass of 9–11 kDa. On the basis of previous findings we wanted to explain what caused the disintegration of a part of fraction M-2 (eluted by 0.2–0.35 M NaCl gradient) and almost the entire M-3 fraction (eluted by 0.35–0.45 M NaCl gradient). It was found that this disintegration is caused by the detergent SDS. Mercaptoethanol (10%) or 8 M urea are without effect. Also, SDS gel filtration on Sephadex G-75 preparative column confirmed the finding. The two fractions obtained (Fig. 6) differed in their molecular mass as determined by SDS-PAGE (Fig. 7). After the removal of SDS, the SDS-2 fraction was again associated into the 67-kDa protein. Both SDS-PAGE and gel filtration analysis showed the SDS-1 fraction as a homogenous protein with a molecular mass 67 kDa. Thus, anorectic protein is partially dissociated in the presence of the detergent SDS into smaller proteins, and both cause the same anorectic effect when administered IP in mice.

Most of our findings support the idea that the protein from urine is similar to satietin. Table 6 summarizes the properties of the isolated protein: its molecular mass was 67 kD. The molecular mass of anorectic proteins of different origin fluctuates between 50 and 70 kDa (1,17,13). This active fraction is a glycoprotein. The data given by different authors are controversial. Ako et al. (1) found no detectable carbohydrates. Chalmers et al. (9) recorded 1% carbohydrate content. On the contrary, satietin and proteins of membranous origin were found to be glycoproteins with 70–75% of carbohydrates (13,18). We used the method of Schiff staining after SDS-PAGE and detected carbohydrates in proteins with a molecular mass of 67 and 9–11 kDa.

There is important information concerning the biological activity of the isolated protein. As opposed to Lee at al. (21), we showed that the anorectic activity is dose dependent in the range of 5–80 mg/kg (Table 2). The dose corresponding to ED_{50} of the M-2 fraction was in agreement with the result of Ako et al. (1), despite different models (mice) using the same way of administration (IP). Comparing the activity of the compound isolated from urine after IP administration in mice and in rat, we found that in mice the substance was four times less active (equipotent doses 40 mg/kg and 10 mg/kg). We compared also the biological activities of anorectic protein fractions with those of cholecystokinin octapeptide and its analogs (24). We studied the time course of anorectic effects of CCK-8 and anorectic protein (see Results). Our finding, that shortly after the administration of anorectic protein animals consume (in a dose-dependent way) certain amounts of food, and then for a long period of time they take minimal amounts of food, is the same as that of Knoll (19). The time course of the anorectic effect of CCK-8 was different (see Fig. 4). The difference could be caused by slower absorption of the

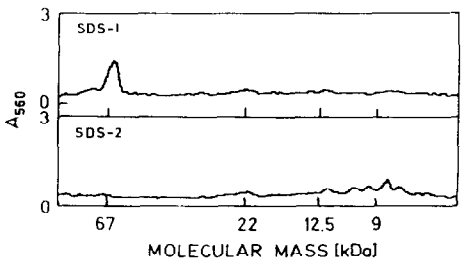

FIG. 7. Denzitometric scanning of SDS-PAGE gels. Fractions SDS-1 and SDS-2 were analyzed by SDS-PAGE in the Laemmli system (18).

relatively large anorectic protein or by its subsequent splitting.

Neither the peripheral nor the central effect of the anorectic protein in mice was the same as that of satietin. In mice, after intracisternal administration, fraction M-2 has similar activity as after IP administration on a dose basis (mg/kg). However, comparing molarities which the substance can achieve after these two ways of administration, then centrally the compound is much less active if at all. The possibility that the anorectic effect after intracisternal administration is due to leakage into the periphery cannot be excluded. Thus, the authors prefer peripheral action. The peripheral effect in rats was found to be about 20 times lower (comparing equipotent doses 10 mg/kg of our compound and 0.5 mg/kg of satietin). A comparison of the central effects showed that the substance from urine was 250 to 1000 times less active than satietin. However, one should keep in mind that different animal species (mice vs. rats), different ways of administration (intracisternal vs. intracerebroventricular), and different feeding models were used for activity determinations by Knoll (18) and Bellinger et al. (5) and the present authors. The activity ratios, however, pointed to the possibility that metabolites of satietin were isolated and tested, which lost the central effect.

As in the case of satietin (18), the anorectic effect of protein from urine is specific, because there is no positive effect in other bioassays with the exception of sedation. This is in line with the fact that other anorectic agents are also sedative. A sedative effect was also found with satietin in rats after intracerebroventricular administration (6). The sedative effect after intracisternal administration was not determined. The sedative effect of the fractions deserves more attention, especially the determination of its time course. This effect was mainly determined in the way usual for CCK-8-like substances, i.e., 10 min after the substance administration when the effect was strongest. However, in this period of time after M-2 administration, mice normally eat even when their exploratory action is reduced according to the test. However, as already mentioned, the time course of the anorectic effect differs from that of CCK-8; thus, one may expect that the sedative effect may also have a different time course. This would be important to be able to exclude the possibility that mice do not eat because they are sedated. However, we observed mice during the whole anorectic test (for 8 h), and it did not seem to be the case.

We conclude that all results point to the relationship of anorectic protein from urine and plasma. Further studies of the qualities of this protein could contribute to the knowledge of respective metabolic pathways and the mechanisms of its action.

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